CHAPTER 2

REVIEW OF LITERATURE

To date, it is not clear whether the reported age-related slowing of the pulmonary oxygen consumption ($\dot{V}O_2$) and muscle oxygenation (mOxy) responses is the result of a prolonged sedentary lifestyle or a significant effect of aging *per se*. From what evidence is available, it appears that previous 'age-related' adaptations are attenuated in older, well-trained individuals compared to age matched sedentary controls. The majority of research detailing the metabolic responses to changes in work intensity has provided information about aged sedentary individuals. However, little is known as to the benefits of physical training into middle-age on these responses. Therefore, this review has several purposes:

- To review the available literature describing the effect of aging on the $\dot{V}O_2$ and mOxy responses to changes in work intensity;
- To discuss the nature of the on- and off-transient $\dot{V}O_2$ and mOxy kinetic responses across increasing exercise intensities;
- To identify and discuss any possible causal or contributing mechanisms of the $\dot{V}O_2$ and mOxy slow component during high-intensity exercise;
- To discuss physiological factors that may influence the nature of the on- and off-transient $\dot{V}O_2$ and mOxy responses; and,
- To review the literature describing the effects of physical training on the $\dot{V}O_2$ and mOxy responses across increasing exercise intensities.

BACKGROUND INFORMATION

Historically, an important area of exercise physiology research has focused upon the adaptation of $\dot{V}O_2$, heart rate (HR) and mOxy to adapt to changes in work intensity (Zoladz, Duda and Majerczak 1998; Tschakovsky and Hughson 1999; Xu and Rhodes 1999; Bangsbo 2000; Borsheim and Bahr 2003). Within this area of research, a topic of much debate relates to the factors associated with the observed exponential increases in $\dot{V}O_2$ and mOxy with changes in work rate, and whether the mechanisms underpinning these are responses are limited by O_2 utilisation or delivery (Richardson, Grassi, Gavin, Haseler, Tagore, Roca and Wagner 1999; Grassi 2000; Grassi 2001; Grassi 2005; Jones and Poole 2005b). Similar debate has focused upon the off-transient $\dot{V}O_2$ and mOxy responses. However, fewer studies have reported on the nature of the off-transient $\dot{V}O_2$ and mOxy responses (Chilibeck, Paterson and Cunningham 1995; Puente-Maestu, Tena, Trascasa, Perez-Parra, Godoy, Garcia and Stringer 2003; duManoir, Delorey, Heenan, Kowalchuk and Paterson 2005).

Substantial research has also reported upon a gradual drift in $\dot{V}O_2$ during high-intensity constant-load exercise which has been termed the $\dot{V}O_2$ slow component (Poole, Schaffartzik, Knight, Derion, Kennedy, Guy, Prediletto and Wagner 1991; Barstow 1994; Poole, Barstow, Gaesser, Willis and Whipp 1994). Such a phenomenon has recently been observed in the mOxy responses to similar exercise intensities (Miur et al. 1999; Demarie et al. 2001). To date, no definitive causal mechanisms have been identified to explain the $\dot{V}O_2$ and mOxy slow components, although a number have been proposed, including increases in muscle temperature, decreases in muscle pH, and/or

shifts in muscle fibre recruitment patterns (Poole 1994; Poole et al. 1994; Whipp 1994; Xu and Rhodes 1999; Borrani, Candau, Millet, Perrey, Fuchslocher and Rouillon 2001; Zoladz and Korzeniewski 2001; Garland, Newham and Turner 2004).

Given that the $\dot{V}O_2$ response to exercise is related to both the extraction and consumption of O_2 within the muscle, more recent and novel research using Near Infrared Spectroscopy (NIRS) has enabled the investigation of changes in mOxy during periods of exercise transition (Mancini 1997; Neary 2004). These studies have provided data to help understand the relationship between pulmonary $\dot{V}O_2$ responses and real-time changes in O_2 content within the working muscle (Bhambhani 2004; Neary 2004). However, despite the recent application of NIRS, limited studies have investigated the relationships between concurrent $\dot{V}O_2$ and mOxy kinetics, particularly within trained and/or aged populations (Babcock et al. 1992; 1994a; 1994b; DeLorey, Kowalchuk and Paterson 2003a; 2003b; Grassi et al. 2003; Stathokostas et al. 2003). Therefore, this review of literature aims to primarily discuss the nature of metabolic adaptation throughout exercise transitions, and secondly, to discuss the effects of physical training and age on these metabolic responses.

Measurement of Oxygen Uptake Responses

The quantification of $\dot{V}O_2$ kinetics is often utilised as a measure of an individual's ability to metabolically adapt to changes in work intensities (Jones and Poole 2005a). Changes in the utilisation and delivery of O_2 are required to allow increased aerobic energy metabolism and maintenance of the cellular homeostatic environment (Tschakovsky and Hughson 1999; Xu and Rhodes

1999; Bangsbo 2000). Measurement of $\dot{V}O_2$ kinetics has historically been performed using mass spectrometers or automated gas analysis systems for over thirty years (Whipp and Wasserman 1972; Xu and Rhodes 1999), and is based upon the measurement of O_2 and CO_2 concentrations of the expired air from the lungs.

At exercise onset, the $\dot{V}O_2$ response follows an exponential path until it reaches a steady-state matched to the required rate of aerobic ATP production of the exercise intensity (Tschakovsky and Hughson 1999; Bangsbo 2000). However, $\dot{V}O_2$ does not instantaneously increase to the amplitude required to fulfil the aerobic metabolism demands (Di Prampero, Boutellier and Pietsch 1983; Barstow, Casaburi and Wasserman 1993; Chilibeck et al. 1998). There is a lagging of the $\dot{V}O_2$ response that has been related to a number of physiological mechanisms including the delay in the return of venous mixed O_2-content blood, muscle phosphocreatine (PCr) kinetics, O_2 delivery to the working muscle and the overcoming of metabolic inertia (Tschakovsky and Hughson 1999; Bangsbo 2000). The magnitude of the mismatch between $\dot{V}O_2$ demand and actual $\dot{V}O_2$ response is termed the O_2 deficit (Linnarsson, Karlsson, Fagraeus and Saltin 1974; Bearden and Moffatt 2000). The nature of the exponential $\dot{V}O_2$ increase at exercise onset is commonly quantified by exponential equations which provide a number of amplitude and speed parameters that can be used for analysis of the on-transient response (Morton 1985; Swanson and Hughson 1988; Barstow 1994; Rossiter, Ward, Doyle, Howe, Griffiths and Whipp 1999; Carter et al. 2002).

The measurement and interpretation of the $\dot{V}O_2$ kinetic response helps to provide a great indication and assessment of cardiorespiratory function (Xu and Rhodes 1999). Through these measures, adaptations in metabolic function and cardiorespiratory capacity can be interpreted and quantified (Whipp and Rossiter 2005). However, the use of $\dot{V}O_2$ kinetics does not provide detailed information on the rate at which metabolic adaptations occur within the working muscle but simply provides an overall indication of systemic adaptation and efficiency (Behnke, Barstow and Poole 2005; Whipp and Rossiter 2005).

The monitoring of mOxy helps to describe changes in both muscle O_2 extraction in response to imposed work bouts and changes in the working muscles capacity for O_2 utilisation (Maikala and Bhambhani 1999; Boushel and Piantadosi 2000; Quaresima and Ferrari 2002a). The concurrent measurement of both $\dot{V}O_2$ and mOxy helps to provide measures of both systemic and peripheral responses to exercise. The on-transient $\dot{V}O_2$ response is multifactorial, and relies upon the capacity to rapidly change the delivery and utilisation of O_2 within the working muscle. However, while the measurement of $\dot{V}O_2$ kinetics provides an adequate measure of such capacity, the monitoring of O_2 utilisation through the use of NIRS within the working muscle may help to identify peripheral limitations to this metabolic adaptation (Mancini 1997; Ding et al. 2001).

Measurement of Muscle Oxygenation Responses

While the description of pulmonary $\dot{V}O_2$ kinetics provides a valid and useful indication of cardiorespiratory function, it does not allow the measurement of changes in the O_2 content of the working muscle. The

monitoring of changes in mOxy during the adaptation to work bouts may provide useful information on both the utilisation of O_2 within the working muscle and peripheral oxygen utilisation limitations (Belardinelli et al. 1995a; 1995b). Such observations may contribute to the O_2 utilisation or delivery limitation debate and provide data to explain the development of the $\dot{V}O_2$ slow component. Changes in mOxy following exercise bouts may also give useful information as to the rate of muscle reoxygenation and recovery (Puente-Maestu et al. 2003; duManoir et al. 2005). Thus, the measurement and quantification of mOxy helps to provide an indirect non-invasive assessment of the rate of O_2 extraction within the working muscle, allowing greater insight into intra-muscular $\dot{V}O_2$ kinetics (Quaresima and Ferrari 2002b; Quaresima, Lepanto and Ferrari 2003; Neary 2004).

NIRS is a relatively recent research technology which allows the real time quantification of mOxy within working muscle during exercise (Quaresima and Ferrari 2002a; Quaresima et al. 2003; Neary 2004). NIRS measures changes in the optical density of light shone into the muscle to quantify changes in mOxy throughout exercise. NIRS systems monitor the optical density of the light reflecting out of the muscle at 760 nm and 850 nm, which correspond to concentrations of Hb/Mb and HbO_2/MbO_2, respectively (Chance et al. 1992). Relative changes in mOxy status are commonly interpreted as the difference between the optical density at the two wavelengths (Δ760-850 nm) (Chance et al. 1992; van Beekvelt, Colier, Wevers and van Engelen 2001). The theoretical basis of NIRS relies upon the Beer-Lambert law modified for scattering media (Schmidt 1999; Boushel and Piantadosi 2000; Quaresima et al. 2003).

The modified Beer-Lambert law states that the amount of light recovered from an illuminated tissue depends upon the intensity of incident light on the tissue, the physical separation of the diodes and photodetectors, the degree of light scattered by tissue, and the amount of tissue absorbency due to chromophore concentration within the tissue (Maikala and Bhambhani 1999). The intensity of the light returning to the photodetectors is dependent upon how saturated the localised muscle and surrounding microvascular structures are with Hb and HbO_2 (i.e. arterioles, capillaries and venules) (DeLorey et al. 2003b).

The valid monitoring of changes in mOxy is subject to a number of methodological limitations which may impact upon the interpretation of the trends in mOxy. Firstly, given that the absorption spectra of myoglobin (Mb) and oxymyoglobin (MbO_2) overlap that of Hb and HbO_2, and therefore the specific concentrations of the two chromophores can not be separated. As such, changes within mOxy are interpreted as changes in the oxygenation state of both HbO_2 and MbO_2 stores (Chance et al. 1992). There is debate as to the contribution of Mb to changes in mOxy, with some researchers suggesting it may contribute as much as 25-35% (Chance et al. 1992). Secondly, the differential path length of the NIR light cannot be measured, and as a result the changes in absolute concentrations in Hb/Mb and HbO_2/MbO_2 are not quantifiable (Bhambhani, Maikala, Jeon and Bell 1998). The majority of investigations report relative changes in mOxy determined through the application of cuff ischemia of the thigh. This technique provides a nadir value of complete tissue deoxygenation (0%) and a hyperaemic response interpreted

as complete reoxygenation (100%) (van Beekvelt et al. 2001; Quaresima and Ferrari 2002a; 2002b).

The thickness of subcutaneous fat below the NIRS probe location may also affect the quality of the NIRS signal within the active muscle (Homma, Fukunaga and Kagaya 1996; Lin, Niwayama, Shiga, Kudo, Takahashi and Yamamoto 2000; Hiroyuki, Hamaoka, Sako, Nishio, Kime, Murakami and Katsumura 2002). Despite reporting this, Homma et al. (1996) observed that NIR light penetrates shallow portions (2-4 cm) of the muscle despite an adipose tissue thickness of up to 15 mm. Hiroyuki et al. (2002) suggested that this limitation of varying subcutaneous fat thickness may be minimised by normalising individual NIRS signals through use of cuff ischemia as discussed above, reporting localised subcutaneous fat measurements, and ensuring homogenous subject characteristics.

Given the small area of muscle monitored by the NIRS probe, the changes observed in mOxy are accepted as being reflective of the whole muscle. Historically, the NIRS probe is positioned over the belly of the VL muscle, 14 cm superior to the patella (Chance et al. 1992; Sahlin 1992; Belardinelli et al. 1995a; Bhambhani, Buckley and Susaki 1997; Costes, Prieur, Feasson, Geyssant, Barthelemy and Denis 2001; Neary, McKenzie and Bhambhani 2002; Grassi et al. 2003). As this point is reported to be a motor point of the VL, it should therefore reflect all recruitment and metabolic activities of the muscle (Kendall et al. 1993). A third limitation of the technology, is that the scattering of light within the muscle may not be consistent within or between

an individual and therefore the use of a constant scattering coefficient may overestimate changes in NIRS variables during exercise (Ferreira et al. 2007).

Despite its limitations, the use of NIRS technology allows the continuous *in vivo* measurement of mOxy during exercise transitions (Chance et al. 1992; Quaresima et al. 2003). Changes in mOxy during exercise reflect the balance between O_2 delivery and utilisation within the localised working muscle, and published data strongly suggest that the use of NIRS is both valid and reliable (Bhambhani et al. 1998). It is suggested that the monitoring of mOxy during exercise transitions provides much more specific and valid measures of changes in the muscle $\dot{V}O_2$, than the systemic $\dot{V}O_2$ measured at the mouth (Maikala and Bhambhani 1999; Boushel and Piantadosi 2000; Neary 2004). However, the concurrent monitoring of both $\dot{V}O_2$ and mOxy kinetics during exercise transitions may allow a greater understanding of the rate at which O_2 is extracted within the working muscle (Quaresima et al. 2003; Neary 2004). The comparison of such systemic and peripheral measures of O_2 consumption or extraction is likely to provide useful information regarding the metabolic adaptation during both the on- and off-transients to an exercise bout.

ON-TRANSIENT KINETIC RESPONSES

The $\dot{V}O_2$ and mOxy responses to SWT are most commonly fitted using either a single or double-exponential function to quantify the magnitude and speed of the response. Whipp and Wasserman (1972) were the first to report upon changes in $\dot{V}O_2$ kinetics in terms of fitting the on-transient response to a single component exponential function. These investigators were the first to use such techniques in order to quantify the nature of such metabolic responses.

Historically, the on-transient $\dot{V}O_2$ response has been reported to comprise three separate components as described below (Whipp and Wasserman 1972; Xu and Rhodes 1999). A typical $\dot{V}O_2$ response to moderate-intensity exercise is presented as Figure 2.1.

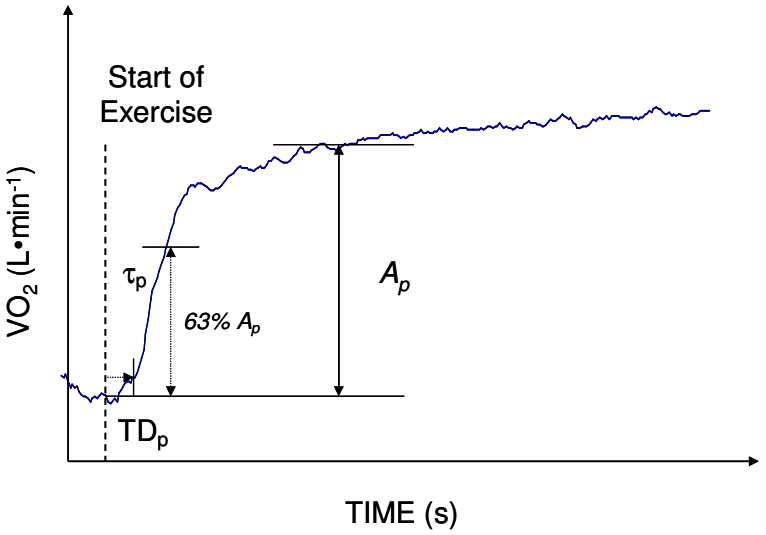

Figure 2.1: Schematic representation of the on-transient $\dot{V}O_2$ response to moderate-intensity steady-state exercise (A_p= Primary component amplitude; TD_p = Primary component time delay; τ_p = Primary component time constant).

1. *Phase I (initial 20 s)*: is termed the *cardiodynamic phase* and represents an initial linear rise in $\dot{V}O_2$ that typical represents the increased ventilation and cardiac responses required of the exercise bout (Jones and Poole 2005).

2. *Phase II*: consists of a rapid exponential increase in $\dot{V}O_2$ in response to the muscle $\dot{V}O_2$ requirements of the working muscle. This increase reflects the influence of the metabolic and O_2 content change within the muscle (Xu and Rhodes 1999). It has also been reported that this phase is reflective of both PCr and muscle O_2 utilisation kinetics (Barstow 1994;

Barstow, Buchthal, Zanconato and Cooper 1994). Phase I and II are often grouped together and comprise the *primary component*.

3. *Phase III:* at intensities <VT it is represented by a steady state $\dot{V}O_2$ matched to the required $\dot{V}O_2$ for maintainance of aerobic ATP production. At exercise intensities >VT, this phase is observed as a decrease in metabolic efficiency or the $\dot{V}O_2$ slow component.

The majority of previous investigations have recommended that the first 20 s of $\dot{V}O_2$ adjustment are removed for analysis as to remove the Phase I dynamics which may influence the fitting of the subsequent functions (Rossiter et al. 1999; Koppo, Bouckaert and Jones 2004). The intensity of the exercise bout determines the nature of the modelling equation. Moderate-intensity (<VT) exercise is universally fitted using a single component function, whereas heavy or severe-intensity exercise (>VT) is fitted using a double component function, assuming a visible slow component is observed (Bearden and Moffat 2001). The use of a double exponential function in order to quantify the $\dot{V}O_2$ slow component has been shown to be a much more valid and accurate quantification method compared to the calculation of the absolute difference between the third and sixth minute of a high-intensity SWT (Bearden and Moffat 2001).

In regards to the adaptation of mOxy at the onset of exercise, the adaptation to the required steady-state values is significantly faster than that of the $\dot{V}O_2$ response (Kawaguchi, Tabusadani, Sekikawa, Hayashi and Onari

2001; DeLorey, Kowalchuk and Paterson 2002; Grassi et al. 2003; DeLorey et al. 2003b; 2004a; 2005).

Research from Chance and others (1992) and Belardinelli and colleagues (1995b) has previously proposed that the on-transient mOxy response to exercise comprises a three phase response (similar to $\dot{V}O_2$):

1. *Phase I:* is represented by an immediate increase in mOxy in comparison to resting baseline values. This is most likely due to an increase in limb blood flow, and HbO_2 concentration (Belardinelli, Barstow et al. 1995b).

2. *Phase II:* is represented by an exponential decline in mOxy from the hyperaemic value which decreased below resting values (in response to a load) until the O_2 utilisation meets the aerobic metabolism demands. This phase coincides with the rapid exponential increase witnessed in $\dot{V}O_2$ measured at the mouth.

3. *Phase III:* is characterised during moderate-intensity constant load exercise by a plateau in mOxy that is equal to the O_2 consumption required for the energy intensity. During high-intensity exercise (>VT), the gradual decrease in mOxy is thought to represent the development of the slow component within the working muscle (Demarie et al. 2001).

The on-transient mOxy response is quantified using similar modelling techniques to those described above for the $\dot{V}O_2$ response. A number of previous investigations have demonstrated that mOxy responses during both

exercise on- and off-transition periods could be fitted to either single or double-exponential component functions (Bhambhani et al. 1999; Grassi et al. 2003; Shibuya, Tanaka and Ogaki 2004).

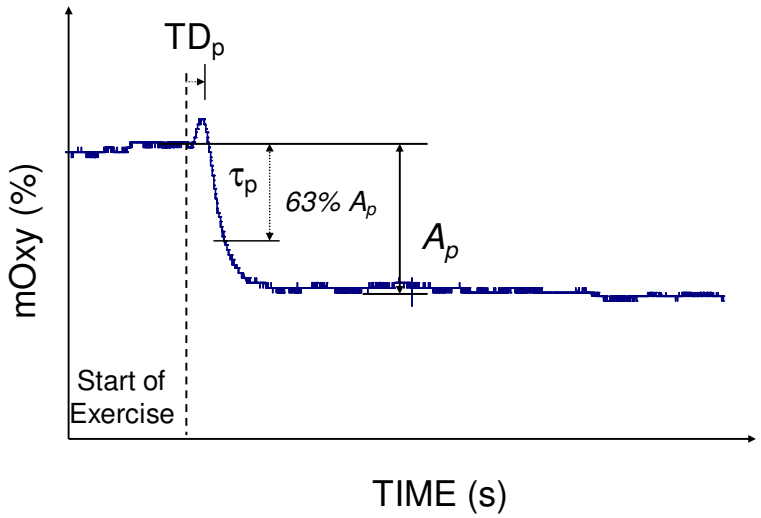

Figure 2.2: Schematic representation of the primary mOxy on-transient response to moderate-intensity exercise (A_p= Primary component amplitude; TD_p = Primary component time delay; τ_p = Primary component time constant).

Another commonly used kinetic parameter to describe the overall response of both the combined primary and slow components is the weighted mean response time (wMRT). The wMRT is representative of the time taken to reach 63% of the total $\dot{V}O_2$ or mOxy amplitude to a SWT, rather than an individual component. This value is of interest to researchers given it is equal to a time constant of the total response, which is a method of quantifying speed, and five time constants should attain ~99% of the total amplitude response (Whipp and Rossiter, 2005). Therefore, quantify the time taken to reach 63% of the response amplitude provides a standardise measure of the speed of adaptation. The wMRT is likely to vary between the $\dot{V}O_2$ and mOxy measures, given the quicker response time in changes of mOxy (DeLorey et al. 2002;

DeLorey et al. 2003b; Grassi et al. 2003). Normal wMRT observed for $\dot{V}O_2$ and mOxy responses throughout the on-transient period range from 30-65 s and 15-60 s, respectively (Stathokostas et al. 2003; DeLorey et al. 2004a; DeLorey et al. 2005; duManoir et al. 2005).

The modelling of both the on-transient $\dot{V}O_2$ and mOxy responses is important to help provide a standardised quantitative measurement of the metabolic adaptation to exercise transitions (Walsh and Lee 1998). These kinetic parameters help researchers to both quantify the rate of metabolic adjustment and investigate methods to manipulate the speed of this adjustment (Whipp and Rossiter 2005). Similarly, the amplitude of the $\dot{V}O_2$ and mOxy responses may help to assess both the muscular energetic and O_2 cost of SWT intensities. These measures may also be useful to track changes in cardiorespiratory function with age, physical training or across intensity domains in normal, clinical and athletic populations. Previously, both the amplitude of the $\dot{V}O_2$ and mOxy primary and slow components has been reported to be influenced by a wide range of factors. For the purpose of the current investigation, these primarily relate to aging (Babcock et al. 1992; Chilibeck et al. 1995; Chilibeck et al. 1998), physical training (Casaburi, Storer, Bendov and Wasserman 1987; Gaesser 1994; Edge, Bishop and Duffield 2003; Saunders, Evans, Arngrimsson, Allison and Cureton 2003; Koppo, Bouckaert et al. 2004; Berger, Rittweger, Kwiet, Michaelis, Williams, Tolfrey and Jones 2006; Berger, Tolfrey, Williams and Jones 2006), and/or exercise intensity (Draper, Wood and Fallowfield 2003; Pringle et al. 2003b; Koppo et al. 2004).

With regards to the speed of the metabolic adaptation, researchers commonly report the time delay (TD) and time constant (τ) of each separate component as indices of the rate of this adaptation. At present, the majority of literature describing $\dot{V}O_2$ kinetics has excluded the Phase I component from the modelled $\dot{V}O_2$ response as a result of the lack of $\dot{V}O_2$ adjustment within this 20 s period (Rossiter et al. 1999; Koppo et al. 2004). Zoladz et al. (1998) have suggested that the omission of this first 20 s also removes any physiological changes which may be incurred during the physical response of adaptation to the work load. The primary component TD (TD_p) is defined as the time taken for $\dot{V}O_2$ to initiate its exponential increase following exercise transition. The slow component time delay (TD_s) is the time taken for the slow component to originate after initial exercise onset (Koga et al. 2005).

Normal $\dot{V}O_2$ TD_p and TD_s range between 10-25 s and 100-150 s for the primary and slow $\dot{V}O_2$ components, respectively (Paterson and Whipp 1991; Pringle et al. 2003b; Koppo et al. 2004). Both the $\dot{V}O_2$ TD_p and TD_s appear to be stable across exercise intensities (Pringle et al. 2003b). The fitting of the mOxy response has previously been shown to display a shorter TD than the $\dot{V}O_2$ response, with mOxy TD_p and TD_s ranging from between 3-8 s, and between 70-80 s for the primary and slow components, respectively (Miura et al. 1999; Demarie et al. 2001). This difference is most likely due to the required transport time of blood from the working muscle where it is deoxygenated to the lungs where the $\dot{V}O_2$ is measured at the mouth. Changes in mOxy within the working muscle are instantaneous and are not subject to such transit delays (Grassi et al. 2003; DeLorey et al. 2002; 2003b; 2005).

The majority of literature examining $\dot{V}O_2$ kinetics has focused upon the rapid exponential increase in the on-transient $\dot{V}O_2$ response (Rossiter et al. 1999; Burnley, Jones, Carter and Doust 2000a; Burnley, Jones, Carter and Doust 2000b; Lucia, Hoyos, Santalla, Perez and Chicharro 2002; Koppo et al. 2004). It is unclear as to whether the primary component on-transient $\dot{V}O_2$ kinetics are limited by the delivery of O_2 to the working muscle, or the ability of the muscle to utilise O_2 during exercise transients (Richardson et al. 1999; Grassi 2000; Grassi 2001). Whilst the majority of empirical evidence suggests that the utilisation of O_2 controls $\dot{V}O_2$ adjustment during the initial adaptation, no definitive explanation has been put forward to support this suggestion (Grassi 2005; Kalliokoski, Knuuti and Nuutila 2005). The speed of the rapid exponential $\dot{V}O_2$ increase (Phase II) is most commonly represented through the primary time constant (τ_p), which represents the time taken to reach 63% of the primary component amplitude (Koga et al. 2005). Normal $\dot{V}O_2$ τ_p values reported within the literature range from 15-30 s for SWT adaptation, or between 30-45 s for ramp test in young healthy subjects (Paterson and Whipp 1991; Pringle et al. 2003b; Koppo et al. 2004). The $\dot{V}O_2$ τ_p values may lengthen with factors such as sedentary aging (Babcock et al. 1992; 1994a; 1994b; DeLorey et al. 2004a; 2005), reductions in O_2 delivery or utilisation (Tschakovsky and Hughson 1999; Xu and Rhodes 1999; Bangsbo 2000; Grassi 2005) or disease states (Bauer, Regensteiner, Brass and Hiatt 1999; Pouliou, Nanas, Papamichalopoulos, Kyprianou, Perpati, Mavrou and Roussos 2001; Puente-Maestu et al. 2003). Normal mOxy τ_p have been reported to be much faster than the $\dot{V}O_2$ τ_p, and are typically between 8-16 s in healthy younger and older subjects (DeLorey et al. 2004a; 2005).

The reporting and comparison of these $\dot{V}O_2$ and mOxy amplitude and speed parameters discussed above help to provide a greater understanding of the metabolic responses to bouts of exercise. To date, the influence of exercise intensity on $\dot{V}O_2$ τ_p remains equivocal. Previous research suggests the $\dot{V}O_2$ τ_p either remains constant (Barstow and Mole 1991; Barstow et al. 1993; Carter, Jones, Barstow, Burnley, Williams and Doust 2000a; Ozyener, Rossiter, Ward and Whipp 2001) or lengthens with increasing exercise intensity (Casaburi, Barstow, Robinson and Wasserman 1989; Paterson and Whipp 1991; Phillips, Green, Macdonald and Hughson 1995; Engelen, Porszasz, Riley, Wasserman, Maehara and Barstow 1996; Jones, Carter, Pringle and Campbell 2002; Koppo et al. 2004). The lengthening of the $\dot{V}O_2$ τ_p has been reported in both trained (Koppo et al. 2004) and untrained (Paterson and Whipp 1991) subjects and is thought to represent an increased delay in the utilisation or delivery of O_2 within the muscle in response to the increased exercise intensity (Koppo et al. 2004). In contrast, the suggestion of a stable $\dot{V}O_2$ τ_p is supportive of O_2 utilisation limitations controlling the speed of $\dot{V}O_2$ adjustment regardless of intensity (Carter et al. 2000; Ozyener et al. 2001).

For the on-transient response, the major limiting factors responsible for the lagging metabolic response remain unknown (Grassi 2000; 2001). However, the on-transient response appears to be limited by either the delayed utilisation or delivery of O_2 within the working muscle (Grassi 2005). In order to investigate these factors limiting the on-transient responses, a number of research investigations have attempted to manipulate the delivery or utilisation of O_2 during adaptations to various exercise intensities or modality, lactate and catecholamine concentrations, muscle temperature, histochemical and

biochemical characteristics, training status and age (Poole et al. 1994; Tschakovsky and Hughson 1999; Bangsbo 2000; Zoladz and Korzeniewski 2001; Borsheim and Bahr 2003). To date, these investigations have failed to identify the major limiting factors for metabolic adaptation to an exercise bout.

As discussed above, the relationship between the $\dot{V}O_2$ and mOxy responses is comparable and appears to be influenced by similar factors. Given the recent introduction of NIRS technology, there is limited research literature which has concurrently examined the concurrent $\dot{V}O_2$ and mOxy responses to an exercise bout (Babcock et al. 1992; 1994a; 1994b; Grassi et al. 2003; Stathokostas et al. 2003; DeLorey et al. 2003a; 2003b; 2004a; 2005; duManoir et al. 2005). To date, no research has fully examined the effect of aging and/or exercise intensity on the on-transient mOxy responses.

DeLorey et al. (2003b) investigated the concurrent $\dot{V}O_2$ and mOxy responses in young (26 ± 3 y) healthy subjects in response to moderate-intensity SWT. In this study, the observed $\dot{V}O_2$ τ_p was 30 ± 8 s, whereas the τ_p of the mOxy response was much faster at 10 ± 3 s, suggesting that the extraction of O_2 within the working muscle is more rapid than that observed at the mouth. The wMRT of the mOxy response (23 ± 4 s) was also significantly faster than the pulmonary $\dot{V}O_2$ wMRT (30 ± 8 s). Later work by DeLorey and colleagues (2004a; 2005) investigated the concurrent $\dot{V}O_2$ and mOxy responses across moderate and heavy-intensity exercise in young (24 ± 4 y) and old (68 ± 3 y) sedentary subjects. The investigators reported that the mOxy kinetics were also significantly faster than the $\dot{V}O_2$ response in both groups.

While the elderly cohort demonstrated a significantly slower $\dot{V}O_2$ response than the young subjects, no difference was observed in the mOxy response.

The difference between the $\dot{V}O_2$ and mOxy responses may be due to the factors associated with the utilisation of O_2 within external processes such as the contraction of stabilising and synergistic muscles, intra-muscular buffering reactions, and unrelated metabolic processes (Tschakovsky and Hughson 1999). These suggestions are supported by the previous work of Bangsbo et al. (2000) who observed an initial delay followed by a rapid increase in O_2 extraction within the quadriceps during high-intensity leg-extension exercise which would support the observations observed in mOxy within the data of DeLorey et al. (2003b; 2004a; 2005) and Grassi et al. (2003). Taken together, the findings of these investigators suggest that the decreases within mOxy occur earlier and at a faster rate than the reported exponential increase in pulmonary $\dot{V}O_2$.

Thus, the metabolic adaptation at the onset of an exercise bout is met with rapid exponential increases in $\dot{V}O_2$ and mOxy until the aerobic demands for ATP production are met. While researchers have investigated the $\dot{V}O_2$ response for a number of decades, the introduction of NIRS to monitor changes in O_2 content within the muscle has been of great significance. The reporting of changes in mOxy helps the understanding of the nature of aerobic metabolic adaptation within the working muscle and may be more valid than measures of pulmonary $\dot{V}O_2$ which are open to a number of external influences. However, this NIRS research still does not conclusively suggest that the utilisation of O_2 within the muscle is the only limiting factor at exercise onset. A number of other

mechanisms have been observed to influence the $\dot{V}O_2$ on-transient response (Tschakovsky and Hughson 1999; Xu and Rhodes 1999; Bangsbo 2000).

Factors Influencing the On-Transient Responses

Previous literature has revealed a large number of factors which may influence the on-transient $\dot{V}O_2$ and mOxy responses (Grassi, Poole, Richardson, Knight, Erickson and Wagner 1996; Bangsbo et al. 2000; Grassi 2000; Grassi 2001; Grassi 2005; Jones and Poole 2005b; Whipp et al. 2005). These potential factors may alter either the delivery (i.e. gas concentration, body posture, muscle capillarisation) or utilisation (i.e. catecholamines, $[BLa^-]$, muscle fibre composition and enzyme activity, prior exercise) of O_2 within the working muscle at the onset of exercise. Valid arguments have supported both the O_2 delivery and utilisation hypotheses, and it appears that exercise intensity may influence the limitations which may influence the on-transient metabolic response.

The delivery of O_2 to the working muscle has been suggested to limit the rate of increase in the $\dot{V}O_2$ response to sudden increases in exercise intensity (Hughson and Smyth 1983; Hughson, Xing, Borkhoff and Butler 1991; Wagner 1995; Tschakovsky and Hughson 1999; Whipp et al. 2005). Tschakovsky and Hughson (1999) suggested that O_2 delivery limitations reflect the resting inertia of transporting the O_2 from the lungs in the blood to the mitochondria within the working muscle. That is, oxidative metabolism and O_2 utilisation can only increase if a higher cellular pO_2 is sustained during exercise on-transitions, suggesting that the pO_2 within the mitochondria is not saturated within all working muscle fibres during the adaptation. This model suggests that an

increased delivery of O_2 to the working muscle would allow sustained utilisation of O_2 through oxidative metabolism through maintaining mitochondrial pO_2 across the metabolic adaptation. In their review, Wagner (1995) identified several factors affecting the delivery of O_2 that may limit the rate of $\dot{V}O_2$ adjustment at exercise onset. These include a reduced inspired pO_2, disease, a reduced cardiac output and muscle blood flow, a reduced Hb concentration, or an impaired diffusion of O_2 between red blood cells and the mitochondria. Whilst these are valid factors, it is unlikely that they will be of significant influence to changes in exercise intensity in healthy athletic populations.

In summary, the on-transient $\dot{V}O_2$ response has been proposed to be limited by the rate of adaptation of the delivery or utilisation of O_2 within the working muscle immediately after load application. While the interaction between these limitations is not fully understood at present, a great deal of research has investigated methods to isolate their effects to improve the on-transient $\dot{V}O_2$ adaptation (Xu and Rhodes 1999; Grassi 2001; 2005; Jones and Poole 2005a). The following section will discuss empirical research that has examined mechanisms that may limit the on-transient $\dot{V}O_2$ response through either O_2 utilisation or delivery limitations.

O_2 Delivery Limitations

Catecholamines

Research has investigated the influence of catecholamines on the O_2 utilisation and delivery limitations previously discussed (Hughson and Morrissey 1983; Hughson and Smyth 1983; Hughson et al. 1991; Tschakovsky and

Hughson 1999). However, such research has not proven any such effect of catecholamine secretion on the on-transient metabolic response.

To date, a slowed $\dot{V}O_2$ kinetics response has been observed through the infusion of β-blockers to slow the HR response and reduce O_2 delivery (Hughson and Morrissey 1983). Hughson and Morrissey (1983) investigated the effect of β-blockade on the on-transient $\dot{V}O_2$ response to submaximal exercise (80% VT) in 17 male subjects (21 ± 1 y; 3.84 ± 0.15 $L \bullet min^{-1}$). While no differences were observed in $\dot{V}O_2$ amplitude or [BLa^-], the speed of the $\dot{V}O_2$ response was significantly slowed with β-blockade. The O_2 deficit was ~200 mL larger and cardiac output significantly reduced in the β-blockade condition. These results suggest that reducing O_2 delivery through slowing the HR responses has a significant slowing effect on the on-transient $\dot{V}O_2$ response.

In their later review, Tschakovsky and Hughson (1999) suggested that the influence of catecholamines on $\dot{V}O_2$ adaptation may be the result of the control of the sympathetic nervous system on factors such as HR and stroke volume (SV) during rest or low-intensity exercise (<100 $b \bullet min^{-1}$). This may be due to the sympathetic nervous system's role as a slow-acting mediator in cardiac adaptation during low intensity exercise transitions (Åstrand, Rodahl, Dahl and Stromme 2003). Further, it may be possible that administration of adrenaline may also be responsible for an increase in O_2 utilisation within the working muscle, given previous observations showed an increased glycolytic capacity and acetyl group availability within muscle infused with adrenaline (Watt, Howlett, Febbraio, Spriet and Hargreaves 2001). At present, no research is available to validate this suggestion. However, no evidence has reported a

speeded $\dot{V}O_2$ response across exercise transitions with adrenaline infusion (Gaesser 1994). In summary, the infusion of β-blockade agents has been reported to retard the on-transient $\dot{V}O_2$ response. This finding suggests other O_2 delivery controlling mechanisms.

Inspired Gas Concentrations

Other research has focused upon the influence of modifying gas concentrations on $\dot{V}O_2$ kinetics during exercise on-transients (Linnarsson, Karlsson et al. 1974; Hughson and Kowalchuk 1995; MacDonald, Pedersen and Hughson 1997; Bell, Paterson, Kowalchuk and Cunningham 1999; Evans, Savasi, Heigenhauser and Spriet 2001; Peltonen, Tikkanen and Rusko 2001). These studies have consistently shown that the breathing of either hypoxic or hyperoxic gas significantly influences the nature of the on-transient $\dot{V}O_2$ response.

Hughson and Kowalchuk (1995) investigated the on-transient $\dot{V}O_2$ kinetics in six healthy volunteers (30.3 ± 3.3 y) during moderate-intensity (<VT) cycling exercise in hypoxic (F_IO_2 = 0.14), normoxic (F_IO_2 = 0.21) and hyperoxic (F_IO_2 = 0.30) conditions. The on-transient $\dot{V}O_2$ speed (τ_p; wMRT) were significantly slowed by hypoxia (26.6 ± 2.9 s; 35.9 ± 1.7 s) compared to the normoxic (16.5 ± 2.8 s; 29.5 ± 1.9 s) or hyperoxic (15.7 ± 2.1 s; 28.6 ± 1.8 s) conditions. Furthermore, the O_2 deficit calculated for the hypoxic conditions (525 ± 24 mL) was significantly larger than that observed for either normoxia (420 ± 29 mL) or hyperoxia (414 ± 25 mL) conditions. Therefore, the proposed increased delivery of O_2 through breathing of a hyperoxic gas mixture appears to have no significant influence on the $\dot{V}O_2$ response during adaptation to

moderate-intensity exercise. In contrast, breathing a hypoxic gas to reduce O_2 delivery to the working muscle was observed to slow the $\dot{V}O_2$ response.

In a later study, MacDonald et al. (1997) examined the effects of breathing normoxic (F_IO_2= 0.21) and hyperoxic (F_IO_2= 0.70) gases during adaptation to both moderate (<VT) and heavy-intensity (>VT) SWT. The $\dot{V}O_2$ wMRT was not significantly improved during the moderate-intensity SWT between the hyperoxic (31.4 ± 1.4 s) and normoxic (31.3 ± 1.3 s) conditions. Interestingly, the $\dot{V}O_2$ MRT was significantly faster in the hyperoxic conditions (44.1 ± 5.2 s) compared to the normoxic (53.9 ± 6.2 s) for the heavy-intensity SWT. No difference was observed in the magnitude of the O_2 deficit at exercise onset. The results of MacDonald et al. (1997) further support the absence of a significant effect of hyperoxia during transitions to moderate-intensity exercise, and may suggest possible O_2 delivery limitations during high-intensity transitions in normoxic conditions.

In summary, the breathing of hypoxic gas has been observed to slow the on-transient $\dot{V}O_2$ response as a result of a reduced O_2 delivery within the muscle. In contrast, the inspiration of hyperoxic gas has been shown to improve the on-transient $\dot{V}O_2$ responses to high-intensity exercise, but not with moderate-intensity exercise. Therefore, these results suggest that the delivery of O_2 to the working muscle may not be an issue at moderate-intensity exercise but may play a role in metabolic adaptations to higher-intensity exercise.

Body Posture

Due to the effects of gravity on the blood flow responses, researchers have investigated alterations in the delivery of O_2 to the working muscle through changing the posture of subjects to augment these gravitational effects (Hughson et al. 1991; MacDonald, Shoemaker, Tschakovsky and Hughson 1998; Sirna, Paterson, Kowalchuk and Cunningham 1998; MacDonald, Naylor, Tschakovsky and Hughson 2001).

Originally, Hughson et al. (1991) investigated the kinetics of ventilation and gas exchange variables during supine and upright cycling in 12 healthy young men during moderate-intensity exerrcise. A significant increase was noted in the speed ($\tau_{p;}$ wMRT) of the $\dot{V}O_2$ response of the upright (26.3 ± 1.9 s; 31.6 ± 1.3 s) compared to the supine (35.1 ± 3.8 s; 40.3 ± 2.3 s) position. Hughson et al. (1991) suggested that these results supported the hypothesis that a reduction in the delivery of O_2 to working muscles is due to a gravitational reduction in blood flow when in the supine position.

MacDonald et al. (1998) later investigated alveolar $\dot{V}O_2$ and femoral artery blood flow during upright and supine leg extensor exercise in seven young healthy volunteers during six minutes of knee extension exercise at 40W. The speed (τ_p; wMRT) of the primary $\dot{V}O_2$ adaptation was significantly slower during the supine (38.7 ± 5.1 s; 39.7 ± 3.8 s) as opposed to the upright (23.1 ± 4.1 s; 29.3 ± 3.0 s) condition. This slowing of the $\dot{V}O_2$ response during supine exercise was matched by a non-significant increase in the wMRT of leg blood flow (27.6 ± 3.9 s) compared to the upright condition (17.3 ± 4.0 s). The wMRT for $\dot{V}O_2$ and leg blood flow were reduced by 35% and 60%, respectively,

during supine versus upright exercise as a result of exercise position. Taken together, these findings support the earlier observation of reductions in O_2 delivery influencing the on-transient $\dot{V}O_2$ time constant. However, these results were contrasted by Sirna et al. (1998) who showed no difference between the supine and upright cycling positions in either healthy sedentary young (n= 8, 20-35 y) and old (n= 8, 60-80 y) subjects.

In summary, it appears that reductions in blood flow and O_2 delivery to the working muscle due to changes in body posture may have a significant influence on the on-transient $\dot{V}O_2$ response. The work of MacDonald et al. (1998) supports that the slower on-transient $\dot{V}O_2$ response is related to reductions in blood flow to the working muscle.

Heart Rate Kinetics

The rate of O_2 delivery to the working muscle also has the capacity to significantly influence the on-transient $\dot{V}O_2$ response, particularly to high-intensity exercise. At exercise onset, it appears that HR increases exponentially before reaching a steady state adequate to match the metabolic demands for the work intensity, and the nature of the HR and $\dot{V}O_2$ responses would be somewhat related (Hughson and Morrissey 1983; Kay, Ashar, Bubien and Dailey 1995).

At present, it appears that the HR wMRT is similar to that of $\dot{V}O_2$ for moderate-intensity exercise, but longer for high-intensity exercise (Sietsema, Daly and Wasserman 1989). Sietsema et al. (1989) investigated the influence of work rate on the early dynamics of both $\dot{V}O_2$ and HR in ten healthy young

male subjects (29-42 y; 47 ± 14 $mL \cdot kg^{-1} \cdot min^{-1}$) across a number of various-intensity six minute SWT (25 W, 50 W, 100 W and 150 W). The researchers suggested that the early dynamics of HR and $\dot{V}O_2$ were dependent on both the exercise intensity and the individual's aerobic fitness level. This observation may be the result of the use of absolute rather than relative work loads in this investigation. Later research by Chilibeck et al. (1996b) reported upon the cardiorespiratory kinetics during bouts of plantar flexion and cycling in young (Y) (26.3 ± 2.5 y) and old (O) (66.7 ± 6.7 y) groups at varying exercise intensities. The younger cohort demonstrated a significantly shorter on-transient HR τ than the elderly cohort for the cycling exercise (Y: 46.4 ± 22.3 s; O: 73.1 ± 36.4 s) but not the plantar flexion exercise (Y: 35.3 ± 28.0 s; O: 32.6 ± 29.3 s) which was also observed in the $\dot{V}O_2$ response. It is likely that this is a result of the cycling exercise being performed at 90% VT, whereas the plantar flexion was performed at 45% peak work rate, suggesting significantly different exercise intensities. The HR τ was strongly correlated to the $\dot{V}O_2$ τ_p during both the cycling and plantar flexion exercise for the young group and the cycling modality for the elderly group (Chilibeck et al. 1996a). The researchers suggested that this may reflect a relationship between the $\dot{V}O_2$ response and the adaptation of O_2 delivery at the onset of exercise.

In summary, these results provide equivocal findings suggesting that HR kinetics influence the on-transient $\dot{V}O_2$ response. A slowed HR response would result in a reduction in the speed of O_2 delivery across exercise intensities. The above evidence suggests O_2 delivery limitations control the speed of metabolic adaptation at exercise onset, in particular to high-intensity constant load exercise.

Lactate

Lactate is a common by-product and widely used measure of anaerobic metabolism, together with decreases in muscle and blood pH (Åstrand et al. 2003). Previous research has observed that [BLa^-] is correlated with a number of speed and amplitude parameters from the $\dot{V}O_2$ and mOxy responses (Roston, Whipp, Davis, Cunningham, Effros and Wasserman 1987; Barstow et al. 1993; Demarie et al. 2001; Jones, Koppo and Burnley 2003). A rightward shift noted in the HbO_2 dissociation curve due to a decrease in muscle and blood pH may help to improve O_2 delivery during exercise transients and act to accelerate the $\dot{V}O_2$ and mOxy responses (Roston et al. 1987; Gaesser and Poole 1996; Grassi et al. 1999; Demarie et al. 2001).

Edge et al. (2003) recently investigated the $\dot{V}O_2$ response at the onset of high intensity exercise and the accumulation of metabolites in the blood and muscle in 17 active young females. A significant correlation was observed between the $\dot{V}O_2$ τ_p and the change in [BLa^-] across the high-intensity exercise bout ($r= 0.60$, $p<0.05$). The O_2 deficit was significantly related to changes in both muscle ATP stores ($r= 0.64$, $p<0.05$) and blood pH ($r= 0.51$, $p<0.05$), but not [BLa^-]. The results of Edge et al. (2003) contradicted previous findings that observed no relationship between the primary $\dot{V}O_2$ response and [BLa^-] accumulation. More recently, Endo, Usui, Fukuoka, Miura, Rossiter and Fukuba (2004) reported that the elevation of [BLa^-] by prior supra-threshold intensity was not significantly related to the on-transient $\dot{V}O_2$ τ_p. These investigators did observe a significant inverse relationship ($r= -0.41$, $p<0.05$) between the $\dot{V}O_2$ τ_p across a wide range of [BLa^-]. Similar results were previously presented by

Burnley, Doust and Jones (2002b) throughout bouts of heavy-intensity and maximal sprint exercise.

Researchers have also suggested that increased [BLa^-] and decreased muscle and blood pH resulting from high-intensity exercise may facilitate a rightward shift in the HbO_2 dissociation curve to increase O_2 delivery to within the muscle cell (Stringer, Wasserman, Casaburi, Porszasz, Maehara and French 1994). Given that the mOxy is calculated as the difference between total Hb and HbO_2 (Chance et al. 1992), any factor which allows greater release of O_2 from Hb will allow greater muscle deoxygenation. To date, no data have demonstrated significant relationships between the on-transient mOxy responses and changes in muscle or blood pH during exercise.

In summary, recent research suggests that the concentration of [BLa^-] either influences or is related to the on-transient $\dot{V}O_2$ and mOxy responses to exercise. The effect of an increased [BLa^-] *per se* on these metabolic responses is unknown, given the additional changes in muscle metabolism that result from prior exercise.

Muscle Temperature

It has been widely suggested that changes in muscle temperature may influence the on-transient $\dot{V}O_2$ response through either the Q_{10} effect, vasoconstriction or vasodilation responses, or the Bohr effect (Beelen and Sargeant 1991; Koga, Shiojiri, Kondo and Barstow 1997; Shiojiri, Shibasaki, Aoki, Kondo and Koga 1997; Binzoni and Delpy 2001; Ferguson, Ball and Sargeant 2002). In further support of this suggestion, previous research has

shown that $\dot{V}O_2$ kinetics are slowed with reduced muscle temperature (Ishii, Ferretti and Cerretelli 1992; Ferretti, Binzoni, Hulo, Kayser, Thomet and Cerretelli 1995). However, no evidence has shown that increases in muscle temperature through hot water convection (Koga et al. 1997) or prior exercise (Koppo, Jones, Vanden Bossche and Bouckaert 2002) speeds the on-transient $\dot{V}O_2$ response.

Ishii et al. (1992) investigated $\dot{V}O_2$ kinetics in response to cycling at 75 W and 125 W following a reduction in muscle temperature from 35.5 ± 1.0 ºC to 28.0 ± 1.6 ºC due to cold water immersion in six untrained men (31 ± 8 y). The results showed that the O_2 deficit for the cold condition was slightly increased compared to normal muscle temperature at both 75 W (803 vs. 714 mL) and 125 W (1360 vs. 1283 mL). The $\dot{V}O_2$ τ_p was slightly longer following the cold-water immersion (41.4 ± 10.0 vs. 36.2 ± 6.7 s @ 75 W; 43.8 ± 14.0 vs. 41.6 ± 8.6 s @ 125 W). In support of these findings, Ferretti et al. (1995) later reported that the half-time (τ ½) of the $\dot{V}O_2$ response (43.4 ± 8.6; 33.3 ± 5.0 s) and O_2 deficit (3.05 ± 1.12; 2.30 ± 0.68 L) were significantly longer and greater in cold (27.5 ± 1.8 ºC) versus normal temperature (34.5 ± 1.2 ºC) conditions during heavy-intensity cycling. The proposed mechanism responsible was a leftward shift in the HbO_2 dissociation curve as a result of a decreased muscle and blood temperature which may have decreased the pO_2 within the working muscle.

Of greater interest is whether the $\dot{V}O_2$ and mOxy kinetic responses are improved through the elevation of muscle temperature. Koga et al. (1997) elevated the temperature of the thigh muscle to ~39ºC in young male subjects

(25.7 ± 9.2 y; 44.5 ± 9.8 $mL \cdot kg^{-1} \cdot min^{-1}$) using hot water pants prior to completing repeat SWT at intensities of 50W and 50% of the difference between VT and $\dot{V}O_2max$ (50%Δ). The results revealed no significant differences in the $\dot{V}O_2$ τ_p, amplitude or economy of exercise between muscle temperature conditions. More recently, Koppo et al. (2002) suggested that the speeding of the on-transient $\dot{V}O_2$ response observed with higher muscle temperatures was through the alleviation of any controlling O_2 delivery or utilisation limitations, despite not supporting this with results. This mechanism was supported by Burnley et al. (2002c) who reported no significant improvement in $\dot{V}O_2$ kinetics through the passive warming of muscle. Therefore, it appears as though the heating of muscle prior to exercise does not facilitate a sufficient shift in the HbO_2 dissociation curve to speed the on-transient metabolic responses.

In summary, a decrease in muscle temperature have been shown to slow the on-transient $\dot{V}O_2$ response, while a rise in muscle temperature appears to have no impact on the $\dot{V}O_2$ response in the studies discussed above. Specifically, the reduction in muscle temperature and decreased O_2 availability has been related to a slowed $\dot{V}O_2$ response, most likely as a result of a lefttward shift in the HbO_2 dissociation curve. In contrast, the on-transient $\dot{V}O_2$ response is not significantly speeded after prior warming of the recruited muscles.

In conclusion, a number of research investigations have suggested that the on-transient $\dot{V}O_2$ response is limited by O_2 delivery factors (Hughson and Smyth 1983; Hughson et al. 1991; Wagner 1995; Tschakovsky and Hughson

1999; Whipp et al. 2005). The significant effect of these O_2 delivery influences have only been observed during adaptation to high-intensity exercise (MacDonald et al. 1997). Alternatively, the majority of contemporary literature has refuted that the on-transient $\dot{V}O_2$ response is limited by the delivery and availability, but rather suggest that the capacity to utilise O_2 within the working muscle controls the speed of adaptation (Grassi et al. 1996; DeLorey et al. 2004a; 2005; Grassi 2005). Factors affecting the utilisation of O_2 within the muscle which may be related to the on-transient responses are discussed below.

O_2 Utilisation Limitations

Prior Exercise

Many studies have supported a faster exponential increase in the $\dot{V}O_2$ response following prior exercise (Gerbino, Ward and Whipp 1996; MacDonald et al. 1997; Bearden and Moffatt 2000; Koppo and Bouckaert 2002; Endo et al. 2004). The majority of literature has shown that prior exercise below VT has no speeding effect on $\dot{V}O_2$ kinetics, whereas a faster on-transient $\dot{V}O_2$ response has been observed following prior high-intensity (>VT) exercise (Gerbino, Ward et al. 1996; MacDonald et al. 1997; Burnley, Doust, Carter and Jones 2001; Koppo and Bouckaert 2001; 2002; Koppo et al. 2002; Jones et al. 2003).

The most likely explanation for the speeded $\dot{V}O_2$ response observed following prior exercise is the reduction of the metabolic inertia required to be overcome prior to the metabolic adaptation (Gerbino et al. 1996; MacDonald et al. 1997; Burnley et al. 2001; Rossiter et al. 2001). This may be due to increases in the activities of several inter- and intra-cellular metabolites and

oxidative enzyme related to muscle $\dot{V}O_2$ (Bangsbo 2000). Previously, a number of investigations noted that oxidative enzyme activities are increased with prior exercise (Tokonogi, Harris and Sahlin 1997; Burnley et al. 2001; Leek, Mudaliar, Henry, Mathieu-Costello and Richardson 2001; Burnley, Doust, Ball and Jones 2002a). Burnley et al. (2002a) highlighted that improved $\dot{V}O_2$ kinetics through prior exercise has only been noted for exercise transients when preceded by exercise of sufficient intensity to decrease blood pH which may increase O_2 availability via the Bohr effect and metabolic vasodilation. Furthermore, Timmons et al. (1998a) hypothesised that the recovery kinetics of other influential muscle hormones (adrenaline, noradrenaline) and metabolites (K^+) may be too quick to influence the $\dot{V}O_2$ response following prior exercise. This suggests that the prolonged increased activities of oxidative enzymes may play a role in the faster adaptation of O_2 utilisation.

To date, only one study by DeLorey, Kowalchuck and Paterson (2004b) has reported upon the effect of prior exercise on the concurrent $\dot{V}O_2$ and mOxy kinetics in response to a moderate-intensity SWT test in both young (25 ± 3 y) and old (68 ± 3 y) healthy male subjects. The researchers reported that following a prior heavy-intensity warm-up (HWU) the $\dot{V}O_2$ τ_p was significantly shorter than no warm-up (NWU) in the older subjects (NWU: 38 ± 5 s; HWU: 30 ± 7 s) but not in the young subjects (NWU: 26 ± 7 s; HWU: 25 ± 5 s). Further, a significant effect of age was observed between the $\dot{V}O_2$ τ_p in the no warm up condition, but was not present in the warm-up intervention. In terms of the mOxy response, the TD_p was significantly shorter in both the young (NWU: 12 ± 2 s; HWU: 10 ± 2 s) and old (NWU: 11 ± 2 s; HWU: 8 ± 2 s) age-groups following the high-intensity warm up. Interestingly, the mOxy τ_p significantly

lengthened following the high-intensity warm-up in both age-groups (Y: NWU: 11 ± 10 s; HWU: 14 ± 4 s: O: NWU: 9 ± 3 s; HWU: 32 ± 17 s). It is likely that the high-intensity warm-up was responsible for an increase in both skin and muscle temperature, which have recently been shown to significantly affect the mOxy response during exercise due to vasodilation responses (Davis, Fadel, Cui, Thomas and Crandall 2006). The high-intensity warm-up may have reduced the metabolic inertia required to be overcome at exercise onset, allowing faster adaptation of muscle O_2 extraction and consumption.

In summary, it is likely that prior exercise may help to alleviate any O_2 delivery or utilisation constraints which limit the $\dot{V}O_2$ and mOxy adjustment to exercise. The speeding of $\dot{V}O_2$ kinetics as a result of prior exercise is most likely facilitated by increased blood flow, enzyme activities, and increased HbO_2 dissociation within the blood (Jones et al. 2003). However, there would appear to be a number of enzymes or reactions which may be responsible for limiting the rate of O_2 extraction and consumption at the onset of exercise by controlling the rate of change in oxidative phosphorylation. The overcoming of the metabolic inertia at exercise onset due to the prior exercise is most likely due to changes in the muscle energetics within the working muscle (Grassi 2005).

Muscle Energetics

It is well established that the utilisation of O_2 and flux of energy pathways is dependent upon the activity of oxidative enzymes within the mitochondria (Sahlin, Ren and Broberg 1988; Greenhaff and Timmons 1998; Timmons et al. 1998a; Timmons, Gustafsson, Sundberg, Jansson, Hultman, Kaijser, Chwalbinska-Moneta, Constantin-Teodosiu, Macdonald and Greenhaff 1998b;

Bangsbo 2000; Bell, Paterson, Kowalchuk, Moy, Thorp, Noble, Taylor and Cunningham 2001; Russ and Kent-Braun 2004). Furthermore, it appears that the initial improvements in muscle energetics are responsible for stimulating ATP production and O_2 utilisation within the muscle which control or influence the $\dot{V}O_2$ and mOxy responses (Barstow et al. 1994; Grassi 2005).

Sahlin et al. (1988) suggested an alternative biochemical explanation for the development of the O_2 deficit at the onset of exercise. They suggested that the O_2 deficit is dependent upon the regulation of cellular changes in muscle metabolites such as Adenosine Diphosphate (ADP), Inorganic Phosphate (P_i), and Nicotinamide Adenine Dinucleotide (NADH). Sahlin, Ren et al. (1988) further suggested that the initial breakdown of ATP at exercise onset stimulates ATP production and PCr breakdown, releasing free P_i to stimulate glycogenolysis and glycolysis due to the increase in the concentration of low level phosphates. They also suggest that this increased anaerobic energy turnover may reduce tissue $\dot{V}O_2$ demands and be evidenced by a decreased or stable $\dot{V}O_2$ at exercise onset. However, when a steady state of ADP and P_i is attained, mitochondrial respiration and $\dot{V}O_2$ will also remain constant at sub-maximal workloads.

Similarly, the oxidative capacity of muscle may also affect the rate at which $\dot{V}O_2$ is able to be utilised within the muscle cell (Grassi 2000; 2005). In particular, this may be reflected by the maximal activities of several oxidative enzymes [pyruvate dehydrogenase (PDH), citrate synthase (CS), and 2-oxoglutarate dehydrogenase (2-OGDH)] which have been proposed to limit the

rate of Tricarboxylic Acid (TCA) cycle flux and muscle $\dot{V}O_2$ (Russ and Kent-Braun 2004).

Pyruvate Dehydrogenase

PDH is considered the rate limiting enzyme for the degradation of pyruvate in skeletal muscle and has been closely linked to muscle $\dot{V}O_2$ (Bangsbo, Gibala, Krustrup, Gonzalez-Alonso and Saltin 2002). The activity of PDH during exercise transients has been suggested to limit the speed of $\dot{V}O_2$ adjustment as it provides acetyl groups to the necessary energy pathways (Greenhaff and Timmons 1998; Bangsbo 2000; Jones et al. 2003).

Originally, Greenhaff and Timmons (1998) suggested that O_2 utilisation is limited during exercise on-transients due to an insufficient production of acetyl-CoA for the TCA cycle. This inadequate supply of acetyl-CoA is most likely the result of the delayed activation of PDH (Greenhaff and Timmons 1998). In support of this suggestion, Parolin et al. (2000) demonstrated that at the onset of exercise ~86% of PDH is activated after 15 s, which is approximately the same length as Phase I of the $\dot{V}O_2$ response, suggesting the kinetics of PDH activation may be closely linked to the exponential increase in $\dot{V}O_2$.

To test this hypothesis, a number of researchers stimulated PDH prior to performing an exercise bout through the infusion of dichloroacetate (DCA) in dogs and humans (Timmons et al. 1998a; 1998b; Howlett, Heigenhauser, Hultman, Hollidge-Horvat and Spriet 1999). The infusion of DCA was linked to a significantly reduced level of cellular level phosphorylation, which is most likely

due to a reduced O_2 deficit as a result of the faster rate of adaptation of muscle $\dot{V}O_2$ at exercise onset (Timmons et al. 1998a; 1998b). In contrast, recent evidence from Bangsbo et al. (2002) reported that enhanced PDH activity does not improve the changes in muscle $\dot{V}O_2$ at the onset of one legged knee-extensor exercise at an work rate of ~110% of peak thigh $\dot{V}O_2$. These researchers elevated PDH activity through DCA administration prior to a 15 s severe-intensity exercise bout and reported that PDH elevation did not significantly increase $\dot{V}O_2$ or a-vO_2diff from the thigh musculature. This finding suggests that PDH may not be related to the on-transient $\dot{V}O_2$ response to high-intensity exercise. This lack of an increase in $\dot{V}O_2$ or O_2 extraction may be the result of the 'anaerobic' exercise intensity employed by Bangsbo et al. (2002). However, limited research is available on the relationship between PDH activity and $\dot{V}O_2$ measured at the mouth in response to more aerobic and submaximal exercise intensities.

In summary, while recent research has produced equivocal results as to the role of PDH on the rate of $\dot{V}O_2$ adjustment at exercise onset, PDH has been linked to the production of the substrates required to maintain the TCA cycle flux which in may play a role as a controlling factor of the $\dot{V}O_2$ response. While some previous research has found such a relationship, the published observations of the PDH effect on the $\dot{V}O_2$ response remains unclear.

Citrate Synthase

Another important mitochondrial respiratory enzyme is CS which is responsible for the combination of acetyl-CoA and oxaloacetate to produce citrate, an important intermediate within the TCA cycle. It has previously been

suggested that CS activity within the muscle is related to aerobic fitness as well as endurance training and performance (Bell et al. 2001; Carter, Rennie, Hamilton and Tarnopolsky 2001; Short, Vittone, Bidelow, Proctor, Rizza, Coenen-Schimke and Nair 2003). A paucity of literature has examined the influence of CS activity or its enhancement following endurance training on the on-transient $\dot{V}O_2$ response to exercise (Bell et al. 2001).

Bell and colleagues (2001) investigated the effects of nine weeks of single leg leg-extension training at an intensity of 75-85% $\dot{V}O_2$max on the relationship between CS activity and $\dot{V}O_2$ kinetics in five elderly male subjects (77 ± 7 y). They observed that the on-transient $\dot{V}O_2$ τ_p was significantly decreased in the trained limb post-training compared to pre-training (92 ± 44 vs. 48 ± 22 s) but not the untrained limb (104 ± 43 vs. 126 ± 35 s). It was also reported that CS activity of the VL significantly increased in the trained leg from 6.7 ± 2.0 to 11.4 ± 3.6 $\mu mol \bullet g_{w.w.}^{-1} \bullet min^{-1}$, but not in the untrained leg (5.9 ± 0.5 to 7.9 ± 1.9 $\mu mol \bullet g_{w.w.}^{-1} \bullet min^{-1}$). Furthermore, Bell et al. (2001) observed no improvement in the kinetics of mean blood velocity within the femoral artery. This suggests that the improvement observed in the on-transient $\dot{V}O_2$ response was most likely due to an increased O_2 extraction. The relationship between the improved $\dot{V}O_2$ on-transient responses and increased CS activity was not reported by the researchers. At present, no data exists on the relationship between enzyme activities and trends in mOxy in response to exercise bouts in any population.

In summary, limited research has examined the relationship between CS activity and the on-transient $\dot{V}O_2$ response. While it appears that an increased

CS activity is related to improved muscle $\dot{V}O_2$ during exercise, further research is required to validate this observation. Nevertheless, the present data are supportive of muscle utilisation limitations controlling the on-transient $\dot{V}O_2$ response to exercise onset.

2-Oxoglutarate Dehydrogenase

Whilst CS and SDH activites are commonly used measures of muscle oxidative capacity, it appears they are not closely related to the TCA cycle flux *in vitro* (Blomstrand, Radegran and Saltin 1997). 2-OGDH is a key regulatory enzyme within the TCA cycle responsible for the conversion of 2-oxoglutarate and coenzyme A into succinyl-CoA and CO_2, allowing NADH to be generated from NAD (Blomstrand, Challiss, Cooney and Newsholme 1983). Blomstrand et al. (1997) have suggested that the maximal flux of the TCA cycle is best related to 2-OGDH activity. Limited evidence has been presented which relates 2-OGDH activity to $\dot{V}O_2$ adjustment in response to imposed workloads (Blomstrand et al. 1997).

Blomstrand et al. (1997) determined the relationship between the maximal activities of a number of oxidative enzymes and the maximal $\dot{V}O_2$ for the quadriceps muscle during incremental leg extension exercise. The researchers observed that the maximum leg $\dot{V}O_2$ was 845 ± 100 $mL \bullet min^{-1}$, or 353 ± 33 $mL \bullet kg^{-1} \bullet min^{-1}$ for the quadriceps muscle, and was significantly correlated to the maximal activities of CS ($r = 0.79$, $p<0.05$) and 2-OGDH ($r = 0.72$, $p<0.05$). The mean exercise $\dot{V}O_2$ corresponded to a TCA cycle flux of 4.6 ± 0.4 $\mu mol \bullet min^{-1} \bullet g^{-1}$, which was similar to that noted for 2-OGDH (5.1 ± 0.3 $\mu mol \bullet min^{-1} \bullet g^{-1}$), but not CS (48 ± 1.5 $\mu mol \bullet min^{-1} \bullet g^{-1}$). The maximal activity

of CS appeared to be 940% higher than the estimated TCA cycle flux. The relationship between the maximal 2-OGDH activity and TCA cycle flux is shown in Figure 2.3. The importance of 2-OGDH activity upon the TCA cycle flux has been identified within several other investigations using animal muscle (Cooney, Taegtmeyer and Newsholme 1981; Blomstrand et al. 1983). However, it has been reported that 2-OGDH activity is not significantly related to exercise performance characteristics (i.e. LT, $\dot{V}O_2max$) of endurance athletes, whereas other oxidative enzymes such as CS and SDH have been related to such performance measures (Bishop, Jenkins, McEniery and Carey 2000). However, limited evidence is available on this relationship at present.

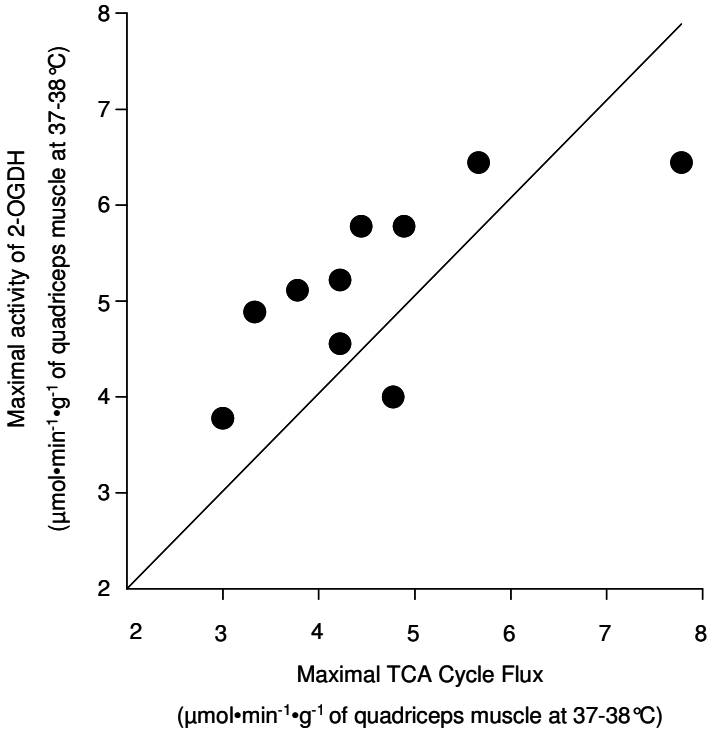

Figure 2.3: Relationship between maximal activity of 2-OGDH and TCA cycle flux within the vastus lateralis during one-legged knee extension exercise at 37-38˚C (Adapted from Blomstrand et al. 1997).

In summary, the activity of 2-OGDH appears to be related to the TCA cycle flux across exercise intensities, and as such may be a more valid oxidative measure than CS or SDH activities (Blomstrand et al. 1997). It is likely that the utilisation of O_2 at exercise onset is linked to the activity of such oxidative enzymes. Therefore, any O_2 utilisation limitations are likely to be representative of the oxidative capacity of the muscle (Grassi 2005). The research discussed above suggests that the rate of adaptation in the metabolic transition at exercise onset is controlled by factors related to O_2 utilisation within the working muscle (Tschakovsky and Hughson 1999; Bangsbo et al. 2000; Grassi 2005). Other factors which may be related to the on-transient $\dot{V}O_2$ response may include histochemical parameters, physical training and aging. These factors may be influential through either changing O_2 utilisation or delivery limitations within the working muscle.

Influence of Histochemical Parameters on the On-Transient Responses

The speed of the $\dot{V}O_2$ response at exercise onset is controlled though either the delivery or utilisation of O_2 within the active muscle cell (Xu and Rhodes 1999; Grassi 2000; 2001). However, both the delivery and utilisation of O_2 within the working muscle is related to muscle fibre type composition and thus muscle capillarisation, enzyme activity and bioenergetics (Bottinelli and Reggiani 2000; He, Bottinelli, Pellegrino, Ferenczi and Reggiani 2000). This supports the earlier hypothesis of Poole (1994) suggesting that the major influencing factors lie within the working muscle.

Previous research has identified that muscle fibre composition has a significant effect on the $\dot{V}O_2$–Work relationship during exercise (Barstow et al.

1996; Barstow, Jones, Nguyen and Casaburi 2000; Pringle et al. 2002; 2003b; Jones, Campbell and Pringle 2004). The metabolic gain of the primary component (G_p), calculated as $\Delta\dot{V}O_2/\Delta W$, has been shown to be an important parameter in describing on-transient $\dot{V}O_2$ response efficiency.

Barstow et al. (2000), Pringle et al. (2002; 2003b) have all suggested that the $\dot{V}O_2$-Work relationship is significantly related to the population of both Type I and II fibre types within the working muscle. Barstow et al. (2000) reported upon the $\dot{V}O_2$-Work relationship for both sub-VT and supra-VT intensity exercise in a group of nine healthy young (31 ± 8y; 3.4 ± 0.5 $L \bullet min^{-1}$) male subjects. Both Jones et al. (2004) and Mallory et al. (2002) reported no significant relationship between Type I fibre population of the VL and the $\Delta\dot{V}O_2/\Delta W$ for sub-VT exercise. They observed no significant relationship between the percentage of Type I fibres and the $\dot{V}O_2$ τ_p for heavy-intensity exercise across a wide range of cadences (45, 60, 75 and 90 RPM). However, they did observe significant ($p<0.05$) correlations between fibre type composition and the $\Delta\dot{V}O_2/\Delta W$ for exercise intensities both below and above VT. Specifically, they reported that the $\Delta\dot{V}O_2/\Delta W$ for sub-VT exercise was ~9 $mL \bullet min^{-1} \bullet W^{-1}$ for individuals with a low percentage of Type I fibres, whereas a high percentage of Type I fibres demonstrated a gain of ~11 $mL \bullet min^{-1} \bullet W^{-1}$ as shown below in Figure 2.4. This difference may be the result of the ~18% lower mitochondrial P_i/O_2 ratio, greater Ca^{2+} ATPase activity and lower energy efficiency previously noted for Type II fibres (Bottinelli and Reggiani 2000; He et al. 2000).

In agreement with Barstow and colleagues (2000), Pringle et al. (2003b) reported that the proportion of Type I muscle fibres and the $\Delta\dot{V}O_2/\Delta W$ were significantly related across moderate ($r= 0.65$, $p<0.05$), heavy ($r= 0.57$, $p<0.05$) and severe-intensity ($r= 0.57$, $p<0.05$) exercise in 14 young (25 ± 4 y; 47.9 ± 2.3 mL•kg^{-1}•min^{-1}) male and female subjects. The $\dot{V}O_2$ G_p was also significantly related to the capillary to fibre ratio (C:F ratio) at both heavy ($r= 0.65$, $p<0.05$) and severe-intensity ($r= 0.63$, $p<0.05$) SWT, and $\dot{V}O_2$max at heavy exercise ($r= 0.67$, $p<0.05$). The $\dot{V}O_2$ τ_p was found to be similar across all three intensities, and significantly related to the percentage of Type I fibres only for heavy-intensity exercise ($r= -0.68$, $p<0.05$). The Type IIx population was significantly related to the $\dot{V}O_2$ τ_p for both heavy ($r= 0.69$, $p<0.01$) and severe-intensity ($r= 0.56$, $p<0.05$) SWT. Importantly, subjects with a low percentage of Type I fibres exhibited a slower $\dot{V}O_2$ τ_p for the heavy and severe-intensity SWT.

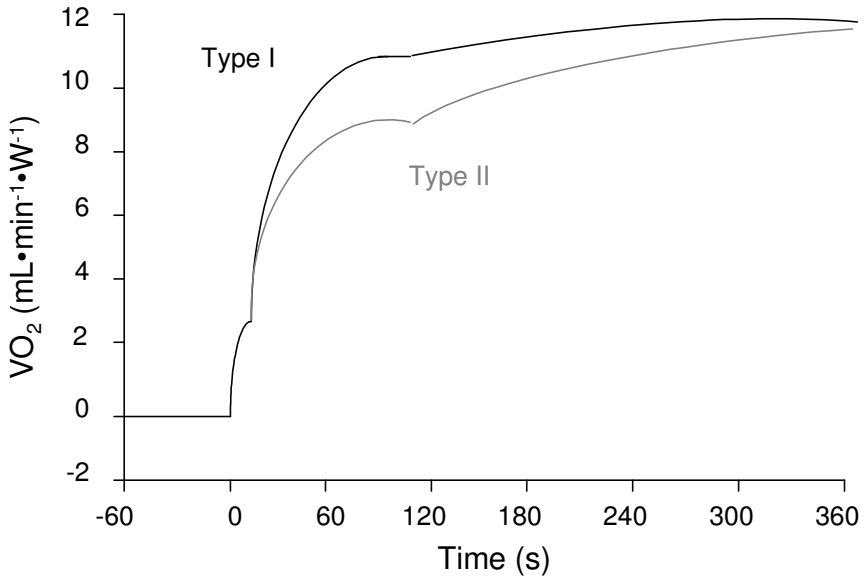

Figure 2.4: The gain for the primary component versus time for moderate, heavy and severe-intensity exercise for subjects with high percentages of Type I and Type II fibres (Adapted from Pringle et al. 2003b).

Pringle et al. (2003b) suggested that the reported relationship between $\dot{V}O_2$ τ_p and percentage of Type I fibres is related to the enhancement of O_2 delivery and oxidative enzymes in comparison to Type II fibres. The increased capillarisation of Type I muscle is also likely to reduce any heterogeneity of blood flow at exercise transients, allowing improved utilisation of O_2 within muscle by reducing the O_2 diffusion distance (Richardson et al. 1999). However, muscle capillarisation does not appear to influence the rate of $\dot{V}O_2$ adjustment during on-transient periods (Chilibeck et al. 1997; Pringle et al. 2003b). Thus, it appears that on-transient $\dot{V}O_2$ responses are not limited by the delivery of O_2 to the cell but rather the actual utilisation of O_2 within the muscle cell. Therefore, the precise mechanism responsible for a faster $\dot{V}O_2$ τ_p across supra-VT exercise on-transients in subjects with a high proportion of Type I fibres is yet to be determined. Possible causal factors might include the increased oxidative enzyme activities, mitochondrial density or capillarisation (Essen-Gustavsson and Borges 1986; Coggan et al. 1992).

The effect of histochemical characteristics on the speed and amplitude of the mOxy response at exercise onset has received little attention. To date, only one investigation has reported the effect of histochemical characteristics of the working muscle on the mOxy responses with exercise (Hamaoka, Mizuno, Katsumura, Osada, Shimomitsu and Quistroff 1998). In this study, the kinetics of the mOxy on-transient response were significantly related to the composition of Type I fibres from the VL. The suggestion that Type I fibres are related to greater and faster decreases in mOxy at exercise onset is most likely due to the greater capacity for blood to be delivered and consumed within the muscle cell. This increased potential for O_2 consumption is due to Type I fibres possessing

greater oxidative enzyme activities, myoglobin stores and capillarisation (Bottinelli and Reggiani 2000). This greater oxidative capacity and capillarisation and aerobic enzyme of Type I fibres would facilitate greater muscle deoxygenation through greater desaturation of HbO_2 and MbO_2 stores.

In summary, it appears that muscle fibre composition and related enzymatic characteristics are significantly related to the speed and amplitude of the on-transient $\dot{V}O_2$ response. The majority of previous literature has suggested that muscle fibre type has a significant influence on the speed (τ_p; wMRT) at which $\dot{V}O_2$ and mOxy adjust to sub-VT and supra-VT intensity exercise intensities (Borrani et al. 2001; Krustrup, Soderlund, Mohr and Bangsbo 2004b). However, limited research has examined the relationship between the on-transient mOxy responses and the histochemical characteristics of muscle. Therefore, a purpose of the present study is to further investigate the relationship between the on-transient $\dot{V}O_2$ and mOxy responses and peripheral muscle histochemical and enzymatic characteristics in well-trained cyclists.

Influence of Physical Training on the On-Transient Responses

Previous investigations have reported significant improvements in $\dot{V}O_2$max (Denis, Dormois and Lacour 1984; Pollock et al. 1987; Katzel et al. 2001), BLa^- thresholds (Sjodin, Jacobs and Karlsson 1981; Masse-Biron et al. 1992) and economy (Millet, Jaouen, Borrani and Candau 2002) with endurance training. The O_2 delivery or utilisation limitations within the working muscle which have previously been discussed may be reduced with physical training of

sufficient intensity and duration given the observed peripheral muscle adaptations (Babcock et al. 1994a; Carter et al. 2000a; Koppo et al. 2004).

Past training studies have observed positive changes in muscle blood flow, capillarisation and HR kinetics with physical training in sedentary subjects, to suggest that improvements in O_2 delivery to the working muscle occur (Denis, Chatard, Dormois, Linossier, Geyssant and Lacour 1986; Grassi 2001). The activity of oxidative enzymes and mitochondrial density related to O_2 utilisation are also increased with endurance training (Green, Thomson, Daub, Houston and Ranney 1979; Gollnick and Saltin 1982; Dawson, Fitzsimons, Green, Goodman, Carey and Cole 1998). It is likely that these peripheral training adaptations are in part responsible for the improved $\dot{V}O_2$ response to exercise (Carter et al. 2000a; Billat, Mille-Hamard, Demarle and Koralsztein 2002).

Billat et al. (2002) observed the effects of a four week endurance-training program on $\dot{V}O_2$ kinetics in seven young (25 ± 1 y; 56.0 ± 6.8 $mL \bullet kg^{-1} \bullet min^{-1}$) male physical education students. They observed significant speeding in the on-transient $\dot{V}O_2$ response after as little as four days of endurance training, and prior to any significant improvement in $\dot{V}O_2max$. They noted that the $\dot{V}O_2$ τ_p for treadmill runs at 90% and 95% $v\dot{V}O_2max$ significantly decreased from 28 ± 5 to 22 ± 4 s (90% $v\dot{V}O_2max$) and 28 ± 5 to 20 ± 7 s (95% $v\dot{V}O_2max$) after the four week training intervention. Similarly, Carter et al. (2000a) observed significant improvements in both LT and $\dot{V}O_2max$ in 23 healthy male subjects after a six-week continuous and interval running training program. However, no adaptations were noted in either the amplitude or the $\dot{V}O_2$ τ_p for the moderate

or heavy-intensity treadmill running, despite a significant reduction in the wMRT of heavy-intensity exercise response.

Recently, Fukuoka et al. (2002) investigated the effects of cycling endurance training on both the on- and off-transient $\dot{V}O_2$ responses in a group of middle-aged (51 ± 3 y) healthy male subjects during repeat SWT at 50% $\dot{V}O_2$max. Subjects performed a 90 day endurance-training program consisting of 30 min of cycling at 50% HR reserve. Fukuoka et al. (2002) observed non-significant improvements in both $\dot{V}O_2$max and peak [BLa^-] during the first 30 days, but significant improvements after both 60 and 90 days of endurance training. The on-transient $\dot{V}O_2$ τ_p improved significantly after seven days (38.1 ± 14.2 s) of training, as well as after 15 days (34.4 ± 12.6 s) of training in comparison to the pre-training values (46.9 ± 18.3 s). No further improvements were observed in the $\dot{V}O_2$ τ_p after 30 (28.8 ± 6.8 s), 60 (30.2 ± 8.0 s) and 90 (30.4 ± 12.4 s) days of training, respectively. Following 90 days of training, the on-transient $\dot{V}O_2$ τ_p of the middle-aged subjects were comparable to the matched younger group (21.6 ± 0.5 y; 29.2 ± 5.3 s) undertaking the same training. However, neither the on-transient $\dot{V}O_2$ amplitude nor TD_p was significantly affected by endurance training in either group. In addition, Fukuoka et al. (2002) showed that the HR $\tau_{½}$ significantly improved after 15 days of training in the middle aged group, but no further improvements were noted after this time. This suggests that the initial speeding of the $\dot{V}O_2$ kinetics response may be due to an enhanced O_2 delivery, and further improvements may be due to peripheral muscle adaptations.

The effect of physical training on changes in mOxy during exercise has only recently been investigated, and limited data are available (Costes et al. 2001; Neary et al. 2002; Puente-Maestu et al. 2003). The limited research suggests that physical training allows greater decreases in mOxy during exercise, most likely as a result of peripheral adaptations in the capacity of muscle to utilise O_2 (Costes et al. 2001; Neary et al. 2002; Puente-Maestu et al. 2003). Costes et al. (2001) investigated the influence of endurance training on mOxy responses during submaximal cycling exercise in seven healthy young (20 ± 2 y) volunteers. The researchers employed a four-week training program consisting of cycling at between 70% and 80% HR_{max} consecutively for two hours per day, six days a week. They investigated the cardiorespiratory, [BLa^-] and mOxy responses to two 15 min submaximal tests at 50% and 80% $\dot{V}O_2max$. The training program was not observed to alter either the amplitude or pattern of the mOxy responses at 50% $\dot{V}O_2max$, but greater muscle deoxygenation was observed across the 80% $\dot{V}O_2max$ SWT. Costes et al. (2001) also reported a weak but significant relationship ($r= 0.42$, $p<0.05$) between the greater mOxy amplitude and lowered [BLa^-]. However, the change in mOxy was significantly related to changes in measures of capillarisation of the VL suggesting an influence on O_2 delivery mechanisms. As a result of the training, the activities of CS and β-HAD were significantly increased, but no significant relationships were observed with the changes in the on-transient mOxy response to a moderate-intensity exercise bout. Therefore, it may be possible that the observed changes in mOxy during exercise are dependent upon the peripheral histochemical and enzymatic adaptations which influence O_2 delivery and utilisation.

In a more recent study, Neary et al. (2002) investigated the effect of short-term endurance training on mOxy responses in a group of eight experienced and well-trained male cyclists (23 ± 5 y, 4.39 ± 0.66 L•min^{-1}). The researchers employed a training program of cycling at an intensity of 85-90% $\dot{V}O_2$max for 1 h a day, four days a week for three weeks. Following the training program, the researchers investigated the cardiorespiratory and mOxy responses during a graded exercise test to exhaustion and a simulated 20 km time trial (20TT). The researchers reported no significant difference in the mOxy observed at $\dot{V}O_2$max and across the 20TT pre- and post-training. At the completion of the 20TT, mean mOxy was significantly decreased following the training, falling from -550 ± 292 to -707 ± 227 mV. Maximal deoxygenation also non-significantly decreased (-807 ± 344 to -1009 ± 331 mV). The researchers suggested that the cyclists who showed the greatest improvement in $\dot{V}O_2$max demonstrated the greatest improvement in 20TT performance and muscle deoxygenation following the three weeks of cycling training. The improvement in 20TT performance was significantly related to the capacity for greater muscle deoxygenation ($r = -0.75$, $p<0.05$). In order to quantify these changes, Neary et al. (2002) used the maximum values of mOxy from the $\dot{V}O_2$max test, rather than the previously validated and favourably employed cuff ischemia method (Sahlin 1992; Bhambhani et al. 1999; Costes et al. 1999; Foster, Rundell, Snyder, Stray-Gunersen, Kemkers, Thometz, Broker and Knapp 1999; Boushel and Piantadosi 2000). It might be suggested that this relationship is a result of adaptations within the peripheral muscle, allowing an increased O_2 extraction and utilisation which allow greater fibre recruitment and force production.

In summary, it appears that endurance training significantly improves both the $\dot{V}O_2$ and mOxy on-transient responses (Costes et al. 2001; Neary et al. 2002). The mechanisms associated with these improvements in the metabolic adaptation are hypothesised to occur within the peripheral muscle histochemical and biochemical parameters. Endurance training has also been observed to improve important physiological characteristics related to the on-transient metabolic responses such as VT, $\dot{V}O_2max$ and BLa^- responses (Midgley, McNaughton and Wilkinson 2006). Importantly, sufficient physical training helps to alleviate the O_2 delivery or utilisation limitations previously discussed to influence the on-transient metabolic responses. Aging has also been shown to alter the histochemical characteristics of peripheral muscle, as well as other physiological responses that may influence the metabolic adaptation at exercise onset.

Influence of Aging on the On-Transient Responses

The speed of the on-transient $\dot{V}O_2$ and mOxy response appears to be slowed with sedentary aging, which most likely reflects either a reduced capacity to deliver or utilise O_2 (Babcock et al. 1994a; Chilibeck et al. 1995; 1998; Bell et al. 1999; Stathokostas et al. 2003; DeLorey et al. 2003a; 2004a; 2005). Age-related changes have been observed in a number of physiological responses that may reduce the capacity to deliver or utilise O_2. These include changes to the initial HR response, muscle capillarisation, changes in muscle fibre composition, and oxidative enzyme activities (Babcock et al. 1994a; 1994b; DeLorey et al. 2003a).

In their classic investigation, Babcock et al. (1994a) investigated the exercise on-transient $\dot{V}O_2$ responses in 46 male subjects aged between 30 and 80 y. Each subject performed repeated six min bouts of cycling at a moderate-intensity (80% VT). The researchers observed that the on-transient $\dot{V}O_2$ τ_p was significantly longer with increasing age, averaging 38.8 ± 9.5 s, 48.6 ± 11.2 s and 60.8 ± 17.6 s for young (30-44 y), middle aged (45-59 y) and elderly (65-80 y) age-groups, respectively. It was also reported that the $\dot{V}O_2$ τ_p was significantly correlated with age ($r= 0.64$, $p<0.05$). The data suggested that $\dot{V}O_2$ τ_p slowed by 0.7 s per year across the age range included in the study. The $\dot{V}O_2$ τ_p was also found to be significantly related to the $\dot{V}CO_2$ τ_p ($r= 0.86$, $p<0.05$), and the $\dot{V}E$ τ_p ($r= 0.58$, $p<0.05$), as well as $\dot{V}O_2max$ ($r= -0.51$, $p<0.05$), but not HR τ_p ($r= 0.21$, $p>0.05$). The researchers hypothesised that the slowing of the $\dot{V}O_2$ τ_p may be the result of a reduced arterial pO_2, poorer vascular conductance or an age-related increase of 'non-muscle' tissue in the active musculature. Orlander and Aniansson (1980) supported these findings and suggested that an age-related increase in the $\dot{V}O_2$ τ_p may be the result of a decreased O_2 utilisation capacity through decreases in mitochondrial density or enzyme activities within the peripheral muscles.

Babcock et al. (1994b) later demonstrated that aerobic endurance training in older men (72 ± 4.4 y: 1.77 ± 0.19 $L \cdot min^{-1}$) significantly improved on-transient $\dot{V}O_2$ kinetic responses to moderate-intensity (80% VT) cycling. Each of the subjects completed a 24 week endurance-training program consisting of three 40 min cycling sessions per week at an intensity of 50%Δ. The $\dot{V}O_2$ τ_p was significantly reduced from 62.2 ± 15.5 to 31.9 ± 7 s following the training program in the aged sedentary males. An age-matched control group showed a

significantly longer $\dot{V}O_2$ τ_p in the post-testing session (53.7 ± 9.9 s), which demonstrates that $\dot{V}O_2$ τ_p is significantly shortened with endurance training. Babcock et al. (1994b) suggested that this improvement may have been the result of increases in mitochondrial density or other histochemical parameters, although these parameters were not measured.

Previous investigations have also reported that the on-transient $\dot{V}O_2$ response is slowed in older subjects due to a delayed O_2 transport from the lungs to the mitochondria (Babcock et al. 1992; Chilibeck et al. 1995; 1996a). Chilibeck and others (1995) reported a significant correlation between the accelerated $\dot{V}O_2$ τ_p and HR τ ($r= 0.78$, $p<0.05$) following endurance-training, suggesting that the faster on-transient $\dot{V}O_2$ response is partially the result of an improved HR response. The speeded HR kinetics, both at heavy submaximal and maximal exercise intensities appear to be explained by an age-related reduction in the adrenergic response together with a reduced sensitivity of cardiac tissue to catecholamines (McCully, Fielding, Evans, Leigh Jr. and Posner 1993; Houmard, Weidner, Gavigan, Tyndall, Hickey and Alshami 1998).

A number of investigations have observed that the oxidative capacity of muscle is maintained in muscles which are used in activities of daily living (e.g. gastrocnemius), as opposed to muscle groups involved in sport-specific activities (e.g. VL) (Chilibeck et al. 1995; 1996b; Russ and Kent-Braun 2004). In a recent review, Russ and Kent-Braun (2004) stated that oxidative capacity decreases with aging, but can be maintained at comparable levels of younger sedentary populations despite advancing age if physical training is maintained.

Limited research has examined the peripheral delivery and utilisation of O_2 in aging populations (Costes et al. 1999; Kutsuzawa, Shioya, Kurita, Haida and Yamabayashi 2001; DeLorey et al. 2003a; Grassi et al. 2003; Stathokostas et al. 2003). Recent data from Grassi and others (2003) suggest that NIRS may be used to detect delayed adjustment of oxidative metabolism *in vivo* throughout exercise transients. This is of great interest, particularly with the alterations in the bioenergetics of skeletal muscle with aging or a prolonged sedentary lifestyle (Hansford 1983). To date, only a handful of empirical studies have investigated the age-associated changes in mOxy responses to exercise (Costes et al. 1999; Kutsuzawa et al. 2001; Stathokostas et al. 2003; DeLorey et al. 2003a; 2004a; DeLorey et al. 2005). Given the age-related changes in $\dot{V}O_2$ kinetics and muscle histochemical and biochemical characteristics (Proctor, Sinning, Walro, Sieck and Lemon 1995; Chilibeck et al. 1997; Houmard et al. 1998), the on-transient mOxy responses may also show a significant effect of age.

Research by Costes et al. (1999) investigated the age-related decrease in cardiovascular function and changes in mOxy during incremental exercise in young (27 ± 4 y) and elderly (67 ± 5 y) healthy subjects. Resting mOxy was significantly lower in the older group (55.0 ± 16.7%) compared to that of the younger group (80.6 ± 20.0%, $p<0.01$). Furthermore, mOxy at $\dot{V}O_2$max was found to be significantly lower in the older (27.7 ± 24.8%) compared to the younger (51.1 ± 21.1%, $p<0.01$) subjects. No significant difference was noted in the amplitude of change in mOxy between the old and young groups (27.3 ± 16.7% vs. 24.3 ± 12.9%). The mOxy τ½ was observed to be similar for both the young (33.5 ± 17.5 s) and old (28.2 ± 10.5 s) groups. Costes et al. (1999)

suggested that impaired O_2 utilisation in the older subjects appeared to be unlikely given the significantly greater desaturation observed at maximal intensity exercise. The researchers suggested that any effect of aging on the metabolic response to exercise is more likely the result of a reduced delivery of O_2 to the working muscle.

More specific to the present thesis is the research of Stathokostas et al. (2003) and DeLorey et al. (2003a; 2004a) who reported upon the mOxy response during the exercise on-transients in aged populations. Firstly, Stathokostas et al. (2003) examined the effect of age on the $\dot{V}O_2$ and mOxy relationship during a ramp cycling test to fatigue in five young (26 ± 3 y) and old (68 ± 3 y) males. They reported a lower $\dot{V}O_2$max in the older subjects, but observed similar changes in Hb across the incremental test between the young (28 ± 12 µM) and elderly (22 ± 7 µM) cohorts. The researchers reported that for a given absolute submaximal $\dot{V}O_2$ (1.5 L•min^{-1}), the amplitude of muscle deoxygenation was significantly higher in the old (64 ± 19%) compared to the younger (27 ± 6%) subjects. This may reflect the higher relative intensity performed by the older subjects at this constant $\dot{V}O_2$.

DeLorey and others (2003a; 2004a; 2005) described the $\dot{V}O_2$ and mOxy responses to moderate (80% VT) and heavy-intensity (50%Δ) cycling in young (25 ± 3 y) and old (68 ± 3 y) healthy subjects. In response to the moderate-intensity SWT, the $\dot{V}O_2$ τ_p was significantly slower in the older cohort (O) (42 ± 9 s; 11 ± 1 s; (τ_p; TD)) compared to the young (Y) (26 ± 7 s; 12 ± 2 s). In contrast, both the TD (Y: 12 ± 2 s; O: 11 ± 1 s) and τ_p (Y: 13 ± 10 s; O: 9 ± 3 s) of the mOxy response were not different between the age-groups.

However, DeLorey et al. (2003a) reported that the older population had a significant increase in mOxy (13 ± 4 $\mu M \bullet L^{-1} \bullet min^{-1}$) for the relative work rate compared to the younger cohort (7 ± 2 $\mu M \bullet L^{-1} \bullet min^{-1}$). In response to the heavy-intensity SWT, DeLorey et al. (2005) reported that the $\dot{V}O_2$ τ_p was significantly slower in the old (49 ± 8 s) compared to the young (29 ± 4 s) research group. While the on-transient mOxy TD_p was similar between age-groups (Y: 7 ± 1 s; O: 8 ± 3 s), the mOxy τ_p was significantly faster in the older (8 ± 2 s) compared to the young (14 ± 2 s).

Taken together, these results suggest that the speed of the metabolic adaptation to moderate-intensity exercise is limited by the delivery of O_2 to the muscle, given the similar rate of adjustment in mOxy (DeLorey et al. 2004b; 2005), despite the slowed HR and $\dot{V}O_2$ kinetic responses in the older exercisers. The researchers highlighted that these results suggest that the capacity for elderly muscle to extract and consume O_2 is maintained with age, and that the slowed $\dot{V}O_2$ response is the result of a decrease in vascular conduction and matching of O_2 delivery to work intensities. However, DeLorey et al. (2004b) concluded that the fundamental limitation to $\dot{V}O_2$ adaptation to moderate-intensity exercise is most likely related to other intra-muscular factors than O_2 delivery. The results from both these previous studies suggest that despite a slowing in the $\dot{V}O_2$ response in older sedentary subjects (Babcock et al. 1994b; DeLorey et al. 2004a), the rate of mOxy adaptation is speeded with aging. This suggests that the capacity to extract and utilise O_2 is maintained or improved within elderly muscle. As such, O_2 delivery limitations may control the speed of the on-transient metabolic adaptation in older individuals.

In summary, the available research suggests that the age-related slowing of $\dot{V}O_2$ kinetics is due to a decreased capacity to match O_2 delivery to the metabolic requirements of the exercise intensity. While a number of hypotheses have been suggested to explain the slowed $\dot{V}O_2$ kinetic response, the monitoring of changes in mOxy during SWT suggests that the capacity to extract and utilise O_2 within the cell is maintained with age (Costes et al. 1999; DeLorey et al. 2004a; 2005). However, the mechanisms associated with the slowed $\dot{V}O_2$ response may not be solely identified through the reported of changes in mOxy within the working muscle. Thus it may be possible that this 'age-related' decline is more the consequence of a prolonged sedentary lifestyle rather than aging *per se*. Thus, a purpose of Study Two of the present series of investigations was to examine the effect of age on the on-transient $\dot{V}O_2$ and mOxy responses in well-trained cyclists.

SLOW COMPONENT DEVELOPMENT

The concept of a metabolic steady-state being attained during constant intensity exercise is only applicable to exercise intensities below VT (Barstow 1994; Poole 1994; Poole et al. 1994; Whipp 1994). Previous research has identified a gradual decrease in the mechanical and/or metabolic efficiency during high-intensity (>VT) exercise, which is observed as an 'additional' rise in $\dot{V}O_2$ visible ~80-180 s after the onset of exercise (Poole 1994; Poole et al. 1994; Xu and Rhodes 1999; Zoladz and Korzeniewski 2001). This gradual increase in $\dot{V}O_2$ is termed the $\dot{V}O_2$ slow component, and is demonstrated in Figure 2.6.

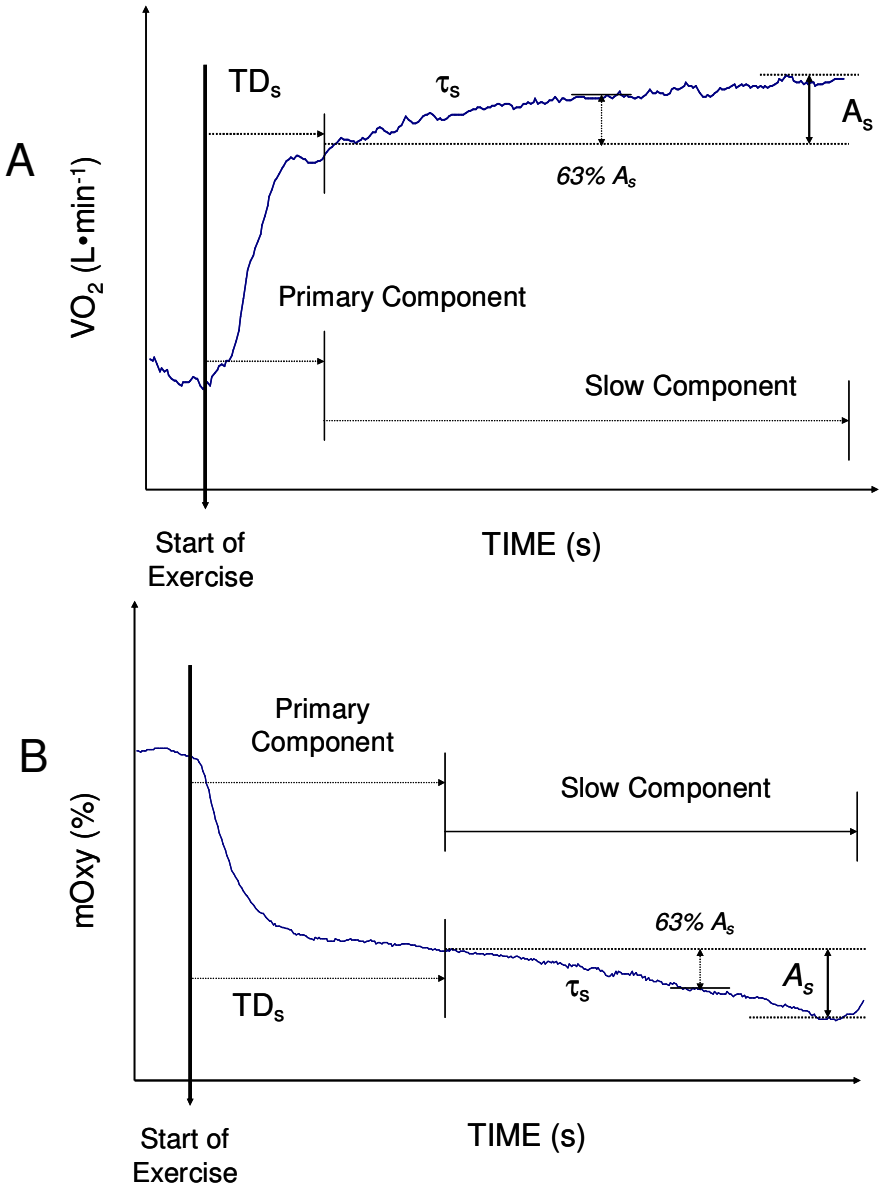

Figure 2.5: Schematic representation of the $\dot{V}O_2$ (A) and mOxy (B) slow component and kinetic parameters during heavy-intensity submaximal exercise (A_s; Slow component amplitude; TD_s; Slow component time delay; τ_s; Slow component time constant).

It is thought that this $\dot{V}O_2$ slow component represents a decrease in efficiency with the energy cost at such exercise intensities appearing to be greater than that predicted from the linear $\dot{V}O_2$–Work relationship observed

during moderate-intensity (<VT) exercise (Zoladz, Rademaker and Sargeant 1995). During heavy- or severe-intensity exercise (>VT), the $\dot{V}O_2$ slow component may demonstrate amplitudes of more than 1 L•min^{-1}, or alternatively may continue to rise until $\dot{V}O_2$max is attained and subsequent fatigue occurs (Poole 1994; Poole et al. 1994). Previous research has suggested a wide range of possible causal mechanisms, but as yet, no definitive primary causal mechanism has been identified. The $\dot{V}O_2$ slow component has been suggested to occur as a result of a decrease in metabolic and/or mechanical efficiency arising from changes in biochemical or neurological factors only facilitated through high-intensity exercise (Xu and Rhodes 1999; Demarie et al. 2001; Zoladz and Korzeniewski 2001).

The $\dot{V}O_2$ slow component was originally measured as the difference in $\dot{V}O_2$ between the 6th and 3rd min of a high-intensity constant load exercise bout (Xu and Rhodes 1999). More recent research has demonstrated that the $\dot{V}O_2$ slow component begins considerably earlier than three minutes after exercise onset (~90-120 s) (Bearden, Henning, Bearden and Moffat 2002; Koppo and Bouckaert 2002). It has more recently become preferred that the slow component is modelled as an additional exponential component to compensate for differences in its TD and amplitude (Bearden and Moffat 2001). Koppo et al. (2002) have also suggested that the classical method of quantification may underestimate the actual magnitude of the $\dot{V}O_2$ slow component during high-intensity exercise.

While the mechanisms responsible for the $\dot{V}O_2$ slow component are not completely understood, it has been suggested that ~86% of the $\dot{V}O_2$ slow

component is developed within the working muscle itself (Poole et al. 1991; Poole 1994). This is supported by Demarie et al. (2001) who reported a gradual decrease in mOxy during high-intensity running. The amplitudes of the mOxy and $\dot{V}O_2$ slow components were significantly correlated in this investigation. Similarly, the work of Belardinelli et al. (1995a) and Grassi et al. (2003) has supported the observation of concurrent $\dot{V}O_2$ and mOxy slow components during high-intensity constant-load exercise. However, the evidence from Grassi et al. (2003) provided no correlation between the respective amplitudes of the $\dot{V}O_2$ and mOxy slow components. Thus, the available evidence suggests that the $\dot{V}O_2$ slow component originates within the working muscle. This suggestion is supported by a small amount of early research also identifying a gradual decrease in mOxy across high-intensity submaximal workloads (Belardinelli et al. 1995a; 1995b; Bhambhani et al. 1997).

Factors Influencing the Slow Component Development

Previous research has linked the $\dot{V}O_2$ slow component to a number of mediating factors including muscle temperature, lactate accumulation, prior exercise, histochemical characteristics, and the change in muscle fibre recruitment patterns (Poole et al. 1994; Saunders, Evans, Arngrimsson, Allison, Warren and Cureton 2000; Koppo and Bouckaert 2002; Koppo et al. 2002). More recent research has suggested that the most likely contributor to the $\dot{V}O_2$ slow component may be the gradual recruitment of Type II muscle fibres in response to the fatigue of Type I fibres (Borrani et al. 2001; Krustrup et al. 2004b), despite contrasting results (Scheuermann, Hoelting, Noble and Barstow 2001).

It would appear that regardless of the causal mechanisms of the $\dot{V}O_2$ slow component, changes within a number of physiological measures have been shown to decrease the magnitude of the $\dot{V}O_2$ slow component through both endurance training (Womack, Davis, Blumer, Barrett, Weltman and Gaesser 1995; Carter et al. 2000a; Edge et al. 2003; Ocel, Miller, Pierson, Wooten, Hawkins, Myers and Herbert 2003; Saunders et al. 2003) and sedentary aging (Chick et al. 1991; Scheuermann, Bell, Paterson, Barstow and Kowalchuk 2002; Sabapathy et al. 2004). A great deal of literature has suggested that development of the $\dot{V}O_2$ slow component is multifactorial in nature (Poole 1994; Poole et al. 1994; Poole, Gladden, Kurdak and Hogan 1995; Bauer et al. 1999; Billat, Morton, Blondel, Berthoin, Bocquet, Koralsztein and Barstow 2000; Carter, Jones, Barstow, Burnley, Williams and Doust 2000b; Koga, Barstow, Shiojiri, Takaishi, Fukuba, Kondo, Shibasaki and Poole 2001; Perrey, Betik, Candau, Rouillon and Hughson 2001; Pringle, Carter, Doust and Jones 2002; Hill, Halcomb and Stevens 2003; Jones et al. 2003; Pringle, Doust, Carter, Tolfrey and Jones 2003a; Pringle et al. 2003b; Tordi, Perrey, Harvey and Hughson 2003).

Muscle Temperature

Muscle temperature has been shown to be related to a number of physiological factors which may affect the $\dot{V}O_2$ slow component during high-intensity constant-load exercise (Koga et al. 1997; 2002). These include an influence of the Q_{10} effect which increases the rate of metabolic reactions, the mechanical efficiency of muscles, or a temperature-regulated rightward shift in the HbO_2 dissociation curve via the Bohr Effect (Poole 1994; Poole et al. 1995).

However, the direct effect of increased muscle temperature on the $\dot{V}O_2$ slow component has not been supported with sufficient research evidence.

In a previous investigation, Koga et al. (1997) elevated thigh muscle temperature to ~39ºC using hot water pants and performed a number of repeated rest to work transitions of moderate- (50W) and high-intensity (50%Δ) exercise in seven untrained healthy young male volunteers (25.7 ± 9.2 y; 44.5 ± 9.8 $mL \cdot kg^{-1} \cdot min^{-1}$). The investigators reported that the $\dot{V}O_2$ slow component was significantly smaller for the heated condition (138 ± 66 $mL \cdot min^{-1}$) than the normal condition (205 ± 70 $mL \cdot min^{-1}$). They stated that this result was inconsistent with the suggestion that increased muscle temperature plays a role in the development of the $\dot{V}O_2$ slow component during heavy-intensity exercise. Similarly, Koppo et al. (2002) investigated the elevation of muscle temperature through prior exercise on $\dot{V}O_2$ kinetics. Koppo et al. (2002) successfully raised the temperature of the VL (35.3 ± 1.0 ºC) through both prior exercise (37.3 ± 0.6 ºC) and passive heating (37.2 ± 0.3 ºC) prior to the completion of a high-intensity (90% $\dot{V}O_2max$) SWT. The researchers observed that beyond the second minute of high-intensity exercise, the $\dot{V}O_2$ response was elevated by prior exercise, and that the $\dot{V}O_2$ slow component amplitude was reduced from 556 ± 72 $mL \cdot min^{-1}$ for the control condition to 241 ± 162 $mL \cdot min^{-1}$ and 461 ± 148 $mL \cdot min^{-1}$ for the prior exercise and passive leg heating interventions, respectively. It was suggested that the elevation of muscle temperature results in an increased metabolic efficiency and smaller $\dot{V}O_2$ slow component rather than actually causing a decrease in either the metabolic or mechanical efficiency. The mechanism responsible for this increase in metabolic efficiency with an increased muscle temperature is not yet fully understood.

To date, three hypotheses have been suggested to explain the above findings of Koga et al. (1997; 2002) and Koppo et al. (2002). Firstly, Brooks et al. (1971) hypothesised that an increase in muscle temperature is associated with an elevated transient and steady-state $\dot{V}O_2$ due to the Q_{10} effect on the metabolism and phosphorylation efficiency (ADP/O_2 molecule) and this may increase metabolic efficiency. It is unlikely that the oxidative phosphorylation within mitochondria becomes uncoupled, causing a decrease in metabolic efficiency, as this only occurs at higher muscle temperatures than those employed within the discussed investigations (≤ 40 ºC) (Gaesser and Poole 1996; Xu and Rhodes 1999; Demarie et al. 2001). Secondly, an increase in muscle temperature is linked to an increase in the mechanical efficiency of the muscle which would decrease the $\dot{V}O_2$ required at any exercise intensity as a result of the lowered viscous resistance of the contractile component of the muscle (Binzoni and Delpy 2001). Finally, increases in muscle temperature may produce a rightward shift in the HbO_2 dissociation curve to allow greater O_2 to be uncoupled from HbO_2 stores, thus increasing the O_2 delivery to the cell (Koga et al. 1997; Koppo et al. 2002). However, this mechanism would only be of benefit if the metabolic efficiency was reduced during the constant-load high-intensity exercise.

In summary, no research to date has definitively identified an elevated muscle temperature as a possible facilitator in the development in the $\dot{V}O_2$ slow component during high-intensity exercise, despite muscle temperature having been shown to change the mechanical and metabolic efficiency of muscle tissue during exercise. Possible casual or influential mechanisms worthy of

discussion may include lactate accumulation or the recruitment of less efficient Type II muscle fibres.

Lactate

The observation that the $\dot{V}O_2$ slow component is only observed at work intensities above VT strongly suggests that the appearance of lactate within the muscle and blood may facilitate the development of the $\dot{V}O_2$ slow and mOxy component. A number of studies have suggested hypotheses for this relationship, including an increased HbO_2 dissociation, an increased $\dot{V}O_2$ cost of lactate oxidation through gluconeogensis, as well as a decrease in mechanical efficiency due to the interference of anaerobic by-prodcuts such as $[H^+]$ with muscle contraction (Roston et al. 1987; Stringer et al. 1994; Gaesser and Poole 1996; Xu and Rhodes 1999; Demarie et al. 2001; Zoladz and Korzeniewski 2001).

Roston et al. (1987) were one of the first to examine the effects of lactate concentration on $\dot{V}O_2$ kinetics during cycling in six healthy young men during repeat six minute SWT at intensities ranging from 80% LT to 80%Δ. The investigators reported that the $\dot{V}O_2$ slow component was significantly correlated ($r= 0.855$, $p<0.05$) with the rise in $[BLa^-]$ from rest to 6 min during the high-intensity cycling. These researchers suggested that the increase in $\dot{V}O_2$ during exercise may be due to the oxidation of lactate, and/or gluconeogenesis of lactate in both the liver and skeletal muscle.

Other researchers have also noted a relationship between the appearance of BLa^- and the onset of the $\dot{V}O_2$ slow component (Casaburi et al.

1989; Whipp and Ward 1990). However, it may be possible that the correlation between increases in $\dot{V}O_2$ and [BLa^-] during heavy-intensity exercise may be coincidental rather than causal. This observation may be more related to the exercise intensity and subsequent mechanical properties of the peripheral muscle rather than [BLa^-] *per se* (Stringer et al. 1994). Poole et al. (1995) have suggested the decrease in blood pH associated with the increases in [BLa^-] causes an increased O_2 delivery via the Bohr effect, which may account for up to ~62% of the $\dot{V}O_2$ slow component amplitude. The rightward shift noted in the HbO_2 dissociation curve from metabolic acidosis may therefore be a primary contributor to the development of the $\dot{V}O_2$ slow component (Stringer et al. 1994).

Other investigators have hypothesised that lactate may be responsible for the $\dot{V}O_2$ slow component due to the extra O_2 requirement for its oxidation (Barstow 1994; Gaesser and Poole 1996). Gaesser and Poole (1996) observed that ~70% of lactate formed during exercise above VT is oxidised within active musculature, while the balance is removed through hepatic gluconeogenesis. Despite the majority of the $\dot{V}O_2$ slow component being developed within the working muscle, (Poole et al. 1994; Poole 1994), it is most likely the increased $\dot{V}O_2$ required to oxidise lactate within skeletal muscle and liver is not a significant contributor to the $\dot{V}O_2$ slow component. Poole (1994) infused isolated canine gastrocnemius with lactate for two 60 min periods during which the muscle was electrically stimulated as to not change blood or muscle pH. The researchers observed a significant decrease in muscle $\dot{V}O_2$, from 5.1 ± 0.3 to 4.1 ± 0.2 $mL \cdot min^{-1} \cdot 100g^{-1}$ across the 60 min in comparison to control values, suggesting that lactate does not facilitate the development of the $\dot{V}O_2$ slow

component. Thus, it may appear that the proposed influence of lactate on $\dot{V}O_2$ kinetics observed in the literature may be due to the influence of other metabolic factors, such as a decrease in pH or recruitment of less efficient Type II muscle fibres during high-intensity exercise (Stringer et al. 1994; Borrani et al. 2001). In summary, it appears that the development and magnitude of the $\dot{V}O_2$ slow component is related to mechanisms observed concurrently with lactate production, however it appears not to be a major causal factor.

More recently, Demarie et al. (2001) reported that the magnitude of the $\dot{V}O_2$ slow component appears related to deoxygenation of the VL (as measured by NIRS) and the increase in [BLa^-] during high-intensity running in eleven young male amateur soccer players (29 ± 2 y; 58.0 ± 4.6 $mL \bullet kg^{-1} \bullet min^{-1}$). Both $\dot{V}O_2$ and [BLa^-] significantly increased, and mOxy decreased between the third and sixth minutes of high-intensity treadmill exercise. There was a significant but weak negative relationship ($r = -0.38$, $p<0.05$) observed between changes in mOxy and [BLa^-] between the third and sixth minutes of exercise. The relationship between changes in $\dot{V}O_2$ and [BLa^-] during the same time period was reported to be non-significant ($r = 0.30$, $p = 0.127$). These findings support Poole (1994) who suggested that the majority of the $\dot{V}O_2$ slow component is developed within the muscle, observed as a gradual deoxygenation of the working muscle.

In conclusion, it appears that the relationship between increases in [BLa^-] and the $\dot{V}O_2$ slow component is equivocal. The secondary effects on the metabolic efficiency of the body suggest that La^- may play a minor role in the development of the $\dot{V}O_2$ slow component. However, the majority of the $\dot{V}O_2$

slow component during high-intensity constant-load endurance exercise is developed from peripheral mechanisms. Since the $\dot{V}O_2$ slow component is only developed at intensities above VT, this suggests that its origin lies within other physiological mechanisms encountered during high-intensity exercise requiring anaerobic metabolism and BLa^- production.

Influence of Histochemical Parameters on the Slow Components

It is well established that Type II fibres have different energetic properties, consume a higher O_2 cost per unit of energy output and higher lactate output than Type I muscle fibres (Bottinelli and Reggiani 2000). At present, equivocal evidence has suggested that the development of the $\dot{V}O_2$ slow component is due to in part an increased recruitment of less efficient Type II muscle fibres during high-intensity exercise (Saunders et al. 2000).

It has been well established that the development and magnitude of the $\dot{V}O_2$ slow component is related to the percentage of Type II fibres within the recruited musculature (Shinohara and Moritani 1992; He et al. 2000; Saunders et al. 2000; Borrani et al. 2001; Pringle et al. 2003b). For example, Pringle et al. (2003b) observed that the $\dot{V}O_2$ slow component amplitude was inversely correlated with the percentage of Type I fibres for both heavy ($r= -0.74$, $p<0.01$) and severe-intensity ($r= -0.64$, $p<0.05$) exercise. Of greater importance, the $\dot{V}O_2$ slow component amplitude was significantly related to the percentage of Type II fibres for heavy-intensity exercise ($r= 0.60$, $p<0.05$), and Type IIx fibres for both the heavy ($r= 0.60$, $p<0.05$) and severe ($r= 0.62$, $p<0.05$) intensity SWT. Further relationships between the $\dot{V}O_2$ slow component amplitude and the combined muscle fibre cross-sectional area to capillary contact ratio for the

heavy (r= 0.63, p<0.05) and severe-intensity (r= 0.74, p<0.01) SWT were observed. Similarly, Russell et al. (2002) observed significant relationships between $\dot{V}O_2$ slow component amplitude and both Type I (r= -0.57, p<0.05) and Type IIa (r= 0.60, p<0.05) composition. These researchers also reported that the amplitude of the $\dot{V}O_2$ slow component was inversely related to $\dot{V}O_2$max (r= -0.73, p<0.01), and maximal CS activity within the VL (r= -0.71, p<0.01), suggesting that aerobically fit subjects will exhibit an attenuated slow component amplitude. Taken together, these studies demonstrate that the decrease in efficiency associated with the slow component is related to muscle fibre histochemical, and perhaps biochemical characteristics. However, it is the recruitment of these fibres which is proposed to be the primary factor responsible for the slow component (Saunders et al. 2000; Borrani et al. 2001; Krustrup et al. 2004b).

While the composition of Type II fibres are related to the $\dot{V}O_2$ slow component amplitude during high-intensity endurance exercise, it is more likely the neuromuscular recruitment of these fibres during the high-intensity submaximal exercise which is responsible for the gradual rise in $\dot{V}O_2$ (Saunders et al. 2000; Borrani et al. 2001; Krustrup et al. 2004b). In a clinical trial, Saunders et al. (2000) investigated the surface electromyographic (sEMG) and magnetic resonance imaging (MRI) changes of the VL and rectus femoris (RF) after 3 and 15 min of moderate (85% VT) and heavy-intensity (60%Δ) cycling. The investigators observed no $\dot{V}O_2$ slow component for the sub-VT work intensity, with no changes in [BLa^-], ventilation or muscle fibre recruitment at that sub-VT intensity. In contrast, high-intensity cycling produced a significant $\dot{V}O_2$ slow component, which was accompanied by significant (p<0.05)

increases in both the mean power frequency and root mean square observed from the VL between 3 (72.1 ± 6.9 mV; 0.19 ± 0.08 mV) and 15 min (76.3 ± 9.0 mV; 0.21 ± 0.07 mV) of cycling. These findings support the suggestion of an increased recruitment of Type II muscle fibres during sustained high-intensity exercise. Other research supporting the Type II fibre hypothesis have suggested that the fatigue Type II fibres may continue to utilise O_2 for recovery (Na^+-K^+ pumping etc.) despite no contribution towards force production (Rossiter et al. 1999; Pringle et al. 2003). This may also contribute to the commonly observed decrease in efficiency.

Further justification of this mechanism was evident through significant increases in muscle activity (as measured by MRI muscle relaxation times) between the 3rd and 15th min for both the VL (33.1 ± 1.6 vs. 36.0 ± 2.9 ms) and RF (29.8 ± 1.8 vs. 30.6 ± 0.8 ms) (Saunders et al. 2000). These increased muscle relaxation times for the recruited muscles were significantly related to the rise in $\dot{V}O_2$ between the third and fifteenth minutes of cycling ($r = 0.63$, $p<0.05$). These findings are supported by a number of sEMG investigations (Shinohara and Moritani 1992; Borrani et al. 2001; Krustrup, Soderlund, Mohr and Bangsbo 2004a; Krustrup et al. 2004b; Sabapathy, Schneider and Morris 2005) but not all (Scheuermann et al. 2001; Cleuziou, Perrey, Borrani, Lecoq, Courteix, Germain and Obert 2004). For example, Cleuziou et al. (2004) reported upon the development of the $\dot{V}O_2$ slow component and changes in fibre recruitment patterns for both moderate- (80% VT) and heavy-intensity (50%Δ) SWT. Cleuziou et al. (2004) reported that the $\dot{V}O_2$ slow component was only observed across the heavy-intensity SWT, despite the changes in EMG activity of the VL being similar between the

different SWT intensities. This finding may suggest that the $\dot{V}O_2$ slow component is related to other intra-muscular mechanisms than changes in fibre recruitment patterns.

The above findings reporting that the increased recruitment of Type II fibres are significantly related to the amplitude of the $\dot{V}O_2$ slow component support the work of Rossiter et al. (2001). These investigators suggested that the $\dot{V}O_2$ slow component is related to a higher phosphate cost with sustained force production at high-intensity submaximal exercise, which can only be maintained through the recruitment of Type II fibres. To test this hypothesis, Pringle et al. (2003a) investigated the $\dot{V}O_2$ slow component across different cadences (35, 75 and 115 RPM) during a high-intensity SWT (50%Δ) in a group of young (26 ± 4 y; 3.58 ± 0.18 L•min^{-1}) well-trained cyclists. They hypothesised that the higher cadences would facilitate an increased recruitment of Type II fibres, given the faster contraction speed required. Such changes in the recruitment pattern would most likely require an additional $\dot{V}O_2$ given the changes in fibre-specific efficiencies across increasing cadences. The researchers reported that the high cadence condition (115 RPM) produced a significantly larger $\dot{V}O_2$ slow component, supporting an increased recruitment of the inefficient Type II fibres (Figure 2.6).

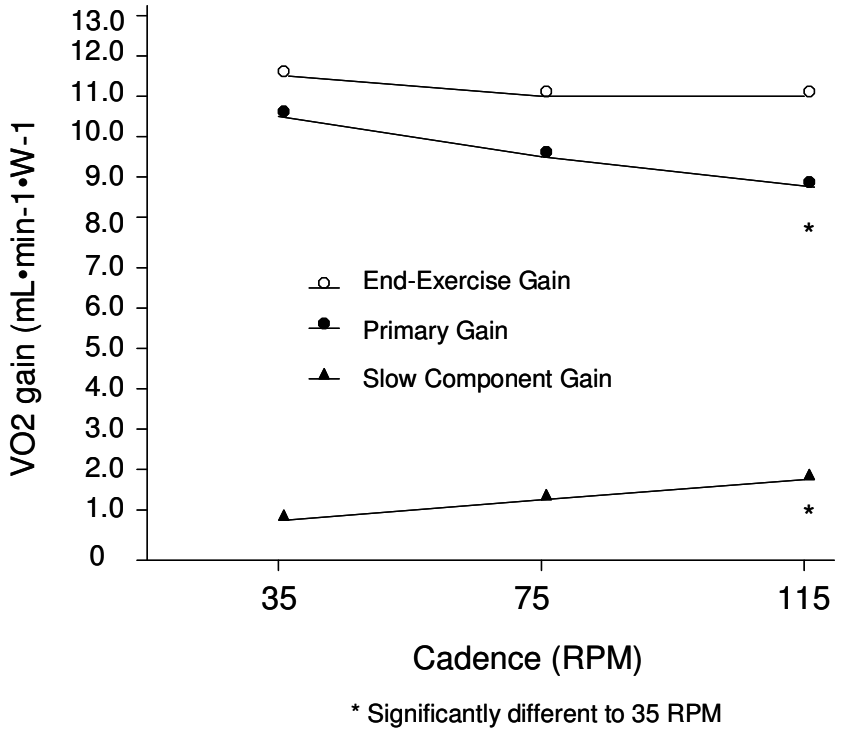

Figure 2.6: The effect of cadence on the primary and end-exercise $\dot{V}O_2$ gains, and $\dot{V}O_2$ slow component magnitude (Adapted from Pringle et al. 2003).

It may also have been possible that the Type II fibres recruited during the faster cadences may have been subject to fatigue, which may have then required Type I fibres to be recruited. Previous evidence has shown that the efficiency of different fibre types to be similar with their preferred recruitment intensities and contractile speed (He et al. 2000). Therefore, the Type I fibres recruited during the high cadence condition may have been equally inefficient and helped to facilitate the development of the $\dot{V}O_2$ slow component. However, the investigation did not employ sEMG techniques to monitor fibre recruitment, and therefore these results suggesting an increased Type II fibre recruitment are speculative.

In order to further test the hypothesis of increased Type II fibre recruitment, recent investigations have attempted to specifically deplete glycogen levels in Type I fibres in order to increase the reliance on Type II recruitment (Bouckaert, Jones and Koppo 2004; Carter, Pringle, Boobis, Jones and Doust 2004; Krustrup et al. 2004a). Carter et al. (2004) employed both low and high-intensity exercise protocols to specifically deplete muscle glycogen stores in Type I and II fibres, prior to completing a six minute high-intensity (50%Δ) SWT in active young men (25.5 ± 4.5 y; 2.95 ± 0.5 L•min^{-1}). No significant differences were observed in either the amplitude or time parameters of the $\dot{V}O_2$ slow component between the control (0.24 ± 0.04 L•min^{-1}; 110.9 ± 6.9 s) or low-intensity (0.27 ± 0.05 L•min^{-1}; 104.8 ± 5.4 s) depletion protocols. However, $\dot{V}O_2$ kinetics following the high-intensity depletion protocol demonstrated a lower amplitude (0.18 ± 0.03 L•min^{-1}) and longer time of onset (127.4 ± 7.9 s) of the $\dot{V}O_2$ slow component than the other two conditions. Therefore, the depletion of glycogen specifically within Type II fibres significantly influenced the nature of the $\dot{V}O_2$ slow component, and supports the hypothesis of altered recruitment patterns being responsible for the decrease in efficiency.

In summary, the most likely mechanism responsible for the development of the $\dot{V}O_2$ slow component lies with the increased recruitment of Type II muscle fibres which are required to sustain power output after Type I fibres fatigue during sustained high-intensity SWT. The magnitude of the $\dot{V}O_2$ slow component has been related to the muscle fibre composition across exercise intensities, which have also been shown to be related to and influenced by both physical training and aging.

Influence of Physical Training on the Slow Components

The mechanisms responsible for the development of the $\dot{V}O_2$ slow component have been shown to lie predominately within the working musculature (Poole 1994). A reduction in the $\dot{V}O_2$ slow component amplitude has strong implications for athletes who compete in constant-load high-intensity endurance events, theoretically through a reduction in metabolic fatigue (Xu and Rhodes 1999). However, the actual effects of physical training on the mechanisms responsible for the $\dot{V}O_2$ slow component remain to be fully evaluated, and it is difficult to suggest what training-related adaptations may affect the $\dot{V}O_2$ slow component.

Carter et al. (2000a) investigated the effects of a six-week combined continuous and interval running training in 23 untrained (22 ± 3 y; 54.9 ± 2.1 $mL \bullet kg^{-1} \bullet min^{-1}$) healthy volunteers. Each subject performed repeat six minute moderate (80% VT) and heavy-intensity (50%Δ) SWT. The researchers observed a significant reduction in the development of the $\dot{V}O_2$ slow component (pre: 321.1 ± 32 $mL \bullet min^{-1}$; post: 217 ± 23 $mL \bullet min^{-1}$) following the training intervention. This decrease in the $\dot{V}O_2$ slow component was significantly related to the reduction in ventilation ($r = 0.46$, $p<0.05$), but not to the reduction in end-exercise [BLa^-] ($r = 0.39$, $p>0.05$).

The observations of Carter et al. (2000a) have been supported by a number of research investigations which have reported similar changes in the $\dot{V}O_2$ slow component with endurance training (Womack et al. 1995; Edge et al. 2003; Ocel et al. 2003; Saunders et al. 2003). For example, Womack et al. (1995) observed the effect of endurance training on the $\dot{V}O_2$ slow component in

a group of untrained males (n = 7, 25.6 ± 1.5 y; 3.20 ± 0.19 L•min^{-1}) during bouts of high-intensity (50%Δ) cycling. As a result of a six week endurance training protocol consisting of four 40-min cycling sessions per week, the researchers reported a significant improvement in $\dot{V}O_2$max (1.57 ± 0.12 vs. 1.97 ± 0.09 L•min^{-1}) and power output at LT (103 ± 11 vs. 132 ± 9 W). They also observed that the amplitude of the $\dot{V}O_2$ slow component was significantly reduced from pre-training levels of 0.42 ± 0.06 L•min^{-1} to 0.20 ± 0.04 L•min^{-1} after two weeks, and was further reduced at the completion of the six-week endurance-training program to 0.15 ± 0.04 L•min^{-1}. This reduction in the magnitude of the $\dot{V}O_2$ slow component was not significantly related to decreases in either end-exercise [BLa$^-$], $\dot{V}E$, plasma adrenaline or plasma noradrenaline. However, the researchers did not specify whether the absolute power output at post-testing was adjusted for training, or was identical to the pre-testing workload. If the workload was not adjusted for the effects of the training intervention, the decreased $\dot{V}O_2$ slow component amplitude may be due to the lower relative exercise intensity.

Additional evidence to support the effect of physical training on the $\dot{V}O_2$ slow component was put forward by Ocel et al. (2003) who examined the effects of six weeks of cycling endurance training on 18 apparently healthy young (23 ± 1 y; 3.18 ± 0.18 L•min^{-1}) men, consisting of moderate (<VT) or high (>VT) intensity exercise. They observed that the magnitude of the $\dot{V}O_2$ slow component was reduced by 44% after one week of high-intensity training, which was significantly higher than the 20% and 12% reductions noted for the moderate-intensity training and control groups, respectively. No significant difference was observed for the reduction in the $\dot{V}O_2$ slow component in the

training group between the moderate- or high-intensity exercise training groups following the six-week training program. However, the $\dot{V}O_2$ slow component amplitude was significantly reduced in comparison to the control group. Ocel et al. (2003) observed that this attenuation of the $\dot{V}O_2$ slow component with moderate and high-intensity training was strongly related to the training-related decreases in both end-exercise [BLa^-] ($r= 0.76$, $p<0.01$) and $\dot{V}E$ ($r= 0.59$, $p<0.05$). Neither [BLa^-] or $\dot{V}E$ have been shown to have a primary role in the development of the $\dot{V}O_2$ slow component (Poole 1994), which suggests that the smaller amplitudes of the $\dot{V}O_2$ slow component were most likely due to increases in aerobic fitness and oxidative capacity of the working muscle.

Thus, it appears that prolonged moderate and high-intensity physical training can significantly alter the development of the $\dot{V}O_2$ slow component (Carter et al. 2000a; Ocel et al. 2003). However, the mechanisms responsible for the decrease in the $\dot{V}O_2$ slow component with training are yet to be elucidated but most likely occur within the peripheral muscle recruited during training and exercise (Poole 1994). It is more likely that the major mechanism for the training-related decrease in the $\dot{V}O_2$ slow component is the more efficient muscle recruitment patterns and improved fatigue resistance of muscle fibres. However, no research investigation to date has fully utilised sEMG to determine whether training-related changes in muscle recruitment patterns are responsible for this improved efficiency across high-intensity exercise. Similarly, no research has reported the training-related effects in mOxy on the concurrent development of the slow component within the working muscle.

In summary, the reduction in the $\dot{V}O_2$ slow component with physical training has wide implications for high-intensity endurance sports performance (Xu and Rhodes 1999; Carter et al. 2000b). While a number of physiological factors may contribute to this training-facilitated attenuation of the $\dot{V}O_2$ slow component, the most likely physiological mechanism may be the changes in the recruitment patterns and efficiency of Type II muscle fibres. However, other factors such as aging and its influence on the innervation and composition of Type II fibres have also been observed to alter the $\dot{V}O_2$ slow component development.

Influence of Aging on the Slow Components

To date, little evidence exists examining the effects of aging on the $\dot{V}O_2$ slow component (Chick et al. 1991; Xu and Rhodes 1999; Scheuermann et al. 2002; Sabapathy et al. 2004). However, sedentary aging has been shown to somewhat influence the peripheral muscle characteristics where the $\dot{V}O_2$ slow component is developed, and therefore the $\dot{V}O_2$ slow component may be subject to such aging effects. Previous data has reported that the $\dot{V}O_2$ slow component is reduced with sedentary aging, (Chick et al. 1991; Scheuermann et al. 2002; Sabapathy et al. 2004), but no investigation has reported on this effect of aging in older trained subjects. It might be suggested that the $\dot{V}O_2$ slow component may be significantly reduced in such a population, given the significant decreases previously discussed to occur with both physical training and aging.

In sedentary aging individuals, Scheuermann et al. (2002) investigated the $\dot{V}O_2$ response to high-intensity (>VT) exercise in eight young (25 ± 3 y), and

nine elderly (71 ± 5 y) healthy volunteers. They observed that the $\dot{V}O_2$ slow component amplitude was not significantly different between the young (175 ± 92 mL•min^{-1}) and elderly (102 ± 70 mL•min^{-1}) cohorts. They also observed that the end-exercise [BLa^-] was significantly reduced in the elderly (5 ± 1 mmol•L^{-1}) group compared to the young (9 ± 2 mmol•L^{-1}). No significant relationship was observed between this difference in [BLa^-] and the reduction in the $\dot{V}O_2$ slow component. In contrast, the observation of an age-related decline in the $\dot{V}O_2$ slow component was previously reported by Chick, Cagle, Vegas, Poliner and Murata (1991). These researchers reported that elderly individuals (68 ± 7.5 y) demonstrated a significantly attenuated $\dot{V}O_2$ slow component amplitude (111 ± 78 mL•min^{-1}) compared to a younger (29.5 ± 6.4 y; 406 ± 172 mL•min^{-1}) cohort. They suggested that the reduced $\dot{V}O_2$ slow component observed in the older population was the result of either a reduced adrenergic response to exercise or the reduced population of Type II muscle fibres widely observed within aging populations (Lexell 1995; Porter, Vandervoort and Lexell 1995; Andersen, Terzis and Kryger 1999). These age-related changes may reduce the glycogenolytic rate during exercise and La^- production which may attenuate the $\dot{V}O_2$ slow component.

It is more likely that changes within the histochemical and neuromuscular systems are responsible for changes in the age-related changes in the $\dot{V}O_2$ slow component. A large number of previous investigations have reported histochemical changes within aged sedentary individuals, with the majority of research reporting atrophy of Type II fibres (Larsson 1983; McCarter 1990; Chilibeck et al. 1995; Lexell 1995; Porter et al. 1995; Proctor et al. 1995; Kirkendall and Garrett Jr 1996; Houmard et al. 1998; Conley, Jubrias

and Esselman 2000; Frontera, Hughes, Fielding, Fiatarone, Evans and Roubenoff 2000; Frontera et al. 2001; Trappe, Lindquist and Carrithers 2001; Thompson 2002; Andersen 2003). Previous research has shown that muscle fibres move towards age-related co-expression of myosin-heavy chain (MHC) contents, suggesting that the muscle fibres are losing their high power and anaerobic capacity, allowing an increased oxidative capacity (Andersen et al. 1999). This co-expression of MHC content has been shown to be reduced through undertaking activity which allows the continued recruitment of Type II fibres through moderate-to-high-intensity resistance training (Williamson, Godard, Porter, Costill and Trappe 2000). An age-related shift towards more oxidative muscle fibres may be the most likely reason that the magnitude of the $\dot{V}O_2$ slow component decreases due to a reduced number and size of the Type II fibres that can be recruited to help maintain power output after Type I fibres fatigue. In contrast, Type II fibres, as well as their force and power characteristics, appear to be maintained in masters athletes who continue high-intensity physical training (Coggan, Spina, Rogers, King, Brown, Nemeth and Holloszy 1990). Therefore, the role of histochemical adaptations with aging on the development of the $\dot{V}O_2$ slow component is not yet fully understood, and future research should attempt to identify differences between trained and untrained older individuals.

Recently, Sabapathy et al. (2004) investigated the nature of the $\dot{V}O_2$ slow component and changes in muscle fibre recruitment patterns in young (21.2 ± 0.9 y; 3.71 ± 0.21 $L \cdot min^{-1}$) and elderly (71.6 ± 0.8 y; 2.12 ± 0.11 $L \cdot min^{-1}$) subjects during heavy-intensity (50%Δ) cycling. The investigators reported that the amplitude and TD_s of the $\dot{V}O_2$ slow component were

significantly higher and faster in the young (595 ± 65 mL•min^{-1}; 118 ± 10 s) than the elderly (223 ± 28 mL•min^{-1}; 178 ± 14 s) subjects, respectively. These discrepancies are not surprising given the significant differences in age and maximal aerobic capacities. Interestingly, when the amplitude of the $\dot{V}O_2$ slow component was made relative to the change in $\dot{V}O_2$ per unit of time, no significant difference was observed between the groups. Sabapathy et al. (2004) also observed that the changes in the mean power frequency (MPF) across the high-intensity SWT were similar regardless of age, with both the young (6.4 ± 1.0%) and elderly (5.4 ± 0.7%) showing significant increases. As such, the researchers suggested that the causal nature of the $\dot{V}O_2$ slow component may not change with age, but rather that the age-related decrease in $\dot{V}O_2$max may be responsible for the significantly lower absolute slow component amplitude. Therefore, the maintenance of $\dot{V}O_2$max with aging through physical training may allow similar $\dot{V}O_2$ slow component amplitudes to be observed in vastly different age groups.

In summary, limited research to date has described the effects of aging on the development of the $\dot{V}O_2$ slow component during high-intensity exercise (Chick et al. 1991; Scheuermann et al. 2002). From the limited evidence available, aging appears to reduce the $\dot{V}O_2$ slow component amplitude, which is most likely due to an age-related denervation and atrophy of low-efficiency high-force Type II muscle fibres noted in sedentary aging individuals (Coggan et al. 1992; Lexell 1995; Porter et al. 1995; Andersen et al. 1999). Therefore, while it is observed that the peripheral muscle characeristics and $\dot{V}O_2$max are significantly changed with aging, the maintenance of such characteristics

through physical training may negate any such effect of aging effect *per se* on the development of the $\dot{V}O_2$ slow component.

In conclusion, the $\dot{V}O_2$ slow component is proposed to represent an 'additional' volume of O_2 consumed within the working muscle during high-intensity exercise (Poole 1994; Poole et al. 1994; 1995; Whipp 1994; Womack et al. 1995; Billat, Richard, Binsse, Koralsztein and Haouzi 1998; Scheuermann, Kowalchuk and Barstow 1999; Lucia, Hoyos and Chicharro 2000; Borrani et al. 2001; Demarie et al. 2001; Demarie, Sardella, Billat, Magini and Faina 2001; Koppo and Bouckaert 2002; Koppo et al. 2002; Borrani, Candau, Perrey, Millet, Millet and Rouillon 2003; Fernandes, Cardoso, Soares, Cascensao, Colaco and Vilas-Boas 2003; Santalla, Perez, Montilla, Vicente, Davison, Earnest and Lucia 2003; Deley, Millet, Borrani, Lattier and Brondel 2005; Sabapathy et al. 2005). It has been suggested that the majority of the decrease in efficiency and appearance of the $\dot{V}O_2$ slow component occurs within the working muscle (Poole 1994). Whilst the majority of available evidence is supportive of this hypothesis, the use of NIRS to monitor changes in mOxy within the working muscle has received minimal attention (Demarie et al. 2001). Such research may help to identify the extent as to which the slow component is developed within active musculature, and other factors influencing a decrease in muscular efficiency within aged and trained populations. Therefore, the purpose of Study Three in the present series of investigations was to examine the effect of age on the development of the $\dot{V}O_2$ and mOxy slow components in well-trained cyclists.

OFF-TRANSIENT KINETIC RESPONSES

The third and final metabolic phase is the off-transient response which details the return of $\dot{V}O_2$ from end-exercise values to resting baseline (Hill and Lupton 1923; Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003). As discussed earlier, a large volume of literature has described the on-transient metabolic responses across exercise intensities. In contrast, a limited amount of research has investigated the metabolic recovery following the completion of exercise (Gaesser and Brooks 1984; Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003).

The rate at which $\dot{V}O_2$ recovers to baseline is of practical significance for a wide range of populations, especially those who suffer from chronic disease or a prolonged sedentary lifestyle as they may endure longer periods of metabolic recovery from activities of daily living (Palange, Galassetti, Mannix, Farber, Manfredi, Serra and Carlone 1995; Nanas, Nanas, Kassiotis, Alexopoulos, Samakovli, Kanakakis, Tsolakis and Roussos 1999; Myers, Gianrossi, Schwitter, Wagner and Dubach 2001; Pouliou et al. 2001; Arena, Humphrey, Peberdy and Madigan 2002). For recreational or competitive athletes, the off-transient $\dot{V}O_2$ response is particularly important for individuals whose sport requires repeated bouts of exercise as seen during interval training or prolonged high-intensity intermittent sports (Gaesser and Brooks 1984; Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003). Therefore, an understanding of the controlling mechanisms of the off-transient $\dot{V}O_2$ response is important in order to attempt to maximise recovery responses.

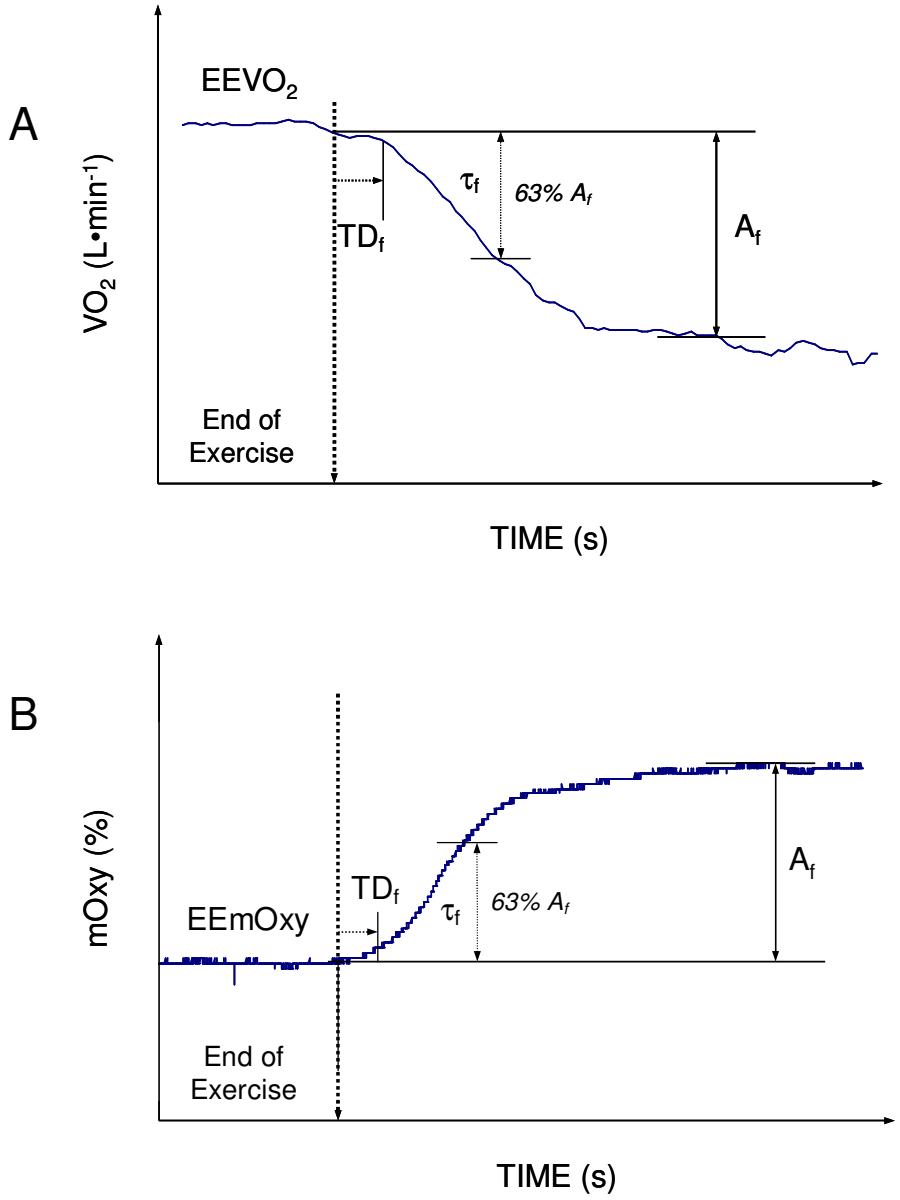

Figure 2.7: Schematic representation of the $\dot{V}O_2$ (A) and mOxy (B) off-transient response and kinetic parameters following heavy-intensity submaximal exercise (EE: End-exercise; A_f: Off-transient amplitude; TD_f: Off-transient time delay; τ_f: Off-transient time constant).

Following the completion of an exercise bout, it is widely accepted that $\dot{V}O_2$ decreases exponentially toward a resting baseline level (Figure 2.7) (Borsheim and Bahr 2003). This elevated $\dot{V}O_2$ following exercise completion

was originally termed O_2 debt (Hill and Lupton 1923; Margaria et al. 1933) but has more recently been labelled excess post-exercise O_2 consumption (EPOC) (Gaesser and Brooks 1984; Bangsbo, Gollnick and Graham 1990; Bahr and Sejersted 1991; Bahr 1992). The off-transient response has been reported to follow either a single or double component path until a resting baseline is reached (Borsheim and Bahr 2003). Previously, Bahr (1992) showed that for heavy-intensity exercise (>VT), the off-transient $\dot{V}O_2$ response could be fitted with a mono-exponential function, despite a double-exponential model being required to fit the on-transient $\dot{V}O_2$ response.

While the $\dot{V}O_2$ off-transient response has been reported in numerous previous investigations (Bahr and Sejersted 1991; Paterson and Whipp 1991; Chilibeck et al. 1995; Chilibeck et al. 1996b, 1997; Borsheim and Bahr 2003), the nature of the mOxy recovery is less documented (Chance, Dait et al. 1992; DeLorey et al. 2003b; Puente-Maestu et al. 2003; duManoir et al. 2005). duManoir, Delorey, Heenan, Kowlachuk and Paterson (2005) investigated the adaptation of the $\dot{V}O_2$, mOxy and leg blood flow responses following moderate-intensity knee-extensor exercise in seven (27 ± 5 y) healthy adults. The investigators reported that the speed of the off-transient $\dot{V}O_2$ τ_f (32 ± 5 s) and leg blood flow τ_f (25 ± 5 s) were significantly quicker than the mOxy τ_f (91 ± 26 s). This observation suggests that the decrease in blood flow to the leg following exercise was greater than the utilisation of O_2 within the muscle, which resulted in a slow increase in mOxy following exercise (duManoir et al. 2005). However, the speed of the $\dot{V}O_2$ and blood flow recovery responses was similar which suggests that the nature of the two responses may be related. The observation of a significantly slower mOxy τ_f compared to either the $\dot{V}O_2$ or

blood flow responses suggests that the reoxygenation of intra-muscular HbO_2 and MbO_2 stores, as well as lactate oxidisation control the metabolic recovery response. The findings of Chance et al. (1992) support this hypothesis by suggesting that the mOxy recovery responses of the working muscle may be dependent upon the muscle fibre type and biochemical environment. These intra-muscular parameters are likely to change with exercise intensity and duration, training status and aging and may influence the kinetics of muscle reoxygenation following constant-load exercise bouts.

In summary, the off-transient response is of both clinical and practical significance to a wide range of populations. However, to date, the off-transient $\dot{V}O_2$ response has received limited scientific interest compared to either the on-transient or slow component responses (Borsheim and Bahr 2003). From the available research, the data suggesting that the off-transient $\dot{V}O_2$ response is dependent upon exercise intensity are unequivocal (Borsheim and Bahr 2003). Similar to the on-transient and slow component $\dot{V}O_2$ responses, the off-transient $\dot{V}O_2$ response is influenced by a number of physiological factors and adaptations that will be discussed below.

Factors Influencing the Off-Transient Responses

The nature of metabolic recovery from a bout of exercise has been suggested to depend upon intra-muscular factors (Bahr 1992). These factors that may influence the off-transient metabolic response include lactate oxidation, exercise intensity and duration, as well as physical training and aging.

Lactate Oxidation

The oxidation of lactate has been suggested as a primary mechanism responsible for the exponential decrease in $\dot{V}O_2$ following exercise (Brooks 1986; Bahr 1992). As observed by Brooks, Hittelman and colleagues (1971) and Bahr and Sejersted (1991) the magnitude of EPOC exhibits a curvilinear relationship with exercise intensity which is most likely related to an elevated [BLa^-]. For example, Brooks (1986) observed that ~70% of lactate accumulated during exercise is oxidised within active muscles, whilst the balance of lactate is converted to glycogen in the liver through the O_2 mediated glyconeogenesis. It may be possible that high-intensity exercise results in an increased amplitude of the off-transient $\dot{V}O_2$ response, due to the increased amount of O_2 required to either oxidise lactate and/or aid in glycogen and pyruvate resynthesis (Bahr 1992).

Bahr and Sejersted (1991) investigated the effect of exercise intensity and duration on the magnitude of the off-transient $\dot{V}O_2$ response in six young (23 ± 0.6 y; 49.9 ± 1.4 $mL \bullet kg^{-1} \bullet min^{-1}$) physically-active male subjects. Each subject completed three 80 min bouts of cycling exercise at 29%, 50% and 75% $\dot{V}O_2max$ at a cadence of 75 RPM. The resulting increase in lactate was small, with [BLa^-] concentrations of 0.87 ± 0.11 $mmol \bullet L^{-1}$, 1.10 ± 0.07 $mmol \bullet L^{-1}$ and 3.83 ± 0.39 $mmol \bullet L^{-1}$, respectively. The investigation revealed a non-significant relationship ($r = 0.65$, $p<0.10$) between the off-transient $\dot{V}O_2$ amplitude and [BLa^-]. No data was provided on the rate at which $\dot{V}O_2$ returned to a baseline condition following the completion of the 80 min exercise bouts. The observation of a non-significant relationship may also be due to the relatively

low [BLa^-] levels observed by the researchers, given that none were above normal anaerobic threshold levels (~4 $mmol \bullet L^{-1}$).

More recently, Billat et al. (2002) investigated the $\dot{V}O_2$ off-transient responses to a series of treadmill runs to exhaustion which elicited intensities of 25%Δ, 50%Δ, 75%Δ and $\dot{V}O_2max$ in nine young healthy endurance-trained males (25 ± 1 y; 56.0 ± 6.8 $mL \bullet kg^{-1} \bullet min^{-1}$). These investigators reported that $\dot{V}O_2$ τ_f was not significantly related to either the running velocity, or end-exercise [BLa^-] at any exercise intensity. Although the majority of the current literature suggests that the $\dot{V}O_2$ off-transient response is lengthened with elevated [BLa^-], it appears that only a small number of research investigations have adequately investigated this relationship. To date, no causal association has been observed between [BLa^-] and the off-transient $\dot{V}O_2$ response (Brooks et al. 1971; Bahr and Sejersted 1991; Bahr 1992; Billat et al. 2002).

In summary, following high-intensity exercise requires an elevated $\dot{V}O_2$ for the oxidation of lactate, although conflicting evidence has questioned this relationship (Gaesser and Brooks 1984; Billat et al. 2002). The link between the off-transient $\dot{V}O_2$ response and [BLa^-] may be dependent upon both the intensity and duration of the exercise, as these factors have been shown to play a significant role in the metabolic recovery responses.

Exercise Intensity and Duration

During on-transient adjustment at the commencement of exercise, the exponential paths of $\dot{V}O_2$ and mOxy continue to meet the oxidative requirements of the energy pathways of the muscle which is dependent upon

the intensity of the prior exercise bout (Bahr and Sejersted 1991; Bahr 1992). Similarly, the duration of exercise is also likely to affect the recovery O_2 response due to factors such as the gradual desaturation of HbO_2 and MbO_2 stores and increased carbohydrate depletion observed with lengthening exercise bouts (Bahr and Sejersted 1991; Bahr 1992; Scheuermann et al. 1999; Borsheim and Bahr 2003).

Previous research has suggested the existence of an exponential relationship between the off-transient $\dot{V}O_2$ response and exercise intensity and a linear relationship with exercise duration, provided that the exercise is performed at intensities of >50% $\dot{V}O_2$max (Borsheim and Bahr 2003). In their recent review, Borsheim and Bahr (2003) suggested that the intensity of exercise accounted for ~46% of the off-transient $\dot{V}O_2$ response, with the duration of exercise, and the interaction between exercise intensity and duration accounting for 8.9% and 7.7%, respectively. Borsheim and Bahr (2003) also suggested that exercise intensity influenced both the amplitude and speed of the off-transient response. Furthermore, these researchers suggested that exercise duration only had the potential to influence the speed of the off-transient response which supports the previous work of Bahr and Sejersted (1991).

Earlier, Billat et al. (2002) investigated the influence of the duration of exercise on the off-transient $\dot{V}O_2$ response during treadmill running to exhaustion in nine endurance-trained males (38 ± 7 y; 57.9 ± 5.6 $mL \cdot kg^{-1} \cdot min^{-1}$) across a range of high intensities (25%Δ, 50%Δ, 75%Δ; and $\dot{V}O_2$max). The EE$\dot{V}O_2$ and the off-transient $\dot{V}O_2$ amplitude were both

significantly increased across the exercise intensities. The $\dot{V}O_2$ TD_f was significantly decreased with increasing exercise intensity. The researchers also observed that the $\dot{V}O_2$ τ_f did not significantly lengthen with increasing intensity treadmill running across the 25%Δ (44 ± 15 s); 50%Δ (47 ± 8 s); 75%Δ (46 ± 10 s); and 100%Δ (53 ± 14 s) SWT. These results support the curvilinear relationship between the $\dot{V}O_2$ response and exercise intensity previously suggested by Bahr (1992). Furthermore, Billat et al. (2002) reported that the off-transient $\dot{V}O_2$ response was best fitted by a mono-exponential function throughout the range of exercise intensities between VT and $\dot{V}O_2$max.

It may also be possible that the $\dot{V}O_2$ slow component may affect the $\dot{V}O_2$ off-transient response following high-intensity exercise bouts, given that it may significantly increase both the EE$\dot{V}O_2$, and off-transient $\dot{V}O_2$ amplitude. For example, Scheuermann et al. (1999) investigated whether the magnitude of the $\dot{V}O_2$ slow component influenced the off-transient $\dot{V}O_2$ response in seven young (25 ± 5 y) male subjects during ramp tests. The researchers reported that as a result of either a fast (65 W•min^{-1}) or slow (8 W•min^{-1}) ramp cycling test that neither the $\dot{V}O_2$ τ_f (29.5 ± 3.5 s vs. 32.1 ± 8.7 s) nor the amplitude (2478 ± 641 mL•min^{-1} vs. 2691 ± 619 mL•min^{-1}) were significantly different between the two ramp protocols. However, the researchers did not quantify any differences in the slow component amplitude between testing protocols.

At present, few studies have examined the effect of exercise intensity on the nature of the mOxy recovery response following various intensity constant-load exercise bouts (Chance et al. 1992; DeLorey et al. 2003b; Puente-Maestu et al. 2003; duManoir et al. 2005). Puente-Maestu et al. (2003) investigated the

effect of intensity on the reoxygenation of muscle in patients with chronic obstructive pulmonary disease. The results from this investigation revealed that the off-transient mOxy τ_f lengthened as exercise intensity increased. The mOxy τ_f lengthened with increasing exercise intensities from 80% LT (63.0 ± 15.0 s), 45%Δ (77.0 ± 26.0 s) and 65%Δ (74.0 ± 15.0 s) prior to a six-week endurance-training program. A similar trend was observed in the post-training mOxy off-transient τ_f across the same exercise intensities (46.0 ± 27.0 s; 57.0 ± 10.0 s; 62.0 ± 26.0 s). The observation of a lengthening mOxy τ_f with increasing exercise intensity suggests an O_2 utilisation mechanism within the working muscle controlling the speed of $\dot{V}O_2$ recovery. From this research, it appears as though exercise intensity has a significant effect on the off-transient mOxy response, while no research has reported the effect of exercise duration.

In conclusion, both the magnitude and speed of the off-transient $\dot{V}O_2$ and mOxy responses is related to both the intensity and duration of the prior exercise bout. The relationship between the off-transient $\dot{V}O_2$ response and exercise intensity appears to be curvilinear across increasing intensities, whereas exercise duration appears to share a linear relationship at moderate to high work intensities (>50% $\dot{V}O_2$max) with the off-transient $\dot{V}O_2$ response (Borsheim and Bahr 2003). The kinetics for the $\dot{V}O_2$ off-transients appear unrelated to either the on-transient response or the appearance of the slow component. However, the off-transient response has been shown to be influenced by both physical training and aging.

Influence of Physical Training on the Off-Transient Responses

It appears that the nature of the off-transient metabolic response is controlled through intra-muscular mechanisms, which may suggest that the off-transient metabolic response is influenced by physical training (Chilibeck et al. 1997; Myers et al. 2001; Billat et al. 2002; Puente-Maestu et al. 2003). Similar to the literature discussed earlier on on-transient $\dot{V}O_2$ kinetics, the speed of $\dot{V}O_2$ off-kinetics is increased by physical training, although evidence elucidating the mechanisms to explain this effect is lacking (Short and Sedlock 1997; Carter et al. 2000a; Billat et al. 2002).

Billat et al. (2002) reported that the off-transient $\dot{V}O_2$ τ_f following runs at 90% (62 ± 19 to 44 ± 11 s) and 95% v$\dot{V}O_2$max (63 ± 22 to 51 ± 9 s) in young males (25 ± 1 ; 56.0 ± 6.8 $mL \bullet kg^{-1} \bullet min^{-1}$) was significantly reduced following four weeks of interval running training. This acceleration of the $\dot{V}O_2$ off-transient response following training was shown to be related to the [BLa^-] at 95% v$\dot{V}O_2$max but not at 90% v$\dot{V}O_2$max. Despite this relationship, no difference was observed in the [BLa^-] and EE$\dot{V}O_2$ between the two exercise intensities. Importantly, the total EPOC during the first three minutes of recovery was not significantly affected by training [(90% v$\dot{V}O_2$max: 4.8 ± 0.5 vs. 4.4 ± 0.6 L)(95% v$\dot{V}O_2$max: 4.7 ± 0.7 vs. 4.6 ± 0.7 L)] for pre- and post-training tests, respectively. Additionally, given that the oxidisation of lactate may be a major contributor to the post-exercise $\dot{V}O_2$ response, it is possible that the reduced [BLa^-] due to the decreased relative intensity may have explained the improved $\dot{V}O_2$ off-transient kinetics, (Gaesser and Brooks 1984; Bahr and Sejersted 1991; Bahr 1992).

Training adaptations in the peripheral muscle fibre composition and capillarisation may also influence the off-transient metabolic response (Denis et al. 1986; Baba, Kawamura, Shibata, Sohirad and Kamiya 1995; Chilibeck et al. 1997). Chilibeck et al. (1997) examined the relationship between the off-transient $\dot{V}O_2$ response following moderate-intensity plantar flexion exercise and measures of capillarisation in both young (25.9 ± 2.1 y) and older (66.0 ± 6.3 y) healthy individuals. The researchers hypothesised that increased muscle capillarisation would allow greater O_2 delivery, and therefore speed the off-transient $\dot{V}O_2$ response following exercise. However, they observed no effect of age or difference in capillarisation between the two age groups. Moreover, Chilibeck et al. (1997) observed that the off-transient $\dot{V}O_2$ τ was significantly related to the capillary density ($r= -0.68$, $p<0.05$), capillary contacts per fibre area ($r= -0.83$, $p<0.05$), as well as the maximal diffusion distance ($r= 0.68$, $p<0.05$) for the two combined groups, but not for either cohort. Moreover, the capillary density and capillary contacts per fibre area measures were significantly related to the off-transient $\dot{V}O_2$ τ when the data were pooled. The results from Chilibeck et al. (1997) suggest that the $\dot{V}O_2$ τ_f was decreased by a training-induced increased capillarisation increasing O_2 delivery to within the muscle cell.

To date, Puente-Maestu et al. (2003) are the only investigators to describe the effect of physical training on the off-transient mOxy responses to both moderate- (80% VT) and high-intensity (45%Δ; 65%Δ) exercise. This study examined the effects of six weeks of cycling at 70% $\dot{V}O_2$max on changes in muscle oxidative capacity and reoxygenation kinetics in patients (63 ± 10 y; 1.33 ± 0.31 $L \cdot min^{-1}$) with chronic obstructive pulmonary disease. Puente-

Maestu et al. (2003) reported that the off-transient mOxy τ_f significantly improved between pre- and post-SWT testing time points at 80% LT (63.0 ± 15 s vs. 46.0 ± 27.0 s), 45%Δ (77.0 ± 26.0 s vs. 57.0 ± 10.0 s) and 65%Δ (74 ± 15 s vs. 69.0 ± 11.0 s). In addition, the researchers reported significant improvements in the oxidative capacity of the VL as evidenced by increased CS activity (20.2 ± 9.9 vs. 29.6 ± 13.1 $\mu mol \bullet g_{w.w.}^{-1} \bullet min^{-1}$), but not β-HAD activity (2.6 ± 0.6 vs. 2.9 ± 0.9 $\mu mol \bullet g_{w.w.}^{-1} \bullet min^{-1}$) as a result of the training regime. Puente-Maestu et al. (2003) reported that the improvement in the time course of muscle reoxygenation was significantly correlated to the change in CS activity following both the 45%Δ and 65%Δ SWT. Therefore, the data presented by Puente-Maestu et al. (2003) suggest that the reoxygenation response after high-intensity submaximal exercise is dependent upon the utilisation of O_2 within the muscle. It may also be possible that the improvement in mOxy recovery kinetics is due to other peripheral training adaptations which were not measured by Puente-Maestu et al. (2003).

In summary, physical training appears to improve the speed but not the amplitude of the off-transient $\dot{V}O_2$ response. It is likely that this improvement in the off-transient $\dot{V}O_2$ response is the result of an increased O_2 delivery to the muscle cell by various physiological and histochemical adaptations which facilitate faster lactate oxidation and gluconeogenesis. It may also be possible that sedentary aging may lead to the slowing of the off-transient $\dot{V}O_2$ response due to the detraining effects that accompany the aging process (Chick et al. 1991; Chilibeck et al. 1995; 1997).

Influence of Aging on the Off-Transient Responses

Finally, it has been suggested that the aging process may influence the off-transient metabolic recovery responses (Chick et al. 1991; Chilibeck et al. 1995; 1997). As previously discussed, sedentary aging has been shown to have an influence in both histochemical and biochemical properties of peripheral muscle fibres (Campbell, McComas and Petito 1973; Houmard et al. 1998; Conley et al. 2000; Frontera et al. 2001). Therefore, changes such as those in lactate responses, capillarisation and fibre composition have the potential to influence the metabolic responses of recovery from constant-load exercise.

Only a few studies have investigated the effects of aging on the off-transient $\dot{V}O_2$ and mOxy responses following constant-load exercise bouts (Chick et al. 1991; Chilibeck et al. 1995; 1997; DeLorey et al. 2003b). For example, Chilibeck et al. (1997) studied nine elderly (66 ± 6.3 y) and eleven young (25.9 ± 2.1 y) volunteers during calf plantar flexion at 45% of peak work rate. The researchers reported that the off-transient $\dot{V}O_2$ τ (44.1 ± 18.8 s) and wMRT (47.0 ± 22.7 s) was significantly slowed in the aged cohort compared to the younger population (36.8 ± 19.0 s; 33.1 ± 16.6 s). They observed weak to moderate significant correlations between the off-transient $\dot{V}O_2$ τ and capillary density ($r= -0.48$, $p<0.05$), and capillary contacts per muscle fibre CSA ($r= -0.59$, $p<0.05$) for the younger group, but not the aged subjects. The researchers proposed that these significant relationships may indicate that the delivery of O_2 to the muscle is of physiological significance during the $\dot{V}O_2$ off-transient response in the young subjects.

An earlier study by the same research group also investigated the $\dot{V}O_2$ kinetics during both cycling and plantar flexion exercise modalities in young (26.9 ± 2.5 y) and old (66 ± 7.7 y) sedentary individuals (Chilibeck et al. 1995). They reported that the older group had a significantly longer $\dot{V}O_2$ τ_f in comparison to the young group (49.7 ± 14.1 s vs. 34.9 ± 5.9 s) following heavy-intensity (90% $\dot{V}O_2$max) cycling exercise. However, following plantar flexion exercise, this significance difference disappeared between the age groups (35.2 ± 12.1 vs. 32.4 ± 13.6 s). Taken together, these findings suggest that the $\dot{V}O_2$ recovery following exercise may also be influenced by the local metabolic activity or histochemical characteristics of the active muscles, and any such local adaptations acquired from physical training or repeated activities of daily living. Similar results were observed by Chick et al. (1991) who reported that sedentary aging demonstrated a slowing effect on the off-transient $\dot{V}O_2$ response, regardless of fitness level. Chick and colleagues (1991) suggested that the delayed transfer of metabolites such at $[H^+]$ and CO_2 from the muscle to the blood and the delayed elimination of CO_2 in respired air due to an age-related reduced ventilatory CO_2 chemosensitivity was responsible for the slowed recovery responses.

More recently, DeLorey et al. (2003a) investigated the relationship between the off-transients for $\dot{V}O_2$ and muscle deoxygenation in sedentary young (25 ± 3 y) and elderly (68 ± 3 y) groups following a moderate-intensity (80% VT) cycling SWT. In this study, DeLorey et al. (2003a) reported that the $\dot{V}O_2$ τ_f was significantly longer in the old (44 ± 9 s) when compared to the young (30 ± 5 s) cohort, despite the mOxy recovery kinetics being non-significantly faster in the older than the younger group (35 ± 24 vs. 51 ± 16 s). These results

suggest that the greater capacity to utilize O_2 within elderly muscle previously discussed may influence the off-transient metabolic response and that age-related changes in the $\dot{V}O_2$ response appear to be related to O_2 transport limitations. The dissociation between the off-transient $\dot{V}O_2$ and mOxy responses suggests a lack of a physiological relationship between these two factors. This lack of a relationship may be due to unrelated O_2 costs within non-working muscle to remove BLa^- and 'repay' HbO_2 stores deoxygenated at exercise onset to help maintain sufficient pO_2 level at the mitochondrial level (Bahr 1992).

In summary, the mechanisms responsible for the age-related slowing of the recovery $\dot{V}O_2$ kinetics remain unknown, but appear to be related to both the utilisation and delivery of O_2 within the muscle cell. The available results suggests that the maintenance of the off-transient $\dot{V}O_2$ responses following activities of daily living (plantar/dorsi flexion), but significant slowing of the off-transient response following unfamiliar exercise such as cycling (Chilibeck et al. 1995). It is presently unknown which aging mechanism is associated with the slowing of the off-transient metabolic responses. However, it appears as though the delivery of O_2 to within the muscle plays a vital role, given that the mOxy recovery kinetics have been shown to be unrelated to the $\dot{V}O_2$ off-transient response. Limited literature has described the concurrent effect of training and aging on the off-transient metabolic responses. Thus, the purpose of Study Four from the present series of investigations is to examine the effect of age on the off-transient $\dot{V}O_2$ and mOxy responses following moderate-, heavy- and severe-intensity SWT in well-trained cyclists.

SUMMARY OF LITERATURE

The present review of literature has attempted to discuss and synthesise the available research regarding the effect of training and aging on the metabolic responses during exercise transitions. Present research has strongly focused upon $\dot{V}O_2$ kinetics, with a large emphasis on the on-transient metabolic adaptation (Whipp and Wasserman 1972; Babcock et al. 1994b; Grassi et al. 1996; Hebestreit, Kriemler, Hughson and Bar-Or 1998; Xu and Rhodes 1999; Bangsbo et al. 2000; Bearden and Moffatt 2000; Grassi 2000; Hughson, O'Leary, Betik and Hebestreit 2000; Grassi 2001; Billat et al. 2002; Carter, Grice, Dekerle, Brickley, Hammond and Pringle 2005; DeLorey et al. 2005; Kilding, Challis, Winter and Fysh 2005). This large body of research has suggested that either O_2 utilisation or delivery limitations are responsible for controlling the rate of metabolic adaptation at the start of exercise (Whipp and Wasserman 1972; Xu and Rhodes 1999; Bangsbo et al. 2000; Grassi 2001; Grassi 2005; Jones and Poole 2005b). Such research has also suggested that the rate of adaptation in response to exercise bouts is influenced by peripheral muscle characteristics (Whipp and Wasserman 1972; Xu and Rhodes 1999; Bangsbo et al. 2000; Grassi 2001; 2005; Jones and Poole 2005b), physical training (Babcock et al. 1994a; Carter et al. 2000a; Koppo et al. 2004) and aging (Babcock et al. 1994a; Chilibeck et al. 1995; 1998; Bell et al. 1999; DeLorey et al. 2003a; 2003b; 2004a; 2005). To date, the consensus is that the on-transient $\dot{V}O_2$ and mOxy responses are slowed with sedentary aging, but improved with physical training. Limited data are available as to whether concurrent physical training with aging has any such effect on these responses (Babcock et al. 1994a).

Secondly, a large body of literature has investigated the $\dot{V}O_2$ slow component observed during exercise of varying intensities (Poole 1994; Poole et al. 1994; Xu and Rhodes 1999; Zoladz and Korzeniewski 2001). In contrast, limited studies have examined the mOxy slow component during high-intensity exercise (Miura et al. 1999; Demarie et al. 2001). The slow component is thought to represent a decrease in either metabolic or mechanical efficiency as observed by a gradual increase in the $\dot{V}O_2$ requirements of the working muscle. From the available data, it appears that the majority of the slow component is developed within the working muscle (Poole 1994; Gaesser and Poole 1996; Demarie et al. 2001). This intra-muscular development has been related to either muscle fibre composition or altered recruitment patterns with sustained high-intensity exercise. While the development of the slow component has been related to a number of other physiological mechanisms, the identified relationships appear to be more coincidental rather than causal. Similarly, the $\dot{V}O_2$ and mOxy slow components have also been observed to be benefited by physical training (Poole 1994; Gaesser and Poole 1996; Carter et al. 2000a; Demarie et al. 2001) and aging (Poole 1994; Gaesser and Poole 1996), but limited evidence has been presented detailing the effects of concurrent aging and physical training on these responses.

Lastly, limited research has investigated the off-transient responses of the $\dot{V}O_2$ and mOxy measures following the completion of various intensity exercise bouts (Bahr and Sejersted 1991; Paterson and Whipp 1991; Chilibeck et al. 1995; Chilibeck et al. 1996b; 1997; Borsheim and Bahr 2003). The available evidence suggests that the elevated aerobic metabolism following exercise is due to the restoration of the working muscle to homeostasis, and

oxidation/removal of intra-muscular metabolites. The nature of the recovery metabolic responses has also been reported to be influenced by both exercise intensity and duration (Bahr and Sejersted 1991; Bahr 1992; Borsheim and Bahr 2003). As with the on-transient and slow component responses, sedentary aging and physical training have been reported to lengthen the off-transient kinetic responses, but no data have reported the effects of concurrent physical training and aging on the off-transient metabolic responses.

In conclusion, while the available evidence suggests that the capacity to adapt metabolically is slowed with aging, this capacity appears more apparent in the $\dot{V}O_2$ kinetics compared to the mOxy kinetics. Physical training has been shown to improve the rate of metabolic adaptation in response to exercise transitions in young subjects, but limited literature has reported the beneficial effects of concurrent training and aging on the $\dot{V}O_2$ and mOxy responses. Therefore, the present study aims to contribute to this small body of literature, and examine the $\dot{V}O_2$ and mOxy responses of young and middle-aged well-trained cyclists across a range of moderate to severe exercise intensities.