Does overload exercise training alter autonomic nervous system activity and hemodynamic regulation in aerobically fit men?

by

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ABSTRACT

In general, chronic endurance training has positive effects on physiological function. However, the performance of training in excess of what an individual is able to recover from can lead to performance, physiological and psychobiological maladaptation. The purposes of this investigation were to assess the temporal changes in: 1) the activity of the sympathetic and parasympathetic branches of the ANS, as well as hemodynamic regulation, at rest, 2) the response of the sympathetic branch of the ANS, and the hemodynamic response, to stress, and 3) psychobiological measures of fatigue associated with a period of overload training and a period of tapered training. It was hypothesized: 1) that resting sympathetic activity would be increased following the period of overload training and return toward baseline levels following the period of tapered training, 2) that the overload period would augment the sympathetic response to a sympathetic stressor and the taper period would return the response toward baseline values, and 3) that the activity of the parasympathetic nervous system at rest would not be altered by the periods of overload or tapered training. Hemodynamic and autonomic activity were assessed at rest and during a graded lower body negative pressure test in young, fit male participants prior to and following one week of intense training and a subsequent week of tapered training. Neither performance nor psychobiological disturbances were observed following either period of training. Blood pressure was reduced and vascular conductance increased following the intense training period, while resting autonomic nervous system activity and the autonomic and hemodynamic responses to stress were not influenced. Thus, the period of intense training performed by participants did not cause performance, physiological or psychobiological maladaptation or alter the activity of the autonomic nervous system.

Preface

This thesis is an original work by William Nathanial Lampe. The research project, of which this thesis is a part, received ethics approval from the University of Alberta Research Ethics Board, Project Name "Quantification of Sympathetic and Parasympathetic Responses to Intense Training and Taper Periods in Male Athletes", Study ID Pro00035038, January 23 2013.

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ANS – autonomic nervous system
BF – brachial artery blood flow
BP – blood pressure
BRS – baroreflex sensitivity
BV – brachial artery blood velocity
DBP – diastolic blood pressure
ECG - electrocardiogram
EPI – plasma epinephrine concentration
FVC – forearm vascular conductance
FVR – forearm vascular resistance
HR – heart rate
HRV – heart rate variability
LBNP – lower body negative pressure
MAP – mean arterial blood pressure
NOREPI – plasma norepinephrine concentration
PPO – peak power output
Q – cardiac output
RER – respiratory exchange ratio
SBP – systolic blood pressure
SV – stroke volume
TPR – total peripheral resistance
TT – Time Trial
VCO_2 – volume of carbon dioxide exhaled
VO_2 – volume of oxygen consumed
VE – minute ventilation
VT1 – aerobic threshold
VT2 – respiratory compensation threshold

Chapter 1: Introduction

1.1 Introduction

1.1.1 Overview

The concept that exercise and physical activity induce a disturbance in cellular homeostasis – thereby providing the stimulus for the initiation of physiological responses to restore homeostasis and inducing positive adaptations – has been defined as the "training principle of overload" (Kuipers, 1998). This principle provides the basis for exercise and physical activity guidelines for improved long term health outcomes, as well as the foundation for physical training to increase athletic performance (Stone, Stone, & Sands, 2007). The ultimate goal of physical training is to improve the individual's physiological capacity and performance (Smith, 2003) by maximizing the physiological adaptations to training stimuli. There is believed to be an optimal range of training stress which elicits the greatest adaptations; training stresses above and below this range do not appear to elicit training benefits to the same extent. This principle – defined as the dose response curve for performance (Busso, 2003) – has provided the foundation for theories regarding maximizing physiological and performance gains, including the concept of "overreaching" (Richardson, Andersen, & Morris, 2008). The distinction between training which is not demanding enough to induce positive physiological or performance changes, that which is appropriate, and that which is overly demanding for the individual is a subtle. In particular, athletes performing intense training in an effort to optimize performance can find themselves straddling the boundary of effective training and excessively taxing training. Such training – defined as overreaching – is achieved through an intensified period of training with inadequate recovery. As overreaching exposes the individual to a training stimulus beyond what is typical for them, periods of overreaching can be used effectively in training programs if they are followed by a recovery period to allow for supercompensation to occur and a new physiological baseline to be achieved. However, if a period of intensified training is not followed by a recovery period and the overreaching exceeds the physical capacity of the individual, the severity of fatigue gradually increases to the extent that prolonged periods of rest are required for recovery (Armstrong & Vanheest, 2002; Kreider, Fry, & O'Toole, 1998). This excessive fatigue has been shown to lead to the persistent unexplained deficits in athletic performance, termed "unexplained underperformance syndrome" (Budgett et al., 2000) and

eventually to the development of overtraining syndrome (Budgett, 1998). Thus, positive physiological and performance adaptations to training are achieved through a balance of training stress – both acute, such as from a single training bout, and chronic, from repeated training bouts – and adequate recovery and adaptation to the training stimuli (Smith, 2003).

As such, the assessment of an individual's response to a training intervention is important in assessing the efficacy of that intervention. However, the measurement and quantification of factors affecting this response is difficult (Smith, 2003). To monitor fatigue and assess the individual's adaptive response to training, many performance measures, such as incremental exercise tests (Pichot et al., 2002), time trial performance tests (Iellamo et al., 2002), and time to fatigue tests (Hedelin, Kentta, Wiklund, Bjerle, & Henriksson-Larsen, 2000) have been investigated. In addition physiological measures have been used including heart rate variability (Hedelin et al., 2000; Uusitalo, Uusitalo, & Rusko, 2000) and endocrine assessments including serum hormone levels (Lehmann, Foster, & Keul, 1993; Steinacker et al., 2000) and pituitary function tests (Lehmann et al., 1993). Additionally, psychobiological measures of fatigue have been recognized as useful tools in monitoring fatigue in athletic populations (Halson & Jeukendrup, 2004; Kreider et al., 1998) and include the Recovery-Stress-Questionnaire for Athletes (RESTQ-Sport) (Kellmann & Gunther, 2000; Steinacker et al., 2000) and a questionnaire developed by the French Society of Sports Medicine (Brun, Varlet-Marie, & Maso, 2003; Varlet-Marie, Maso, Lac, & Brun, 2004). Although these measures often serve to assess whether a training intervention is beyond what is optimal for the individual – that is, to assess whether the individual is overly fatigued or overreached – they can also be useful in sport science contexts to ensure that an individual is able to cope with the intervention. For example, positive changes in these performance, physiological and psychobiological measures suggest that the individual has responded well to the intervention. Similarly, if unfavourable changes in those assessments are not observed despite a demanding training intervention, it would suggest that the intervention has not resulted in maladaptation and is not excessively demanding for the individual.

Assessment of the autonomic nervous system (ANS) and ANS regulation of the cardiovascular system has been used to monitor individuals' responses to training (Earnest et al.,

2004; Iellamo et al., 2002; Pichot et al., 2000). As changes in cardiovascular regulation reflecting favourable ANS adaptations are associated with exercise training (Aubert, Seps, & Beckers, 2003), while changes reflecting unfavourable ANS adaptations are associated with the overtrained state (Lehmann, Foster, Dickhuth, & Gastmann, 1998), the assessment of the ANS regulation of the cardiovascular system may provide important insight into the physiological adaptation to the training stimuli. However, at present there is no general consensus or set of guidelines for what measures of ANS control are most sensitive, appropriate, or valid with respect to stress-recovery balance associated with exercise stressors.

1.1.2 Exercise Training, Overreaching, and the Taper

A fundamental component of exercise is that the physical training causes a perturbation of cellular homeostasis, which provides the stimulus ultimately responsible for the physiological adaptations to training which occurs when the individual recovers and homeostasis is restored (Kuipers, 1998). The recovery process does not stop with the restoration of homeostasis but continues, resulting in a small supercompensation. These positive physiological adaptations are often accompanied by improvements in fitness (Kuipers, 1996). Short-term periods of purposeful overreaching are a central feature of well-designed training programmes and are key in eliciting physiological adaptations and producing peak performance. However, it is important that the overreaching period is followed by a period of tapered training – described as "a specialized exercise training technique which has been designed to reverse the training-induced fatigue without a loss of the training adaptations" (Neary, Martin, Reid, Burnham, & Quinney, 1992) – to allow for recovery and supercompensation to occur. Prolonged periods of excessive training stress and inadequate recovery can lead to an overtrained state (Kentta & Hassmen, 1998; Kuipers, 1998).

Conceptually, it is generally accepted that there is a continuum of training stress-recovery imbalance. On one end of the continuum is the balance of training stress and recovery, where the individual is able to recover completely from the training stress. Overreaching exists further along the continuum and involves the incomplete recovery from acute training stresses. There is

a gradual transition from overreaching to overtraining, which exists at the far end of the continuum (Kentta & Hassmen, 1998; Kuipers, 1998; Lehmann et al., 1993).

1.1.3 Autonomic Regulation of the Cardiovascular System

The role of the ANS in the beat-to-beat adjustment of hemodynamic parameters is vital to cardiovascular function in healthy individuals (Aubert et al., 2003), and the sympathetic and parasympathetic nervous systems are considered to be the principal systems involved in the short-term regulation of cardiovascular control (in the time frame of seconds to minutes). The ANS reacts rapidly to beat-to-beat perturbations in cardiovascular homeostasis, with the efferent limbs of the sympathetic and parasympathetic systems acting to alter heart rate, atrioventricular conduction, cardiac contractility, and arterial and venous motor tone to maintain cardiovascular homeostasis (Akselrod et al., 1981) through both baroreflex and non-baroreflex responses (Blaber, Yamamoto, & Hughson, 1995).

Postganglionic sympathetic fibres supply the heart and entire peripheral circulation. In the heart, the sympathetic system innervates the SA and AV nodes and myocardium, and sympathetic stimulation increases heart rate and conduction velocity, and augments myocardial contractility. The peripheral vasculature is innervated by the sympathetic nervous system, with sympathetic activity acting to cause constriction of the blood vessels. The influence of the parasympathetic system on the cardiovascular system is largely limited to the heart, with the vagus nerve innervating the SA and AV nodes, and exerting an inhibitory effect on the heart by slowing heart rate and AV conduction velocity and decreasing cardiac contractility, principally in the atria (Smith & Kampine, 1990).

The arterial baroreflex acts to regulate the activity of the sympathetic and parasympathetic branches of the autonomic nervous system in response to acute changes in blood pressure. Arterial baroreceptors respond to changes in stretch – reflecting changes in arterial blood pressure – with afferent baroreceptor discharge being relayed to the nucleus of the solitary tract in the medulla. Changes in afferent baroreceptor discharge alter the efferent parasympathetic and sympathetic nervous system activity. Increases in blood pressure act to

increase the afferent baroreceptor discharge. With regard to the cardiovagal branch of the arterial baroreflex, increases in afferent baroreceptor discharge act to increase efferent cardiovagal discharge and reduce heart rate, whereas reductions in afferent baroreceptor discharge act to reduce efferent cardiovagal discharge, thereby increasing heart rate (Rowell, 1993). Thus, the assessment of the spontaneous cardiovagal baroreflex sensitivity (BRS) provides a non-invasive measure of the arterial baroreceptor reflex. Using the sequence method, sequences of 3 or more consecutive arterial pulses with both HR and SBP either increasing or decreasing are identified, with the average slope of the linear SBP-HR relationships providing a measure of the BRS. As the spontaneous BRS reflects primarily cardiovagal activity it provides a non-invasive measure of parasympathetic activity, whereby increases in the BRS are associated with augmented parasympathetic activity and decreased BRS is associated with decreased parasympathetic activity (Chapleau & Sabharwal, 2011).

With regard to the arterial baroreceptor regulation of the sympathetic branch of the autonomic nervous system, increases in afferent baroreceptor discharge act to reduce efferent sympathetic activity, whereas reductions in afferent baroreceptor discharge act to increase efferent sympathetic activity. In regulating the sympathetic outflow to the blood vessels and the heart, the arterial baroreceptors influence vascular tone, heart rate, and intrinsic contractility of the heart. For the purposes of this investigation, the cardiovagal branch of the arterial baroreflex was assessed while the sympathetic branch was not.

Circulating epinephrine and norepinephrine can originate either from the terminal nerve endings of sympathetic postganglionic neurons or from the adrenal medullae. In the sympathetic nervous system, norepinephrine acts as neurotransmitter and is secreted into the blood by the terminal nerve endings of sympathetic postganglionic neurons, usually remaining very active for only 10 – 30 seconds after which its activity declines over 1 to several minutes. Norepinephrine is also secreted from the adrenal medullae as a hormone. The release of circulating epinephrine and norepinephrine originating from the adrenal medullae occurs in response to stimulation by sympathetic nerve terminals innervating the adrenal medullae, and usually exert their effect for approximately 5 – 10 times as long as when released as neurotransmitters, as they are removed from the blood more slowly over a period of 1 – 3 minutes (Hall & Guyton, 2011). The

quantities of epinephrine and norepinephrine secreted from the adrenal medullae under normal resting conditions are substantial and are responsible for much of the overall tone of the sympathetic nervous system at rest (Hall & Guyton, 2011). As such, resting plasma catecholamine concentrations, which should reflect primarily the catecholamines secreted by the adrenal medullae which are longer-acting than those secreted as a sympathetic neurotransmitter, have been useful in assessing resting sympathetic, independent of an acute stressor, in exercise physiology investigations (Halson et al., 2002; Hooper, Mackinnon, Howard, Gordon, & Bachmann, 1995; Hooper, Mackinnon, Gordon, & Bachmann, 1993; Lehmann et al., 1991; Dressendorfer, Hauser, & Timmis, 2000).

1.1.4 Lower Body Negative Pressure

The application of lower body negative pressure (LBNP) is an effective stressor of the cardiovascular stressor which results in the pooling of blood in the lower limbs accompanied by a central hypovolemia. The reduction in central blood volume initiates neurocirculatory and humoral reflexes which act to compensate for the loss and maintain perfusion of the heart and brain (Wolthuis, Bergman, & Nicogossian, 1974). The baroreceptor unloading – primarily the unloading of the cardiopulmonary baroreceptors up to -15 to -20mmHg of LBNP, and the unloading of both cardiopulmonary and aortic baroreceptors at greater levels of LBNP – acts to increase muscle sympathetic nerve activity, vasoconstriction of the peripheral vasculature, and increase total peripheral resistance as well as elicit an increase in heart rate in order to maintain arterial blood pressure and prevent the onset of syncope (Sundlof & Wallin, 1978; Wolthuis et al., 1974). As central venous pressure decreases with increasing LBNP intensity, progressively unloading the aortic and cardiopulmonary baroreceptors, MSNA increases and stroke volume decrease progressively, so that the level of sympathetic vasomotor activity elicited in response to the orthostatic stress reflects the extent to which the aortic and cardiopulmonary baroreceptors are unloaded (Taylor, Halliwill, Brown, Hayano, & Eckberg, 1995; Pawelczyk & Raven, 1989; Convertino, Ludwig, & Cooke, 2004).

Owing to the controlled nature of the LBNP test and the ability to accurately and reliably expose participants to varying degrees of stress, as well as the benefit of not introducing

cardiovascular reflexes present during other forms of stress such as exercise, the use of a graded LBNP test during which participants are exposed to increasing levels of LBNP offers a way to assess the hemodynamic and autonomic responses to a stressor across a range of levels of stress.

1.1.5 Effect of Endurance Training on the Response to Lower Body Negative Pressure

Individuals with histories of endurance training have been shown to have impaired orthostatic tolerance and cardiovascular regulation during orthostatic stress (Raven, 1993; Stevens, Foresman, Shi, Stern, & Raven, 1992; Levine et al., 1991; Raven & Pawelczyk, 1993; Morikawa et al., 2001). It appears that the adaptations to prolonged endurance training such as that performed by endurance athletes, such as increases in compliance of the vasculature of the lower limbs, altered Frank-Starling relations with respect to left ventricular pressure and volume, and altered baroreflex function – rather than simply aerobic fitness itself – are responsible for these impairments (Raven & Pawelczyk, 1993; Levine, Lane, Buckey, Friedman, & Blomqvist, 1991; Levine et al., 1991). Additionally, shorter periods of endurance training in individuals with average fitness have been shown to alter the hemodynamic response to LBNP in individuals with low tolerance to orthostatic stress. Nazar et al. found that the decline in stroke volume during LBNP was reduced following training, and the accompanying tachycardia response was also lessened, following six weeks of training. This, combined with a vasoconstrictor response which was not altered by the training, led to improved LBNP tolerance (Nazar et al., 2006).

1.1.6 Effect of Endurance Training on the Parasympathetic Nervous System

Chronic endurance training is associated with a variety of benefits with regard to health and cardiovascular fitness, among which are favourable autonomic adaptations. Cross-sectional investigations have demonstrated enhanced levels of parasympathetic activity in endurance-trained compared to sedentary individuals (Barney et al., 1988; Dixon, Kamath, McCartney, & Fallen, 1992; Goldsmith, Bigger, Steinman, & Fleiss, 1992; De Meersman, 1993; Seals & Chase, 1989). Longitudinal training studies using HRV measures have confirmed that endurance training can increase the activity of the parasympathetic nervous system (Seals & Chase, 1989; De Meersman, 1992; Levy et al., 1998; Yamamoto, Miyachi, Saitoh, Yoshioka, & Onodera,

2001) and VO_2max – a commonly-used measure of aerobic fitness – is highly correlated with indices of parasympathetic activity (Kenney, 1985) and in sedentary individuals.

Even in individuals with high levels of aerobic fitness and extensive endurance training histories, the relationship between training and parasympathetic activity has been observed. Parasympathetic indices of HRV and BRS have been found to increase in rowers as the intensity of their training increased over the course of season of training and competition (Iellamo et al., 2002; Iellamo et al., 2006b). Similarly, endurance-trained cross-country skiers and canoeists whose VO_2max had improved following 7 months of training and competition relative to prior to pre-season testing have also been observed to have increases in parasympathetic indices of HRV (Hedelin, Bjerle, & Henriksson-Larsen, 2001).

In contrast to the increases in parasympathetic activity typically associated with chronic endurance training which improves aerobic fitness, Sedentary individuals who performed one month of endurance training were found to have increased parasympathetic indices of HRV. A further increase in training load, essentially resulting in overreaching, was accompanied by a stagnation in the increase in parasympathetic HRV measures(Pichot et al., 2002).

Similarly, parasympathetic indices of HRV have been found to increase in rowers over a 6-month period of training at 75% of their maximum training load, while they were reduced following a 3-month period of training at 100% of their maximum training load (Iellamo et al., 2002).

The analysis of HRV of professional male cyclists in the Tour of Spain suggests that changes in resting HRV are inversely correlated to training stress (Earnest et al., 2004). HRV was monitored prior to the start of the race, after 9 days of racing, and after 15 days of racing, and changes in parasympathetic HRV indices were inversely related with training stress during the subsequent days of racing, suggesting attenuated resting parasympathetic activity due to high cumulative physical effort in the cyclists. Similarly, three weeks of intense training in elite middle-distance runners has been shown to decrease HF indices of HRV (Pichot et al., 2000).

1.1.7 Effect of Endurance Training on the Sympathetic Nervous System

There is disagreement in the literature as to the ability of HRV analysis to provide insight into both the sympathetic and parasympathetic control of HR. It is accepted that parasympathetic activity can be quantified using the HF component of HRV (Aubert et al., 2003). However, to what extent HRV is able to quantify sympathetic activity or distinguish it from parasympathetic activity is less clear. Variability in HR in the LF band has been found to reflect primarily parasympathetic activity, with rather indirect sympathetic influence (Grasso, Schena, Gulli, & Cevese, 1997; Jokkel, Bonyhay, & Kollai, 1995; Pomeranz et al., 1985; Weise, Heydenreich, & Runge, 1987). Thus, although the effects of endurance training on the parasympathetic nervous system can be ascertained from HRV indices, the findings related to the sympathetic branch should be interpreted with caution. Although it is commonly used as such, it does not appear that the LF component of HRV is an appropriate measure of sympathetic activity.

A review of investigations using microneurography to investigate the sympathetic adaptations to exercise training in humans found that most cross-sectional studies report no difference in resting MSNA between trained and untrained subjects, while longitudinal training studies report increases, decreases, and no change in resting MSNA (Ray & Hume, 1998).

Longitudinal studies investigating the effect of endurance training on sympathetic nervous system activity using microneurography to assess muscle sympathetic nerve activity have shown mixed results. No change in resting MSNA was elicited by 8 weeks of cycling training (Svedenhag, Wallin, Suldlof, & Henriksson, 1984), 6 weeks of single-leg cycling training (Ray, 1999), or 12 weeks of high-intensity run training (Sheldahl, Ebert, Cox, & Tristani, 1994) . However, resting MSNA was found to be lower after 10 weeks of intensive run training than before in young untrained individuals, which was suggested to demonstrate that a reduction in sympathetic nerve activity originates from the central effect of training – that is, by a reduction in neural sympathetic discharge and not by the release of norepinephrine from nerve terminals (Grassi et al., 1994). In each of these investigations, VO_2max was significantly increased following the training intervention.

Longitudinal investigations of the physiological effects of overload training periods in endurance-trained individuals have shown evidence increased sympathetic activity following such training interventions. Such findings have used low-frequency power of HRV measures in overtrained athletes (Uusitalo, Uusitalo, & Rusko, 1998a; Mourot et al., 2004), resting catecholamine concentrations (Hooper et al., 1995; Hooper et al., 1993; Lehmann et al., 1991), and reports of clinical features which appear to be consistent with elevated sympathetic activity in overreached and overtrained athletes (Israel, 1958). Even intense training interventions which do not result in overreaching have been found to increase LF heart rate variability in elite rowers (Iellamo et al., 2002) and increases in normetanephrine, a metabolite of circulating norepinephrine, in national-level road cyclists (Filaire et al., 2002).

However, other overload training investigations have found that resting plasma catecholamine concentrations were not altered by overload training, even when it resulted in overreaching (Billat, Flechet, Petit, Muriaux, & Koralsztein, 1999; Halson et al., 2002; Hedelin et al., 2000; Urhausen, Gabriel, & Kindermann, 1998).

Previous investigations have demonstrated that the sympathetic response to stressors may be augmented by overload training interventions. Middle- and long-distance runners who performed one week of overload training which did not result in overreaching showed increased plasma norepinephrine responses to a maximal exercise test, despite no change in resting plasma catecholamine concentrations (Billat et al., 1999). Marathon runners who performed 2 weeks of intensified training which resulted in overreaching have also been found to have increased plasma catecholamine responses to submaximal and maximal exercise (Dressendorfer et al., 2000).

Thus, while it is unclear whether overload training interventions alter resting sympathetic activity, they appear to augment the sympathetic response to stress.

1.2 Purpose

Sufficiently demanding periods of overload training are a normal and necessary part of effective exercise training programs and are important in eliciting positive physiological adaptations (Smith, 2003). However, periods of overload training combined with inadequate recovery can lead to overreaching. The overreached state has been associated with altered autonomic nervous system activity (Billat et al., 1999; Brooks & Carter, 2013; Mackinnon, 2000; Smith, 2003; Lehmann et al., 1998). The autonomic changes associated with periods of overload training which do not exceed the individual's capacity to recover, and therefore do not result in overreaching, have been investigated in longitudinal studies with sedentary participants (Pichot et al., 2002; Uusitalo, Laitinen, Vaisanen, Lansimies, & Rauramaa, 2002; Yamamoto et al., 2001). The differences in ANS activity between fit and sedentary individuals has also been examined in cross-sectional investigations (Pichot et al., 2000). However, a gap exists in the literature in that the changes in ANS activity have not been thoroughly investigated in individuals with high levels of aerobic fitness, or the changes in autonomic nervous system activity which may occur as a result of exercise training which and which are not attributable to improvements in aerobic fitness.

Additionally, planned periods of reduced training stress often follow overload training periods, ideally reversing fatigue associated with the overload training period while maintaining or furthering the positive adaptations which occur as a result of exercise training. Changes in hormonal, hematological, and performance parameters associated with periods of tapered training have been previously described (Mujika, Padilla, Pyne, & Busso, 2004) although their effects on the autonomic nervous system remain unclear.

Thus, the purposes of this investigation were:

1. To assess the temporal changes in the activity of the sympathetic and parasympathetic branches of the ANS, as well as hemodynamic regulation, at rest associated with a period of overload training and a period of tapered training in aerobically fit individuals,

2. To assess the temporal changes in the response of the sympathetic branch of the ANS, and the hemodynamic response, to stress associated with a period of overload training and a period of tapered training in aerobically fit individuals.

3. To assess the temporal changes in psychobiological measures of fatigue associated with a period of overload training and a period of tapered training in aerobically fit individuals.

The activity of the parasympathetic branch of the ANS was assessed by the analysis of heart rate variability (HRV) and arterial baroreflex sensitivity (BRS) in this thesis. The activity of the sympathetic branch of the ANS was assessed using resting plasma catecholamine concentrations. Finally, hemodynamic measures were assessed using Doppler Ultrasonography imaging of the brachial artery and the Finapres beat-to-beat blood pressure cuff. Graded lower body negative pressure tests were used as a stressor stimulus. Participants were healthy young males with a history of endurance training. Assessments were performed prior to the commencement of an overload training period, following 7 days of overload training, and following 5 days of tapered training.

1.3 Hypotheses

It was hypothesized that resting plasma catecholamine concentrations would be elevated following the overload training intervention and return toward baseline levels following the period of tapered training – indicating elevated sympathetic activity at rest following the overload intervention and restoration of baseline levels of sympathetic activity following the period of tapered training.

It was also hypothesized that the peripheral vasoconstriction and augmented plasma catecholamine concentrations in response to graded LBNP would be greater following the overload intervention compared to baseline, and would return towards baseline levels following the period of tapered training – indicating an elevated sympathetic response to a sympathetic stressor following the overload intervention and return toward baseline levels following the taper period.

With respect to the parasympathetic branch of the ANS, it was hypothesized that changes in HRV and spontaneous baroreflex at rest would not be observed between baseline, post-overload intervention, and post-taper periods – indicating that parasympathetic activity at rest would not be altered by the training interventions.

1.4 Significance

Physiological differences between aerobically fit and sedentary individuals have been detailed, as have the adaptations of previously sedentary individuals both to training interventions producing positive changes in cardiovascular fitness and overtraining interventions. The physiological adaptations of endurance athletes to overtraining have also been documented. However, the adaptations to periods of overload training in aerobically fit individuals – that is, the adaptations attributable to the overload training period and not to differences in aerobic fitness – are less clear.

The ANS is essential in the regulation of the cardiovascular system. As such, understanding changes which occur in the ANS during periods of intensive training may advance our understanding of cardiovascular regulation in aerobically fit individuals and the effects of exercise on the autonomic nervous system. Because periods of intense training, tapered training, and pre-competition training strategies to optimize athletic performance are common and important in endurance sports, this investigation may have practical applications to the paradigm of sport science and coach education in endurance sports.

1.5 Delimitations

Aerobically fit men (VO_2peak of 50 mL/kg/min or greater on a cycle ergometer) with a history of endurance exercise training and participation in endurance sports, without any known medical conditions, and who were not taking any medications which may have influenced the autonomic nervous system were recruited as participants for this investigation. Men were selected for this investigation due to the confounding effect of menstrual cycle hormone phases

on autonomic nervous system activity (Hirshoren et al., 2002). Participants of ages 18-45 were selected for this investigation to minimize the confounding effects of aging on sympathetic nervous system activity and peripheral vascular resistance (Iwase, Mano, Watanabe, Saito, & Kobayashi, 1991; Narkiewicz et al., 2005; Sundlof & Wallin, 1978). This level of aerobic fitness and training history were selected to reduce the risk of participant mortality during the investigation due to the demanding nature of the overload training intervention.

Participants were asked to perform a standardized training protocol for three days before the investigation. All participants performed the same exercise interventions during the investigation.

1.6 Limitations

Inherent limitations within the investigation include participants' volitional adherence to pre-testing restrictions regarding the consumption of food, caffeine and alcohol, as well as the restriction of training performed during the investigation to that which was prescribed.

A limitation of this investigation is the participants' level of aerobic fitness and training history. Training history and aerobic fitness are important factors in endurance exercise performance and the response to training (Smith, 2003), while factors associated with endurance exercise training affect hemodynamic conditions at rest as well as the autonomic and hemodynamic responses to lower body negative pressure (Raven & Pawelczyk, 1993). Thus, the applicability of the findings of this investigation is more pertinent to athletic populations, and their direct relevance for other populations may be limited.

1.7 References

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Chapter 2: Does Overload Exercise Training Alter Autonomic Nervous System Activity and Hemodynamic Regulation in Aerobically Fit Men?

2.1 Introduction

The balance of the physiological stress imposed by exercise training and an individual's recovery are important in determining the ultimate adaptation to training. In order for exercise to induce positive adaptations and improve physiological capacity and performance, it must impose a strain sufficient to act as a perturbation to cellular homeostasis (Kuipers, 1998). If the individual is able to recover from this overload training then homeostasis can be restored and ideally a new, improved physiological baseline achieved. If the individual is not able to recover and further overload training is performed, a situation of physiological maladaptation, impaired athletic performance, and general fatigue may result (Richardson et al., 2008).

In general, exercise training is associated with positive health and cardioprotetive effects. Specific to the autonomic nervous system and its regulation of the cardiovascular system, chronic endurance exercise is associated with reductions in sympathetic activity and augmented parasympathetic activity (Leosco et al., 2013). However, situations of excessive exercise stress or inadequate recovery have been purported to alter the activity of the ANS unfavourably. Increases in sympathetic activity (Uusitalo, Uusitalo, & Rusko, 1998b; Hooper et al., 1993; Lehmann et al., 1991; Mourot et al., 2004) and reductions in parasympathetic activity (Iellamo et al., 2002; Pichot et al., 2000) in overreached and overtrained athletes have been previously reported.

The importance of understanding the effects of a period of overload training ANS activity and hemodynamic regulation is twofold. Insight into the response of the ANS to such training would allow for ANS activity to be monitored, and changes in ANS activity to be used to assess whether the balance of training stress and recovery is appropriate or whether there is a stress-recovery imbalance. Additionally, understanding the effects of overload training on the ANS and hemodynamic regulation may contribute to the current understanding of the physiological effects of exercise training. Given the inherent role of the sympathetic and parasympathetic branches of the ANS in the beat-to-beat adjustment of the cardiovascular system in response to changes in

blood pressure, and the integrated nature of the cardiovascular and autonomic nervous systems in this response, the investigation of the influence of overload training on both the autonomic nervous system and on hemodynamic regulation – specifically, the regulation of heart rate and vascular resistance, is important.

Thus, the purposes of this investigation were: to assess the temporal changes in the activity of the sympathetic and parasympathetic branches of the ANS, as well as hemodynamic regulation, at rest associated with a period of overload training and a period of tapered training in aerobically fit individuals; to assess the temporal changes in the response of the sympathetic branch of the ANS, and the hemodynamic response, to stress associated with a period of overload training and a period of tapered training in aerobically fit individuals; and to assess the temporal changes in psychobiological measures of fatigue associated with a period of overload training and a period of tapered training in aerobically fit individuals.

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It was hypothesized that resting plasma catecholamine concentrations would be elevated following the overload training intervention and return toward baseline levels following the period of tapered training – indicating elevated sympathetic activity at rest following the overload intervention and restoration of baseline levels of sympathetic activity following the period of tapered training.

It was also hypothesized that the peripheral vasoconstriction and augmented plasma catecholamine concentrations in response to graded LBNP would be greater following the overload intervention compared to baseline, and would return towards baseline levels following the period of tapered training – indicating an elevated sympathetic response to a sympathetic stressor following the overload intervention and return toward baseline levels following the taper period.

With respect to the parasympathetic branch of the ANS, it was hypothesized that changes in HRV and spontaneous baroreflex at rest would not be observed between baseline, post-

overload intervention, and post-taper periods – indicating that parasympathetic activity at rest would not be altered by the training interventions.

2.2 Methods

2.2.1 Participants

Eleven young (20 to 33 year-old), healthy males volunteered and gave written informed consent to participate in this study. Participants completed a PAR-Q and the Preparticipation Physical Evaluation (American Academy of Family Physicians et al., 2005) forms prior to participating in the investigation. Participants had a history of at least 5 years of training for endurance sports and a history of participation in endurance sports. All participants cycled as a mode of exercise training and had a VO_2peak of at least 50 mL/kg/min on a bicycle ergometer. This training history and level of aerobic fitness were selected in an effort to ensure that participants would be able to tolerate the training intervention – described below – and reduce the risk of participant mortality due to the inability to complete the overload training intervention. Previous cycling experience was included in the inclusion criteria in order to reduce the learning effect, or an improvement in cycling performance due to increased experience and familiarity with exercising on a cycling ergometer, as a confounding variable in this investigation. No individuals who volunteered for this investigation were taking medication which may have influenced ANS activity or regulation of the cardiovascular system.

2.2.2 Sample Size

The sample size for this investigation was determined based on previous research examining the effect of physical training on changes in ANS regulation of the cardiovascular system. These select investigations have had a median of 9.5 participants in the intervention group, with a range of 7-34 participants Sample size calculations based on the plasma catecholamine concentrations during stress (submaximal exercise) produced samples sizes of 3 and 22 participants. As number of participants in similar previous investigations has had a large range and been variable, and given the large range of participants produced by the sample size calculations performed, 11 participants were recruited for this study. This exceeds the median number of participants in previous investigations, and is in the range of participants from the sample size calculations (Appendix A).

2.2.3 Study Design

All testing and training was completed at the University of Alberta Integrative Human Exercise Physiology (IHEP) laboratory (E-439) in the Faculty of Physical Education and Recreation. Participants reported to the IHEP laboratory on fourteen consecutive days, and one additional day prior to beginning the investigation.

This investigation used a single group design to investigate the effects of 2 periods of training performed serially. Participants performed 7 days of overload training followed by 5 days of less demanding training, and were asked to perform 3 tests of the ANS regulation of the cardiovascular system, to complete 2 questionnaires 3 times each, to perform a test of endurance cycling performance 6 times, and to perform 3 VO_2peak tests on a cycle ergometer. Participants were asked to standardize their training during the 3 days prior to the investigation to ensure that training prior to the intervention is not overly taxing and did not cause them to be excessively fatigued at the beginning of the training intervention, and in order to standardize participants' training leading up to the investigation.

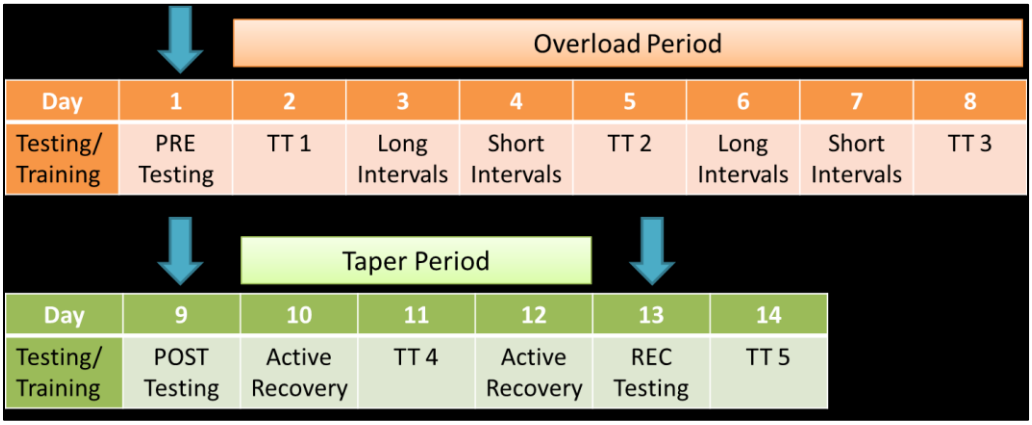

Figure 1. Study timeline. The study timeline was created around two periods of training: an overload period consisting of intense training greater than what was typical for participants, and a period of tapered training to allow for recovery. Autonomic, hemodynamic and graded exercise testing was scheduled around these periods, and Time Trial tests were performed every three days over the course of the study to evaluate the timecourse of changes in athletic performance.

2.2.4 Training Interventions

Cycling VO_2peak was assessed using an incremental exercise test to exhaustion on an electrically braked cycle ergometer (Ergoselect 200 K, Ergoline, Bitz, Germany), and performance testing and training were performed on participants' personal bicycles mounted on a CycleOps PowerBeam Pro bicycle trainer (CycleOps, Madison, WI). In addition to functioning as a resistance unit and allowing participants to use their own bicycle for the cycling performance tests and training, the CycleOps PowerBeam Pro bicycle trainer is able to accurately and reliably measure power and work output (Bertucci, Duc, Villerius, Pernin, & Grappe, 2005; Siedlik et al., 2013), in addition to heart rate, pedaling cadence, and wheel speed. Participants' heart rates, cycling speed, cadence, and power output were monitored and recorded during all cycling testing and training.

Participants were asked to perform a standardized training regimen of 75 minutes of cycling per day at 65-70% of their reported peak heart rate, for the three days preceding the start of the investigation. Participants reported understanding, and adhering to, this training prescription. The training prescription was determined based on consultations with experienced endurance sport coaches, and was intended to be less demanding than participants' typical daily training routine while still providing adequate training stimulus to avoid detraining.

Participants reported to the IHEP laboratory at a date prior to the first laboratory assessment in order to perform a familiarization 10 km Time Trial on a road bicycle and CycleOps PowerBeam Pro bicycle trainer. The purpose of this visit was both to familiarize participants Time Trial protocol – the approximate duration, perceived effort, and physical exertion involved – and to attempt to reduce the potential influence of the learning effect as a confounding factor in Time Trial performance during the investigation. In the investigators' experience, an individual who changes their pacing strategy for performance tests such as Time Trial most often does so following the first one that they perform compared to following subsequent performances. A potential confounding factor in Time Trial performance would be introduced if a participant adopted a different pacing strategy for the final four Time Trial tests than for the initial test. Thus, the familiarization Time Trial provided the opportunity to alter pacing strategy without influencing the Time Trial assessments.

An outline of the study timeline is presented in Appendix E. The day preceding the start of the intensive training period, participants performed an ANS test and a VO_2peak test. On the 1st, 4th and 7th days of intensive training participants performed a warm-up followed by a test of cycling performance (a 10 km Time Trial (TT) test repeated twice, with 15 minutes of easy cycling between tests) and a cool-down. The warm-up performed by each participant on the day of the first TT was replicated each subsequent TT testing session to reduce potential differences during the warm-up as a confounding factor in influencing TT performance. On the 2nd and 5th days of overload training, participants performed a warm-up followed by 5 repetitions of 4 minute intervals at 80% of their peak aerobic power (PPO, determined from the initial VO_2peak test) with 90 seconds of easy cycling between repetitions, repeated twice with 10 minutes of recovery between sets of intervals. On the 3rd and 6th days of intensive training participants performed a warm-up followed by 10 repetitions of 1 minute at PPO with 2 minutes of easy cycling at 50W between repetitions, repeated twice with 10 minutes of easy cycling at 50W between sets of intervals. The timeline for these interval training days is outlined in Appendix E.

This training intervention was based on interventions which have been demonstrated to be effective at improving cycling performance in participants with a similar training history and aerobic fitness level to those recruited for this investigation, and which have been published in peer-reviewed journals (Stepto, Hawley, Dennis, & Hopkins, 1999; Lamberts, Swart, Noakes, & Lambert, 2009; Lindsay et al., 1996; Laursen, Blanchard, & Jenkins, 2002).

Immediately following the intense training period, participants performed 5 days of training at a reduced training load, designed to reduce the fatigue accumulated during the intense training period while maintaining the physiological adaptations incurred from the intense training. On the 1st and 5th days of reduced training participants performed the same ANS and VO_2peak protocols as on the day preceding the training intervention. On the 2nd and 4th days of reduced training participants performed 45 minutes of cycling at an intensity eliciting a heart from 65-70% of the peak HR achieved during the prior VO_2peak test. On the 3rd day of reduced training participants performed the same TT testing protocol performed during the intensive

training period. The day following the last day of the reduced training period, participants performed a final TT test.

The intense period of training was designed in consultation with experienced coaches and sports scientists to simulate training that would typically be performed by endurance athletes during overload training periods such as training camps. This period of training was designed to induce a training stimulus greater than that induced by participants' normal daily training, and was intended to result in an accumulation of fatigue over the intense training period. The period of reduced training load was designed to provide adequate training stimulus to maintain the physiological adaptations while reducing the fatigue accumulated during the intense period of training (Neary et al., 1992).

At the beginning of each Time Trial and training session, participants' rear bicycle tires were inflated to the same pressure and the CycleOps PowerBeam Pro trainers were calibrated according to the manufacturer's specifications.

2.2.5 Testing

2.2.5.1 Autonomic Regulation of the Cardiovascular System Tests

Participants reported to the IHEP laboratory at approximately the same time of the morning on each day that ANS testing was performed, and were asked to refrain from caffeine and alcohol consumption for 12 hours prior to testing as each have been found to alter the ANS regulation of the cardiovascular system (Nurminen, Niittynen, Korpela, & Vapaatalo, 1999; Romanowicz, Schmidt, Bostwick, Mrazek, & Karpyak, 2011). Participants were asked to refrain from consuming any food within 3 hours prior to testing to reduce the effect of digestion on the ANS and cardiovascular systems during testing, as is standard practice investigations of the ANS and cardiovascular systems. An intravenous cathelon was inserted into the antecubital vein and a 10 mL blood sample was taken. The intravenous cathelon was kept patent with sterile saline (0.9% NaCl), and 5mL of blood was drawn and discarded to remove any saline present in the cathelon before each blood sample was drawn. After all pre-test tasks and instrumentation were completed (approximately 20 minutes) participants lay supine with their lower body in the lower

body negative pressure (LBNP) chamber, which was sealed at the level of the iliac crest, as sealing at the iliac crest avoids the possibility of the bladder or lower intestinal region being compressed by the seal or splanchnic blood flow being influenced and is usually used as the default location for LBNP (Goswami, Loeppky, & Hinghofer-Szalkay, 2008). Participants then rested in the supine position for 10 minutes. During the last 5 minutes of rest, baseline ECG and BP and Doppler ultrasonography data were recorded, after which a 10mL blood sample was drawn and the LBNP protocol was initiated.

Participants were submitted to serial LBNP at -15 mmHg, -30 mmHg and -50mmHg for approximately 6 minutes at each stage or until the onset of pre-syncopal signs or symptoms, including light-headedness, nausea, sweating, narrowing of vision, a rapid drop of systolic BP by more than 20 mmHg and bradycardia (Nazar et al., 2006). ECG and BP measures were recorded continuously during the LBNP testing protocol. At the end of the each of the -15mmHg, -30mmHg and -50mmHg stages of LBNP, 10mL blood samples were drawn and 10mL of saline was injected into the cathelon. The increase in suction between levels of LBNP occurred over approximately a 15 second period.

Upon completion of the test the level of negative pressure was slowly reduced in the LBNP chamber until it reached atmospheric pressure, and the participant was removed from the chamber.

2.2.5.2 Graded Exercise Test to Exhaustion

Approximately 60-90 minutes following the completion of the LBNP test, participants performed a graded exercise test to exhaustion. Snack foods and energy bars and drinks were made available to participants for consumption following the LBNP test, and participants were given the option of consuming a meal or snack of their choosing. The amount of each food and drink consumed by each participant on the first testing day was recorded, and participants consumed the same food and drink between the LBNP and graded exercise tests on each of the subsequent LBNP and graded exercise testing days.

After all pre-test tasks were completed a 10 mL blood sample was drawn from the intravenous cathelon. The workrate was increased to 30 Watts, and at the end of each minute of the test the workload was increased by 30 Watts. At the end of every second workload completed by (every 60W, or 2 minutes, during the test) and immediately following the termination of the test, a 10mL blood sample was drawn from the cathelon.

Peak aerobic power (PPO) was defined as the last completed work rate in Watts plus the fraction of time spent at the final non-completed work rate multiplied by 30W (Stepto et al., 1999; Halson et al., 2002; Weston et al., 1997). Criteria for determination of a valid VO_2peak included volitional exhaustion as well as at least 2 of the following:

- a plateau or decrease in VO_2 with increasing intensity
- attainment of age predicted maximum heart rate (220 – age)
- RER >1.15

2.2.5.3 Time Trial Performance Test

After all pre-test tasks and a warm-up were completed, participants cycled for 1 minute gradually increasing their cadence and power output, and the 10km Time Trial test began at the end of this minute. Participants were free to adjust the resistance and cadence, and were able to see the distance remaining in the Time Trial. Verbal encouragement was provided by the same investigator during each Time Trial. The test was complete when the participant has cycled the equivalent of 10km from the start of the 2nd minute of the test.

Upon completion of the Time Trial, participants had a 15 minute break during which they could consume water and sport drinks and recover either actively (easy cycling, or dismounting from the bicycle and walking) or passively (sitting on the bicycle or on a chair). The type and amount of fluid consumed, and the activity performed by participants during the recovery period, during the first Time Trial test was recorded and replicated during each of the following Time Trial tests. Following the 15 minute break, participants repeated the 10km Time Trial protocol.

The warm-up protocol used by each participant on their first day of Time Trial testing was recorded, and the protocol replicated for each Time Trial test. Thus, a standardized warm-up protocol was not used for all participants, but the warm-up protocol used for each test, by each participant, was consistent throughout the investigation.

2.2.5.4 Psychobiological Assessments of Fatigue

Participants were asked to complete the French Society of Sports Medicine questionnaire, which is designed to detect a mismatch in training stress and recovery in athletes and allow for the early detection of poor adaptation to training and insufficient recovery (Appendix C) (Bricout, 2003). In the case that a participant's Time Trial performance was impaired following the overload training intervention and had not returned to initial levels following the taper period, this questionnaire was to be evaluated to provide a secondary measure of overtraining. Participants were also asked to complete the RESTQ-Sport questionnaire, which provides a quantitative measure of individuals' perceived stress and recovery from exercise (Appendix B). This questionnaire was used to assess participants' perceived training stress-recovery balance (Kellmann & Gunther, 2000). Participants were asked to complete both questionnaires on each of the 3 ANS and graded exercise testing days.

2.2.5.5 Pre-Test Tasks

Prior to the VO_2peak and 10km TT tests, participants were instrumented with heart rate (HR) monitors (Suunto, Finland) which were used to measure and record HR for the duration of each test.

Prior to ANS tests, participants were instrumented with a three-lead ECG and beat-to-beat BP cuff (Finapres Medical System, Amsterdam).

Prior to VO_2peak tests, participants were instrumented with a NIRO 200 (Hamamatsu Photonics, Japan) spatially resolved near infrared oxygenation monitor, and a mouthpiece to through which to breathe.

2.2.6 General Design Comments

The laboratory environment was maintained at approximately 20°C and approximately the same humidity for each participant's ANS assessments, graded exercise tests, and 10km Time Trial tests. Participants were given the option of using fan during the first Time Trial tests. Whether a fan was used, and fan's intensity setting, were replicated for each of the subsequent Time Trial tests.

Participants used their own bike pedals and shoes for all testing and training.

2.2.7 Research Measures

2.2.7.1 Anaerobic Threshold Detection from Graded Exercise Testing

Determination of AT was performed using expiratory gas exchange analysis, where expired gas values were analysed both in 20 sec averages and 1 minute averages. The method for each threshold was determined via the following published protocols.

VT1 (Aerobic Threshold):

1. Primary verification was determined with the V-slope method, determined as an increase in slope greater than 1 for VCO_2 compared to VO_2 (Wasserman, Whipp, Koyl, & Beaver, 1973).
2. Secondary verification was determined by VE/VO_2 where the lowest value indicates VT1.
3. Tertiary verification was determined by increases in the $P_{ET}O_2$ without a decrease in $P_{ET}CO_2$.

VT2 (Respiratory Compensation Threshold):

1. Primary verification was done with the VE/VCO_2 where the lowest value indicates respiratory compensation.

2. Secondary verification was determined as a systematic increase in VE compared to VCO_2 indicates respiratory compensation.
3. Tertiary verification was determined by a systematic decrease in $P_{ET}CO_2$.

2.2.7.2 Cardio-Respiratory Measures

Pulmonary gas exchange (VO_2, VCO_2, RER) and minute expired ventilation (VE) were measured on a breath-by-breath basis by "open-circuit" method (Vmax 229d, Viasys Healthcare, Palm Springs, CA) while participants breathed through a mouthpiece and low-resistance mass-flow meter with their nose occluded by a nose clip. Before each test, the flow-meter was calibrated by pumping a 3-liter syringe at a range of flow rates expected during the graded exercise tests, and the O_2 and CO_2 analysers were calibrated with gases of known concentrations. The breath-by-breath data was averaged in 20 second intervals for determination of VT1 and VT2, and for the purposes of data presentation for statistical analysis. The three-lead ECG signal was sampled at a frequency of 4k Hz and amplified (ADInstruments BioAMP ML 132, Colorado Springs, CO). Beat-by-beat BP was measured using a Finometer Pro system (Finapres Medical Systems, Amsterdam). The above cardiorespiratory signals were collected using a data acquisition system (ADInstruments Powerlab ML 880, Colorado Springs, CO) and analyzed using Lab Chart 7.1 software (ADInstruments, Colorado Springs, CO) and Beatscope software (Finapres Medical Systems, Amsterdam). Frequency-domain measures of heart rate variability were calculated according to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). The spontaneous baroreflex response was determined from the resting supine ECG and BP data. Sequences of 3 or more beats in which SBP and HR of the same (lag 0), following (lag 1), or next following (lag 2) beat changed in the same direction were identified and linear regressions performed (Blaber et al., 1995). The spontaneous BRS were calculated as the mean of the slope of the linear SBP-HR relationships (Chapleau & Sabharwal, 2011). The assessment of spontaneous BRS was performed using HemoLab software (http://haraldstauss.com/HaraldStaussScientific/hemolab/).

2.2.7.3 Muscle Spatially Resolved Spectroscopy Measures

Muscle spatially resolved spectroscopy measures were collected using a NIRO 200 (Hamamatsu Photonics, Japan) spatially resolved near infrared oxygenation monitor, allowing for the assessment of muscle average O_2 saturation (TOI) and total Hb concentration (THI) during the incremental exercise tests. The NIRO 200 is a 2 channel SRS oxygenation monitor utilizing an emission probe (light source) made of fiber optics which irradiates laser beams, and a detection probe, placed 5 cm from the light source. The probes are affixed in a black probe holder to ensure maintenance of distance between light source and detection probe. The distal probe was placed in the distal third region of the right legs vastus lateralis muscle with the center of the probe approximately 10 – 12 cm above the knee joint (Grassi et al., 2003; Quaresima, Komiyama, & Ferrari, 2002). The center of the proximal probe was placed 10 cm from the center of the distal probe. These placements were made while seated on the cycle ergometer with the knee at a 90 degree angle. The area was shaved or relatively hairless to minimize any influence that the hair may have on light transmission. The right leg was wrapped with tensor bandages to affix the probes and eliminate ambient light from contaminating the SRS signal.

2.2.7.4 Brachial Blood Flow Measures

Brachial artery blood velocity (BV) was assessed using pulsed-Doppler ultrasonography (GE Vivid I, WI, USA; Multigon 500M, NY, USA) during the LBNP tests. Brachial artery data was acquired continuously with a 7.5 MHz probe with a 45° angle of insonation placed on the skin over the brachial artery. The artery diameter was measured during diastole in triplicate at rest and during each of the -15mmHg, -30mmHg, and -50mmHg stages, and the average of the triplicate measures was used to obtain a brachial artery diameter for each subject during each condition. Mean blood velocity (BV) was calculated on a beat-by-beat basis. Brachial blood flow (BF) was calculated as: $BF\ (mL/min) = BV*\pi r^2*60$, where r is the radius of the brachial artery. Forearm vascular conductance was calculated as: FVC = BF/MAP.

2.2.7.5 Psychobiological Measures

Participants were asked to complete the French Society of Sports Medicine and RESTQ-Sport questionnaires each day that ANS testing was performed. For the French Society of Sports Medicine questionnaire, participants were asked to provide a "yes" or "no" response to questions regarding early clinical symptoms of overtraining. The number of questions answered "yes" gave a score between 0 and 54, with a score greater than 20 highly suggestive of overreaching or overtraining (Varlet-Marie et al., 2004). The RESTQ-Sport questionnaire was developed to measure athletes' perceptions of stress and recovery, including general and sport-specific components. The questionnaire uses a Likert-type scale with values ranging from 0 (never) to 6 (always) indicating how often the individual participated in various activities in the past 3 days. High scores in stress-related activities reflect high stress, and high scores in recovery-related activities reflect higher recovery (Kellmann & Gunther, 2000).

2.2.7.6 Catecholamine Measures

Blood samples from the antecubital vein were collected in a 10mL syringe. Five mL of blood were transferred into a test-tube coated in Ethylenediaminetetraacetic acid (EDTA) and five mL of blood were transferred into a test-tube coated with clot activator. The blood tubes were centrifuged for 10 minutes at 1500 x gravity and then decanted into microcentrifuge tubes and frozen immediately at -20°C and then transferred to a -80°C ultra low freezer until the laboratory analyses were performed. Epinephrine and norepinephrine concentrations were determined in duplicate using an enzyme-linked immunosorbent assay kit (ELISA) (Rocky Mountain Diagnostics Inc. BA E-6500, Colorado Springs, CO).

A total of 256 blood samples were collected and analyzed for plasma catecholamine concentrations. Of the 512 concentrations determined for each of catecholamine assessed, 9 epinephrine and 16 norepinephrine concentrations were greater than 2 standard deviations from the mean for a given level of LBNP. However, all of the epinephrine and 15 of the norepinephrine measures were consistent with the other measures for the relevant participant and

level of LBNP, and 1 norepinephrine concentration was considered as an outlier and excluded from further analysis.

Four of each of epinephrine and norepinephrine assay kits were used for plasma catecholamine analysis. For each participant, all blood samples collected from them at rest and during LBNP were assessed in the same assay kit.

Intra-assay coefficient of variation (CV) was calculated for each assay kit used, using the data from the duplicate plasma catecholamine concentration determined for each blood sample.

Intraclass correlation coefficient (ICC) was calculated for plasma epinephrine and norepinephrine concentrations. The ICC for epinephrine and norepinephrine were 0.23403 and 0.110345, respectively.

The standard curve fit was determined for each assay kit used using a 4 parameter standard curve.

Table 1. Intra-Assay Coefficients of Variation and Standard Curve Fits

Catecholamine	**Assay Kit**	**Coefficient of Variation (%)**	**Standard Curve Fit (R^2)**
Epinephrine	A	25.65179	1.0000
	B	3.91260	0.9990
	C	2.23006	1.0000
	D	27.39306	1.0000
Norepinephrine	E	19.20305	0.9996
	F	10.13270	0.9971
	G	3.35149	1.0000
	H	3.64304	0.9990

2.2.8 Data Analysis

2.9.8.1 Resting Hemodynamic Measures, Autonomic Measures and Blood Sampling

Resting heart rate variability was assessed from the ECG signal from the 5-minute period preceding the draw of a venous blood sample using Lab Chart 7.1 software (ADInstruments,

Colorado Springs, CO). Resting BRS was assessed using systolic blood pressure and R-R interval data from the beat-by-beat BP signal from the same 5-minute period and was calculated as the mean of the slope of the linear SBP-HR relationships (Chapleau & Sabharwal, 2011) using HemoLab software (http://haraldstauss.com/HaraldStaussScientific/hemolab/). Measures derived from Doppler ultrasonography, ECG and beat-by-beat BP signals were averaged over the 3-minute period preceding the draw of a venous blood sample, with the exception of brachial artery diameter measures, which were taken immediately following this period and prior to the sample being drawn.

2.2.8.2 Hemodynamic Measures and Blood Sampling during LBNP

After approximately 4 minutes at each level of LBNP the brachial artery diameter was measured, after which the venous blood sample was drawn. Measures derived from Doppler ultrasonography, ECG and beat-by-beat BP cuff signals were averaged over the 30 second period preceding the assessment of brachial artery diameter. Cardiovascular measures were taken at approximately 3 minutes and 30 seconds into each LBNP stage in an effort to ensure that adaptation to that level of LBNP was complete, without subjecting participants to excessive LBNP exposure. Cardiovascular changes occur within 3 minutes of LBNP application and the plasma catecholamine response to LBNP is almost immediate (Goswami et al., 2008).

2.2.8.3 Metabolic Measures and Blood Sampling during Graded Exercise Tests

Pulmonary gas exchange and ventilatory measures were recorded on a breath-by-breath basis. For the purposes of the determination of VT1, VT2, and VO_2peak, 20-second averages of pulmonary gas exchange and ventilatory measures were assessed. For the purposes of statistical analysis and data presentation, the measures were averaged for the entire stage (1 minute). Heart rate for each stage was assessed by the heart rate at the end of the stage. Attempts were made to draw venous blood samples during the final 20 seconds of every other stage of exercise (60 Watt increments between blood samples).

2.2.8.4 Measures during Time Trial Tests

Data was collected in real time, and heart rate and power output measures were averaged over the duration of the Time Trial. Analysis of these measures was done using CycleOps PowerAgent 7 Software (http://www.powertap.com/collections/software/products/poweragent).

2.2.9 Statistical Analysis

2.9.9.1 LBNP Testing

To assess the interaction of the LBNP and training interventions, two-way RM ANOVA was performed on data collected during the LBNP tests, with the training condition (PRE – prior to the overreaching training intervention, POST – following the overreaching training intervention, and REC – following the period of tapered training) and level of LBNP (0, -15, -30 and 50mmHg) as factors. Post-hoc Fisher LSD tests were performed. Analysis was performed using the absolute values at each of 0, -15, -30 and -50mmHg.

To assess whether differences revealed by performing two-way RM ANOVA on the absolute values at 0, -15, -30 and -50mmHg of LBNP were due to differences at baseline or due to differences in the response to the graded LBNP test, one-way RM ANOVA was performed on data collected from the LBNP tests at 0mmHg and on the magnitude of the change from 0mmHg to each level of LBNP. Fisher LSD post-hoc testing was performed to assess differences between PRE, POST and REC conditions.

Measures of the Peripheral Vasoconstriction Response to LBNP: To assess the peripheral vasoconstriction response to LBNP, the Vasoconstrictor Index was calculated as:

$$\frac{brachial\ artery\ diameter\ (LBNP) - \ brachial\ artery\ diameter\ (baseline)}{brachial\ artery\ diameter\ (baseline)} * 100$$

where a negative Vasoconstrictor Index value indicates a reduction in brachial artery diameter at a given level of LBNP relative to at rest.

To assess the magnitude of the peripheral vasoconstriction response relative to the increase in sympathetic activity, the Vasoconstrictor Index was normalized for the plasma norepinephrine response to a given level of LBNP. This was calculated as:

$$\frac{Vasoconstrictor\ Index}{NOREPI\ (LBNP) - NOREPI\ (baseline)}$$

where negative values for the Vasoconstrictor Index / NOREPI Response indicate that the change in plasma NOREPI and in brachial diameter from baseline were in the same direction – that is, both NOREPI and brachial diameter were reduced, or both were increased at a given level of LBNP.

Finally, the change in FVC from baseline to a given level of LBNP was normalized to the change in plasma norepinephrine from baseline to the corresponding level of LBNP to assess the effect of changes in sympathetic activity on the conductance on the peripheral vasculature. This was calculated as:

$$\frac{FVC(LBNP) - FVC(baseline)}{NOREPI\ (LBNP) - NOREPI\ (baseline)}$$

The sign of the change in FVC from baseline was reversed, so that positive values for the change in FVC/ NOREPI Response indicate a vasoconstrictor response (reduction in FVC) with an increase in plasma NOREPI.

2.2.9.2 Graded Exercise Testing

One-way RM ANOVA was performed on data collected during the graded exercise tests, with post-hoc Fisher LSD tests performed to evaluate differences between PRE, POST and REC tests.

2.2.9.3 Time Trials

One-way RM ANOVA was performed on data collected during the Time Trials, with post-hoc Fisher LSD tests performed to evaluate differences between PRE, POST and REC tests. This analysis was performed for the absolute values and the percent of values relative to the first

Time Trial – for example, analysis was performed for time taken to complete each Time Trial in seconds, and as a % of the first Time Trial performance.

Relationships between parameters assessed during the graded exercise tests and Time Trials were determined by Pearson product moment correlation. Correlation coefficients of 0.36-0.67 were interpreted as modest, and correlations of 0.68-1.00 were interpreted as strong (Taylor, 1990).

2.2.9.4 RESTQ-Sport Questionnaires

For most questions in the RESTQ-Sport questionnaire, a higher score reflects greater stress than a lower score. For some questions, the opposite is the case – a higher score reflects lower stress, a lower score reflects higher stress. For the purposes of addressing the research question addressed with this thesis, the scores were inverted for such questions. Thus, higher scores reflect greater stress for all questions.

In the case of items not completed by participants, the mean stress score for that item, for that training condition (PRE, POST or REC) was determined and rounded to the nearest integer, and the missing score replaced with that number. Out of a total of 1848 items, 25 were missing values.

The RESTQ-Sport questionnaire is comprised of 19 stress scales, each comprised of several items in the questionnaire. The sum of the scores of all questions within a scale was determined, and one-way RM ANOVA performed for: each of the scales within the questionnaire; and the total score for the questionnaire. Post-hoc Fisher LSD tests were performed to evaluate differences between PRE, POST and REC tests.

2.2.9.5 Catecholamine Assays

Plasma epinephrine and norepinephrine concentrations were assessed in duplicate for each blood sample drawn at rest and during LBNP, and the mean of the duplicate measures used

in reporting catecholamine concentrations and statistical analysis. To detect the presence of outliers, the mean and standard deviation for each level of LBNP were determined – that is, the mean and standard deviation for samples from all participants, from PRE, POST and REC tests, for each of 0mmHg, -15mmHg, -30mmHg and -50mmHg LBNP. For each level of LBNP, plasma concentrations greater than 2 standard deviations from the mean concentration for that level of LBNP were excluded. An exception was made if a concentration was consistent with the other concentrations determined for a participant for a given level of LBNP in which case, despite being greater than 2 standard deviations from the mean of all measures for that level of LBNP, the concentration was not treated as an outlier.

2.2.9.6 Statistical Significance

A P-value ≤ 0.05 was considered statistically significant.

With respect to the one-way RM ANOVA analysis performed for autonomic and hemodynamic measures at rest and the two-way RM ANOVA analysis performed for LBNP, the assumption of normality was satisfied for all measures assessed during LBNP with the exception of: MAP at -15mmHg during POST; SV at 0mmHg during PRE; SV at -50mmHg during REC; FVR at 0mmHg and -15mmHg during POST; TP at -15mmHg, -30mmHg and -50mmHg at PRE, at 0mmHg and -30mmHg during POST; LF at -15mmHg and -30mmHg during POST, and at 0mmHg and -15mmHg during REC; HF at 0mmHg, -15mmHg and -30mmHg during Pre, AND AT -30mmHg during REC; LFnu at -50mmHg during REC; LF/HF at 0mmHg during PRE and -15mmHg during POST; EPI at -15mmHg during PRE and 0mmHg during REC; and NOREPI at -50mmHg during PRE (Kolmogorov-Smirnov P-value <0.05). The assumption of equal variance was satisfied for all measures assessed during LBNP with the exception of EPI ($P<0.05$).

With respect to the one-way RM analysis performed for the autonomic and hemodynamic responses to LBNP, the assumption of normality was satisfied for all measures with the exception of: the HF response at -15mmHg; the vasoconstrictor response normalized to NOREPI at -15mmHg; the FVC response normalized to NOREPI at -15mmHg and -30mmHg; the TP and

HF responses to -30mmHg and -50mmHg; the HR response to -50mmHg; the DBP and MAP responses to -50mmHg; and the vasoconstrictor response to -50mmHg (Kolmogorov-Smirnov P-value <0.05). The assumption of equal variance was satisfied with the exception of the EPI response to -30mmHg ($P<0.05$).

With respect to the one-way RM ANOVA analysis performed for performance measures, he assumption of normality was satisfied for all measures assessed during graded exercise testing and Time Trial testing (Kolmogorov-Smirnov P-value >0.05). The assumption of equal variance was satisfied for all measures assessed during graded exercise testing and Time Trial testing with the exception of: power output at VT2 ($P<0.05$).

With respect to the one-way RM ANOVA analysis performed for the RESTQ-Sport Questionnaire, the assumption of normality was satisfied for each subscale assessed as well as the total score (Kolmogorov-Smirnov P-value >0.05). The assumption of equal variance was satisfied for all measures with the exception of Scale 18 ($P<0.05$).

2.3 Results

2.3.1 Participant Characteristics

Nine participants completed the investigation, while two additional participants started but did not complete the investigation. For one of participant who did not complete the investigation, the physiological responses to the PRE LBNP test were not consistent with what has been reported in young, healthy adults – there was minimal evidence of a peripheral vasoconstriction response to LBNP, arterial blood pressure was not maintained, and the test was terminated upon the onset of pre-syncopal symptoms during the -30mmHg stage – and the participant was referred to a physician for follow-up testing. The second participant who did not complete the investigation withdrew during the overload training intervention for personal reasons unrelated to the investigation.

All participants met the VO_2peak cut-off for this investigation – 50mL/kg/min – with PRE values ranging from 50.7 – 70.2 mL/kg/min. Participants ranged in age from 20 – 33 years and all had histories training for and participating in endurance sports at either recreation or competitive levels. One-way RM ANOVA performed for participants' weight at PRE, POST and REC revealed that weight was not different at PRE, POST or REC testing (P=0.980).

Table 2. Participant Characteristics

Participant	**Age (years)**	**Height (cm)**	**Weight (kg)**	**VO_2peak (mL/kg/min)**
1	20	185	83.2	58.3
2	25	173	84.6	50.7
3	33	176	74.1	69.3
4	21	168	65.6	61.4
5	24	188	89	66.5
6	33	182	85.8	63.8
7	29	188	85.3	62.8
8	23	170	69	70.2
9	23	183	70.6	59.6
Mean±SD	**25.7±4.9**	**179.2±7.7**	**78.6±8.7**	**62.5±6.0**

2.4 Discussion

The activity of the sympathetic and parasympathetic branches of the autonomic nervous system have been purported to be altered by changes in training stress – in particular, by increases in training stress which can lead to overreaching and overtraining, and by reductions in training stress such as those associated with periods of tapered training. The purposes of this investigation were: 1) to assess the temporal changes in the activity of the sympathetic and parasympathetic branches of the ANS, as well as hemodynamic regulation, at rest associated with a period of overload training and a period of tapered training in aerobically fit individuals, 2) to assess the temporal changes in the response of the sympathetic branch of the ANS, and the hemodynamic response, to stress associated with a period of overload training and a period of tapered training in aerobically fit individuals, and 3) to assess the temporal changes in psychobiological measures of fatigue associated with a period of overload training and a period of tapered training in aerobically fit individuals.

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Three hypotheses were proposed: 1) that resting sympathetic activity would be increased following the period of overload training and return toward baseline levels following the period of tapered training, 2) that the overload period would augment the sympathetic response to a sympathetic stressor and the taper period would return the response toward baseline values, and 3) that the activity of the parasympathetic nervous system at rest would not be altered by the periods of overload or tapered training.

2.4.1 Overreaching Measures

As periods of overload training, which combined with inadequate recovery, can lead to overreaching – a state of performance maladaptation which previous investigations have demonstrated to be associated with physiological and psychobiological maladaptation – it is important to address whether participants experienced overreaching in the present investigation with respect to the framework within which the results are interpreted.

Although participants reported verbally to the investigators that the overload training intervention was more demanding than their typical training and that they were fatigued as a result of the overload intervention, the overload period did not cause overreaching as defined by current overreaching as defined by the European College of Sport Science and American College of Sports Medicine, and based on the definition from Kreider, Fry and O'Toole which has been widely adopted in the areas of exercise physiology and sport science (Kreider et al., 1998). Overreaching is defined as:

> an accumulation of training and/or non-training stress resulting in *short-term* decrement in performance capacity with or without related physiological and psychological signs and symptoms of maladaptation in which restoration of performance capacity may take from *several days to several weeks (Meeusen et al., 2006)*.

The primary marker of overreaching is thus a short-term decrement in performance – assessed in this investigation by Time Trial performance. As the physiological and biochemical changes associated with increased exercise stress are often accompanied by psychological changes (Fry, Morton, & Keast, 1992), and as psychological disturbances have been observed in overreached individuals (Halson et al., 2002; Iellamo et al., 2006a), a psychological index of perceived stress (the RESTQ-Sport questionnaire) was used in this investigation as a secondary marker of overreaching. Neither decrements in Time Trial performance nor in ratings of stress on the RESTQ-Sport questionnaire were observed, despite reports of fatigue from participants. Thus, although the overload intervention was reported to be more demanding than the training typically performed, it does not appear to have exceeded participants' adaptive capacity or have resulted in maladaptation due to an excessive imbalance of training stress and recovery.

The discrepancy between participants' verbal reports of fatigue and the investigators' perceptions of their general behaviour and demeanour over the course of the investigation, compared with the results of the RESTQ-Sport questionnaire were surprising. Participants' reports of physical and general fatigue increased over the course of the overload training period and decreased during the taper training period. This was consistent with the investigators' perceptions of participants' demeanour. However, these changes were not reflected in the

RESTQ-Sport questionnaire results. Thus, the subjective feeling of participants during the investigation and the quantitative assessment of stress and fatigue appear to be inconsistent. Given the reported validity and reliability of the RESTQ-Sport Questionnaire as measure of psychobiological stress and fatigue, and its wide use in applied sport science and research (Kellman & Kallus, 2001), this would suggest the need the interpretation of athletes' verbal reports and inferences derived from their behaviour and demeanour to be used with taken with a certain degree of skepticism.

Although the overload training intervention used in this investigation was anticipated to cause overreaching in participants, based on the current investigation and similar training investigations performed by other investigators it appears to be difficult to judge prospectively whether an overload intervention will result in overreaching, or whether participants' performance capacity will not be impaired. Jeukendrup et al. investigated the physiological responses to two weeks of intensified training in trained cyclists, and found that participants were overreached following one week of intensified training (Jeukendrup, Hesselink, Snyder, Kuipers, & Keizer, 1992). The participants – competitive male cyclists – performed "heavy training predominantly consisting of high intensity interval training", and intended to induce overreaching, for two weeks. The number of hours of training per week were approximately 12.5 hours and 17.5 hours during the period preceding the intensified training, and during the 2 weeks of intensified training, respectively. However, the amount of time spent performing "endurance training" was lower, and the amount of time spent performing intensive interval training was greater during the intensive training period. Compared to baseline tests, participants' time trial performances were impaired after 1 week of heavy training and remained impaired after the second week, with a rebound in performance occurring following a 2-week "recovery phase".

Similarly, Halson et al. investigated the time course of changes in performance and in markers of fatigue during intensified training, and found that one week of intensified training was sufficient to induce overreaching in trained participants (male endurance cyclists; VO_2max = 58.0±1.73 mL/kg/min) (Halson et al., 2002). Endurance-trained males were subjected to a 2-week period of intense training, during which the amount of training performed above 82% of participants' maximum heart rate was increased compared to what they were accustomed to. This

was achieved through an increase in the volume of training performed above participants' lactate threshold. In addition to the volume of training at a high intensity being increased during this 2-week period, the volume of training at a lower intensity was also increased. Evidence of overreaching was observed 3-5 days into the 2-week intense training period, with the severity of overreaching unaffected by the additional week of overload training (Halson et al., 2002).

Likewise, a 6-day training camp during which the total amount of training was increased compared to participants' typical training, was sufficient to reduce performance (both the time to exhaustion during a graded exercise test on a treadmill, and VO_2max, were reduced) in 9 international-level canoeists from the Swedish national team. Although the composition of athletes' training prior to the training camp is not reported, and the relative changes in high- and low-intensity training are not clear, the 13±1.6 hours of training performed during the 6-day training camp is reported to be equivalent to an average increase in training load of approximately 50% compared to the weekly training during the preceding month (Hedelin et al., 2000).

Thus, it is apparent that overreaching can be achieved within one week of overload training, even in aerobically fit individuals with endurance training histories, and that the duration of the overload training period in the present investigation was appropriate to induce overreaching in participants.

Similar to the present investigation, several other investigations in which participants performed periods of overload training have not resulted in overreaching. Despite resulting in increases in muscle soreness, poor sleep quality, and an increased plasma norepinephrine response to maximal exercise treadmill testing, one week of training during which the exercise intensity was increased compared to what was typical for participants did not cause performance impairment in middle- and long-distance runners. Participants first performed 4 weeks of normal training with one high-intensity interval session per week, followed by 4 weeks of overload training with 3 interval sessions performed each week (Billat et al., 1999). Ten days of increased training volume in highly trained swimmers has been shown not to impair swimming performance (Costill et al., 1988). Even 21 days of intensified training – consisting of a cycle

tour during which participants' training volume was increased compared to their typical volume – did not produce overreaching. Participants were trained cyclists and triathletes, and performance was assessed by 1 hour time trials repeated every 3 days throughout the investigation (Slivka, Hailes, Cuddy, & Ruby, 2010).

An investigation by Lehmann and colleagues into the influence of an increase in training volume compared to an increase in training intensity on performance and catecholamine measures may provide insight into the overreaching caused by some overload training interventions but not by others. Middle- and long-distance runners were recruited as participants for both training studies, which each consisted of 3 weeks of increased training stress. For the intense training study, speed-endurance, high-speed and interval runs increased from an average of 9km prior to the study to 22.7km during the 3rd week of the study, with a the volume of long-distance runs increasing slightly from 52.5km prior to the study to 62km during the 3rd week. For the high-volume study, the amount of training for each participant increased by one-third each week, approximately doubling the initial training volume during the 3rd week of the study. Resting norepinephrine was elevated after the high-volume study compared to initial levels, as were the epinephrine and norepinephrine concentrations at the same submaximal running speed. Resting epinephrine was lower and norepinephrine was not changed at the end of the intense training study compared to at the beginning, while epinephrine and norepinephrine concentrations at the same submaximal running speed were both lower at the end of the study. The high-volume training resulted in a decrease in endurance performance measures in 6 of the 8 participants, whereas 7 of the 9 participants in the intense training study showed improvements in endurance performance measures (Lehmann et al., 1991).

A potentially important similarity between the high-volume study of Lehmann and colleagues and the investigations of the Hedelin, Jeukendrup and Halson groups discussed above are the combination of an elevated training stress and an increase in the volume of training. It is possible that the increase in training stress combined with the reduction in the time to recover before the next demanding bout of training contributes to the development of performance and physiological maladaptation. In contrast, participants in the investigation by the Billat group performed 3 intense training sessions per week and did not experience maladaptation. Despite

the substantial increase in high-intensity training that participants in the Lehmann group's intense training study performed, the volume of training at a lower intensity was similar during the overload training period to what it had been prior to the study. Similarly, the majority of participants' training in the present investigation was performed at a very high intensity while relatively little low-intensity training was performed. It is possible that, despite the demands of the intense training sessions, participants' recovery was not negatively affected by the relatively low volume of training performed at a lower intensity, allowing participants to adequately recover and avoid performance and physiological maladaptation.

A principal limitation in exercise training studies is the underlying supposition that the adaptation to training is predictable and follows a determinable training pattern, such that interventions can be adequately planned in advance and the outcome of the interventions can be predicted (Kiely, 2012). It is important, however, to recognize the diversity of individual responses to the same training intervention, even in individuals with similar levels of fitness and training experience.

The smallest worthwhile change in fitness test results for elite athletes has been reported to be 0.2 x the between-subject standard deviation of that event or test, based on the concepts of Cohen's Effect Size (Pyne, 2003). The mean between-subject standard deviation and smallest worthwhile changes in this investigation were 6.72% and 1.34% for the average power output and 2.51% and 0.50% for the average time. However, Bertucci et al. have reported that the PowerTap cycling powermeter – which uses the same power measurement hardware as the PowerBeam Pro trainer unit used in this investigation, according to the manufacturer – has an accuracy of ±2-3% at power outputs between 100W and 420W (Bertucci et al., 2005). Thus, a 2% change in power output and time were determined to be smallest worthwhile changes in this investigation.

In this investigation the changes in performance over the course of the overload and tapered training periods was varied, reflecting the range of each individual's capacity to adapt to the training stimulus. Following three days of overload training – the mid-way point of the overload intervention – 2 participants' Time Trial performance was impaired compared to the

first test, while 5 participants' performance was unchanged and 2 participants' performance was improved. At the end of the overload training period, only one of the participants whose performance was impaired midway through the overload period remained impaired, while the had returned to baseline levels. One participant whose performance was improved after 3 days of intense training still demonstrated improved performance at the end of the overload period, while the other's performance had returned to its initial level. One participant whose performance was unchanged at the midway point of the overload period had impaired performance at the end of the period, while another had improved performance. A total of 2 participants' performance was improved, and 2 participants' performance was impaired at the end of the overload training period, while the performance of 5 participants was unchanged. At the final Time Trial, one participant – whose performance had improved during the overload period – took longer to complete the test than at the beginning of the investigation. Three participants showed performance improvements, and the performance of 5 participants was unchanged from its initial level.

While it is evident that participants' response to the overload and tapered training interventions as well as their capacity to manage the interventions varied, it remains unclear why similar overload training interventions performed by participants with similar levels of aerobic fitness and similar training backgrounds sometimes result in overreaching and sometimes do not. This investigation reinforces the importance of recognizing that the response to training interventions is not straightforward and predictable, and that it is difficult to predict the effect that a training intervention will have on a given individual. Specifically, investigation emphasizes the difficulty in judging prospectively whether an overload training intervention which will or will not cause overreaching in participants.

2.4.2 Influence of Training Intervention Exercise Performance

Time Trial performance was not different at any point in the present investigation, suggesting that neither the overload nor the tapered training interventions influenced cycling performance.

Despite no differences in cycling performance, participants' power output at aerobic threshold (VT1) increased by approximately 1 workload (30 Watts) following the overload training period, and remained elevated after the tapered training. This increase in power output was mirrored by an increase in oxygen consumption at the aerobic threshold. Although the power output at the respiratory compensation point (VT2) was unchanged following the overload training, it increased by approximately 1 workload following the taper period compared to either the PRE or POST tests. This increase in power at VT2 was accompanied by an increase in oxygen consumption compared to during the PRE condition. Neither the maximum power output nor VO_2 were altered following the overload or tapered training periods. Interestingly, the maximum heart rate was lower during the POST condition than either the PRE or REC, despite power output and VO_2 being unchanged.

The average power output and heart rate during the Time Trials was approximately the same as those at VT2 during the graded exercise tests. Although the average power output was not different between Time Trials, the average heart rate was lower during the POST than during the PRE Time Trial. Interestingly, although the Time Trial power outputs were similar to the power output at VT2 during the graded exercise tests – with no difference observed in the heart rate or power output between the PRE and POST tests – the average heart rate was lower during the POST compared to during the PRE Time Trial.

Also interesting is the discrepancy between participants' performance during VO_2peak testing and Time Trial tests. Given the moderate positive correlation observed between the average Time Trial power outputs and power at VT2 for all tests ($R=0.596$), and the PRE and POST Time Trials being performed at an average power output near the average power output at VT2 – with the power at VT2 being approximately 1 stage, or 30 Watts, higher at REC than at either PRE or POST – it was unexpected that average power output during the REC Time Trial would be only 5 Watts and 3 Watts higher than during the PRE and POST Time Trials, respectively. This, combined with the finding that 35.5% of the variation in average Time Trial power could be explained by power at VT2 ($R^2=0.355$), would suggest either that improvements in endurance exercise performance lagged behind physiological adaptations which are theoretically favourable for performance, or that non-physiological factors acted to counter the

physiological improvements. It is possible, for example, that participants' effort during the REC Time Trial was less than during the earlier Time Trials, resulting in similar performances despite physiological improvements.

The reduced maximum heart rate during the POST compared to the PRE graded exercise test, and the lower average power output during the POST compared to the PRE Time Trial, suggest that the heart rate response to similar absolute exercise intensities was blunted following the period of intense training. This blunted heart rate response appears to have been limited to near maximal intensity exercise, and was not observed at lower workloads.

Given that VO_2peak was not different between the PRE and POST tests, the metabolic demands and cardiac output required to meet those demands should have been similar during the two tests. As such, two potential factors which may have lowered the heart rate at VO_2peak are differences in the autonomic regulation of the heart and differences in blood volume, as the difference in heart rate was not due to different metabolic demands at maximal exercise. As discussed above, it is possible that the overload training intervention caused an increase in plasma volume. A scenario of increased plasma volume would allow for the exercising heart rate to be lower, while maintaining cardiac output to meet those metabolic demands, via an increase in stroke volume. As the increase in heart rate at high exercise intensities is attributable primarily to increases in sympathetic stimulation of the heart (Rowell, 1993), an increase in heart rate during maximal exercise at POST compared to at PRE would support the hypothesis that sympathetic activity would be elevated following the overload training period. The reduction in maximal heart rate which was observed does not support this hypothesis and may suggest that the sympathetic response to exercise stress may have been reduced following the overload training period.

Reductions in maximal heart rate have been reported in previous investigations in which overload training interventions were performed. Similar to what was observed in the present investigation, a ten-day period of overload training in a group of highly-trained swimmers, during which participants' typical training volume was doubled but performance in neither sprint nor endurance events was impaired, reduced the swimmers' maximum heart rate during a

VO_2max test but did not alter maximal oxygen consumption (Costill et al., 1988). Similarly, endurance cyclists who performed two weeks of intensified training had reduced maximal heart rates during VO_2max tests both half-way through and at the end of the intense training period. Maximal plasma epinephrine and norepinephrine concentrations during the VO_2max test were not different either after the first week of intensified training or after the second week, compared with levels prior to the training intervention, suggesting that the magnitude of the sympathetic response to maximal exercise was not altered by the training intervention. However, the lower heart rates were observed in conjunction with reduced maximal power outputs during the VO_2max tests, and participants' cycling performance – assessed by time trial performance – was also reduced (Halson et al., 2002). Thus, it is possible that the reduced maximum heart rate was, at least in part, a function of the reduced maximum workrate, and it is unclear whether the plasma catecholamine concentration would have been different following the training intervention were the participants able to achieve the same maximal workrates. Similarly, overreaching in elite canoeists was found to reduce maximum heart rate and as well as reduced oxygen consumption during a VO_2max test, although the authors did not report whether the maximal workrate was different before and after the training intervention (Hedelin et al., 2000). As participants' heart rates during submaximal exercise were reduced as well following the training camp, and plasma volume was found to be increased by 7%, the authors suggest that the plasma volume was responsible for the observed reduction in exercising heart rate.

A potential practical application of this finding is that maximal heart rate is not necessarily an indicator of overreaching or of maladaptation to training, as has been proposed (Mackinnon, 2000; Hedelin et al., 2000). In the present investigation, maximum heart rate was reduced without performance impairment being observed. The interpretation of a reduction in maximum exercising heart rate as an indicator of overreaching may lead coaches or athletes to reduce the athlete's training unnecessarily, reducing the potential physiological and performance improvements from the training.

With regards to the efficacy of the overload training intervention used in this investigation as it applies to athletic training, the intervention did not elicit improvements in cycling performance or VO_2peak either immediately following the overload training period or

after the subsequent period of tapered training, although the power output and rate of oxygen consumption at VT1 and VT2 were greater following the training.

Previous investigations using relatively short periods of overload training have had similar findings with regards to the efficacy of the training interventions. Halson and colleagues found that trained cyclists who performed two weeks of overload training, resulting in overreaching and reduced Time Trial performance and oxygen consumption and power output at VO_2max, these measures were all restored to pre-overload training levels after two weeks of recovery, but that the measures had not improved compared to prior to the training period (Halson et al., 2002).

On the other hand, similar bouts of training as those performed during the overload period of the present investigation – that is, high-intensity training sessions consisting of interval training – are effective at improving aerobic fitness and endurance performance. It may be that the intense training sessions, when spread over a longer period of time and separated by an adequate amount of time to recover more fully from the training, are more effective at improving fitness and performance than the training sessions grouped together during an overload training period. Weston et al. showed that six sessions of 5-8 repetitions of 5-minutes cycling at 80% of PPO separated by 1 minute of recovery, spread over a 28-day period, improved the time to fatigue at PPO, 40km Time Trial performance, and power output at VO_2max in highly trained cyclists (Weston et al., 1997; Lindsay et al., 1996). The same intervals, performed more frequently and for more weeks (12 sessions over 6 weeks rather than 6 sessions over 4 weeks) have also been shown to improve power at VO_2max and 40km Time Trial performance (Westgarth-Taylor et al., 1997). Similarly, eight sessions spread over 4 weeks of eight 4-minute intervals performed at 80% of PPO with 90 seconds of recovery between intervals was found to increase power output at VO_2max – despite no change in the maximum rate of oxygen consumption – as well as 40km Time Trial performance in well-trained cyclists (Lamberts et al., 2009). As few as four sessions, spread over 2 weeks, of 20 intervals 60 seconds long performed at PPO, with 2 minutes between intervals, has been shown to improve VO_2 at VT1, VT2 and maximum in highly trained cyclists (Laursen et al., 2002).

2.4.3 Cardiovascular and Autonomic Responses to LBNP

The cardiovascular, sympathetic and parasympathetic responses to LBNP observed in the present investigation are generally consistent with what has previously been described. In the context of the present investigation, this is important given the role of graded LBNP testing as a stressor stimulus to assess influence of the training intervention on the hemodynamic and autonomic responses to stress.

In the present investigation, exposure to LBNP at -30mmHg reduced participants' stroke volume by 11.4mL±8.1mL, 3.8mL±4.9mL and 12.8mL±9.3mL compared to at rest during PRE, POST and REC, respectively, while exposure to LBNP at -50mmHg reduced participants' stroke volume by 27.5mL±9.6mL, 19.5mL±8.5mL and 19.5mL±8.5mL at PRE, POST and REC. Interestingly, although these results are consistent with previous investigations in that they are an indication of conditions of central hypovolemia and a reduction in stroke volume during LBNP (Convertino & Cooke, 2002; Wolthuis et al., 1974; Cooke, Ryan, & Convertino, 2004), these reductions in stroke volume are less than has been previously reported. For example, reductions of 26±7.3mL from baseline to -30mmHg have been reported in young, endurance-trained males (Esch et al., 2010) and reductions to -50mmHg in young, fit males of approximately 50mL from baseline have been reported (Levine et al., 1991; Raven, Rohm-Young, & Blomqvist, 1984). The difference between the magnitude of the change in stroke volume during LBNP observed in the present investigation and those previously reported may be attributable in part to the different methods of assessment used in the various investigations. The present investigation used stroke volume measures derived from Modelflow calculations based on the signal recorded by the Finapres device, while Esch and colleagues assessed cardiac output and stroke volume using cardiac magnetic resonance imaging, Levine and colleagues used the acetylene rebreathing technique, and Raven and colleagues utilized CO_2 rebreathing.

The central hypovolemia produced by the application of LBNP provides a stimulus for cardiovascular compensation via reflexive hemodynamic responses which act to maintain blood pressure and cerebral perfusion (Cooke et al., 2004). One such reflex is an increase in sympathetic mediated by unloading of the cardiopulmonary and aortic baroreceptors, which acts to increase peripheral vascular resistance (Sundlof & Wallin, 1978). Both sympathetic activity

and peripheral resistance increase progressively as the intensity of LBNP increases (Pawelczyk & Raven, 1989; Convertino et al., 2004) and reflect the extent to which the aortic and cardiopulmonary baroreceptors are unloaded as a result of central hypovolemia (Taylor et al., 1995).

In the present investigation, Doppler ultrasonography revealed increases in FVR and reductions in FVC at an LBNP intensity of -50mmHg during the PRE and REC tests. FVR was 1480.0±1576.5, 567.30±1183.7 and 1705.7±2264.1mmHg/L/min greater and FVC was 0.0003±0.0003, 0.0002±0.0003 and 0.0003±0.0002L/min/mmHg lower at -50mmHg than at rest during PRE, POST and REC, respectively. The observed increases of approximately 87%, 26% and 112% of FVR from baseline to -50mmHg at PRE, POST and REC, respectively – with a mean increase of approximately 75% for all trials – is similar to what has been previously reported in healthy males at the same LBNP intensity. Pawelczyk and Raven, for example, observed an increase in FVR of approximately 78% at -50mmHg (Pawelczyk & Raven, 1989).

Interestingly, evidence of increased total peripheral resistance derived from beat-by-beat blood pressure waveforms was only apparent during the PRE test. One possible reason for this discrepancy between the indices of peripheral vasoconstriction derived from Doppler ultrasonography and from the pressure waveform detected by the Finapres device is the quality of the signal to each device. The Beatscope software used in this investigation relies on the Modelflow method to calculate hemodynamic parameters based on arterial pressure waves, which were collected non-invasively using the Finapres beat-by-beat blood pressure cuff. As such, the quality of the arterial blood pressure signal is fundamental in obtaining accurate measures derived from that signal. Although arterial blood pressure measures derived from Finapres measurement have been used in previous LBNP investigations which have included the application of -100mmHg of LBNP (Lee, Buchanan, Flatau, & Franke, 2004; Convertino, Rickards, & Ryan, 2012), some investigations have used Finapres to assess arterial BP while using other methods to assess hemodynamic parameters such as SV and cardiac output in place of using calculations derived from the arterial pressure waves (Lee et al., 2004; Raven, Welch-O'Connor, Shi, & Blomqvist, 1998). However, there are recent publications which have

presented hemodynamic parameters derived from Modelflow calculations and Finapres instrumentation during LBNP (Convertino et al., 2012).

In our laboratory's experience, the pressure waveforms detected by the Finapres device in situations in the perfusion of the fingers is reduced – such as individuals with cold fingers or poor circulation to the fingers – are improved when the hand is warmed either by covering it or by holding a warm object for a period of time, and when the height of the hand relative to the heart is lowered and finger perfusion is presumably increased. Although visual inspection of the pressure waveforms during from LBNP tests did not suggest that the quality of the waveform signal changed significantly during LBNP testing, it is possible that the perfusion of the fingers was reduced to some extent during LBNP compared to at rest due to the pooling of blood in the lower extremities, thereby influencing the hemodynamic measures derived from the waveform. Thus, although there is some discrepancy between the evidence of peripheral vasoconstriction during LBNP based on the reduction in FVC and increase in FVR observed in this investigation, and the Finapres-derived TPR which was not significantly altered by LBNP during POST and REC testing, it appears more likely than not that there was a vasoconstrictor response of the peripheral vasculature during LBNP.

The plasma catecholamine response, to high levels of LBNP, as well as the changes in the LF component of HRV and the LF/HF ratio, supported the Doppler ultrasonography observations in demonstrating LBNP intensity-dependent increases in sympathetic activity, and provided further evidence of an increase in sympathetic activity in response to LBNP. Plasma epinephrine concentrations were increased at -50mmHg compared to at baseline during PRE and REC testing by 0.062±0.101, 0.008±0.195 and 0.093±0.082nmol/L respectively, while plasma norepinephrine concentrations were increased at the same level of LBNP by 0.768±0.888, 1.119±1.041 and 0.646±0.690nmol/L during PRE and POST testing. Exposure to similar LBNP protocols as the one used in this investigation have shown similar, progressive increases in plasma norepinephrine with increasing LBNP intensity (Nazar et al., 2006; van Hoeyweghen et al., 2001; Goldsmith, Francis, Cowley, & Cohn, 1982). The observed increase of both the LF component of HRV as well as in the LF/HF ratio with increasing LBNP intensity is consistent

with the plasma catecholamine evidence of the LBNP protocol acting to elevate sympathetic activity.

Specific to the parasympathetic branch of the ANS, the observed reduction in both HF and HFnu HRV indices at an LBNP intensity of -50mmHg compared to at baseline, during both POST and REC testing, indicate a reduction of parasympathetic activity at the heart during LBNP. This evidence of parasympathetic withdrawal is consistent with what has been previously reported during LBNP (Lee et al., 2004).

Thus the evidence of central hypovolemia, increased sympathetic activity, parasympathetic withdrawal, and of the tachycardia and peripheral vasoconstrictor responses to LBNP observed in the present investigation are consistent with has been previously reported. These findings are important with regard to the present study in that they are indicative that the LBNP protocol used was effective in evoking a sympathetic stress response and challenging hemodynamic homeostasis. It is important that the LBNP protocol elicited the physiological responses which have been previously reported and which are consistent with the current understanding of the effects of LBNP because of its role in assessing the activity of the sympathetic nervous system and cardiovascular regulation. That the LBNP protocol elicited the physiological effects which it was expected to gives credence to its validity and reliability in its role in the present investigation.

2.4.4 Influence of Training Interventions on Parasympathetic Measures

It was hypothesized that neither the overload nor the taper training intervention would alter resting parasympathetic activity, assessed by BRS and the frequency-domain indices of HRV under parasympathetic regulation – total power of HRV, power in the high-frequency domain, and normalized power in the high-frequency domain. Although the absence of evidence of altered parasympathetic activity is not itself evidence that parasympathetic activity was the same, this suggests that parasympathetic activity was not altered by the training intervention and does not refute the hypothesis.

Based on findings from previous investigations, exercise interventions can influence resting parasympathetic activity: in situations where aerobic fitness is enhanced by the exercise intervention; and in situations where there is maladaptation to the exercise intervention which manifests, among other ways, in altered autonomic nervous system activity.

Increases in parasympathetic indices of HRV have been observed to accompany improvements in aerobic fitness in individuals without histories of endurance training or high initial levels of aerobic fitness (Hautala et al., 2004; Sloan et al., 2009; Tulppo et al., 2003) – an adaptation which is thought to have cardioprotective benefits (Goldsmith, Bloomfield, & Rosenwinkel, 2000). Increases in parasympathetic indices of HRV have also been observed following prolonged periods of training sufficient to improve fitness or performance – athletic training in preparation for competition, for example – in aerobically fit individuals (Iellamo et al., 2006a; Hedelin et al., 2001). The improvements in aerobic fitness which are accompanied by augmented resting parasympathetic activity can occur fairly rapidly in moderately-fit individuals. For example, Kiviniemi and colleagues have recently shown that six exercise sessions in two weeks – either 4-6 all-out 30 second sprints on a cycle ergometer, or 60 minutes of continuous cycling at 60% of VO_2max workload – are sufficient to increase both aerobic fitness and parasympathetic indices of HRV in healthy, middle-aged sedentary men (Kiviniemi et al., 2014). On the other hand, such rapid improvements in aerobic fitness appear to be uncommon in aerobically fit individuals. As participants in the present investigation had high levels of aerobic fitness and extensive training histories, and as the short nature of the intervention was not expected to be sufficient to improve aerobic fitness, increases in parasympathetic activity attributable to improvements in aerobic fitness were not anticipated. Indeed, no changes in VO_2peak were observed in the present investigation.

Based on the findings of the present investigation and those previously reported in overload training studies, it appears that short periods of overload training do not alter resting parasympathetic activity. No differences in frequency-domain HRV indices of parasympathetic activity – HF, HFnu or total power of HRV – were observed in our participants following either the overload or tapered training periods. This is consistent with what has previously been reported in elite canoeists who underwent testing before and after a 6-day training camp. Despite

the overload intervention being sufficient to cause overreaching, parasympathetic indices of HRV at rest were found to be unaltered by the training intervention (Hedelin et al., 2000). Similarly, resting HRV indices were not found to be different at the beginning of, 9 days into, or 16 days into a 3-week stage race in professional cyclists, despite the race being described as a condition of "chronic exposure to heavy exertion" (Earnest et al., 2004).

In contrast to the positive, cardioprotective increases in parasympathetic activity which tend to accompany improvements in aerobic fitness, and the lack of influence of short periods of overload training on parasympathetic activity, prolonged overload training periods which result in a maladaptation to training may either reduce the increases in parasympathetic activity which would otherwise be expected or, to a certain extent, act to reverse them. Uusitalo and colleagues showed that 4 weeks of endurance training at participants' usual training load increased TP HRV at rest in female athletes, whereas female athletes who were overtrained following that period did not have increases in TP or any parasympathetic indices of HRV assessed (Uusitalo et al., 1998a). On the other hand, Le Meur and colleagues have recently reported augmented parasympathetic activity in overreached male triathletes following a three-week overload training period, during which training was increased by 40% compared to participants' typical training load. Autonomic changes were not observed in triathletes who continued their regular training rather than increase their training load (Le Meur et al., 2013). Conversely, Pichot et al. have demonstrated a progressive reduction in parasympathetic indices of HRV during 3 weeks of overload training in highly trained middle-distance runners and that these changes were reversed after 1 week of tapered training, suggesting that the alterations in ANS activity which may occur with overload training are transient. A control group of runners who continued their regular training for that period did not show evidence of altered autonomic nervous system activity. Performance measures were not reported, so it is not clear whether or participants in the experimental groups were overreached (Pichot et al., 2000).

Although it may be that overreaching is associated with augmented parasympathetic activity in endurance trained individuals whereas these increases are reversed in cases of more severe training-recovery imbalance, such as overtraining, the results of the present investigation suggest that overload training is itself not responsible for these alterations in parasympathetic

activity. Rather, the results of the present investigation and previous overload training investigations suggest that parasympathetic activity may be altered when the imbalance of training stress and recovery is sufficient that maladaptation occurs – that is, that the maladaptation is manifested, among other ways, in changes in the activity of the parasympathetic branch of the ANS.

2.4.5 Influence of Training Interventions on Sympathetic Activity at Rest

The hypothesis that resting plasma catecholamine concentrations would be elevated at rest following the overload training period and return toward baseline levels after the tapered training period – reflecting an increase in resting sympathetic activity following the overload period, followed by a return toward baseline levels following the tapered period – was not supported by the by the findings of the present investigation. This hypothesis was based on the findings of previous investigations which have found evidence of increased sympathetic activity after overload training including low-frequency power of HRV measures in overtrained athletes (Uusitalo et al., 1998a; Mourot et al., 2004), resting catecholamine concentrations (Hooper et al., 1995; Hooper et al., 1993; Lehmann et al., 1991), and reports of clinical features which appear to be consistent with elevated sympathetic activity in overreached and overtrained athletes (Israel, 1958). In the present investigation, plasma catecholamine concentrations at rest were not different between PRE, POST and REC conditions, suggesting that resting sympathetic activity was not altered by the overload or tapered training interventions. This is in disagreement with previous reports of increased sympathetic activity following overload training – even that which does not result in overreaching – such as increased LF heart rate variability in elite rowers following a period of overload training (Iellamo et al., 2002) and increases in normetanephrine, a metabolite of circulating norepinephrine, in national-level road cyclists following just 4 days of overload training (Filaire et al., 2002). However, as other groups have reported that, even when it results in overreaching, resting plasma catecholamine concentrations are not altered by periods of overload training (Billat et al., 1999; Halson et al., 2002; Hedelin et al., 2000; Urhausen et al., 1998), it is not necessarily surprising that the overload training intervention in the present investigation, which did not induce overreaching, did not appear to elevate sympathetic activity at rest.

2.4.6 Influence of Training Interventions on Sympathetic Response to Stressor Stimuli

Several physiological and structural changes associated with endurance exercise training appear to contribute to impaired blood pressure regulation and tolerance to LBNP, including: increased vascular compliance; eccentric ventricular hypertrophy; increased total blood volume, which could reduce cardiopulmonary baroreflex responsiveness and shift ventricular function to a steeper portion of the ventricular compliance curve; and reduced carotid and aortic baroreflex responsiveness (Raven & Pawelczyk, 1993). Of these factors, the period of overload training in the current investigation was expected to partially reverse the reduction in carotid and aortic baroreflex responsiveness, but not to influence the other factors due to the relatively short duration of the training intervention.

Investigations of the baroreflex-mediated regulation of sympathetic nerve activity and the vasoconstrictor response to orthostatic stress have shown that aerobic fitness – specifically the physiological adaptations to aerobic exercise associated with improvements in aerobic fitness – diminishes the typical cardiovascular response to orthostatic stress. Fadel et al. have shown that individuals with high fitness experience greater drops in MAP in response to acute hypotension than those with average fitness (Fadel et al., 2001). An arterial thigh cuff – the release of which produced an acute drop in MAP – was used investigate the arterial baroreflex control of MSNA during acute hypotension in males who were classified as having high fitness (those involved in competitive sports; VO_2max of 67.8±2.3 mL/kg/min) or average fitness (those not performing exercise training on a regular basis; VO_2max of 49.4±2.1 mL/kg/min). The participants with high fitness were found to experience greater falls in MAP compared to those with average fitness, despite having greater baroreflex control of MSNA compared to participants with average fitness. Despite the increase in MSNA for a given drop in diastolic blood pressure being greater in participants with high than with average fitness, but they were less able to prevent a drop in MAP. The authors suggest that this may be due to training-induced changes in central neural processing or impaired control of vascular reactivity (Fadel et al., 2001).

The same group investigated the role of endurance training in LBNP tolerance and cardiovascular regulation during LBNP. A longitudinal study of male participants prior to and following 8 months of endurance training, consisting of approximately 45 minutes of exercise

just below ventilatory threshold performed 4 times per week, found that both LBNP tolerance and the hemodynamic response to LBNP were altered following the training intervention. LBNP tolerance was reduced following training, as was the increase in peripheral vascular resistance for a given increase in LBNP intensity. The authors suggested that the reduced vasoconstrictor response suggest a possible resetting of the cardiopulmonary baroreceptor's operating point, altered central integration of the afferent information acting to reduce efferent input, and possible alterations in alpha-receptor sensitivity or responsiveness to stimulation (Stevens et al., 1992). Similarly, a cross-sectional investigation of hemodynamic regulation during LBNP in fit (VO_2max = 70.2±2.6 mL/kg/min) and average fit (VO_2max = 41.3±2.9 mL/kg/min) found that fit participants had a greater reduction in arterial blood pressure, reduced tachycardia response to LBNP, and a reduced peripheral vasoconstriction response to LBNP compared with the average fit group (Raven et al., 1984).

With regard to endurance training and the sympathetic response to orthostatic stress, soldiers who jogged for 30-50 minutes three times per week, at an intensity of 60% of heart rate reserve, for 12 weeks had a lower increase in plasma norepinephrine during a tilt-table test (30 minutes at a 75° angle) compared to soldiers who were not prescribed the jogging intervention (Winker et al., 2005).

The findings of an augmented MSNA response for a given reduction in DBP in, conjunction with the blunted increase in peripheral vascular resistance for a given change in LBNP, suggest that the greater increase in MSNA may act to compensate for the reduced vasoconstrictor response.

Additionally, as increases in the catecholamine response to stressors have been observed following periods of overload training, it was hypothesized that the sympathetic response to LBNP would be augmented following the week of overload training in the present investigation, reversing, to a certain extent, the blunted sympathetic response to LBNP which may have affected participants' PRE LBNP test as a result of the structural and physiological adaptations to endurance training. Billat et al. have shown that, even without maladaptation to intensified training – that is, following one week of intensified training which did not cause overreaching in

highly trained middle- and long-distance runners – overload training increased the plasma norepinephrine in response to a maximal exercise test on the treadmill (Billat et al., 1999). This was observed without changes in resting plasma catecholamine concentrations, suggesting that even if sympathetic activity at rest is not altered by a period of overload training the sympathetic response to stress may be augmented, and suggesting that the sympathetic response to stressors can be altered even when the training-recovery imbalance is not sufficient to result in maladaptation. Dressendorfer et al. submitted male marathon runners to 2 weeks of intensified training, during which participants' regular training volume was doubled, and found that the plasma epinephrine concentrations at rest, during submaximal exercise, and at maximal exercise intensity were all increased following the training intervention for runners whose performance (15km run time) was impaired (Dressendorfer et al., 2000). It may be that the inconsistency between the sympathetic response to LBNP in the present investigation and to exercise in the studies discussed above stems from the different nature of the stressor used. Although the arterial baroreflex is involved in the neural responses to both LBNP and exercise, the response to LBNP does not include the central command or exercise pressor reflex contributions to altered neural activity which is involved during exercise. It is possible that the baroreflex response to sympathetic stressors is not altered by overload training while the central command or exercise pressor reflexes are.

Thus, the finding of the present investigation that plasma catecholamine concentrations were not altered at rest or during LBNP by the training interventions, and the lack of an interaction effect of the training intervention and the sympathetic response to LBNP, do not support the hypothesis that the sympathetic response to LBNP would be augmented following the overload training period and return toward baseline levels following the period of tapered training.

That resting BRS was not different at any testing occasion, nor was the plasma norepinephrine response to graded LBNP, suggest that the afferent signals stimuli from the arterial baroreceptors to the nucleus of the solitary tract (NTS) were not altered by the training interventions, nor was the efferent sympathetic activity from the intermediolateral nucleus (IML). If the baroreceptor-mediated sympathetic response to LBNP was not altered by the

training, then any changes in the hemodynamic response or response at the level of the peripheral vasculature should be attributable to changes in the response of the vasculature to sympathetic stimulation.

Although MAP was greater at PRE than at POST or REC at rest and during LBNP, and FVC was lower at PRE than at POST at rest and during LBNP – that is, differences in MAP (Figure 7) and FVC (Figure 9) under resting conditions were maintained during LBNP – the magnitude of the changes in MAP and FVC in response to LBNP were not different between the training conditions (Table 6). Given that the sympathetic stimuli to the peripheral vasculature appear to have been similar during the PRE, POST and REC conditions – given the similar plasma catecholamine responses to LBNP – this suggests that the response of the peripheral vasculature to a given stimulus was also similar during each of the PRE, POST and REC conditions. This is supported by the similar change in brachial artery diameter for a given change in plasma norepinephrine observed during LBNP during each of PRE, POST and REC, and the similar change in FVC for a given change in plasma norepinephrine during LBNP during all three conditions.

Thus, the hemodynamic and plasma catecholamine responses to LBNP during the PRE, POST and REC conditions do not support the hypothesis that the sympathetic response to LBNP would be increased following the overload training period and return toward initial levels following the period of tapered training. Based on these findings and those of previous overload training investigations, it appears that it is not overload training itself, but rather the ultimate maladaptation to overload training which occurs when individuals are unable to fully recover from the exercise stress, may lead to a situation of an altered sympathetic nervous system response to a stressor.

2.4.7 Influence of Training Interventions on Arterial Blood Pressure

In the present investigation, reductions in arterial blood pressure at rest and during LBNP were observed at POST compared to PRE, and this change was maintained at the time of REC testing. It would appear that the mechanism or mechanisms underlying the reduction in arterial

blood pressure and the increase in vascular conductance observed following the overload training intervention would have similar effects at rest and during orthostatic challenge, as an interaction effect between the training intervention and the autonomic and hemodynamic responses to LBNP was not observed in the present investigation. Although there was not evidence of an effect of the training intervention on the cardiovagal branch of the baroreflex, neither the baroreflex set-point nor the sympathetic branch of the baroreflex were assessed in the present investigation. Thus, based on this investigation it is not possible to ascertain whether the difference in arterial blood pressure and vascular conductance may have been due to changes in either the sympathetic branch or the setpoint of the baroreflex. However, it is possible that the overload training intervention caused an expansion in plasma volume, ultimately influencing arterial blood pressure.

Longitudinal endurance training studies have shown expansions in blood plasma volume following training (Convertino, 1991), and the expansion in plasma volume associated with the early stages of endurance training – such as those in untrained individuals who perform several months of endurance training – have been associated with improved hemodynamic regulation during orthostatic stress and improved orthostatic tolerance (Winker et al., 2005). This initial volume expansion has been demonstrated to limit the reduction in stroke volume and compensatory increase in heart rate during orthostatic stress (Convertino, 1993; Nazar et al., 2006; Winker et al., 2005). In contrast to the initial effects of plasma volume expansion, it appears that chronic plasma volume expansion such as that associated with long-term endurance training or athletic training increase arterial compliance (Blomqvist & Saltin, 1983), a result of which is a greater reduction in end-diastolic volume for a given reduction in end-diastolic pressure, and therefore a greater reduction in stroke volume, during orthostatic stress (Bergenwald, Freyschuss, & Sjöstrand, 1977). The net result of chronic plasma volume expansion is then, generally, impaired hemodynamic regulation during orthostatic stress. Given the training histories of the participants in the present investigation, it is likely that they would have adaptations consistent with chronic plasma volume expansion.

Curiously, following the overload training period of the present investigation the reduction in stroke volume at high LBNP intensity was less, and the accompanying tachycardia

response was reduced, compared to before the overload period. These changes are consistent with those in untrained individuals who experience plasma volume expansion at the onset of an exercise intervention, as described above. Although it is more often reported as a response to endurance training in individuals who are no already highly fit, Hedelin et al. have reported a 7% increase in plasma volume in elite canoeists following a 6-day training camp which induced overreaching (Hedelin et al., 2000) and Uusitalo et al. have also reported a 7% increase in plasma volume, although in overtrained endurance athletes following 6-9 weeks of overload training (Uusitalo et al., 1998a). It is possible, then, that the overload training period in the present investigation caused an expansion in plasma volume, thereby influencing the cardiovascular response to LBNP. In addition to explaining, at least in part, the smaller decrease in stroke volume and the smaller tachycardia response during LBNP, plasma volume expansion could explain the observed reduction in arterial blood pressure at rest and during LBNP following overload training. It is possible that, contrary to what had generally been thought about baroreceptor resetting and the role of baroreceptors in long-term arterial blood pressure regulation, carotid baroreceptor resetting does not necessarily occur during prolonged periods of baroreceptor loading or unloading (Thrasher, 2004). If carotid baroreceptor resetting did not occur, and there was an expansion of plasma volume, the afferent activity from the baroreceptors should be elevated and the efferent activity acting to cause constriction in resistance vessels would be reduced, offering a potential explanation for the lower arterial blood pressure following overload training in the present investigation.

Due to the metabolic and thermal demands of exercise, the circulating blood volume is acutely decreased after exercise, accompanied by an increase in electrolyte concentrations and osmolality which act to initiate the renin-angiotensin-aldosterone cascade and increase vasopressin. Renal water and sodium retention are increased, and urine output is reduced during the 24 hours after exercise. The magnitude of this response is mediated by the degree of metabolic and thermal stress. If the water intake during this time exceeds the volume of water lost during exercise and the urine output, there is a net increase in water balance and subsequent blood volume expansion (Convertino, 2007). It is possible that this acute response to exercise, which could act to increase blood volume following a training bout, was great enough in magnitude and sustained for a long enough period of time over the course of the overload

training period – due to the demanding nature and close timing of the training sessions – that it was sufficient to increase plasma volume beyond baseline levels.

In cases where arterial blood pressure or vascular capitance are not changed in concurrence with increased plasma volume, central venous pressure is elevated (Convertino, 2007), likely resulting in an increase in stroke volume. That a change in resting stroke volume was not observed in the present investigation may be attributable to the reduction in arterial blood pressure which was observed. It would be expected that an increase in vascular capitance would result in a greater reduction in central venous pressure, and thus stroke volume, during LBNP. That the reduction in stroke volume in response to LBNP was not different between the PRE, POST and REC conditions suggests that vascular capitance was not influenced by training, or not to the extent that it altered the pooling of blood in the lower extremities during LBNP. Evidence of augmented vascular capitance could have provided a possible mechanism for the reduction in arterial blood pressure observed following the overload training intervention. Thus, based on the measures recorded during the present investigation, it remains unclear why arterial blood pressure would have been reduced following the period of overload training.

As plasma volume was not assessed in the present investigation, this explanation for the observed differences in the hemodynamic response to LBNP and the differences in arterial blood pressure following the overload training period are speculative. However, such a change would appear to explain the observed changes in arterial blood pressure and in the stroke volume and heart rate responses to LBNP.

Another potential cause of the observed reduction in arterial blood pressure following the overload training intervention is the concept of post-exercise hypotension. Conditions of post-exercise hypotension are characterized by a well-regulated reduction in arterial blood pressure which lasts for several hours following a bout of exercise. This reduction in pressure is similar in magnitude in the supine and upright conditions. During the recovery from a bout of exercise, an immediate post-exercise hyperemia effect as well as sustained post-exercise vasodilation of the skeletal muscle vascular beds which were used during the exercise bout. Whereas the immediate post-exercise hyperemia lasts for up to 20 minutes following a bout of exercise, the sustained

post-exercise vasodilation lasts for two hours or longer appears to be dependent on histamine H_1- and H_2-receptor activation (Halliwill et al., 2014). As histamine is a potent vasodilator, the increased intramuscular histamine present following a bout of exercise have a vasodilator effect (Halliwill, Buck, Lacewell, & Romero, 2013).

Halliwill and colleagues have demonstrated that in sedentary and active individuals, the increase in peripheral vascular conductance observed following a bout of exercise which contributes to post-exercise hypotension is mediated by histamine 1 and histamine 2 receptors in the vasculature, with H_1 receptors mediating the short-term elevation in conductance and H_2 receptors mediating the long-term increase. The group has reported a reduction of approximately 4mmHg in MAP at 30, 60 and 90 minutes after the completion of 1 hour of exercise at 60% of VO_2peak, and a corresponding increase of approximately 6% and 7% increases in forearm and thigh vascular conductance at 30 minutes and at 60 minutes post-exercise in endurance trained men. These changes in blood pressure and conductance were abolished under conditions of H_1 and H_2 receptor blockade. Thus, the phenomena of post-exercise hypotension occurs in endurance trained individuals and is mediated, at least in part, by H_1 and H_2 receptor-mediated vasodilation (McCord & Halliwill, 2006). In the present investigation, reduction in resting MAP of approximately 7.66mmHg from PRE to POST as well as an increase in FVC of approximately 82%. While the magnitude of the increase in FVC observed in the present investigation far exceeds that reported by McCord and Halliwill, the intensity of each bout of exercise performed by the participants of the present investigation was also far greater. Additionally, it is possible that the cumulative effect of 7 consecutive days of intense training bouts acts to heighten the post-exercise hypotension effects by enhancing the exercise-induced activation of histamine receptors, the amount of histamine present, or some combination of these factors.

With respect to the time course of the return of arterial blood pressure to initial levels following an exercise bout, many investigations have been limited to several hours following a bout of exercise, and the temporal effects of post-exercise hypotension remain unclear. However, Pescatello and colleagues have reported that 30 minutes of cycling at 40% or at 70% of VO_2peak reduced diastolic blood pressure and mean arterial pressure by 8mmHg and 7mmHg respectively for nearly 13 hours in a group of hypertensive individuals (Pescatello, Fargo, Leach,

& Scherzer, 1991). In the present investigation, POST testing was conducted between 12 and 24 hours after the last Time Trial was performed during the overload training period. However, given the intensity of the exercise bouts performed during the overload period, as well as the cumulative effect of 7 consecutive days of intense training, it is possible that exercise-induced vasodilation and hypotension could have persisted at the time of testing.

2.5 Conclusions

In summary, this investigation examined the effect of a period of overload exercise training and a period of tapered training on autonomic nervous system activity at rest and in response to acute physiological stress, as well as hemodynamic parameters at rest and in response to acute physiological stress, and on tests of aerobic fitness and athletic performance. The training interventions did not alter autonomic activity either at rest or in response to stress and, although arterial blood pressure was lower following the overload training, the hemodynamic response to acute stress was not altered by training. Overload training led to favourable cardiovascular adaptations with regard to the graded exercise test, and the period of tapered training led to further favourable adaptations. Despite these physiological improvements, athletic performance was unchanged by either training intervention. Overall, these findings indicate that short periods of intense training do not alter the activity of the autonomic nervous system or its regulation of the cardiovascular system during stress.

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Chapter 3: General Discussion

3.1 Main Findings

This thesis aimed to evaluate the effect of periods of overload and tapered training on the autonomic regulation of the cardiovascular system, and produced a number of important findings. First, contrary to what was hypothesized, neither the period of overload nor the period of tapered training were found to alter the activity of the sympathetic nervous system either at rest or in response to a sympathetic stressor. Second, the overload training period was found to reduce arterial blood pressure and increase forearm vascular conductance at rest, but not to alter the response to a sympathetic stressor. Third, consistent with what was hypothesized, neither period of training was found to alter the activity of the parasympathetic nervous system at rest. Finally, the overload training intervention performed by participants did not impair cycling performance or increase psychobiological measures of stress, nor cause overreaching based on the currently accepted definition.

The results of this investigation are consistent with previous investigations which have not shown short periods of overload training to induce physiological changes with respect to resting sympathetic (Billat et al., 1999; Halson et al., 2002; Hedelin et al., 2000; Urhausen et al., 1998) or parasympathetic activity (Hedelin et al., 2000; Earnest et al., 2004). These results are, however, inconsistent with previous findings of elevated sympathetic responses to stress following short periods of overload training (Billat et al., 1999; Dressendorfer et al., 2000; Halson et al., 2002). Finally, these results suggest that short periods of overload training do not alter the hemodynamic response to stress and do not alter cycling performance. The results of the this and previous investigation suggest that a combination of increased training stress and reduced recovery may be important in an overload training intervention leading to maladaptation, whether it is in the performance, physiological or psychobiological domains. Overload training interventions consisting primarily of high-intensity training may not impede individuals' recovery from training to the same extent that interventions which include a high volume of training do. It is possible that the additional time spent exercising, by allowing for less time for recovery between training bouts, is an important factor in leading to maladaptation. It may also be that there is a threshold of training-recovery imbalance below which maladaptation

does not occur, and that it is only when the threshold is exceeded and there is an inability to balance the recovery with the training stress, that physiological, performance, and psychological maladaptation are likely to occur.

In this regard, it appears that short periods of overload training are more similar to acute training bouts than to overreaching, in that individuals are able to recover from the stress of the training bout before the onset of the next training bout.

This investigation may have practical implications for athletes and individuals who regularly participate in strenuous physical exercise. In particular, the findings that the overload period did not disturb the autonomic nervous system and did not alter the cardiovascular response to stress suggests that there may be little risk of maladaptation to short periods of intense training, such as a training camp or several days of intense exercise, so long as the individual's recovery is adequate. Given the important role of short periods of intense training in training plans, and this could have practical applications for athletes, coaches, and sport scientists in planning training programs.

In summary, this thesis demonstrated that periods of overload training which do not cause overreaching may alter hemodynamic regulation at rest, but do not alter the activity of the autonomic branch of the nervous system at rest or the hemodynamic or autonomic responses to lower body negative pressure stress.

3.2 Experimental Considerations and Limitations

A major strength of the present experimental approach was the prescription of a standardized training intervention and the use of an ergometer to ensure that the training performed by participants was consistent with what was prescribed. Previous overload training investigations have monitored training, among other ways, by: categorizing training into classifications such as "endurance training", "sprint, muscular, and extensive training sessions" and "maximal exhaustive training sessions" (Pichot et al., 2000); prescribing and quantifying training based on distance, such as distance run by runners (Dressendorfer et al., 2000); prescribing training using heart rate zones, and quantified the training using heart rate-based

calculations (Dressendorfer et al., 2002; Halson et al., 2002). The categorization of training classifications is, to a certain extent, subjective and, as was observed in the present investigation, the heart rate at a given exercise intensity can vary over a period as short as one week (Buchheit, 2014). The prescription and quantification of training based on volume – such as distance run – neglects the contribution of exercise intensity to the training stimulus. Thus, key strength of the present investigation was the use of an ergometer to ensure that the exercise intervention performed by participants was consistent with what was prescribed.

Another strength of the present experimental approach was the use of a stressor which could reliably subject participants to graded levels of stress, when assessing the sympathetic and hemodynamic responses to stress. More often, either resting catecholamine concentrations alone (Filaire et al., 2002; Hooper et al., 1993; Hooper et al., 1995) or catecholamine concentrations at rest and during graded exercise tests (Dressendorfer et al., 2000; Halson et al., 2002; Lehmann et al., 1991) have been used to assess sympathetic activity and the sympathetic response to a stressor. The assessment of plasma catecholamine concentrations both at rest and during graded LBNP testing has the benefit of allowing both resting sympathetic activity and the sympathetic response to a valid and reliable challenge to the sympathetic nervous system to be assessed, to determine wither training altered ANS and hemodynamic parameters at rest and in response to stress.

A final strength of the present experimental approach was the use of plasma catecholamine concentrations of epinephrine and norepinephrine as indices of sympathetic activity. Previous investigations of the autonomic nervous system with regard to training interventions have often used heart rate variability measures as indices of sympathetic nervous system activity (Hynynen, Uusitalo, Konttinen, & Rusko, 2006; Iellamo et al., 2006a; Atlaoui et al., 2007; Pichot et al., 2002; Pichot et al., 2000; Uusitalo et al., 2000), although it appears that heart rate variability measures are not ideal indices of sympathetic activity (1996). The assessment of plasma catecholamine concentrations in the present investigation offers a more valid assessment of the potential sympathetic changes associated with the training intervention.

Due to the nature of heart rate variability indices and plasma catecholamine concentrations, the variation between these measures among participants was substantial, thus increasing the chance of Type I statistical error and reducing the likelihood of statistical significance being achieved in these measures.

The respiratory cycle influences autonomic rhythms, including heart rate fluctuations (Eckberg, 2000). Although human respiratory rate is generally similar to that of the HF range of heart rate variability (0.15 – 0.4 Hz; approximately 9 – 24 cycles per minute) and the investigators did not observe noticeable differences or anomalies in participants' respiration during the periods that heart rate variability was assessed, having participants breath at a controlled rate and depth may have minimized any potential respiratory effects on heart rate variability measures.

Additionally, while the use of LBNP as a sympathetic stressor allowed for the controlled application of a known intensity of stress, the hemodynamic and sympathetic responses to LBNP are largely mediated by the arterial baroreflex. It is possible that investigation of the sympathetic and hemodynamic responses to other forms of sympathetic stress such as exercise, the response to which also encompasses altered central command and exercise pressor reflexes, may reveal differences in the sympathetic and hemodynamic responses to these forms of stress attributable to the overload and tapered training interventions.

Finally, a limitation of this investigation is the use of aerobically fit participants with extensive endurance training histories. Training history and aerobic fitness play important roles in an individual's response to training and their capacity to manage training interventions (Smith, 2003). It is possible that evidence of maladaptation to training was not observed following the overload training period due, in part, to the participants being highly fit and having performed intense periods of training prior to this investigation. Similar training interventions performed by individuals with lower levels of fitness or less endurance training experience may be more likely to lead to maladaptation, performance impairment, autonomic disturbances, and psychobiological changes. Additionally, the structural and functional changes associated with chronic endurance training are include changes which alter autonomic activity and hemodynamic

conditions at rest, as well as the autonomic and hemodynamic responses to lower body negative pressure (Raven & Pawelczyk, 1993). Thus, the applicability of the findings of this investigation is more pertinent to athletic populations, and their direct relevance for other populations may be limited.

3.3 Future Directions

As discussed in this thesis, it is possible that plasma volume expansion occurred over the overload training period in the present investigation which could have contributed to the difference observed in the heart rate at high exercise intensity following the overload training period. In this regard, the assessment of the plasma volume response to the overload training intervention would provide further insight.

The overload training intervention performed did not cause decrements in cycling performance, nor alter psychobiological measures of stress and fatigue, and did not result in overreaching. To determine whether autonomic and hemodynamic changes are induced in the overreached state – in addition to the performance decrements and fatigue which are, by definition, present in the overreached state – future investigations could implement overload training interventions which are performed until participants' performance begins to decline, or could utilize training interventions which were even more demanding than those used in the present investigation.

Finally, the present investigation demonstrated that the sympathetic response to LBNP was not altered by the periods of overload or tapered training. The assessment of the plasma catecholamine response to a graded exercise test could reveal whether the sympathetic response to other forms of stress is altered by such training interventions. The results of this investigation suggest that the baroreflex-mediated sympathetic response to LBNP was not altered by the overload or taper training interventions. However, Billat et al. showed that the norepinephrine response to maximal exercise – which involves the central command and exercise pressor responses, in addition to a baroreflex-mediated response – is increased following overload training which does not cause overreaching, even without changes in resting sympathetic activity

(Billat et al., 1999). Venous blood samples were collected during the graded exercise tests performed in the present investigation and stored for future analysis; evaluation of these samples could provide insight into whether the sympathetic response to exercise was altered in the present investigation.

3.4 Conclusion

The purposes of this thesis were to assess the temporal changes in the activity of the sympathetic and parasympathetic branches of the autonomic nervous system as well as hemodynamic regulation: 1) at rest, and 2) in response to lower body negative pressure stress, as well as 3) changes in psychobiological measures of fatigue associated with a period of overload training and a period of tapered training in aerobically fit individuals. The results demonstrate that the periods of overload and tapered training, during which the exercise stress is greater than and less than what is typical for an individual, respectively, did not alter autonomic activity at rest or the sympathetic response to stress. The results also demonstrated that the overload training period reduced arterial blood pressure and increased vascular conductance, but that neither overload nor tapered training influence the cardiovascular response to stress.

Adaptations to exercise training appear to exist on a dose-response curve – there is a range of exercise stress which elicits the greatest adaptations, while training stress above or below this range does not elicit positive adaptations to the same extent (Busso, 2003). Among the positive adaptations to regular endurance exercise are improvements in athletic performance and positive, cardioprotective changes in autonomic activity (Goldsmith et al., 2000). While overreaching and overtraining have been found to alter autonomic activity, it appears that this is not disrupted by overload training which does not exceed the individual's ability to recover.

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Appendices

Appendix A. Sample Size

Previous investigations of the influence of training stress on ANS activity in endurance athletes have had the following number of participants in the intervention group: Baumert et al. – 10; Iellamo et al. – 7; Iellamo et al.– 34; Mourot et al. (2004) – 7; Uusitalo, Uusitalo, & Rusko – 9; Winsley, Battersby, & Cockle – 20; Dressendorferer et al. (2000) – 12; Lehmann et al. (1991) – 8. The median number of participants was 9.5 and the range was 7 to 34 (Baumert et al., 2006; Iellamo et al., 2002; Iellamo et al., 2006a; Uusitalo et al., 1998a; Winsley, Battersby, & Cockle, 2005; Dressendorfer et al., 2000; Lehmann et al., 1991).

The primary outcome measure selected for this thesis was the plasma catecholamine response to stress. The assessment of autonomic and performance changes in trained male runners over 14 consecutive days of unaccustomed high training load revealed an increase in plasma epinephrine concentrations during fixed-rate submaximal cycling following the training intervention in a subgroup of participants who also demonstrated other signs of maladaptation to training (Dressendorfer et al., 2000).

Calculation of sample size:

$$\frac{n}{group} = \frac{2\sigma^2}{(\mu_2 - \mu_1)^2} * f(\alpha, \beta)$$ (Warren, 1997)

Where σ is the standard deviation of the comparison group, μ_1 and μ_2 are the means of the comparison and intervention groups, respectively, and f is the f-value. For $\alpha = 0.05$ and $\beta = 0.2$, $f = 7.9$.

Using the plasma epinephrine data from Dressendorferer and colleagues:

$$\sigma = 3.6 ng/dL, u_1 = 15.3 ng/dL, \text{ and } u_2 = 31.0 ng/dL$$

$$\frac{n}{group} = 3$$

A similar study by Lehmann and colleagues found that, following 3 weeks of unaccustomed high training stress in trained runners, plasma catecholamine concentrations at the

same submaximal workload were also elevated (Lehmann et al., 1991). Using the plasma norepinephrine data from Lehmann and colleagues:

$\sigma = 3.04\ nmol/, u_1 = 8.41 nmol/L$, and $u_2 = 10.99 nmol/L$.

$$\frac{n}{group} = 22$$

Appendix B. RESTQ-Sport Questionnaire

The RESTQ-Sport questionnaire is a method of quantitatively assessing an individual's balance of stress and recovery, and to monitor changes in their stress-recovery balance over time. The questionnaire is based on the hypothesis that an accumulation of stress in different areas of life, paired with insufficient recovery, leads to a changed psychobiological state. For this thesis, the original format of the RESTQ-Sport questionnaire (Kellman & Kallus, 2001) was replicated using the SurveyMonkey online survey and questionnaire software, allowing participants to complete the questionnaire by computer or smartphone (www.surveymonkey.com).

The RESTQ-Sport questionnaire consists of 77 statements, asking participants to use the scale below to inventory the following items, "In the past (3) days/nights":

0	1	2	3	4	5	6
Never	Seldom	Sometimes	Often	More often	Very often	Always

1. I watched TV
2. I did not get enough sleep
3. I finished important tasks
4. I was unable to concentrate well
5. Everything bothered me
6. I laughed
7. I felt physically bad
8. I was in a bad mood
9. I felt physically relaxed
10. I was in good spirits
11. I had difficulties in concentrating
12. I worried about unresolved problems
13. I felt at ease
14. I had a good time with friends
15. I had a headache
16. I was tired from work
17. I was successful in what I did
18. I couldn't switch off my mind
19. I fell asleep satisfied and relaxed
20. I felt uncomfortable
21. I was annoyed by others
22. I felt down
23. I visited some close friends
24. I felt depressed
25. I was dead tired after work
26. Other people got on my nerves

27. I had a satisfied sleep
28. I felt anxious or inhibited
29. I felt physically fit
30. I was fed up with everything
31. I was lethargic
32. I felt I had to perform well in front of others
33. I had fun
34. I was in a good mood
35. I was overtired
36. I slept restlessly
37. I was annoyed
38. I felt as if I could get everything done
39. I was upset
40. I put off making decisions
41. I made important decisions
42. I felt physically exhausted
43. I felt happy
44. I felt under pressure
45. Everything was too much for me
46. My sleep was interrupted easily
47. I felt content
48. I was angry with someone
49. I had some good ideas
50. Parts of my body were aching
51. I could not get rest during the breaks
52. I was convinced I could achieve my set goals during performance
53. I recovered well physically
54. I felt burned out by my sport
55. I accomplished many worthwhile things in my sport
56. I prepared myself mentally for performance
57. My muscles felt stiff or tense during performance
58. I had the impression there were too few breaks
59. I was convinced that I could achieve my performance at any time
60. I dealt very effectively with my teammates' problems
61. I was in a good condition physically
62. I pushed myself during performance
63. I felt emotionally drained from performance
64. I had muscle pain after performance
65. I was convinced that I performed well
66. Too much was demanded of me during the breaks
67. I psyched myself up before performance
68. I felt that I wanted to quit my sport
69. I felt very energetic
70. I easily understood how my teammates felt about things
71. I was convinced that I had trained well
72. The breaks were not at the right times

73. I felt vulnerable to injuries
74. I set definite goals for myself during performance
75. My body felt strong
76. I felt frustrated by my sport
77. I dealt with emotional problems in my sport very calmly

The 77 items of the RESTQ-Sport questionnaire are grouped into 19 stress scales:

Scale 1: General Stress
(22) I felt down
(24) I felt depressed
(30) I was fed up with everything
(45) Everything was too much for me

Scale 2: Emotional Stress
(5) Everything bothered me
(8) I was in a bad mood
(28) I felt anxious or inhibited
(37) I was annoyed

Scale 3: Social Stress
(21) I was annoyed by others
(26) Other people got on my nerves
(38) I was upset
(48) I was angry with someone

Scale 4: Conflicts/Pressure
(12) I worried about unresolved problems
(18) I couldn't switch my mind off
(32) I felt I had to perform well in front of others
(44) I felt under pressure

Scale 5: Fatigue
(2) I did not get enough sleep
(16) I was tired from work
(25) I was dead tired after work
(35) I was overtired

Scale 6: Lack of Energy
(4) I was unable to concentrate well
(11) I had difficulties in concentrating
(31) I was lethargic
(40) I put off making decisions

Scale 7: Somatic Complaints
(7) I felt physically bad
(15) I had a headache

(20) I felt uncomfortable
(42) I felt physically exhausted

Scale 8: Success
(3) I finished important tasks
(17) I was successful in what I did
(41) I made important decisions
(49) I had some good ideas

Scale 9: Social Relaxation
(6) I laughed
(14) I had a good time with my friends
(23) I visited some close friends
(33) I had fun

Scale 10: Somatic Relaxation
(9) I felt physically relaxed
(13) I felt at ease
(29) I felt physically fit
(38) I felt as if I could get everything done

Scale 11: General Well-being
(10) I was in good spirits
(34) I was in a good mood
(43) I felt happy
(47) I felt content

Scale 12: Sleep Quality
(19) I fell asleep satisfied and relaxed
(27) I had a satisfying sleep
(36) I slept restlessly
(46) My sleep was interrupted easily

Scale 13: Disturbed Breaks
(51) I could not get rest during the breaks
(58) I had the impression there were too few breaks
(66) Too much was demanded of me during the breaks
(72) The breaks were not at the right times

Scale 14: Burnout/Emotional Exhaustion
(54) I felt burned out by my sport
(63) I felt emotionally drained from performance
(68) I felt that I wanted to quit my sport
(76) I felt frustrated by my sport

Scale 15: Fitness/Injury

(50) Parts of my body were aching
(57) My muscles felt stiff or tense during performance
(64) I had muscle pain after performance
(73) I felt vulnerable to injuries

Scale 16: Fitness/Being in Shape
(53) I recovered well physically
(61) I was in a good condition physically
(69) I felt very energetic
(75) My body felt strong

Scale 17: Burnout/Personal Accomplishment
(55) I accomplished many worthwhile things in my sport
(60) I dealt very effectively with my teammates' problems
(70) I easily understood how my teammate felt about things
(77) I dealt with emotional problems in my sport very calmly

Scale 18: Self-Efficacy
(52) I was convinced I could achieve my set goals during performance
(59) I was convinced that I could achieve my performance at any time
(65) I was convinced that I performed well
(71) I was convinced that I had trained well

Scale 19: Self-Regulation
(56) I prepared myself mentally for performance
(62) I pushed myself during performance
(67) I psyched myself up before performance
(74) I set definite goals for myself during performance

Appendix C. French Society of Sports Medicine Questionnaire

The following questionnaire, developed by the French Society of Sports Medicine, was to be used to assess overtraining syndrome if participants' performance was impaired following the overload training period and remained impaired during the final Time Trial test. The questionnaire is used by sports doctors and coaches to detect overtraining in athletes, and has been shown to be sensitive to the development of overtraining (Bricout, 2003). This English translation of the questionnaire has been used and in peer-reviewed publications (Brun et al., 2003; Varlet-Marie et al., 2004). The number of questions answered "yes" gives a score between 0 and 54, with scores greater than 20 consistently found in overtrained individuals (Varlet-Marie et al., 2004). For this thesis, the questionnaire was replicated using the SurveyMonkey online survey and questionnaire software, allowing participants to complete the questionnaire by computer or smartphone (www.surveymonkey.com).

Overtraining questionnaire

Sex	M	F	
Date of birth:			
What is your profession?			
If you are a student, are you in period of examinations?	Yes	No	
What is your main sport or game?			
How many hours do you practice per week?	6–8 h	8–10 h	more than 10 h
If you practice other sports or games, write them:			
This month, has there been any significant event which may have disturbed your private or professional life?	Yes	No	
This month:			
1 - My level of sport performance/my state of form has decreased:	Yes	No	
2 - I am not as attentive as before:	Yes	No	
3 - My close friends think that my behaviour has changed:	Yes	No	
4 - I have a sensation of oppression in my chest:	Yes	No	
5 - My heart seems to beat faster:	Yes	No	
6 - I have a lump in my throat:	Yes	No	
7 - I have less appetite than before:	Yes	No	
8 - I eat more:	Yes	No	
9 - I do not sleep as well as before:	Yes	No	
10 - I drowse and yawn in the daytime:	Yes	No	
11 - The lapse of time between training sessions seems to me too short:	Yes	No	
12 - My sexual appetence has decreased:	Yes	No	
13 - My performances are poor:	Yes	No	
14 - I frequently catch a cold:	Yes	No	
15 - I have put on weight:	Yes	No	
16 - I have memory problems:	Yes	No	
17 - I often feel tired:	Yes	No	
18 - I underestimate myself:	Yes	No	
19 - I often have cramps, muscular pain:	Yes	No	
20 - I suffer from headaches more frequently:	Yes	No	
21 - I do no feel fit:	Yes	No	
22 - I sometimes feel dizzy, on the point of fainting:	Yes	No	
23 - I do no confide in others so easily:	Yes	No	
24 - I am often seedy:	Yes	No	
25 - I have a sore throat more often:	Yes	No	
26 - I feel nervous, insecure, anxious:	Yes	No	
27 - I do no bear training so well:	Yes	No	
28 - At rest, my heart rate is faster than before:	Yes	No	
29 - During exercise, my heart rate is faster than before:	Yes	No	
30 - I often feel rotten:	Yes	No	
31 - I get tired more easily:	Yes	No	
32 - I often have digestive disorders:	Yes	No	
33 - I feel like staying in bed:	Yes	No	
34 - I am not so confident in myself:	Yes	No	
35 - I get injured more easily:	Yes	No	
36 - I have more difficulties in organizing my thoughts:	Yes	No	
37 - I have more difficulties in concentrating in my sports activity:	Yes	No	

38 - My sporting gestures are less precise, less skilful:	Yes	No
39 - I have lost force and aggressiveness:	Yes	No
40 - I feel as if I had no one to talk to:	Yes	No
41 - I sleep longer:	Yes	No
42 - I cough more often:	Yes	No
43 - I do not enjoy practicing my sports as much:	Yes	No
44 - I do not enjoy my leisure activities as much:	Yes	No
45 - I get irritated more easily:	Yes	No
46 - I am less efficient in my school or professional activity:	Yes	No
47 - People around me think that I have become less pleasant:	Yes	No
48 - Training seems harder and harder:	Yes	No
49 - It is my fault if my results are worse:	Yes	No
50 - My legs feel heavy:	Yes	No
51 - I lose my personal things more easily (wallet, keys, etc.):	Yes	No
52 - I am pessimistic, I have the blues:	Yes	No
53 - I have lost weight:	Yes	No
54 - My motivation, will and tenacity are weaker:	Yes	No

Put a cross to range between these two opposite states

My physical level:
Great form ⟷ Bad form
I feel fatigued:
More slowly ⟷ More quickly
I recover from my state of tiredness:
More quickly ⟷ More slowly
I feel:
Very relaxed ⟷ Very anxious
I have the feeling that my muscular strength has:
Increased ⟷ Decreased
I have the feeling that my endurance has:
Increased ⟷ Decreased

Have you had any difficulties in understanding some of the questions? Yes No
If yes, which questions did you find difficult to understand (write the numbers)?

(Varlet-Marie et al., 2004)

Appendix D: Assays Completed

Epinephrine and Norepinephrine Assays: General Principle.

Epinephrine and norepinephrine are extracted by using a cis-diol-specific affinity gel, acylated and then derivatized enzymatically.

The competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples and the solid phase bound analytes compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

Appendix E: Study Timeline

Study Timeline:

Day		1	2	3, 4	5	6, 7	8	9	10	11	12	13	14
Description	Familiarization Time Trial	PRE	TT 1	Interval Training	TT 2	Interval Training	TT 3	POST	Taper Training	TT 4	Taper Training	REC	TT 5

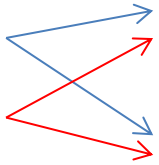
Timeline for PRE, POST and REC experimental days:

Time after arrival (hours:minutes)	**Description**
0:00	Arrival and intravenous catheterization
0:30	LBNP protocol commenced
1:00	Break
2:30	VO_2peak test commenced

Timeline for Time Trial days:

Time after arrival (approximate; varied by participant)	**Description**
0:00	Warm-up
0:20	10 km Time Trial
0:40	Break
0:55	10 km Time Trial
1:15	Cool-down

Timeline for Interval Training days:

Time after arrival (approximate; varied by participant)	**Description**		
0:00	Warm-up		*Days 3, 6:* 5 repetitions of 4 minutes at 80% PPO; 90 seconds active recovery *Days 4, 7:* 10 repetitions of 1 minute at 100% PPO; 2 minutes active recovery
0:20	Intervals (1st set)		
0:50	Break		
1:00	Intervals (2nd set)		
1:30	Cool-down		

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