Autonomic dysfunction and perioperative outcome

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I, John Oliver Rafe Whittle, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed……..

Abstract

Objective: To examine the relationship between established autonomic dysfunction, measures of cardiopulmonary physiology and perioperative outcome in high-risk patients presenting for major surgery.

Summary background data: Experimental data demonstrate that autonomic activity is a key modulator of both cardiovascular and immune function following tissue injury and inflammation. Autonomic dysfunction is associated with adverse outcomes across several medical populations. Whether pre-existing autonomic dysfunction is detrimental following controlled tissue injury (surgery) in humans is unknown.

Summary of studies: Parasympathetic autonomic dysfunction (PAD), defined by impaired heart rate recovery after exercise, was associated with a distinct physiological profile in patients presenting for preoperative Cardiopulmonary Exercise Testing (CPET). This comprised impaired cardiac performance at peak exercise, reduced peak oxygen uptake and anaerobic threshold as well as chronotropic incompetence. Levels of GRK2, a regulator of beta adrenoreceptor and immune/inflammatory activity, in circulating lymphocytes were raised in cells derived from individuals with PAD.

Retrospective analysis of outcomes from two prospectively collected colorectal surgical cohorts (n=1047) revealed PAD to be common (>30%) and associated with an increased length of hospital stay (12 days (95% CI: 9-16) vs. 8 days (95% CI: 6-8.5), p=0.01), as well as increased risk of significant Clavien-Dindo defined morbidity, postoperative gastrointestinal function, sepsis and increased 90-day mortality (RR 1.1 (1.007-1.41), p=0.008).

Intraoperative haemodynamic data indicated impaired cardiac contractility and increased risk of intraoperative hypotension, possibly contributing to detriments in postoperative outcome.

Abstract

Sympathetic autonomic hyperactivity, defined by excessive anticipatory heart rate rise prior to starting loaded exercise was associated with a different CPET profile to that seen in PAD, defined by evidence of cardiac ischaemia during exercise, resulting in impaired cardiac contractile function at peak effort, but also associated with increased hospital length of stay. Patients with PAD did not necessarily demonstrate sympathetic hyperactivity, but when both were present, physiological performance and postoperative outcomes were further impaired.

Conclusions: Both preoperative parasympathetic and sympathetic autonomic dysfunction are associated with impaired perioperative outcomes. These data demonstrate in high risk surgical patients that established autonomic dysregulation is associated with the development of sepsis, myocardial ischaemia, critical illness and mortality following major elective surgery. The autonomic nervous system represents an underexplored target for therapies aimed at reducing the morbidity burden of major surgery.

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Abbreviations

ACE	Angiotensin converting enzyme
ACh	Acetylcholine
AD	Autonomic dysfunction
AHRi	Anticipatory heart rate increase
AKT	Alpha threonine serine protein kinase
ANS	Autonomic Nervous System
ASA	American Society of Anesthesiology
AT	Anaerobic Threshold
ATS	American Thoracic Society
α7nAChR	Alpha-7 nicotinic acetyl choline receptor
BAR	Beta adrenoreceptor
BMI	Body Mass Index
BP	Blood pressure
bpm	Beats per minute
CAN	Cardiovascular autonomic neuropathy
c-AMP	Cyclic adenosine monophosphate
CI	Cardiac Index
CI (statistics)	Confidence Interval
CN	Cranial nerve
CNS	Central Nervous System
CO	Cardiac Output
COPD	Chronic Obstructive Pulmonary Disease
COMPETE-C	Cardiac output monitoring and preoperative exercise testing trial
CPET	Cardiopulmonary Exercise Test
CRP	C reactive protein
DHP	Derriford Hospital Plymouth
DMEM	Dulbecco's Modified Eagle Medium
DO_2	Oxygen Delivery
EHRi	Excessive heart rate increase
ERAS	Enhanced Recovery after Surgery
FBC	Full blood count
fMRI	Functional Magnetic resonance imaging
FTc	Flow Time corrected
GDT	Goal Directed Therapy
GPCR	G-protein coupled receptor
GRK	G-protein receptor kinase
GWAS	Genome wide association study
HF	High frequency
HIF	Hypoxia inducible factor
HLA-DR	Human Leucocyte Antigen D-Related
HMGB-1	High motility group box protein-1
HR	Heart rate
HRg	Heart rate gradient
HRR	Heart rate recovery (abnormal)
HRV	Heart rate variability
IL-1	Interleukin 1
Il-10	Interleukin-10
IL-10R	Interleukin-10 receptor
iNOS	Inducible Nitric Oxide Synthase
IQR	Interquartile range
LF	Low frequency
LF/HF	Low frequency to high frequency ratio

LOS	Length of stay
LPS	Lipopolysaccharide
LV	Left ventricular
KO	Knock out
MA	Mean Acceleration
mMol	MilliMole
mRNA	Messenger Ribonucleic Acid
NF-kB	Nuclear Factor kappa beta
NIGB	National Information Governance Board
NLR	Neutrophil to Lymphocyte ratio
NN50	The number of R-R interval differences of >50 ms
NYHA	New York Heart Association
OD/ODM	Oesophageal Doppler (monitor)
PAD	Parasympathetic Autonomic Dysfunction
PGID	Postoperative gastrointestinal tract dysfunction
PI3	Phospho-inositide 3 kinase
POM	Perioperative Morbidity Score
POM-HR	Perioperative Morbidity – Heart Rate trial
POM-O	Perioperative Morbidity – Oxygen delivery trial
PV	Peak Velocity
QSART	Quantitative sudomotor axon reflex test
RAAS	Renin-Angiotensin-Aldosterone System
RCRI	Modified Lee Cardiac risk index
RMSSD	Square root of mean square differences of successive RR intervals
ROS	Reactive Oxygen Species
RR	Relative risk
R-R	Beat to beat
SAD	Sinoaortic Denervation
SD	Stroke Distance
s.d.	Standard Deviation
SDANN	Standard deviation of the N-N interval averaged over 5 minutes
SDNN	Standard deviation of the N-N interval
SV	Stroke Volume
TLR	Toll-like receptor
TNFα	Tumour Necrosis Factor alpha
TST	Thermoregulatory stress test
UCLH	University College London Hospital
UCL	University College London
UKCRN	United Kingdom Clinical Research network
U&Es	Urea and Electrolytes
VE/VCO_2	Ventilatory equivalents for Carbon Dioxide
VE/VO_2	Ventilatory equivalents for Oxygen
SSR	Sympathetic skin response
5-HT3	5-hydroxytryptophan 3

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1 Introduction

1.1 Introduction

Postoperative morbidity is a significant public health burden, which results in individual suffering and exhibits a strong relationship with reductions in longer term quality of life, morbidity and mortality (Moonesinghe et al. 2011). To date, whilst common co-morbidities associated with impaired postoperative outcomes have been well described (Khuri et al. 2005; Story 2011), biological mechanisms as to how and why higher risk patients develop postoperative critical illness have remained underexplored. This thesis examines the potential contribution of one candidate mechanism for the development of postoperative morbidity: established autonomic dysfunction.

In this chapter, I will discuss the importance of postoperative morbidity and mortality as a public health problem and explore common mechanisms by which postoperative morbidity develops. I will then describe how intact autonomic function is essential for biological stability and introduce the concept of autonomic dysfunction. I will subsequently outline why autonomic dysfunction is likely to be highly prevalent in the high risk surgical population and explore potential mechanisms by which autonomic dysfunction might drive the development of postoperative morbidity, multi-organ dysfunction and critical illness. Finally, I will explore commonly used methods to assess autonomic function and propose the use of heart rate recovery after exercise as the most appropriate measure of established parasympathetic autonomic in the surgical population.

1.1.1 Post-operative morbidity and mortality are important and incompletely understood issues in healthcare

Over 300 million surgical procedures are performed worldwide each year (Weiser et al. 2016). In Europe in 2016, around 100 patients per 100,000 population underwent colectomy (Eurostat 2016). Inpatient mortality after surgery varies widely, but across Europe around 4% of patients die before hospital discharge (Pearse et al. 2012). Of

those undergoing major surgery, between and 10% & 17% suffer major postoperative complications (Khuri et al. 2005; Ghaferi et al. 2009; McNicol et al. 2007, Ferraris et al. 2014) and up to half suffer short-term minor postoperative morbidity (Grocott et al. 2007; Bennett-Guerrero et al. 1999) resulting in increased usage of healthcare resources and delayed or failed recovery to preoperative function. Of those patients who go on to develop serious postoperative complications, between 10% & 20% go on to die within 30 days (Ferraris et al. 2014).

Postoperative morbidity further carries implications for long-term health. In a landmark study of 105,951 patients, those who survived to hospital discharge but had suffered a postoperative complication in the first 30 days after surgery, suffered up to a 20% increase in mortality up to 10 years postoperatively. This may reflect both the premorbid state of patients presenting for surgery influencing the likelihood of development of post operative complications, as well as the impact of these complications themselves on long term health (Khuri et al. 2005). Similar to other hospital-treated diseases such as community acquired pneumonia, many patients may leave hospital with ongoing subclinical inflammation, which may result in longer term morbidity and mortality despite apparent clinical recovery at discharge (Ackland & Edwards 2010). These findings have been supported since by several other studies (Toner & Hamilton 2013).

Within the cohort of patients who are deemed at highest risk, a significant minority appear to suffer the majority of post-operative complications (Pearse et al. 2006, Ferraris et al. 2014). Identifying which individuals from this high risk group are at greatest risk, and indeed refining our definitions and scoring systems for perioperative risk has been a focus of much interest in recent years (Moonesinghe et al. 2009, Ferraris et al. 2014).

The potential of accurately identifying those at the highest risk has only been partially recognised and incompletely realised to date. Many of the risk factors used today to identify those at increased risk of postoperative morbidity have been derived from relatively underpowered cohort studies that are able to identify broad groups of

patients and individual risk factors for perioperative complications, but lack plausible biological mechanisms to explain the development of specific postoperative morbidity.

Aside from better informing the consent process; directing potentially beneficial treatment strategies towards those most likely to benefit thereby promoting appropriate allocation of healthcare resources, a more complete mechanistic understanding of drivers of perioperative morbidity will help development of new therapeutic and management strategies in addition to better definitions of perioperative risk.

1.1.2 Mechanisms of postoperative morbidity

Morbidity after surgery often affects multiple systems, including those remote from the operative site (Bennett-Guerrero et al. 1999). The incidence of gastrointestinal dysfunction after lower limb surgery, for example, may be as high as 17% (Grocott et al. 2007). Evidence is accumulating for the existence of multisystem drivers for the development of pathology remote from the operative site, which are influenced by disruption in physiological control and regulation pathways (Edwards et al. 2015; Karmali et al. 2015; Sultan et al. 2014). This implies that multisystem pathophysiological processes underlie much of postoperative morbidity. The link between pre-existing systemic pathophysiology and the development of new pathologies in the postoperative period has not been well elucidated.

Surgical trauma activates multiple metabolic, bioenergetic, immunological and neuro-humoral processes that interact closely in ways still not well understood. Additional complexity is contributed by pre-existing pathology and subsequent and diverse physiological stressors applied during the operative period and beyond.

Surgery of moderate to major severity induces neurological, immune and inflammatory changes, both through direct tissue trauma and through secondary pathways (Kohl & Deutschman 2006). Hypothalamo-pituitary activation, increases in sympathetic nervous activity, vagal withdrawal, hormonal and metabolic changes

associated with catabolism and increases in the metabolic demand and usage of oxygen have all be described; and dysregulation of all these systems has been associated with perioperative morbidity (Pearse et al. 2005; Shoemaker 1988; Desborough 2000; Ackland et al. 2015).

Why in patients seemingly matched in terms of commonly recorded risk factors, the cardiometabolic, neurological and immune/inflammatory response to surgery in certain individuals can vary so widely from that in others is not fully understood. These variations, particularly in the higher risk cohort, result in the development of multi-organ dysfunction and increased susceptibility to complications such as sepsis in seemingly otherwise matched patients.

Dysfunction of the autonomic nervous system (ANS), by virtue of the central role of the ANS in the regulation of multiple core biological processes is a strong candidate mechanism for the promotion of and development of postoperative morbidity.

1.2 The Autonomic Nervous System in health and disease

1.2.1 Intact autonomic function is central to maintaining biological stability

The autonomic nervous system is that part of the nervous system responsible for the control of biological systems that are not normally consciously regulated or directed. Autonomic functions include the control of respiration, as well as cardiovascular and gastrointestinal regulation (Figure 1-1).
Classically, the autonomic nervous system is described as a component of the peripheral nervous system consisting of two branches; parasympathetic (predominantly cholinergic) and sympathetic (predominantly adrenergic), which are both anatomically and functionally differentiated. Commonly, the two systems are conceived as having opposite actions where activation of one limb will result in an inhibitory response from the other. However, current understanding offers a more nuanced interpretation of function, where one limb may directly support or enable function in the other. A third system of neurons which are non-adrenergic and non-cholinergic have relatively recently been described that are integral to autonomic function and may act in part to help integrate activity of the two limbs.

Central regulation of autonomic function is integrated by the hypothalamus, which receives input from multiple specialised centres (such as the vasomotor, apneustic and pneumotactic centres and the dorsal vagal nucleus) as well as regulatory input from the limbic and somatosensory systems, including sensory input from both peripheral afferent sympathetic and parasympathetic neurons (Hall & Guyton 2006).

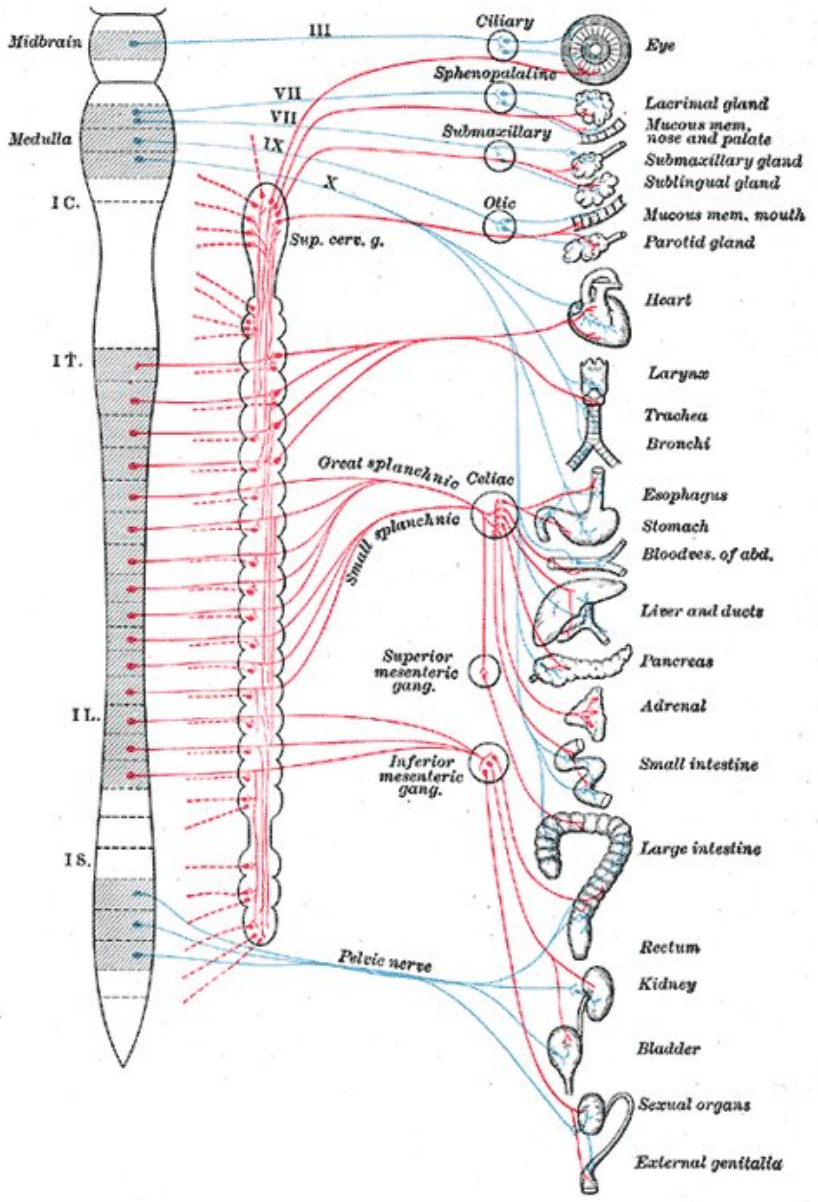

Figure 1-1: The Autonomic Nervous System

Red represents the sympathetic nervous outflow (thoraco-lumbar), whereas blue represents parasympathetic outflow (cranio-sacral), including cranial nerve X (the vagus nerve). Current understanding could include other interfaces, such as that between the autonomic and immune systems. Reproduced from Henry Carter (1918), Public Domain.

In health, the autonomic nervous system is both responsible for biological stability through homeostasis (the maintenance of equilibrium between interdependent elements permitting optimal function of multiple physiological processes, e.g. the maintenance of organ perfusion pressure through the regulation of blood pressure) as well as the maintenance of stability through change by appropriate responses to intrinsic and environmental physiological stressors (allostasis; e.g. an increase in heart rate in response to a demand such as exercise, an important component of homeostasis; McEwen & Wingfield 2003). Allostatic changes are in general adaptive in the short term (Schulkin 2004), though in the long term can contribute to the pathogenesis of common chronic diseases (e.g. the role of chronic sympathetic activation secondary to psychological stress in the pathogenesis of end-organ damage secondary to hypertension; McEwen 1998; McEwen 2004).

Dysfunction in autonomic controlled allostatic mechanisms may lead to disease through several mechanisms. These include the frequent activation of allostatic mechanisms, failure to terminate an allostatic response after a stressor has been removed and the inadequate response of an allostatic mechanism to another normally counter-regulated allostatic mechanism (e.g. failure of vagal activity to adequately counter elevated sympathetic outflow; McEwen 1998). The maintenance of biological variability is therefore key to health and the maintenance of intact organ function, loss of which may result in multi system pathology (Godin & Buchman 1996).

By virtue of the multisystem connectivity resultant from autonomic innervation, the autonomic nervous system provides a conduit for inter-organ cross-talk which in health promotes adaptive function, but in disease may promote multi-organ dysfunction (Brumovsky & Gebhart 2010; Dobson et al. 2006; Davenport 2008; Kociol et al. 2009; Zoccali et al. 2014; Mastitskaya et al. 2012). Diseases such as cardiac failure, hypertension and diabetes cause chronic physiological stress to the patient resulting in measurable damage to end-organ systems. Autonomic dysfunction, possibly secondary to this load, is common in these conditions and plays a mechanistic role in their pathophysiology and impaired patient outcomes (Lauer 2009).

1.2.2 Autonomic dysfunction

Human autonomic dysfunction has been variously described by alterations in heart rate variability parameters (Lauer 2009), detriments in baroreceptor function and altered cardiovascular reflexes (Latson et al. 1994), altered skin (sudomotor) responses to cardiovascular testing (Guinjoan et al. 1995) and altered heart rate dynamics in response to exercise (Jouven et al. 2009; Jouven et al. 2005; Cole et al. 1999; Barbato1999).

The sympathetic and parasympathetic nervous systems do not always work in opposition, but rather interact in a complex, dynamic manner. These interactions are modulated in part by second messenger systems. Both sympathetic and parasympathetic systems are able to inhibit reciprocal traffic presynaptically (Olshansky et al. 2008).

At rest, vagal tone is dominant. Indeed, under normal physiological conditions, a response known as accentuated antagonism, where intense vagus discharge overrides increases in sympathetic discharge (e.g. at times of stress or exercise), maintains appropriate balance between the two limbs of the ANS (Olshansky et al. 2008).

Parasympathetic Autonomic Dysfunction (PAD) is a condition where resting vagal tone and vagal reflexes, including accentuated antagonism, are reduced. This may be accompanied by chronic elevations in sympathetic activity and/or sympathetic hyperactivity in response to stressors. Baroreceptor dysfunction, commonly described in diabetes mellitus, heart failure, ischaemic heart disease and hypertension, is a prototypical example of PAD pathophysiology and is associated with chronic renin-angiotensin-aldosterone system (RAAS) activation (Yee & Struthers 1998).

Both established and acquired impairments in vagal activity are likely to predispose to poorer outcomes after surgery through a variety of mechanisms including an exaggerated inflammatory response, impaired cellular immunity (Czura & Tracey

2005), reduced cardiac function (Kakinuma et al. 2009; De Ferrari 2014), and impaired post operative gastrointestinal function (Lubbers et al. 2010).

1.3 Autonomic Dysfunction and the surgical patient

1.3.1 Autonomic Dysfunction is likely to be common in higher risk surgical patients presenting for major surgery

Of those patients who die following surgery, the vast majority (~96%) has significant medical co-morbidities at the time of surgery. Many have a co-existing cardiovascular (76%) or respiratory pathology (56%) at time of presentation (National Confidential Enquiry into Perioperative Deaths, Outcomes & Efficiency, 2010).

In the UK, the EUSOS study (Pearse et al. 2012) revealed that, over the seven day study period, 2.5% of all patients presenting for surgery had a diagnosis of heart failure, 14.5% COPD, 13.4% Coronary artery disease and 10% either type-1 or 2 diabetes; all conditions associated with autonomic dysfunction (Filipovic et al. 2003; Tang et al. 2009; Vinik et al. 2011; Ponikowski et al. 1997; Thayer et al. 2010).

The burden of comorbidity in cancer patients, who frequently present for surgery, is particularly high. Up to 63% of those aged over 75 years old present with at least one co-morbid condition, the most common of which include cardiac or vascular disease (30%), diabetes (25%) and hypertension (25%; Coebergh et al. 1999). Again, in all of these pathologies, autonomic dysfunction is prevalent (Lauer 2009).

Additionally, the demographic of patients presenting for major surgery is changing to reflect the aging population (Etzioni et al. 2003, Ngaage et al. 2008, Kordatou et al. 2014, Linkhorn et al. 2016); the fastest growing segment being individuals aged over 65, projected to increase in numbers by 53.2% by 2020 (Etzioni et al. 2003). Significant changes in autonomic function occur with age that are responsible for an impaired ability to adapt to environmental or intrinsic stressors (Hotta & Uchida

2010), with potentially important implications for the elderly patient presenting for surgery.

In the postoperative period, severe morbidity may result in critical illness, intensive care unit admission and multi-organ dysfunction leading to death. Again, the majority of patients in the intensive care unit already exhibit established features of chronic diseases commonly associated with autonomic dysfunction prior to requiring critical care (Angus et al. 2001; Angus et al. 2006). Autonomic dysfunction itself, in the context of critical illness, is strongly associated with impaired outcome (Schmidt et al. 2008; Toner et al. 2013; Gang & Malik 2002).

1.3.2 Major surgery presents a severe challenge to the autonomic nervous system

Major surgery presents an enormous physiological challenge (Bennett 2005). The autonomic nervous system is central to a regulated response in this context, since sympathetic activation and parasympathetic withdrawal are core features of the immediate inflammatory response to tissue injury (Desborough 2000).

Alterations in blood volume, fluid shifts, adrenocortical and renin-angiotensin-aldosterone axis activation result from, and cause, large shifts in autonomic outflow (Desborough 2000). Similarly, many drugs (such as epinephrine, ephedrine, beta-blockers) administered and interventions (such as neuraxial anaesthesia, regional blockade and intubation) carried out in the perioperative period, have a profound effect on autonomic activity (Licker et al. 2003; Hirayama et al. 2006; Desborough 2000; Heller et al. 1984; Mazzeo et al. 2011).

Individuals with established autonomic dysfunction may be at greater risk of perioperative morbidity as a result. Intraoperative hypotension (Huang et al. 2006) and cardiovascular instability (Knuttgen et al. 1990), altered pain responses (Heller et al. 1984) and an increased risk for postoperative myocardial ischaemia and myocardial death, prolonged hospitalisation and surgical resource usage have all been described in patients presenting for surgery with impaired autonomic function

as defined by reduced heart rate variability (Knuttgen et al. 1990; Mazzeo et al. 2011; Huang et al. 2006; Latson et al. 1994; Laitio et al. 2007).

1.4 Autonomic Dysfunction as a key mechanism underlying acute and chronic disease

1.4.1 Autonomic Dysfunction drives the development of critical illness

Critical illness after major surgery remains an important cause of postoperative morbidity and mortality. The development of sepsis and multi-organ dysfunction are key features of postoperative critical illness, and are the chief causes of death in this period.

The critical illness phenotype is characterised by features including sustained inflammation, cardiovascular dysfunction and immunosuppression. It is becoming increasingly evident that deranged autonomic function provides the motor for its development (Toner 2014).

Both clinical and experimental studies have demonstrated rapid and profound alterations in autonomic activity following a range of disparate pathologies, including surgery, resulting in critical illness. In the first instance, alterations in autonomic activity in response to surgery and immune challenge may be adaptive, exaggerated, maladaptive or sustained changes subsequently resulting in pathology.

1.4.2 Sympathetic nervous activation provides an adaptive response to physiological challenge

Sympathetic nervous system activation is central and essential to a coordinated cardiorespiratory, metabolic and immune response to tissue injury and infection. Blood volume loss from surgery or trauma and signalling provoked by direct tissue damage result in sympathetic activation, maintaining blood pressure through increases in cardiac pre- and afterload, cardiac contractility and through renin-angiotensin-aldosterone system (RAAS) activation (Desborough 2000). However, as

will subsequently be described, excessive or prolonged sympathetic activity can be harmful.

1.4.3 Autonomic activation is rapid and occurs in response to peripheral immune challenges

The central nervous system (CNS) receives sensory input from the immune system through both humoral and neural routes (Czura & Tracey 2005). Cytokines and other immunological mediators directly influence CNS centres devoid of blood-brain barrier (circumventricular regions) or via active transport systems resulting in classical hypothalamic-pituitary responses to illness and injury (Tracey 2010). Similarly, some mediators (e.g. IL-1B and endotoxin) are directly sensed by peripheral nerve endings resulting in the sickness syndrome (fever, anorexia, social isolation behaviour etc.).

Whilst humoral immune to brain communication remains the predominant conduit when cytokine levels are high, the CNS can be informed of peripheral injury and inflammation via afferent nerve traffic, predominantly travelling in the vagus, when circulating cytokine levels are low. Sensory information about the status of tissue injury is transmitted from immune modulating organs (liver, kidney, spleen, lungs, gut) via afferent vagal ascending fibers along with pain and other information. The precise mechanism by which the vagus senses inflammation is not yet known, but IL-1 receptor mRNA and discrete IL-1 binding sites have been found in neurons and glomus cells in the vagus nerve itself (Watkins & Maier 1999).

The importance of peripheral neural sensors in the mediation of the immune response lies in the speed of response elicited. Peripheral infusion of endotoxin to healthy volunteers rapidly results in an hyperadrenergic response characterised by rapid several fold increases in the levels of circulating catecholamines, and rapid, parallel reductions in heart rate variability.

This sympathetic drive in response to immune challenge is both adaptive and essential, maintaining blood pressure and hence organ perfusion. Early survival

from endotoxaemic shock is dependent on the maintenance of an elevated sympathetic drive. Aside from the peripheral circulatory benefits in terms of increased cardiac output and salt/water retention of increased catecholamine release and RAAS activation, heat shock protein-70 prevents circulatory collapse through inhibition of inducible nitric oxide (iNOS) gene expression in the rostral ventrolateral medulla, the brain area responsible for elevations in sympathetic activity (Chan et al. 2004).

1.4.4 Loss of crucial signalling and receptor activity in autonomic dysfunction impairs the physiological response to critical illness

The concept that impaired autonomic activity is central to the development of the critical illness phenotype, and for impaired survival from critical illness, has been demonstrated in laboratory models where the carotid sinus nerve is transected, preventing transduction of crucial biological information from carotid body chemoreceptors and carotid sinus baroreceptors to brain-stem control centres. Intact carotid body chemoreceptors are able to detect circulating inflammatory mediators, enhancing hypoxic sensitivity, thereby contributing to the ubiquitous features of tachycardia and tachypnoea, seen at the start of critical illness, often in the absence of hypoxia or acidosis (Ackland et al. 2007).

Transection of the carotid sinus nerves results in reduced survival from endotoxaemic sepsis. This is one demonstration of a central role for afferent autonomic signal transduction from chemo/immune sensors in the integrated immune response to sepsis (Tang et al. 1998).

Sepsis is associated with and perhaps causes an intrinsic failure in autonomic control, as demonstrated by post mortem data from septic patients, where CNS autonomic control areas in patients who had died from septic shock showed evidence of ischaemia and apoptosis as compared with those who had died from other causes (both non-septic shock and extra cranial injury; Sharshar et al. 2003). This failure of autonomic control is compounded by many of the conditions and therapies common in the critical care unit, which profoundly alter, if not ablate,

autonomic, baroreflex and chemoreceptor control. These include sedation, neuromuscular blockade, immobilisation, bed rest and anxiety amongst others (Akada et al. 2008; Eriksson et al. 1993; Ebert 2005; Hughson et al. 1994; A. Toner et al., 2013).

1.5 Autonomic neural regulation of inflammation

The organisation of the ANS into a series of neural reflex circuits allows a rapid and profound influence on the immune response to various stressors, not just in terms of sensing and afferent signalling as described previously, but also in terms of a direct influence on the function of circulating immune cells.

The inflammatory response is central to regulated antimicrobial defence and healing after tissue damage. Excessive inflammation, or failure to resolve the initial inflammatory phase after tissue injury however, results in excessive tissue destruction, circulatory collapse and potentially death (Gentile 2012). Additionally, immune changes due to autonomic dysfunction in chronic conditions such as cardiac failure, relevant to the high-risk surgical patient, demonstrate the implications of established disease on the response to immune or inflammatory challenge (Jankowska 2006, Florea 2014).

The description of a series of neural inflammatory reflexes is key to understanding the role of autonomic activity in the real time sensing and regulation of inflammation and immunity. A summary of autonomic-immune interactions is found in Table 1-1.

1.5.1 Evidence for an autonomic–immune interaction: anatomical and cellular

Evidence for cytokine-induced activation of the hypothalamic-adrenal axis provided the original evidence for bidirectional linkage of the autonomic and immune systems. Subsequent work has implicated corticosteroid-independent, circulating humoral and autonomic mechanisms in the mediation of stress related immune depression (Toner et al. 2013). Evidence of extensive sympathetic and parasympathetic innervation of the thymus, spleen and other lymphoid tissue has been demonstrated in

neuroanatomical studies, additionally providing fixed loci for neuroimmune interaction (Rosas-Ballina & Tracey 2009; Nance & Sanders 2007; Stevens-Felten & Bellinger 1997; Anagnostou et al. 2007).

In vitro work has indicated that both sympathetic and parasympathetic neurotransmitters can modulate all aspects of the immune response, including cytokine production, lymphocyte proliferation and antibody secretion (Tracey 2010).

1.5.2 Sympathetic neural influence on immune function

Alterations in circulating immune cell activity, specifically monocyte deactivation, have been identified as sharing pivotal mechanistic links in the pathogenesis of both critical illness and cardiac failure with the development of autonomic dysfunction. Immune dysregulation is a key feature of heart failure, a condition where chronic sympathetic hyperactivity is pathognomonic. Absolute numbers, as well as function, of monocytes and T-cells are markedly altered in cardiac failure. High levels of circulating endotoxin and lymphopaenia are also noted in heart failure resulting in down regulation of monocyte $CD14^{+}$ expression and adversely altered Neutrophil to Lymphocyte Ratios (NLR) (Apostolakis et al. 2010).

Sympathetic hyperactivity appears to further result in immune impairment through various additional pathways. In parallel with reductions in cardiac performance (Silverman et al. 1993), lymphocytes exposed to tumour necrosis factor (TNF)-α (Singh et al. 1993) or serum obtained from septic patients (Bernardin et al. 2003) exhibit impaired production of cyclic adenosine monophosphate (c-AMP) following β-adrenergic receptor stimulation, the levels of which help to determine the balance between cellular activation and suppression.

Monocyte deactivation, common in cardiac failure, and characterised by reduced HLA-DR expression, diminished antigen presentation and bactericidal killing, is associated with increased risk of infection and higher mortality (Monneret et al. 2008). This deactivation may be reproduced by catecholamine inhibition of TNFα

production associated with increased release of the anti-inflammatory cytokine IL-10 (Meisel & Meisel 2011).

Deleterious alterations in immune activity have been described in $CD14^+$ monocytes isolated from patients with septic shock. These monocytes demonstrate β-adrenergic desensitisation after catecholamine stimulation in a parallel manner to the decrease in β-adrenergic sensitivity seen in non-survivors of critical illness (Link et al. 2008). This monocyte response has been subsequently reproduced *ex vivo* by incubation of monocytes from healthy volunteers with Lipopolysaccharide (LPS) and isoproterenol (a β-adrenoreceptor agonist) (Ackland et al. 2015).

Host-pathogen interactions are also altered in the presence of sympathoexcitation. Established sympathetic nervous system activation predisposes to the accumulation of bacteria in tissues and therefore increases the likelihood of sepsis (Straub et al. 2000). Both endogenous and exogenous catecholamines stimulate bacterial growth and increase the virulence of bacteria (Link et al. 2008).

Sympathoexcitation acting both through humoral and direct neural pathways is, under normal circumstances, an adaptive response to acute tissue injury. However, excessive or chronic activity is injurious through the promotion of an excessive inflammatory response, impairment of immune cell function, promotion of bacterial growth and alterations in host-pathogen interaction. Dysregulated sympathetic activity as a component of autonomic dysfunction, in the perioperative period, is therefore likely to result in impaired immune/inflammatory outcomes, resulting in morbidity and likely mortality.

1.5.3 Parasympathetic neural influence on immune function

The vagus (CN X, or the 'wandering' nerve) is the longest cranial nerve and innervates the majority of the visceral organs with both sensory and motor fibres, the majority being sensory. Importantly, these sensory fibres are able to rapidly monitor and subsequently control peripheral inflammatory responses.

Early evidence for this monitoring role was provided by the observation that sub-diaphragmatic vagotomy abolished the expected stress and febrile responses to interleukin-1B (Watkins & Maier 1999). This relationship was subsequently confirmed through observations of dose dependent increases in hepatic vagal nerve afferent activity following intraportal administration of both IL-1B and bacterial products. It has been suggested that the vagus may be able to convey cytokine or bacterial product specific information to the brain stem, allowing differential responses to individual immune challenges (Watkins 2016).

Similarly, the carotid body, acting through the carotid sinus and glossopharyngeal nerves as described previously, in addition to sensing oxygen, carbon dioxide and pH, acts as a sensor organ for peripheral inflammation, intact function of which is essential for survival from sepsis (Zapata et al. 2011). For example, a significant increase in basal chemosensory discharge has been reported after IV Lipopolysaccharide infusion in cats. This increase in discharge appears essential for survival from experimental sepsis (Fernández et al. 2008). Importantly, section of the carotid and aortic nerves (sino-aortic denervation, SAD) prevents increases in respiratory rate normally seen in response to sepsis (Fernández et al. 2008).

The precise mechanism of this sensing role has not been described, however, specific sensory neurons in the dorsal root and trigeminal ganglia of the peripheral nervous system are capable of directly sensing inflammatory mediators through the expression of toll-like sensors and immune receptors tailored to specific bacterial motifs (Ackland et al. 2007; Huston 2012). Microarray analysis of both human and mouse carotid bodies have demonstrated expression of many key genes involved in the immune and inflammatory response (including many pro-inflammatory cytokines, NF-kB, IL-10R and HMGB-1, Mkrtcian et al. 2012).

The vagal inflammatory reflex is central to the neural regulation of inflammation. Early studies demonstrated reduced levels of circulating TNFα after vagal stimulation in an inflammatory model (Borovikova et al). This observation was extended by the demonstration of reduced inflammatory cytokine production in stimulated

macrophages in the presence of acetylcholine, the prototypical parasympathetic neurotransmitter.

Evidence for an anatomical site for the interaction of the vagus nerve and immune cells comes from the description of splenic nerve terminals ending near an acetylcholine releasing population of T-cells. This regional acetylcholine release appears to act on splenic immune cells (including macrophages) via the nicotinic acetylcholine receptor α7 (α7nAChR), resulting in reduced nuclear factor kB assembly as well as inhibiting inflammasome formation via mitochondrial α7nAChR activity. α7nAChR knockout mice do not reduce TNFα production in response to vagal nerve stimulation. Importantly, these same knockout animals demonstrate elevated systemic TNFα in response to endotoxaemia, and increased mortality, pin-pointing the vagus as an essential regulator of the systemic inflammatory response (Parrish et al. 2008; Tracey 2002).

Further evidence for vagal involvement in the systemic inflammatory response comes from the intestinal immunoendocrine axis. Enteroendocrine cells, dispersed throughout the intestinal epithelium, collectively form the largest endocrine system in humans, and secrete more than 20 peptide hormones, classically studied for their roles in the postprandial uptake of nutrients (Worthington 2015). Vagal activation is the prototypical response in response to gut hormone release after feeding (Dockray 2014).

Human and rodent studies have demonstrated alterations in both the number and activity of enteroendocrine cells in the gut during inflammation (Harrison et al. 2013). Enteroendocrine cells possess functional toll like receptors which can directly respond to both pathogens and commensal bacteria, thereby helping regulate the intestinal immune response through release of immunomodulators such as cytokines and direct orchestration of immune cell function (Bogunovic et al. 2007; Cani et al. 2013; Worthington 2015).

Immune cells also express a range of receptors for enteroendocrine hormone peptides (Genton & Kudsk 2003). Interaction of gut hormones with these receptors

produces a wide range of effects varying from altered cellular recruitment, activation, phagocytosis, antigen presentation and cytokine secretion (Shajib & Khan 2015).

Branch	Effect	Afferent/Efferent
Sympathetic	Hypothalamic-adrenal axis activation by cytokines	Afferent
	Decreased monocyte numbers	Efferent
	Reduced monocyte function	Efferent
	Reduced monocyte HLA-DR expression	Efferent
	Reduced Neutrophil to Lymphocyte ratio	Efferent
	Altered immune cell activation/suppression	Efferent
	Reduced antigen presentation	Efferent
	Reduced bactericidal killing	Efferent
	Altered host-pathogen interaction	Efferent
	Accumulation of bacteria in the tissues	Efferent
	Excessive inflammatory response	Efferent
Parasympathetic	Carotid body sensing/modulation of inflammation	Afferent
	Differential vagal sensing of cytokine/bacterial products	Afferent
	Vagal inflammatory reflex	Afferent/Efferent
	Intestinal immunoendocrine axis	Afferent/Efferent

Table 1-1 Key autonomic influencers

Aside from direct interaction with immune cells, enteroendocrine peptides also modulate the vagal inflammatory response. Cholecystokinin, produced in response to a fat rich diet, triggers increased vagal activity resulting in reduced inflammatory cytokine release from macrophages (Luyer et al. 2005). These observations have been reproduced in a variety of inflammatory conditions (Matteoli & Boeckxstaens 2013). The vagal anti-inflammatory response and the direct influences of intestinal peptides on immune function, provide potential pathways for dietary modulation of the inflammatory response highly relevant to the surgical patient (Martindale et al. 2013).

1.5.4 Clinical implications of the vagal inflammatory reflex in surgical patients

The vagal inflammatory reflex has been identified as a potential therapeutic target across a wide variety of inflammatory conditions. Nonselective α7nAChR blockade in experimental sepsis increases survival, and in aseptic surgery modulates neuroinflammation and preserves cognitive performance (Eriksson et al. 2016).

Early changes in heart rate variability, associated with impaired cardiac contractility, in incipient sepsis point to early vagal nerve withdrawal. Indeed, these early changes, even before the onset of severe sepsis or septic shock, are predictive of later mortality. Prolonged or profound ongoing loss of heart rate variability is also associated with impaired survival from sepsis and critical illness (Toner et al. 2013).

On the other hand, evidence for a protective role for intact vagal activity in sepsis is provided by increased survival and a reduced inflammatory burden in studies where the vagus nerve is directly stimulated or α7nAChR agonists are administered. Some evidence exists in models of acute lung injury, with and without barotrauma, for a protective role of both vagal stimulation and α7nAChR agonism, potentially carrying implications both for, (1) intraoperative ventilator strategies (supported by clinical studies that show pulmonary benefits of intraoperative lung protective ventilation), and (2) post operative pulmonary complications in higher risk patients (Kessler et al. 2012; Huston 2012).

Taken together, intact or preserved, or even augmented, parasympathetic activity in the perioperative period could be predicted to have a protective effect in terms of the immune response to surgical trauma. Regulation of an appropriate inflammatory response is the central mechanism underlying any proposed benefit. Abrogation of excessive sympathetic activity, reduced septic, infectious and inflammatory complications (such as post operative pulmonary complications, myocardial ischaemia and gastrointestinal dysfunction) would be predicted in individuals with intact vagal function when compared with those with PAD.

Chronic vagal withdrawal has been associated with enhanced cytokine release and tissue damage in chronic inflammatory diseases (Tracey 2010; Straub et al. 1997; Tan et al. 1993; Toussirot et al. 1993). The impact of established parasympathetic autonomic dysfunction on immune responses to sterile trauma can be hypothesised from vagotomy and pharmacological blockade studies, but has yet to be described in human surgical patients.

1.6 Autonomic Dysfunction and the heart

Both ischaemic heart disease and cardiac failure are common in the high risk surgical population and are associated with materially worsened outcomes (Fleisher et al. 2014).

Whilst in particular, myocardial injury associated with troponin leak is associated with morbidity and mortality (Devereaux et al. 2012), the issue as to whether ischaemia rather than inflammation is the dominant pathophysiological mechanism remains unresolved (Moonesinghe et al. 2011).

Autonomic dysfunction, both in terms of sympathetic over activity and parasympathetic withdrawal, is clearly associated with a dysregulated inflammatory response to tissue injury and may underlie some of the observations of increased cardiac risk in higher risk patients both through functional impairment of cardiac function (Olshansky et al. 2008) and through increased local inflammation resulting in myocardial cellular injury and death (Pavlov & Tracey 2005). These observations, if translated to perioperative cardiac injury, would be associated with materially worse perioperative outcomes in patients with established autonomic dysfunction.

1.6.1 Parasympathetic Autonomic Dysfunction and cardiac injury

Aside from the clear benefits of preserved vagal activity in terms of slowing heart rate, allowing improved coronary perfusion, better diastolic filling time, and reduced cardiac work, key roles in the modulation of myocardial inflammation and cellular

responses to ischaemia have been described both in acute and chronic contexts (Paton et al. 2006; Rosas-Ballina & Tracey 2009).

In patients with known ischaemic heart disease, reduced heart rate variability and other markers of parasympathetic autonomic dysfunction are strongly associated with increased risk for sudden death, arrhythmia and myocardial infarction (Huikuri & Mäkikallio 2001; Jouven et al. 2005). Indeed, reduced heart rate variability and reduced heart rate recovery after exercise, a marker of impaired vagal activity, have both been correlated with both the extent and severity of coronary arterial disease (Rich et al. 1988; Ghaffari et al. 2011).

Preserved vagal activity is protective in myocardial injury through several mechanisms, and its role in the moderation of reperfusion injury is of great importance, since reperfusion after infarct contributes up to 50% of final infarct size (Shinlapawittayatorn et al. 2014, Mastitskaya et al. 2012).

Local inflammation, arrhythmia (in part through preservation of connexin-43; Ando et al. 2005), oxidative stress and cardiomyocyte calcium overload are key components of reperfusion injury, all of which may be ameliorated or even prevented through increased cardiac vagal activity before, during and after myocardial ischaemia (Kakinuma et al. 2013; Zhang et al. 2014; Xiong et al. 2009; Johnston & Webster 2009; De Ferrari 2014; Shinlapawittayatorn et al. 2014).

Intermittent vagus nerve stimulation for example, in one model, decreased myocardial infarct size in an atropine-dependent fashion (Uitterdijk et al. 2015). Increased activation of the PI3/AKT/hypoxia-inducible factor (HIF) 1a cell survival pathway, improved glucose usage and angioneogenesis with an associated reduced infarct size and preserved cellular function in an atropine dependent manner in animal models of cholinergic stimulation and dysfunction (Kakinuma et al. 2013; Kakinuma, Ando, Kuwabara, Rajesh G Katare, et al. 2005).

Aside from a uniquely cardiac influence on myocardial responses to ischaemia, intact parasympathetic function plays a multi-system role. Inter-organ cross-talk

mediated neurally by the parasympathetic nervous system further limits myocardial damage following coronary ischaemia-reperfusion injury (Mastitskaya et al. 2012). This is most clearly represented by the observation that remote ischaemic preconditioning, which dramatically attenuates myocardial damage following transient coronary artery ligation, is strongly dependent on intact vagal function (Mastitskaya et al. 2012).

In the perioperative period, established or acquired autonomic dysfunction may therefore be deleterious through several mechanisms including: promotion of myocardial inflammation, worsening of established myocardial ischaemia through impaired cell survival, worsening of myocardial ischaemia-reperfusion injury, impaired angioneogenesis, increased mitochondrial ROS formation, arrhythmogenesis both through influence on sino-atrial and atrio-ventricular node activity and through altered expression of connexins.

1.6.2 Sympathetic Autonomic Dysfunction and cardiac injury

Sympathetic activation is provoked by both myocardial ischaemia and reperfusion and is mechanistically linked to the development of myocardial injury (Longhurst et al. 2001; Heusch et al. 1986; Minisi & Thames 1991; Schömig 1990; Kolettis et al. 2016).

Aside from the deleterious pro-inflammatory immune effects engendered by sympathetic activation, the accumulation of intracellular calcium, triggering myocardial necrosis, can be induced by excessive sympathetic activity (Dünser & Hasibeder 2009; Ellison et al. 2007). This effect is potentiated both by metabolic dysregulation and a prothrombotic state, and may underlie, or at least exacerbate, the elevated troponin levels frequently seen in critically ill patients and in the perioperative period (Devereaux, Chan, et al. 2014; Sear et al. 2008).

Acute psychological stress is associated with increased sympathetic activity and such stress alone can, in susceptible individuals, provoke excessive sympathetic outflow, representing sympathetic autonomic hyperactivity, and trigger endothelial

cell activation and coronary artery vasospasm, effects which importantly can be abolished by beta blockade (Nagele & Liggett 2011). Excessive sympathetic activation, prominent in several illnesses characterised by increased levels of psychological stress, is strongly associated with increased risk of myocardial ischaemic events and sudden cardiac death (Dishman et al. 2000; Treiber et al. 2001; McCraty et al. 2001; Ponikowski et al. 1997).

Increased sympathetic activity and renin-angiotensin-aldosterone system activation in both chronic heart failure and at times of acute physiological stress promote reactive oxygen species production, myocardial cellular damage and mitochondrial dysfunction (Hubens et al. 2013; Sam et al. 2005).

Aside from direct myocardial damage and promotion of inflammation, sympathetic activation promotes arrhythmogenesis both in the context of acute ischaemia and in chronic heart diseases and forms the basis for several therapeutic stratagies (Hou et al. 2016; Wengrowski et al. 2015).

Abrogation of excessive sympathetic drive, most commonly through beta adrenoreceptor blockade, has therefore taken a central role in the management of acute myocardial ischaemia (Welch et al. 2012; Roffi et al. 2015). A reduced heart rate allows coronary filling and reduces myocardial shear stress in a manner parallel to and complementary of preservation of vagal activity. Further beneficial effects include reduction of myocardial cellular damage, decreases in ROS release, preservation of mitochondrial function and mitigation of tachyarrhythmias.

A great deal of attention has been paid over the past decade to the issue of perioperative sympatholysis in an attempt to reduce cardiac risk through prevention and mitigation of cardiac ischaemia and through treatment of intraoperative tachycardia and hypertension which are associated with increased length of stay and complications after non-cardiac surgery (Reich et al. 2002).

The most recent series of randomised trials (POISE 1 & 2) demonstrated that whilst beta blockade and alpha 2 blockade do appear to reduce the risk of perioperative

myocardial ischaemic events and arrhythmia, these benefits occur at the expense of greater risk of stroke and hypotension (Devereaux, Sessler, et al., 2014; Fleisher et al., 2014; POISE Group, 2008; Sear et al., 2008). Haemodynamic instability due to sympatholysis may result in reduced cardiac contractility and rate with subsequent impaired organ perfusion and resultant triggering of pathophysiological cascades leading to multi-organ failure.

The potential therapeutic benefits of sympatholysis, therefore, need to be balanced against the potential harms. Preoperative identification of individuals who may come to harm due to exaggerated sympathetic outflow in the perioperative period could aid in the targeted application of sympatholysis.

1.6.3 Autonomic Dysfunction and Cardiac Failure

Autonomic dysfunction is strongly associated with and prognostically linked with cardiac failure. Reduced heart rate variability has been associated with NYHA grade of heart failure (Adamson et al. 2004; Wijbenga et al. 1998), likelihood of death (Jiang et al. 1997) and even mode of death (Sandercock & Brodie 2006).

Sympathetic activation is ubiquitous, likely compensatory for impaired ventricular function and linked with pathophysiological consequences (as outlined previously) (Floras 2009; Floras & Ponikowski 2015). Ultimately, chronic sympathetic nervous system activation is associated with accelerated disease progression, dysregulation of adrenoreceptor signalling and transduction and ventricular remodelling (Triposkiadis et al. 2009; Sigurdsson & Swedberg 1994).

As a result, beta adrenoreceptor blockade and modulators of the renin-angiotensin-aldosterone axis have become the mainstays of cardiac failure management (Floras 2009). Autonomic influences on cardiac function are summarised in Table 1-2.

1.6.4 The Parasympathetic Nervous System and Heart Failure

Traditionally, sympathetic nervous outflow has been held solely responsible for modulating positive inotropy (Hall & Guyton 2006). The influences of

parasympathetic activity on cardiac chronotropy are well described (G.G. et al. 1992), but an increasing body of laboratory evidence is accumulating to indicate that parasympathetic muscarinic activity is essential for increasing cardiac contractility at times of increased demand (Hussain et al. 2009; Kitazawa et al. 2009; Kakinuma et al. 2009; Wang et al. 2007). Indeed, reductions in vagal tone (Floras & Ponikowski 2015; Ferrari 1993) and specifically, impaired cardiac muscarinic activity (Vatner et al. 1988; Kakinuma et al. 2009; Z. Wang et al. 2004) are core features of cardiac failure and are likely mechanistically linked to the reductions in cardiac performance (Olshansky et al. 2008).

Branch	Process	Outcome
Sympathetic/ Parasympathetic	Local inflammation	Myocardial cellular injury Troponin release
	Reduced heart rate variability	Worsened Cardiac Failure
Parasympathetic	Reduced heart rate	Improved coronary perfusion
	Reduced vagal outflow	Increased severity coronary arterial disease Increased infarct size Increased oxidative stress/calcium overload Accelerated reperfusion injury Impaired ventricular remodelling
	Reduced connexin -43 expression	Increased risk of arrhythmia
	PI3/AKT/HIF-1a activation	Cell survival/reduced infarct size
	Improved glucose usage	
	Modulation of remote ischaemic conditioning	
	Muscarinic activity	Increased inotropy
Sympathetic	Accumulation of myocardial calcium	Myocardial ischaemia/injury
	Metabolic dysregulation	
	Activation of clotting cascade	
	Endothelial activation	
	Coronary artery vasospasm	
	Renin-angiotensin-aldosterone axis activation	Reactive oxygen species production Mitochondrial dysfunction Myocardial cellular damage Pathological ventricular remodelling
	Myocardial conducting system	Arrhythmia
	Chronic activation	Accelerated progression of cardiac failure
	Adrenoreceptor signalling	Dysregulated receptor expression and signalling

Table 1-2. Autonomic influences on and associations with cardiac function

Parasympathetic activation is reduced even in very early heart failure, and may provide prognostic information on patients at increased risk of developing progressive myocardial dysfunction (Kinugawa & Dibner-Dunlap 1995). Lack of attenuation of sympathetic activity through increases in vagal tone is also common in cardiac failure and is associated with baroreceptor dysfunction, as well as central changes in sympathetic/parasympathetic interaction.

Chronic vagal withdrawal, as described previously, is associated with inflammation which appears to play a pathogenic role in the progression of left ventricular dysfunction and remodelling in cardiac failure (Li & Olshansky 2011).

Several therapies targeted at improving parasympathetic activity have shown beneficial effects in established cardiac failure. These include Angiotensin Converting Enzyme (ACE) inhibitors, beta blockade, statins and vagal stimulation, (Zannad et al. 2015; Osterziel & Dietz 1996; Kubo et al. 2005; Pliquett et al. 2003). Impaired cardiac function in itself is a significant risk factor for the development of postoperative complications (Sabat et al., 2011). Indeed, impaired intraoperative oxygen delivery due to impaired cardiac output is the focus of many perioperative goal directed therapy stratagies (Aya et al. 2013). Despite this, there remains a cohort of patients who fail to respond to oxygen delivery driven (using fluids ± inotropes to achieve prescribed targets) goal directed therapy (Ackland et al. 2015). It could be hypothesised that vagal withdrawal may underlie these observations due to its negative influence on cardiac performance.

Given the high incidence of morbidity associated with clinical and subclinical autonomic dysfunction in the high risk surgical population (e.g. hypertension & diabetes) it would appear likely that a proportion of these patients will suffer from consequent cardiac impairment, presenting the possibility of a previously unidentified cohort who may be at greater perioperative risk, but in whom targeted therapy directed at autonomic dysfunction may be of benefit.

1.7 G-protein receptor kinases and autonomic function

1.7.1 G-protein receptor kinases play a key role in the pathophysiology of cardiac failure

G protein-coupled receptors (GPCRs) are diverse, therapeutically important and play multiple central roles in the physiology of various organs, including the heart. The beta adrenoreceptor and angiotensin II type-1 receptor are key and relevant examples.

Autonomic dysfunction, resulting in enhanced catecholamine release and RAAS activation, is strongly associated with increases in G-protein coupled receptor phosphorylation, mediated mainly through the G-protein receptor kinases GRK2 and GRK5, as well as increased Gi protein expression (Eschenhagen et al. 1992; Lymperopoulos et al. 2013; Lymperopoulos et al. 2007; Lymperopoulos 2011; Lymperopoulos et al. 2007). These changes have functional implications including downregulation and functional uncoupling of the beta adrenoreceptor as well as other direct pathophysiological consequences in the heart and other organ systems. GRK2 elevation is canonical in human heart failure.

Agonist binding of a GPCR promotes GRK phosphorylation. This phosphorylation enhances the affinity of the receptor for binding with adaptor proteins (B-arrestins), which sterically prevent further GPCR agonist binding and signalling. B-arrestin binding further promotes internalisation of the activated GPCR for either recycling (resensitisation) or degradation (downregulation); these processes are central in health to adaptive regulation of sympathetic stimulation, but in cardiac failure are key to disease progression. The predominant consequence of chronic GRK2 activation is a reduction in numbers of expressed beta adrenoreceptors with resultant reductions in beta adrenoreceptor inotropic reserve (Woodall et al. 2014) (summarised in Figure 1-2).

Impaired cardiac responses to beta adrenoreceptor stimulation are an important negative prognostic factor for survival in critical illness, where autonomic dysfunction is prototypical (Rhodes et al., 1999). Similarly, reduced inotropy in response to

catecholamine stimulation is ubiquitous in established cardiac failure, and is associated with reductions in both the numbers of beta adrenoreceptors expressed on the failing myocardium and the maximal inotropic response possible (Lymperopoulos et al. 2013). In sepsis-related myocardial dysfunction, as in cardiac failure, myocardial beta adrenoreceptor density and consequently signal transduction is reduced (de Montmollin et al. 2009). Reductions in beta adrenoreceptor density in these conditions are modulated in large part by GRK2 (Evron et al. 2012; Lymperopoulos 2011; Iaccarino et al. 2005).

In addition to roles in GPCR recycling and destruction, GRK2 and consequently beta-arrestins are strongly implicated in mitogenic signalling and RAAS activation in the failing heart, promoting cardiac hypertrophy, cardiomyocyte apoptosis and impaired contractility (independent of its GPCR desensitising activity)(Xiang & Kobilka 2003). Further non-classical roles for GRK2 in heart failure include negative regulation of insulin signalling, altered cardiomyocyte metabolism, promotion of reactive oxygen species production through mitochondrial co-location and accelerated atherogenesis (Kim et al. 2008; Woodall et al. 2014).

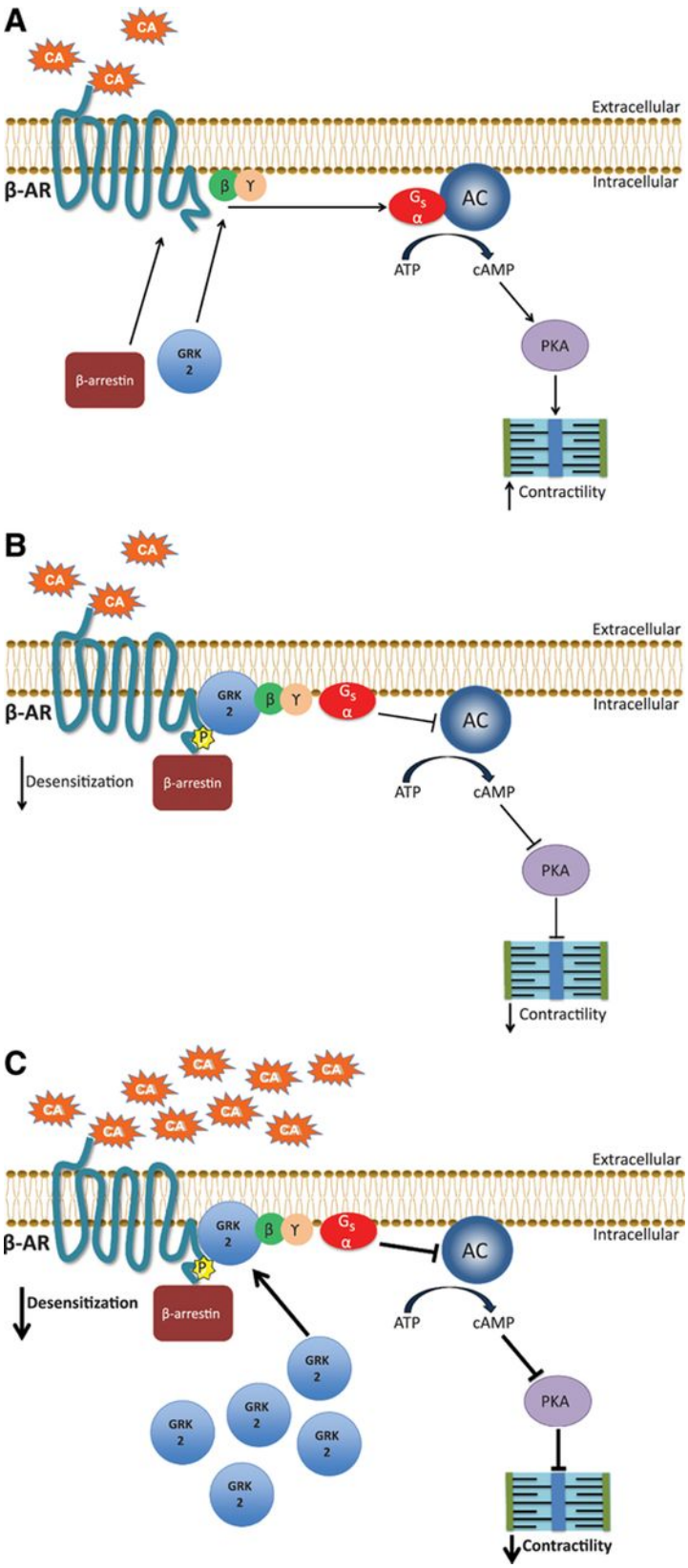

Figure 1-2: The classical actions of G protein–coupled receptor kinase 2 (GRK2) on cardiac β-adrenergic receptors (βARs) during physiological conditions and disease states

A. Under normal conditions, catecholamines (CA) activate the βAR, stimulating downstream signalling through Gαs that ultimately leads to increased contractility by activating adenylate cyclase (AC) and protein kinase A (PKA).

B. As receptor activation occurs, signal desensitisation is triggered simultaneously by translocation of GRK2 to the membrane and binding to Gβγ where it is able to interact with the agonist-occupied receptor and phosphorylate it, starting the G protein uncoupling process. G protein activation is blocked by recruitment of β-arrestin to the phosphorylated receptor. β-arrestins also start the receptor internalization and downregulation process.

C. After cardiac injury, GRK2 levels are increased due to stress-induced increases in catecholamines. This increase in GRK2 helps to prevent overstimulation of the βAR initially, but over time a cycle of chronically increased βAR desensitization occurs contributing to the progression of heart failure.

Reproduced with permission from (Woodall et al. 2014)

1.7.2 The extra-cardiac GRK2 interactome is central to multiple pathophysiological processes

Outside of the heart, GRK2 and beta arrestins are implicated in multiple pathophysiological processes. These include impaired immune cell chemotaxis and function (Liu et al. 2013; Lafarga et al. 2012), increased reactive oxygen species production (Ciccarelli et al. 2013) and altered innate immune responses (Loniewski et al. 2008). As such, the GRK2 interactome is recognised as a key factor in the regulation of neuroendocrine-immune communication (Vroon et al. 2006; Heijnen 2007).

GRK2 has been implicated in the regulation of both severity and duration of acute inflammatory pain (N Eijkelkamp et al. 2010; Niels Eijkelkamp et al. 2010; Ferrari et al. 2012) as well as in the regulation of brain injury after ischaemia (Nijboer et al. 2013). Chronic GRK2 elevation in the adrenals secondary to prolonged BAR stimulation results in exaggerated catecholamine secretion, contributing to a positive feedback loop in the pathogenesis of heart failure (Lymperopoulos et al. 2007). Relevant physiological roles of GRK2 are summarised in Table 1-3.

1.7.3 Lymphocyte GRK2 as a biomarker in parasympathetic autonomic dysfunction

GRK2 is a useful biomarker in cardiac failure (Lymperopoulos 2011; Woodall et al. 2014; Liggett 2005a). Circulating lymphocyte GRK2 levels reflect those expressed in the cardiomyocyte (both cardiomyocytes and lymphocytes are subjected to a similar neurohormonal milieu), and are related to ejection fraction, NYHA class and prognosis (Liggett 2005a).

Vagal withdrawal is strongly associated with and likely mechanistically linked to human cardiac failure. Neurohormonal activation, secondary to cardiac impairment and associated with vagal withdrawal (including elevations in both catecholamine release and activation of the renin-angiotensin-aldosterone axis (RAAS) (Anker 1998)), would be expected to be associated with alterations in the dynamics of beta adrenoreceptor recycling mediated through GRK2 and its downstream effects.

System:	Process:
Cardiovascular	Reduced contractility Mitogenic signalling Cardiac hypertrophy Cardiomyocyte apoptosis Impaired apoptosis Negative regulation of cardiomyocyte metabolism Negative regulation of insulin signalling Promotion of Reactive Oxygen Species release Accelerated atherogenesis **Biomarker in cardiac failure:** related to degree of failure & prognosis
Immune	Increased ROS expression Impaired innate immunity Altered immune cell chemotaxis Altered expression of beta adreno and other receptors on lymphocyte cell surfaces
Central Nervous	Inflammatory pain modulation. Increased ischaemic pain Regulation of ischaemic brain injury
Neurohormonal	RAAS activation Modulation of neuroendocrine-immune communication
Second Messenger	Beta adrenoreceptor downregulation Adrenal promotion of catecholamine secretion

Table 1-3: Physiological roles of GRK2 relevant to the high-risk surgical patient

It is therefore highly likely that lymphocytes derived from individuals with established parasympathetic autonomic dysfunction will exhibit alterations in GRK2 expression. It is also possible that the total expression of GRK2 in these lymphocytes will reflect the degree of PAD in the individuals from whom they are taken. Elevations in GRK2 levels would further implicate PAD mechanistically in the development of cardiac impairment as well as neuro-immune dysfunction. Lymphocyte GRK2 levels will therefore be explored as a potential biomarker for PAD in this thesis.

1.8 Parasympathetic Autonomic Dysfunction and Postoperative Gastrointestinal Dysfunction

The classical role for parasympathetic nervous activation resides in the regulation of gastrointestinal function as part of the 'rest and digest' paradigm (Hall & Guyton 2006). Parasympathetic withdrawal then, would be predicted to be associated with impairment of gastrointestinal function.

Postoperative gastrointestinal dysfunction (PGID) is a common complication after all types of moderate to major surgery, not being limited to surgery on the gut or abdominal viscera. PGID is associated not only with patient suffering and other morbidity, but also with increased hospital length of stay (Mythen 2009). PGID is the most common postoperative morbid event in major non-cardiac surgery to be associated with a length of stay prolonged beyond seven days (Bennett-Guerrero et al. 1999).

The pathogenesis of PGID is complex and multifactorial, though the disruption of neural reflexes that modulate coordinated bowel motility and/or inflammation of the intestinal muscularis is thought to be central (Tache et al. 1993; Mythen et al. 1993).

Direct bowel manipulation influences local and systemic autonomic signalling, both through direct myenteric neural control (causing corticotrophin releasing factor release in the dorsal vagal complex with resultant non-cholinergic motor neuron activation through vagal and splanchnic routes) and through the influences of local and systemic inflammation (De Winter et al. 1997; Tache et al. 1993).

Similarly, autonomic signalling is altered in non-gastrointestinal surgery, predominantly through the influences of inflammation on sympathetic activation and parasympathetic withdrawal. Impaired gut mucosal perfusion, acting through hypoxia-induced local 5-HT3 activation of caudal vagal afferents and local inflammation, is also strongly implicated in the development of PGID, and is clearly related to impaired macrocirculatory parameters common in reduced cardiac output states, such as cardiac failure (Mythen 2009; Mythen et al. 1993).

Aside from interventions such as adequate filling, reduced bowel manipulation and balanced analgesia regimens, that in themselves are likely to have some beneficial autonomic influence, direct augmentation of vagal outflow has been successfully investigated both as prophylaxis for and as a potential therapy in PGID (The et al. 2011; Lubbers et al. 2010; Karmali et al. 2015). These interventions include sham feeding, early enteral feeding and direct vagus nerve stimulation. Improved or preserved heart rate variability, as a marker of parasympathetic tone, is associated with reduced time to becoming morbidity free (Karmali et al. 2015).

The role of established parasympathetic autonomic dysfunction in the promotion of PGID is not known, though it could be predicted to represent a greater risk for its subsequent development in the postoperative period.

1.9 The influence of Autonomic Dysfunction on the physiological response to anaesthesia

To date, many studies examining the influence of autonomic dysfunction in the perioperative period have predominantly focused on the cardiovascular consequences of the class of gross dysfunction noted in cardiovascular autonomic neuropathy (CAN) secondary to diabetes mellitus (Vohra et al. 1993; Knuttgen et al. 1990; Knüttgen et al. 1990; Lankhorst et al. 2014). CAN may however be caused by multiple disorders, including paraneoplastic syndromes and chemotherapy or radiotherapy (Lankhorst et al. 2014). These latter conditions are of clear relevance to the surgical oncology population and such patients form the majority of the population to be explored in this thesis.

Studies exploring the impact of CAN on patients undergoing surgery have demonstrated increased risks of perioperative cardiac complications, but have not examined both the potential consequences of subclinical autonomic dysfunction or predominantly parasympathetic autonomic dysfunction and its likely impact on multi-system pathology (including immune/inflammatory dysfunction and PGID). It is however highly likely that individuals exhibiting cardiovascular autonomic dysfunction will also display central and systemic signs of multi-system dysfunction (De Angelis et al. 2009).

Similarly, systematic exploration of the impact of autonomic dysfunction on cardiac function and cardiopulmonary physiology in the perioperative period has not previously been performed. Studies to date looking at perioperative AD have been further hampered by the use of heterogeneous techniques used to assess cardiovascular autonomic function in the perioperative period which has lead to great variability in consequent reported morbidity and mortality (Lankhorst et al. 2014).

The presence of cardiovascular autonomic neuropathy (caused by damage to the autonomic nerve fibres that innervate the heart and blood vessels, causing abnormalities in heart rate control and vascular dynamics; Lankhorst et al. 2014) is associated with both perioperative haemodynamic instability and postoperative complications.

Anaesthesia, both general and regional, has major influences on perioperative autonomic function. These changes may be particularly pronounced in diabetic patients, who are more prone to the development of intraoperative bradycardia and hypotension during anaesthesia; in particular during induction (Knuttgen et al. 1990; Vinik & Ziegler 2007; Burgos et al. 1989). Intraoperative haemodynamic instability in CAN appears related to the severity of autonomic dysfunction (Knüttgen et al. 1990), and the degree of derangement on preoperative HRV analysis is related to the greatest drop in blood pressure during surgery. Pharmacological intraoperative blood pressure support is more likely to be employed more frequently in patients with autonomic dysfunction (Burgos et al. 1989).

Reduced preoperative heart rate variability predicts intraoperative hypotension, even when age, history of diabetes and ASA class are controlled for (Huang et al. 2006).

Similarly, perioperative risks are increased: the risk of intraoperative hypothermia, itself associated with significant morbidity, is raised in CAN, suggesting autonomic involvement beyond the cardiovascular system (Kitamura et al. 2000).

Increased risk for perioperative myocardial infarction, myocardial ischaemia and heart failure and arrhythmia have all been reported associated with autonomic dysfunction (Laitio et al. 2004; Vinik & Ziegler 2007). Importantly, the incidence of cardiac arrest at induction and in the perioperative period is much increased in patients with CAN (Vinik & Ziegler 2007; Burgos et al. 1989; Lankhorst et al. 2014). Aggregate perioperative cardiovascular morbidity and mortality may be increased up to three times in these individuals (Kitamura et al. 2000; Vinik & Ziegler 2007; Lankhorst et al. 2014). Parasympathetic withdrawal in particular, as assessed by preoperative frequency domain analysis, appears highly predictive of one year mortality after major non-cardiac surgery (Filipovic et al. 2003).

In the postoperative period, reduced heart rate variability, increased age and diabetes mellitus are all independent predictors of prolonged hospital length of stay (Gang & Malik 2002).

1.9.1 Commonly used drugs in anaesthesia may exacerbate existing autonomic dysfunction

Anaesthetic agents and drugs used commonly in the perioperative period have differing and profound effects on autonomic activity, which could potentially influence outcome in patients with established autonomic dysfunction. The choice of anaesthetic technique therefore may have some bearing on perioperative outcome.

During anaesthesia, both vagal and sympathetic activity may be suppressed, and the pattern is dependent on the agents used and the depth of anaesthesia (Mazzeo et al. 2011). It would appear that patients with pre-existing autonomic dysfunction may be at greatest risk (Mazzeo et al. 2011; Lankhorst et al. 2014). For illustration, the autonomic effects of several (but not all) commonly used drugs in the perioperative period are outlined subsequently.

1.9.1.1 Inhalational anaesthetic agents

In general, heart rate increases, and is associated with a decrease in heart rate variability, during inhalational anaesthesia. Corresponding decreases in time domain measures such as SDNN, High Frequency and Low Frequency power at have also

been demonstrated. With increasing doses of inhalational anaesthetic agent (different agents affecting activity to different extents), cardiac vagal activity, reflected in reduced high frequency power, decreases (Picker et al. 2001).

1.9.1.2 Intravenous anaesthesia

Propofol (a commonly used intravenous anaesthetic agent) administration is associated with hypotension, suppresses both baroreceptor sensitivity and peripheral sympathetic nervous activity (Ebert & Muzi 1994). These changes are accompanied by reductions in high frequency power on heart rate variability measurement, reflecting reductions in cardiac parasympathetic activity (Deutschman et al. 1994).

Propofol administration is associated with blocks in cardiac conduction (Liu et al. 2011). Whether this is due to autonomic nervous system influence is controversial. One study indicated that propofol administration does not affect cardiac conduction when autonomic nervous activity is completely blocked (Ikeno et al. 1999).

1.9.1.3 Anticholinergic agents

Administration of the commonly used muscarinic antagonists glycopyrrolate and Atropine result in reductions in both baroreceptor sensitivity and heart rate variability (with specific decreases in the high frequency or vagal component on frequency domain analysis) for several hours after administration (J. L. Parlow et al. 1997; J. Parlow et al. 1997). These reductions persist beyond any measurable influence on heart rate or blood pressure (van Vlymen & Parlow 1997).

At low dose, however, atropine administration results in an increase in central vagal tone and abrogation of peripheral, efferent, sympathetic nerve activity (Montano et al. 1998; Yuasa et al. 2000). It is at higher doses, that the muscarinic effect at the sinoatrial node predominates.

1.9.1.4 Opiates

In general, opiate medications reduce low frequency power (reflecting reduced sympathetic cardiac activity). Remifentanil in particular is associated with increases

in both heart rate variability and high frequency power, not blocked by atropine administration (Tirel et al. 2005).

1.9.1.5 Neuraxial blockade

Limited studies have been carried out directly examining the influence of regional anaesthesia on autonomic function, though neuraxial blockade inhibits efferent sympathetic outflow. Thoracic blockade has greater effect on cardiovascular function than lumbar possibly due to involvement of cardiac acceleratory fibres, as well increased vasodilatation of splanchnic vessels resulting in venous pooling. In labouring parturients, epidural block to T6 increased parasympathetic outflow, and decreased sympathetic power on HRV analysis. Epidural analgesia is also associated with improved postoperative recovery of heart rate parameters (Licker et al. 2003). Under spinal anaesthesia, preoperative increases in LF/HF ratio, thought to represent established sympathetic dominance, are predictive of hypotension (Hanss et al. 2006).

1.9.2 Autonomic dysfunction is likely to result in impaired outcomes both during and after anaesthesia

A clear signal appears to exist then relating cardiac autonomic neuropathy and impaired perioperative haemodynamic and cardiac outcome. Perioperative cardiac risk is increased, as is the risk of haemodynamic instability, morbidity and mortality in the postoperative period.

However, no attention has yet been paid to perioperative infectious/immune outcomes, integrated cardiovascular physiology or morbidity specific to either sympathetic or parasympathetic autonomic dysfunction. Furthermore, a large cohort of patients who do not necessarily exhibit overt cardiac autonomic neuropathy, but are at risk of covert autonomic dysfunction associated with common conditions such as hypertension, ischaemic heart disease and undiagnosed cardiac failure, may have been missed in studies conducted to date. This is despite plausible mechanistic processes that should link autonomic dysfunction to impaired postoperative outcome. Similarly, global outcomes have not been examined in high-risk patients with existing autonomic dysfunction presenting for surgery. This thesis

therefore explores these outcomes in this important population. How to define dysfunction in either limb of the ANS during the preoperative period is therefore an important question, which is addressed subsequently.

1.10 Techniques for assessing autonomic function

Various techniques have been used to detect alterations in autonomic function in a wide range of diseases and contexts. The wide variety of techniques employed and variable cut-offs used to define AD have hampered comparison of outcomes between studies (Colombo et al. 2015). In anaesthesia and critical care, commonly used modalities have chiefly focused on the autonomic modulation of heart rate (heart rate variability) and the arterial baroreflex (Mazzeo et al. 2011; McGrane et al. 2014). Interpretation of studies looking at autonomic function has been hampered by the lack of population norms, variable acquisition and analysis techniques, and in many cases, lack of long term follow up (Keet et al. 2011; Keet et al. 2014).

There are a large number of tests of autonomic function available. These tests variably describe different aspects of autonomic activity, covering parasympathetic/sympathetic, afferent/efferent and integrated reflex activity. The majority of tests focus either on end organ 'read-outs' of autonomic activity (e.g. sudomotor skin tests) or integrated reflexes (such as blood pressure responses to head-up tilt). The individual clinical or research question or paradigm determines the most appropriate approach to test selection for contextual assessment of autonomic function (Zygmunt & Stanczyk 2010).

Many tests are affected by the state of hydration of the patient, or the medications that individual may be taking. Several tests require a degree of control of environmental or patient factors (such as the need for paced breathing in accurate assessment of heart rate variability).

The majority of tests use end organ function and responsiveness (e.g. heart rate dynamics) as a read out, rather than measuring central autonomic neural activity directly (e.g. using neuroimaging techniques such as fMRI), due to the technical

complexities of so doing in the intact individual (Zygmunt & Stanczyk 2010). These difficulties include physiological noise artefacts affecting brainstem neuroimaging data, as well as difficulty in deriving non-invasive continuous assessments of autonomic modulation correlated with peripheral indicators of physiological response (Macey et al. 2016; Napadow et al. 2008).

These challenges may lead to problems with interpretation, but in context, where clinical outcome correlates exist, end-organ readouts can supply large amounts of useful information (Zygmunt & Stanczyk 2010). Over the past five years, over 1000 publications have been produced where autonomic function has been assessed using cardiovascular testing and associated with altered outcomes in a variety of clinical conditions (Lankhorst et al. 2014).

Broadly, autonomic function tests may be divided into the following categories:

1. **Cardiovagal** innervation (parasympathetic): Heart rate and heart rate variability dynamic responses to deep breathing, the Valsalva manoeuvre and postural change. Heart rate recovery after exercise.
2. **Adrenergic** (sympathetic): Blood pressure responses to the Valsalva manoeuvre, sustained hand grip and postural change.
3. **Mixed** (parasympathetic and sympathetic): Heart Rate Variability, static and dynamic heart rate changes (such as resting heart rate and chronotropic incompetence) in response to stressors.
4. **Sudomotor**: quantitative sudomotor axon reflex test (QSART), thermoregulatory sweat test (TST), sympathetic skin response (SSR) and silastic sweat imprint. These tests are in general used in the context of peripheral autonomic neuropathies and will not be discussed further.

1.10.1 Selection of an appropriate autonomic testing modality for use in the high-risk surgical patient

Various techniques have been employed in the assessment of autonomic activity in surgical populations (Mustafa et al. 2012). For the purposes of this thesis a testing modality was required that would measure the effects of either parasympathetic *or* sympathetic autonomic dysfunction on perioperative outcome.

Such a test should be well established and validated in the general medical literature, both in terms of specific patient outcomes and in terms of physiological reliability. It should be specific and reproducible and ideally be easily carried out in the preoperative period without extra inconvenience as part of the preoperative work-up of the high-risk surgical patient. The test selected should have been well explored in the contexts of cardiac risk and cardiac insufficiency in the wider population, and be well related to other established measures of cardiac function. Further considerations, for wider applicability in future studies and clinical practice, should ideally minimise the need for complex or expensive equipment and avoid over-reliance on proprietary software or mathematical algorithms.

Several candidate tests, each with their potential limitations, which have previously been applied in the preoperative period and/or in the high-risk cardiac population, are outlined below:

1.10.2 Heart Rate Dynamic Changes (deep breathing, Valsalva, postural change, sustained hand grip)

Heart rate responses during controlled deep breathing, the Valsalva manoeuvre and during changes in posture are commonly used to assess autonomic integrity, in particular parasympathetic function. There are only a limited number of reports detailing reference values for cardiovascular autonomic function in healthy subjects. (Keet et al. 2011; Keet et al. 2014; Voss et al. 2012; Ewing & Clarke 1982).

In controlled deep breathing, the maximum and minimum ECG R-R intervals during each breathing cycle are measured. This consists of 6 cycles of 5 seconds of inspiration and 5 seconds of expiration during a minute. The R-R intervals during inspiration and expiration are subsequently determined and expressed as a ratio.

A similar technique may be employed using a Valsalva manoeuvre, where a subject is asked to produce an intrathoracic pressure of 40 mmHg for 15 seconds by blowing into a manometer. The Valsalva ratio is defined as the ratio between the longest and the shortest R-R intervals immediately post test.

R-R interval changes during postural change have also been used to assess autonomic cardiovascular integrity. After standing up quickly from the sitting position, the R-R intervals at 15 and 30 beats are measured. In healthy subjects, the ratio of the longest and shortest R-R intervals should be >1.04 (Keet et al. 2014; Keet et al. 2011; Ewing & Clarke 1982; Lankhorst et al. 2014).

Whilst these tests are useful for the clinical assessment of cardiovascular autonomic neuropathy, they lack precision in terms of identifying specific impairments in parasympathetic or sympathetic function.

1.10.3 Blood Pressure Responses

Blood pressure responses during sustained handgrip have also been use to assess cardiovascular autonomic function, in particular sympathetic nervous activity. One method employs a sustained contraction (at 30% of maximum contraction for 5 minutes). The diastolic blood pressure should rise by >16 mmHg in the opposite arm in healthy subjects.

In the Schellong (quick-standing) test, systolic blood pressure is first measured in the supine resting state. Two minutes after rapidly standing up, the systolic blood pressure is re-measured. In healthy individuals, a drop in blood pressure of <10 mmHg is expected. In individuals who exhibit a drop of more than 20 mmHg in systolic blood pressure after standing, impaired autonomic cardiovascular function is indicated (Lankhorst et al. 2014).

These tests are specific for sympathetic autonomic dysfunction, but are not very sensitive. Ideally, the modality of testing used in the perioperative context would allow assessment of both parasympathetic and sympathetic autonomic testing in one sitting.

1.10.4 Heart Rate Variability Analysis

Heart Rate Variability (HRV) is commonly used in the assessment of cardiac autonomic dysfunction, but poses many challenges in interpretation. In the general

population, a low HRV is associated with both morbidity and mortality from many causes, not all cardiac (Billman 2011).

HRV refers to physiological variability in the timing between individual heartbeats (NN periods/RR intervals) and reflects oscillations between individual heartbeats. It is measured over short term (classically 5 minutes) or longer (up to 24 hours) time periods. Prior to the identification of beat-to-beat heart rate variability, the ability to estimate cardiac autonomic function was limited (Monfredi et al. 2014). Two modalities of assessment are commonly used: frequency domain and time domain analysis. Standard values exist and are widely accepted (Malik et al. 1996).

Multiple papers (>17,000) encompassing a variety of methods for HRV analysis have been published indicating impaired outcomes associated with reduced variability in a wide array of pathology (Billman 2011; Monfredi et al. 2014), including in surgical patients (Mazzeo et al. 2011). Measurement and interpretation of heart rate variability measures remain the basis of a significant volume of translational science publications to date (Monfredi et al. 2014).

Some controversy remains over the exact interpretation of HRV measures, especially in relation to the precise limb of the ANS affecting change in variability parameters, and even whether or not HRV is a pure marker of cardiac autonomic activity (Monfredi et al. 2014).

1.10.4.1 Time Domain Measures

Time domain measures of heart rate variability reflect sinus node depolarisation or variations in the intervals between normal QRS complexes. Various measures are in common use:

- SDNN - standard deviation of normal RR intervals
- SDANN - standard deviation averaged over 5 minutes
- RRMSD - square root of mean square differences of successive RR intervals
- NN50 - the number of interval differences of >50ms

Time domain measures are most frequently employed over shorter recordings (five minutes being the standard recording length). Whilst alterations in variability of a variety of time domain measures has been implicated in poor prognosis and increased mortality across a wide range of disease states, the exact contributions of the parasympathetic and the sympathetic divisions of the autonomic nervous system to this variability are controversial and remain the subject of active investigation and debate (Billman 2011).

Over time it has become apparent that variability in heart rate is not the product simply of a regular periodic oscillator (i.e. a sine wave), but actually exhibits complex, non-linear behaviour (Guevara et al. 1981). This limits the applicability of simple statistical approaches to HRV to analyse non-linear changes. Approaches employing fractal mathematics have been attempted with some success to overcome these limitations. These approaches measure system complexity rather than HRV magnitude. In several contexts, these approaches have proved superior to traditional methods in predicting outcome in the clinical context (Billman 2011; Pikkujämsä et al. 1999). They are, however, mathematically complex and not immediately accessible or available to all researchers. Pragmatic application of standard time domain measures therefore remains the default usage in clinical and research practice.

1.10.4.2 Frequency Domain Measures

Frequency domain measures are calculated either through the application of a fast Fourier transform to an NN series or by autoregressive spectral estimation (the maximum entropy method). High frequency variations in sinus rhythm NN intervals (0.12 to 0.4 Hz) are thought to predominantly reflect vagal activity, whereas slower variations are thought to reflect a combination of both sympathetic and parasympathetic modulation, as well as non-autonomic factors. The very low frequency band (0.00 to 0.04 Hz) is thought to most clearly represent sympathetic function and fluctuations in vasomotor tone. Ascribing a single limb of the autonomic nervous system to any band of frequency domain analysis remains difficult for similar reasons to that used in time domain analysis.

The low frequency band (0.04-0.15 Hz) includes physiological oscillations associated with the baroreflex, whereas the high frequency band (0.15 to 0.40 Hz) encompasses respiratory sinus arrhythmia. Interpretation of frequency domain powers must therefore be carried out with some caution as either arm of the autonomic nervous system may cause low frequency band power changes. Despite widespread and popular usage, frequency domain descriptions of sympatho-vagal balance are increasingly thought not to accurately describe actual autonomic activity in patients (Billman 2013; Milicević 2005; Antelmi et al. 2008; Reyes del Paso et al. 2013).

Shorter recordings (most commonly over five minute periods) can and have been used and been thought to be adequate, in both research and clinical contexts, with both time and frequency domain analysis. Very low frequency analysis is however thought to be inaccurate over shorter time periods (Malik et al. 1996).

The application of five-minute recordings to other bands remains a topic of discussion in research and clinical practice. The optimum sampling period for frequency domain analysis therefore remains elusive. It is highly unlikely that an individual will remain in steady state over a 24-hour period, casting doubt on the applicability of these longer-term recordings. Indeed, the real-time capture of changes in autonomic parameters under altering conditions could be considered the holy grail of autonomic research. Proprietary software exists that allows shorter-term frequency domain analysis (e.g. over five minute periods), but the utility of such data remains a moot point (Nunanet al. 2010; Keet et al. 2011).

In one analysis of over 3100 papers dealing with all measures of heart rate variability, only 44 papers were found to be in agreement with Task Force recommendations for signal quality and normal values for healthy individuals. Heart rate variability in these studies showed wide variability between healthy controls (Nunan et al. 2010).

1.10.5 . Heart Rate Variability as a measure of cardiac autonomic system activity

A variety of confounders exist in the interpretation of heart rate variability, especially in the clinical context. Respiratory changes can alter HRV independent of autonomic regulation; this creates the requirement for controlled or paced breathing for accurate interpretation. It is not always possible to fulfil this requirement in the clinical context, where many recordings are analysed from Holter monitor tracings, with the subject going about their daily activities (Brown et al. 1993).

HRV only provides an indirect readout of cardiac sympathetic and parasympathetic function in the absence of measures of central neurological activity (e.g. fMRI). This is important, since a portion of heart rate variability occurs as a consequence of mechanical events, such as atrial stretch (e.g. the Bainbridge reflex). In transplanted hearts, for example, a small (2-8%) change in HRV is detectable in concert with the respiratory cycle (Bernardi et al. 1989).

Recent evidence derived from intact humans and animals (Langendorff-perfused hearts and single sinoatrial nodal cell preparations) further demonstrate that heart rate variability is not predominantly related to cardiac autonomic activity, but rather to primarily to underlying heart rate (i.e. there is a universal exponential decay-like relationship between HR and HRV, so as heart rate rises, R-R interval drops and vice-versa) (Monfredi et al. 2014).

Conventionally, heart rate variability is calculated from differences between a sequence of R-R intervals rather than the underlying heart rate. When HRV is calculated from HR, it depends less on the underlying heart rate for accuracy (Stauss 2014). This key observation suggests that observed differences in morbidity and mortality due to alterations in HRV could just reflect the underlying heart rate; an increased heart rate is associated with increased mortality. Certainly, any study using heart rate variability as a measure of cardiac autonomic activity should control for underlying mean heart rate during the recording period before interpretation.

Further complexity is added when a biophysical model of sinoatrial node depolarisation is considered. This model demonstrates that the HR: RR interval relationship is not the only determinant of HRV, rather that changes in autonomic outflow at the sinoatrial node on heart rate depend on underlying heart rate.

For a given perturbing ion current (generated by the autonomic nervous system) acting on phase 4 (diastolic depolarization) of the sinoatrial action potential, the resulting change in HR depends on the prevailing slope of the diastolic depolarization in a nonlinear manner (Stauss 2014; O Monfredi et al. 2014). At lower heart rates, a change in perturbing current (which changes the slope of diastolic depolarization by a given amount) causes a greater effect on cardiac cycle length and heart rate than the same perturbation would at higher heart rates. This phenomenon is independent of the inverse relationship between R-R interval and heart rate (Monfredi et al. 2014).

Whilst, therefore, heart rate variability analysis is widely used as a measurement of cardiac autonomic control, and likely in many circumstances remains reflective of autonomic activity (Stauss 2014), many potential confounders exist that limit its use in the preoperative assessment of predominant parasympathetic or sympathetic autonomic dysfunction. Monfredi et al. state that HRV “cannot be used in any simple way to assess autonomic nerve activity to the heart” (Monfredi et al. 2014). Other testing modalities that are less influenced by other physiological parameters than autonomic activity may therefore be more accessible and applicable for more precise identification of dysfunction of either limb of the autonomic nervous system prior to major surgery. Candidate modalities include dynamic heart rate responses elicited largely due to alterations in autonomic activity.

1.10.6 Resting Heart Rate

Resting heart rate is directly linked to survival. This relationship is preserved across species, with life expectancy being close to 1,000,000 heart beats (Lauer 2009). In humans, large volumes of epidemiological data links resting heart rate to mortality (Wilhelmsen et al. 1986; Levine 1997; Eijgelsheim et al. 2010). Increases in resting

heart rate in ischaemic heart disease are associated with morbidity and mortality, whilst decreases (e.g. with beta-blockade and ivabradine) are associated with improved survival. Improved outcome is linked to the magnitude in reduction of heart rate in response to the intervention (Reil & Böhm 2007; Swedberg et al. 2010). It is not known whether resting heart rate directly impacts mortality or rather reflects other underlying subclinical pathophysiological processes.

Resting heart rate is closely related to parasympathetic tone, as well as aerobic capacity (both modifiable; Kenney 1985). Up to 32% of resting heart rate variation in the general population might be explained by heredity (Wilk et al. 2002), with twin studies suggesting an even greater influence (>50%; Dalageorgou et al. 2008).

Recent genome-wide association studies (GWAS) have identified common variation at or near *MYH6*, CD34 & *GJA1* (myosin heavy chain, a protein involved in bone marrow stem cell differentiation & connexin 43) associated with heart rate control. These chromosomal loci may represent novel risk factors for cardiovascular disease outcomes (Cho et al. 2009; Holm et al. 2010).

In one genome wide study, several genes associated with resting heart rate are associated with cardiac ion channels and their regulatory proteins. One locus encodes a thyroid receptor-interacting protein, and another (*AChE*) encodes acetylcholinesterase, which provides a potential link between parasympathetic function and resting heart rate at a genome level (Eijgelsheim et al. 2010). Other genetic determinants have been associated with resting heart rate (also linked to risk of cardiovascular disease) which are related to other factors including basal metabolism, which is also under strong autonomic influence (Mezzavilla et al. 2014). The differentiation between epigenetic and genetic phenomenon affecting genetic studies (such as the influences of environmental factors such as training or anxiety) add noise to the phenotype, limiting utility in population risk prediction.

In the perioperative population, a raised resting heart rate is strongly associated with perioperative adverse cardiac events (Abbott et al. 2016; Foëx & Higham 2016). Unfortunately, as a clinical measure of parasympathetic tone, resting heart rate

reflects environmental influences such as stress, dehydration and metabolism, limiting usage to large population studies not easily extrapolated to an individual at any particular point in time. Raised resting heart rate may itself be causative of adverse outcomes, and/or be a marker of an underlying disease such as heart failure.

1.11 Heart rate recovery after exercise: a measure of parasympathetic autonomic function

In light of the previously discussed potential limitations inherent in other common measuring modalities used to measure autonomic function, I considered heart rate recovery after exercise as a candidate test for the purposes of assessment of parasympathetic activity in the surgical patient. This test was considered since it is very clearly representative of parasympathetic activity, is non-invasive, can be carried out as part of the normal work up of the high-risk surgical patient and has, in comparable populations, been associated with impaired short and long term morbidity and mortality.

Heart rate recovery after exercise, which depends on the reactivation of parasympathetic tone (Imai et al. 1994), is a robust physiologic marker of established parasympathetic autonomic dysfunction (Lauer 2009). Abnormal heart rate recovery is independently associated with long-term cardiovascular morbidity and all-cause mortality in the general medical population – independent of pathology conventionally associated with autonomic dysfunction (Jouven et al. 2005). Key studies examining the relationship between abnormal heart rate recovery and outcome, including all cause mortality are summarised in Table 1-4. Methods of determining abnormal heart rate recovery in these populations are also outlined in the same table.

Heart rate variability measures require Holter ECG monitoring as well as dedicated interpretative software, whereas measurement of heart rate recovery is more straightforward to perform.

1.11.1 Abnormal heart rate recovery is due to impaired parasympathetic autonomic function

During ramped exercise (where the external work performed is increased in a graded manner), heart rate increases in parallel with exertion, due initially to parasympathetic withdrawal at onset of exercise, followed by elevations in sympathetic tone as well as parasympathetic withdrawal during more vigorous activity. In the first 30 to 120 seconds after cessation of exercise, heart rate drops rapidly due initially mostly to reinstitution of parasympathetic tone, followed by subsequent sympathetic deactivation (Lauer 2009; Pierpont et al. 2000; Figure 1-3).

In healthy individuals and athletes, a biexponential drop in heart rate is observed at cessation of activity. This comprises a log-linear decrease in heart rate over the first 30 to 120 seconds that is not dependent on the intensity of exercise, followed by a slower latter recovery that is thought to be work load dependent (Lauer 2009; Buchheit et al. 2007). This biexponential decline is abolished when atropine is administered (atropine as a muscarinic antagonist blocks vagal activity), and a more linear decline in heart rate is observed. This pattern closely matches the expected heart rate recovery profile of individuals with heart failure (Imai et al. 1994; Savin et al. 1982).

Heart rate recovery after exercise may be quantified by: (1) measuring the absolute difference between heart rate at the cessation of exercise and after 60 to 120 seconds of recovery, (2) measuring the time constant of heart rate decay through fitting early heart rate recovery after exercise to a first order exponential decay curve, or (3) analysis of the first 30 seconds of post exercise heart rate recovery with semilogarithmic regression analysis (Buchheit et al. 2007).

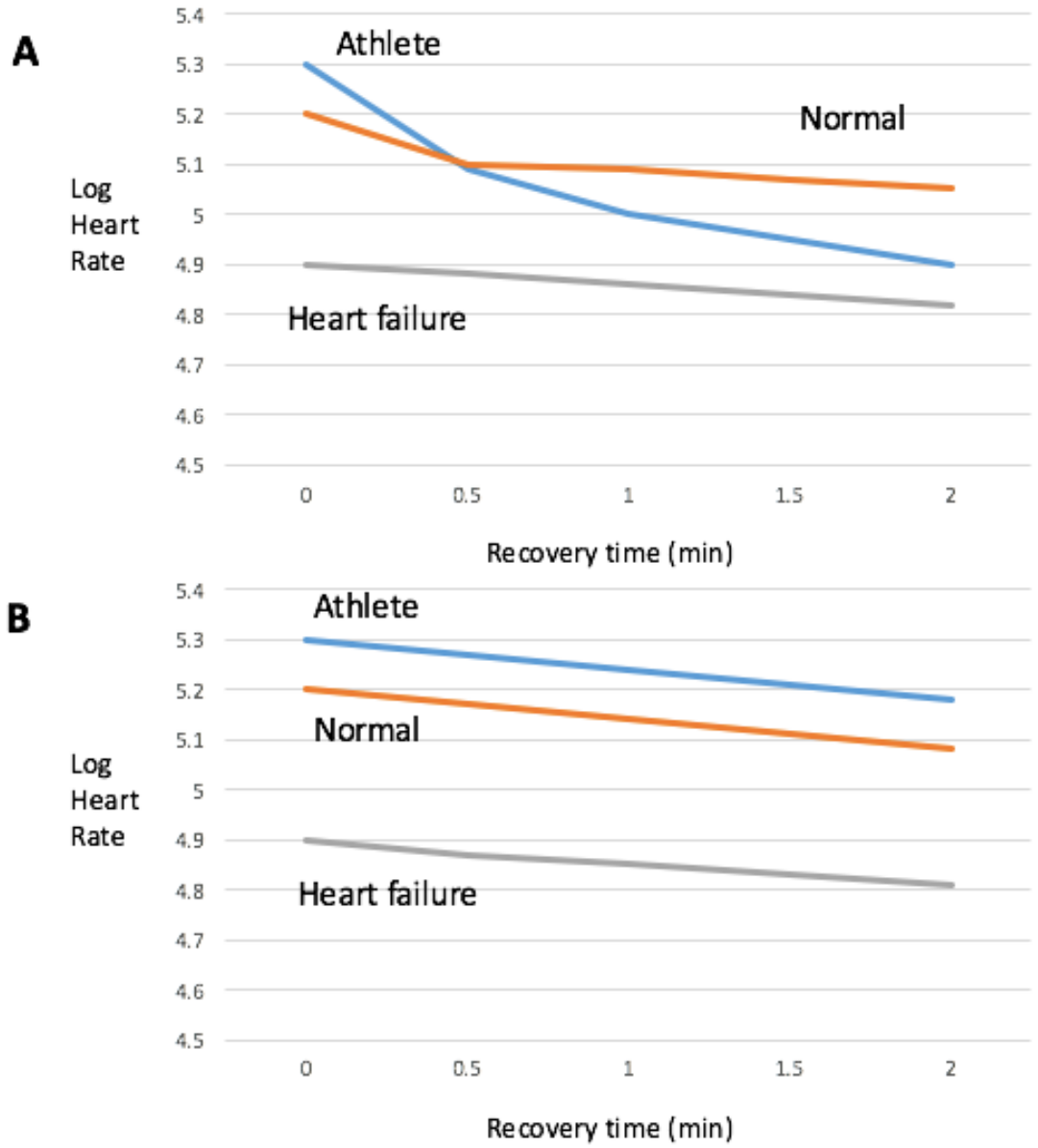

Figure 1-3: The association between parasympathetic tone and heart rate recovery (A) Absolute heart rate recovery (log transformed) in the first two minutes after cessation of graded exercise. A biexponential heart rate recovery profile is seen in both normal individuals and athletes, which is absent in heart failure. (B) Heart rate recovery after administration of atropine. The initial rapid reduction in heart rate is lost. Redrawn from (Lauer 2009).

When pyridostigmine (an acetylcholinesterase inhibitor which increases the availability of acetylcholine) is administered to healthy untrained controls, but not athletes, immediate post exercise heart rate recovery is augmented, but not late heart rate recovery (Dewland et al. 2007).

Further evidence for return of parasympathetic activity predominating over sympathetic withdrawal in early recovery for exercise comes from the observation of no improvement in initial heart rate kinetics after beta blockade (Crouse et al. 1989).

Known plasma norepinephrine kinetics also decrease the likelihood of sympathetic withdrawal underlying early heart rate recovery. Levels of circulating catecholamines are not affected by the duration of exercise and in fact peak after the cessation of activity. The plasma half-life of norepinephrine is around 3 minutes, easily outlasting the classically observed rapid initial recovery of heart rate, suggesting that late rather than early heart rate recovery is related to sympathetic withdrawal (Hagberg et al. 1979; Pierpont et al. 2000; Perini et al. 1989).

When compared with other commonly used measures of resting parasympathetic withdrawal, heart rate recovery performs well. Abnormal heart rate recovery has been associated with resting heart rate variability indices of parasympathetic withdrawal in patients with known coronary arterial disease, diabetes and healthy middle aged subjects (Evrengul et al. 2006; Copie et al. 2003; Nunan et al. 2010; Turker et al. 2013).

Before graded exercise, rMSSD and High Frequency Power used to capture instantaneous levels of parasympathetic reactivation have been directly related to the degree of heart rate recovery after exercise. In these same individuals, parasympathetic withdrawal at all time points derived from HRV parameters appears greater in individuals with reduced HRR (Danieli et al. 2014; Chen et al. 2011).

Although HRV measures such as HF-power and rMSSD appear to be predominantly generated by respiratory modulation of parasympathetic outflow (Malik et al. 1996), more recently it has become accepted that dynamic vagal-related HRV measures better reflect modulation of parasympathetic activity than overall vagal tone (Hedman et al. 1995).

Since both rMSSD and HRR are thought to represent parasympathetic activity, it might be expected that the two measures be always correlated. This, despite

evidence from studies such as those previously referenced, is not always the case (Buchheit, Papelier, et al. 2007). After very intense exercise, heart rate recovery may be preserved, despite minimal parasympathetic reactivation (Buchheit, Laursen, et al. 2007). HRV measures may be affected by respiratory parameters, underlying heart rate and the degree of sympathetic activation. Indeed, HRV and HRR might characterise independent aspects of cardiac parasympathetic function (Buchheit, Papelier, et al. 2007).

1.11.2 Abnormal heart rate recovery is predictive of mortality

Established parasympathetic autonomic dysfunction (PAD) is not only related to morbidity and mortality, but is highly likely to provide the mechanism for the development of organ dysfunction. Epidemiological studies using heart rate recovery as a marker of established PAD strongly support this assertion.

Jouven et al., in 5,713 asymptomatic working men, referred for routine annual medical screening, found an increase in risk of sudden death of 2.1 times in subjects with a heart rate recovery of less than 25 beats per minute (the lowest quintile of the studied population), when compared with the highest quintile (representing a heart rate recovery of greater than 40 beats per minute) (Jouven et al. 2005).

Cole et al. described a 1.5 times increase in likelihood of death in a population of 5,234 individuals without known cardiovascular disease for subjects with a heart rate recovery at one minute of at less then 42 beats per minute (the lowest quartile in that population) (Cole et al. 2000; Iyer et al. 2009). When conventional cardiovascular risk factors and exercise test parameters including aerobic capacity, chronotropic incompetence and abnormal heart rate recovery were examined across a population of 6,546 individuals without known cardiac disease, only aerobic capacity and abnormal heart rate recovery were predictive of 8 year mortality. Predictive power was further increased when the two variables were combined (Dhoble et al. 2014). Across a further 9,454 patients referred for exercise testing, both exercise capacity and abnormal heart rate recovery were predictive of 5 year mortality. No interaction was noted between these two measures (Nishime et al. 2000). This independent

predictive capacity is preserved when impaired left ventricular systolic function is controlled for (Watanabe et al. 2001).

In a 3,043 patient, 22-year outcome study, designed to identify exercise parameters that could be related to lower socioeconomic status and mortality, heart rate recovery and reduced functional status correlated best with mortality (Shishehbor et al. 2006).

Heart rate recovery is linked with aerobic fitness. Athletes tend to exhibit higher resting vagal tone and faster heart rate recovery than untrained individuals (Imai et al. 1994; Pierpont et al. 2000). It is not, however always possible to improve heart rate recovery with aerobic exercise training (Danieli et al. 2014; Pierpont et al. 2000; Jolly et al. 2011). Jolly et al. in their study of >1,300 patients referred for cardiac rehabilitation found that of those patients who presented with abnormal heart rate recovery, 41% improved (i.e. their HRR increased above 12bpm at 1 minute after cessation of exercise). Those patients that entered and exited the study with an abnormal heart rate recovery exhibited the highest mortality, whereas those who improved their heart rate recovery after cardiac rehabilitation also improved their 10-year mortality (HR 0.92). Predictors of failure to improve heart rate recovery with training included advanced age, failure to increase exercise capacity, diabetes mellitus, prior congestive heart failure, use of nitrates, and peripheral arterial disease (Jolly et al. 2011).

Author & Year	Population	Outcome	Heart rate recovery threshold/limit	Conclusions
(Cole et al. 1999)	Patients without known heart disease referred for diagnostic thallium scan + exercise testing 2,428 patients	All cause mortality (6 years)	1 minute after peak exercise </= 12 bpm The maximal value for the log-rank chi-square test statistic for all possible cut-off points between the 10th and 90th percentiles for the study cohort.	A delayed decrease in the heart rate during the first minute after graded exercise is a predictor of overall mortality, independent of workload, the presence or absence of myocardial perfusion defects, and changes in heart rate during exercise.
(Cole et al. 2000)	Patients with no known heart disease enrolled in a Lipid research cohort study. Submaximal exercise testing. 5,234 patients	All cause mortality (12 years)	2 minutes after peak exercise <24 bpm	When other known risk factors for cardiac death are controlled for, impaired HRR predicts death
(Shetler et al. 2001)	Patients referred for treadmill stress testing and coronary angiography 2,193 patients	All cause mortality (6 years) findings on coronary angiography	12,18,42 bpm at 1, 2 and 3 minutes post peak exercise (published criteria) 2 minutes after peak exercise HRR and outcome in proportional hazards model <22 bpm	HRR independently predicted death at all time points and in all models Did not perform better than other traditional variables Failed to discriminate coronary angiography findings
(Watanabe et al. 2001)	Patients referred for exercise echocardiography with no history of cardiac disease 5,438 patients	All cause mortality (3 years)	1 minute after peak exercise </=18 bpm A series of Kaplan-Meier curves was constructed describing mortality rates above and below all heart rate recovery values between the 10th and 90th percentiles of the entire cohort. A cut-off value of	After accounting for left ventricular function, heart rate recovery remains predictive of all cause mortality

			</=18 beats per minute was considered abnormal, this value yielded the highest log-rank score	
(Jouven et al. 2005)	Asymptomatic working men without cardiovascular disease 5,713 patients	All cause mortality (5 years)	1 minute after peak exercise, <26 bpm (lowest quintile and 2, 3 minutes after exercise)	All cause mortality and mortality from sudden death increased in lowest quintile at each recovery time point. No excess incidence of myocardial infarction
(Shishehbor et al. 2006)	Patients referred for treadmill stress testing 30,043 patients	All cause mortality (6.5 years) Estimated functional capacity and heart rate recovery, physiologic characteristics that are determined directly from exercise testing and socioeconomic status	1 minute after peak exercise <12 bpm Based on published criteria	Impaired functional status and heart rate recovery associated with low socioeconomic status and account for much of its relationship with mortality
(Kubrychtova et al. 2009)(Sacre et al. 2012)	Heart Failure (Ejection Fraction <45%) 712 patients	All cause mortality (6 years)	1 minute after peak exercise <5, <6, <10 bpm (Quartiles)	Ranked HRR independently predicted mortality
(Dhoble et al. 2014)	Patients referred for exercise testing without known cardiac disease 6,546 patients	All cause mortality (8 years)	1 minute after peak exercise <12 bpm Based on published criteria	Both functional aerobic capacity and heart rate recovery predicted mortality in the study period

Table 1-4 Large-scale epidemiological studies where morbidity and mortality have been related to heart rate recovery after exercise. A significant degree of variation in measuring method for heart rate recovery is observed across these studies. Several studies use the lowest quartile of recovery observed in the population studied. Others use fixed definitions of abnormal HRR (such as 12bpm at one minute of recovery). Others stratify according to eventual outcome. The range of measuring modalities and differing populations renders comparison more difficult, but across all the studies presented, impaired heart rate recovery after exercise is associated with worse outcomes both in terms of mortality and morbidity.

Age is another key consideration in terms of autonomic health. With increasing age, autonomic dysfunction becomes more likely. This is manifest in both reduced heart rate variability and impaired heart rate recovery regardless of aerobic fitness level. However, at any given age, improved aerobic fitness is associated with increased HRV and a more rapid heart rate recovery when compared with matched controls (Trevizani et al. 2012).

Ultimately though, autonomic function and aerobic fitness do not represent the same, but rather interactive, physiological processes. A role for intact parasympathetic activity can be hypothesised in the promotion and maintenance of cardiac contractility, which in turn will affect VO_2 peak. This relationship may become most important at the lower end of cardiac performance. However, in fit individuals there appears to be either no relationship between aerobic endurance and cardiovascular autonomic control (Bosquet et al. 2007), or at least a variable relationship (Sugawara et al. 2001).

1.11.3 Abnormal Heart rate recovery is predictive of impaired outcome across a range of pathologies

The predictive value of HRR for morbidity, disease progression and mortality is sustained across a variety of pathologies. PAD is strongly implicated in the development of cardiac dysfunction. In heart failure, an abnormal heart rate recovery is associated with disease progression, mortality and elevated levels of serum inflammatory markers (Tang et al. 2009; Ritt et al. 2012; Arena et al. 2006; Arena & Sietsema 2011; Kubrychtova et al. 2009; Yilmaz et al. 2013; Bilsel et al. 2006). During cardiopulmonary exercise testing in individuals with known heart failure, an abnormal heart rate recovery is able to provide additional discriminatory predictive value for mortality risk over and above aerobic capacity (Ritt et al. 2012; Arena et al. 2006).

Intact or augmented parasympathetic function is protective during myocardial ischaemia. In patients with known ischaemic heart disease and patients referred with chest pain for exercising testing, an abnormal heart rate recovery is discriminatory for the presence of angiographic disease (Shetler et al. 2001) and

predictive of coronary arterial disease severity (Ghaffari et al. 2011). Furthermore, along with chronotropic incompetence, abnormal heart rate recovery is predictive of abnormalities in myocardial perfusion (Georgoulias et al. 2003).

In diabetes, an abnormal HRR is also related to reduced HRV, mortality, glucose control and inflammatory parameters (Turker et al. 2013; Cheng et al. 2003) and is therefore thought to represent, or at least predict, Cardiac Autonomic Neuropathy (Sacre et al. 2012).

1.11.4 Heart rate recovery after exercise as a measure of established parasympathetic autonomic dysfunction in the perioperative period

To date, no specific exploration of the impact of established parasympathetic autonomic dysfunction has been attempted in the high-risk surgical population. Studies examining autonomic dysfunction have suffered methodologically and in terms of content as follows:

- The use of heart rate variability (the most commonly employed assessment technique in perioperative studies) to define autonomic dysfunction is burdened both with technical and interpretative difficulty.
- Little or no corroborating physiological data has previously been collected that describes the impact of established PAD on cardiopulmonary fitness or intraoperative cardiac performance.
- No systemic description of postoperative morbidity has been carried out.
- Patient selection has focused on medical diagnosis (e.g. diabetes) rather than the high-risk surgical population as a whole.

Planned surgery offers a unique opportunity to systematically phenotype an individual prior to a timed, predictable insult (Cain et al. 2015). Cardiopulmonary exercise testing (CPET), a routine preoperative investigation for higher risk patients in many centres provides the means by which to achieve this goal. By using CPET, a comprehensive battery of physiological parameters may be additionally described in the context of normal and abnormal heart rate recovery.

Heart rate recovery shows potential as a powerful, but low cost, low technology tool for the assessment of risk in the cardiac patient. This thesis will explore its use in the surgical population.

1.12 Sympathetic Autonomic Dysfunction

The interaction between parasympathetic and sympathetic autonomic neural activity is complex and influenced by a multitude of different factors. It is possible that 'pure' parasympathetic or sympathetic autonomic dysfunction does not exist, but rather the evolution of individual pathologies and disease states are influenced by differing degrees of predominantly parasympathetic or sympathetic impairment.

As outlined previously, excessive sympathetic outflow may result in a variety of pathophysiological changes including cardiac dysfunction and immune paralysis (Ackland et al. 2015), myocardial cellular injury, perioperative haemodynamic instability and prolonged postoperative myocardial ischaemia (Ellison et al. 2007; Huang et al. 2006; Laitio et al. 2007). Perioperative beta blockade has shown some promise in the reduction of major adverse cardiac events (MACE), though at the expense of an increased risk of perioperative CVA and hypotension in the surgical population at risk of adverse cardiac events (Devereaux et al. 2008; Fleisher et al. 2014).

Identification of surgical patients who demonstrate preoperative evidence of sympathetic autonomic hyperactivity may therefore be of benefit in terms of determining individuals who may, or may not benefit from pharmacological intervention such as beta-blockade.

Impaired heart rate recovery after exercise and reduced heart rate variability may be influenced by high levels of sympathetic autonomic activity (Buchheit, Papelier, et al. 2007). It is important therefore to determine whether or not the phenotype of individuals with PAD, and any detriments in perioperative outcome associated with it, is the same as that seen in individuals who demonstrate excessive sympathetic

activity. It is also important to determine whether or not these two populations are, in fact, composed of the same individuals.

Anxiety disorders are associated with increased cardiac risk, thought to be due to chronic and cyclical sympathetic activation as evidenced by reduced heart rate variability and increased levels of circulating catecholamines in response to stressors (Rutledge et al. 2009; Rutledge et al. 2013; Dishman et al. 2000; Fiedorowicz et al. 2011). In the cardiac surgical population, anxiety is associated with impaired perioperative cardiac outcomes (Tully et al. 2011).

Heart rate increases during mental stress, mostly as a result of sympathetic activation and is associated with reductions in heart rate variability (Taelman et al. 2009). Mild mental stress associated with impending exercise and resulting in an excessive heart rate rise is associated with an increased risk of sudden cardiac death (Jouven et al.2009). No correlation has been described between pre-exercise anticipatory heart rate rise and heart rate dynamics during exercise. However, in those individuals who demonstrate chronotropic incompetence, cardiac risk is further elevated (Jouven et al. 2009). Anticipatory heart rate rise before exercise can therefore be used as a marker of sympathetic autonomic hyperactivity.

The final results chapter of this thesis examines this relationship, in the light of findings in the same population of patients presented in preceding chapters dealing with PAD represented by impaired heart rate recovery after exercise.

1.13 Conclusion

Postoperative morbidity is a major issue in global healthcare and is associated with increased short and long term mortality. A minority of 'higher risk' individuals suffers the majority of adverse postoperative outcomes. Accurate and individualised identification of these at-risk patients remains the subject of much research. Mechanistic explanations for the development of multi-system pathology are urgently needed, both in terms of the prediction of individual risk and to aid in the development of new therapeutic and management strategies to mitigate risk.

One candidate mechanism for the production of postoperative morbidity is established autonomic dysfunction. Both parasympathetic and sympathetic autonomic dysfunction could be hypothesised to predispose to adverse perioperative outcomes via independent and interdependent mechanisms. Autonomic dysfunction is known to be materially related to prognosis and mechanistically underlie disease progression in a variety of multisystem pathologies relevant to the high-risk surgical patient.

Heart rate recovery after exercise represents return of parasympathetic tone and is prognostic in a variety of relevant disease states as well as having potential as an appropriate preoperative test for the identification of PAD in high-risk surgical patients. Other heart rate dynamic changes, such as anticipatory heart rate rise prior to exercise, which can also be measured at the time of exercise testing, can also be used to assess sympathetic autonomic dysfunction in the same patients.

Whether established autonomic dysfunction is associated with perioperative cardiac impairment and impaired outcomes after surgery is unknown and warrants further exploration.

1.14 Hypothesis

Preoperative autonomic dysfunction is associated with increased postoperative morbidity and mortality in high-risk patients undergoing major surgery.

1.15 Plan of Investigation

1.15.1 The impact of Parasympathetic Autonomic Dysfunction on exercise physiology

Parasympathetic autonomic dysfunction, through a variety of mechanisms, would be predicted to result in impaired performance at Cardiopulmonary Exercise Testing. Impaired exercise performance is strongly associated with adverse perioperative outcomes.

The first results chapter will examine the exercise physiology associated with PAD, as defined by impaired heart rate recovery, during formal cardiopulmonary exercise testing in a high-risk population presenting for major colorectal surgery.

Abnormal heart rate recovery will be defined in this population according to population distribution. Next, heart rate variability parameters will be examined in the same population and parameters currently associated with PAD in the general literature will be sought in patients with abnormal heart rate recovery. Similarly, markers of cardiac dynamic performance during exercise testing associated with adverse outcomes, such as chronotropic incompetence, will be examined in individuals with and without PAD.

Since PAD has previously been associated with cardiac ischaemia at stress testing, markers of ischaemia will be further examined in the same surgical population with PAD.

Established PAD is associated with chronic physiological stress, including renin-angiotensin-aldosterone system activation in both animal and human models. One marker of this with important physiological associations, GRK2 in circulating lymphocytes, will be examined in this patient population.

1.15.2 The impact of Parasympathetic Autonomic Dysfunction on perioperative outcome

Parasympathetic Autonomic Dysfunction, or vagal withdrawal has been associated with various adverse perioperative outcomes. Similarly, acting through several plausible mechanisms including immune/inflammatory dysregulation and modulation of cardiac function, perioperative vagal withdrawal could be predicted to underlie the development of postoperative morbidity. The second results chapter will examine the relationship between PAD as defined by impaired heart rate recovery and relevant perioperative outcomes in the same population examined in the first results chapter. Specific attention will be paid to other established predictors of impaired perioperative outcome, including a reduced anaerobic threshold.

1.15.3 The impact of Parasympathetic Autonomic Dysfunction on intraoperative haemodynamics

Impaired performance at preoperative exercise testing is thought to translate into reduced intraoperative global oxygen delivery, which in turn is thought to result in increased postoperative morbidity. It is not known whether any impairment in preoperative exercise performance in individuals translate to the intraoperative period.

The third results chapter will investigate the relationship between preoperative exercise physiology and intraoperative cardiac output measured using the oesophageal Doppler monitor in a high-risk surgical population undergoing major colorectal surgery. This will comprise a reanalysis of data from a previously published trial of intraoperative goal directed therapy and will therefore afford the opportunity to assess the response to fluid resuscitation in patients with and without PAD and relate intraoperative cardiac output to preoperative exercise physiology.

To date, established cardiac autonomic dysfunction has been associated with intraoperative hypotension. Confirmation of these findings in other surgical populations will be sought in this population with PAD defined through impaired heart rate recovery after exercise.

1.15.4 Sympathetic Autonomic dysfunction and perioperative outcome

The first three results chapters focus on the impact of parasympathetic autonomic dysfunction on perioperative physiology and outcome. What is not clear, however, is whether PAD always results in unopposed sympathetic activity. Similarly, the impact of preoperative sympathetic hyperactivity, predicted to be associated with immune/inflammatory dysregulation and increased cardiac risk in particular, is not known.

The final results chapter will define increased sympathetic activity by an excessive heart rate increase prior to commencing loaded exercise at time of preoperative CPET. Whether or not individuals with PAD, as defined by impaired heart rate recovery, also demonstrate sympathetic hyperactivity will be examined and the impact of sympathetic hyperactivity on perioperative outcomes will be examined, focusing on cardiac ischaemic pathophysiology most commonly associated with increased sympathetic outflow.

2 General Methods

2.1 Clinical Studies

Clinical data presented in this thesis are derived from that recorded in patients at two centres (University College London Hospital (UCLH) and Derriford Hospital Plymouth (DHP)) presenting for major non-cardiac surgery who had undergone preoperative Cardiopulmonary Exercise Testing (CPET) and who were enrolled in the observational Perioperative Morbidity: Heart rate recovery (POM-HR: UKCRN 13695) study which was composed of retrospective and prospective limbs.

The Derriford Hospital Plymouth cohort results, incorporated in the retrospective limb of POM-HR, were derived from a novel analysis of data available from the Cardiac Output Monitoring and Pre-operative Exercise Testing for Colorectal surgery (COMPETE-C: UKCRN 7285) trial (Challand et al. 2012).

Blood derived immune cells collected prospectively were evaluated in patients enrolled in the prospective limb of POM-HR at UCLH.

Additional heart rate variability data was available for comparison from a subset of patients enrolled on the Perioperative Morbidity: Oxygen Delivery (POM-O: UKCRN 8132) trial who had also undergone CPET as part of their routine clinical care.

At UCLH, medical history and CPET data for patients referred for exercise testing based on local clinical and protocol grounds is collected in a database prospectively for research, audit and service improvement reasons. This data was analysed for the retrospective limb of POM-HR.

All patients enrolled in the studies described were seen in a nurse-led preoperative assessment clinic, typically two to four weeks pre-operatively. Here, physical examination and investigations were carried out according to local protocol. Where clinically necessary, a consultant anaesthetist review was undertaken and additional

relevant preoperative treatments and investigations were instigated according to local protocol.

Routine blood samples including full blood count with differential white cell count (FBC) and renal function testing (U&Es) were carried out at this time.

Consultants undertook surgery and anaesthesia. Intraoperative fluid management depended on the individual study protocol, but in those studies where no protocol was stipulated, therapy was at the discretion of the treating anaesthetist. No limitations were placed on the choice of anaesthesia or the usage of regional anaesthetic techniques though local consensus guidelines were available. Antibiotics and thromboprophylaxis were prescribed according to local practice and guidelines.

The patients' treating medical team provided postoperative care. Admission to critical care was at the discretion of the surgeon or anaesthetist. Postoperative blood testing was carried out according to local protocol and at the discretion of the treating medical team. Routine physiotherapy was given according to local practice and acute pain team review was available for advice, all patients on parenteral analgesia were reviewed daily.

Readiness for discharge, postoperative morbidity (Clavien-Dindo) (Clavien et al. 2009) and return of bowel function data were collected prospectively at DHP only. Hospital length of stay data and mortality data were prospectively collected at both sites.

A summary of the patient populations studied and the relevant thesis chapters in which these data are analysed is presented in Table 2-1.

Study title:	Site:	Number of patients:	Heart Rate Parameters reported:	Thesis results chapter:
POM-HR (Retrospective limb)	**DHP** (COMPETE-C) Derivation **UCLH** Validation	814 235	HRR, aHRi HRR, aHRi	3,4,5,6 3,4,6
POM-O	**UCLH**	46	HRR, HRV	3,6
POM-HR (Prospective limb)	**UCLH**	20	HRR, aHRi	3

Table 2-1: Populations studied in this thesis. DHP: Derriford Hospital Plymouth, UCLH: University College London Hospitals, HRR: Heart rate recovery, aHRi: abnormal heart rate increase, HRV: Heart Rate Variability.

2.2 Perioperative Morbidity: Heart rate recovery (POM-HR) Study

The Perioperative Morbidity: Heart rate recovery (POM-HR) study (UKCRN: 13695) is a multi-centre observational study of exercise-induced heart rate dynamics and their relationship to post operative morbidity in elective surgical patients undergoing cardiopulmonary exercise testing (CPET) as part of their routine preoperative workup.

It is composed of both a retrospective limb (comprising a derivation (Derriford Hospital, Plymouth, DHP); and validation (University College London Hospital, UCLH) cohort) and a prospective limb (University College London Hospital, Derriford Hospital, Royal Bournemouth Hospital, Royal Surrey Hospital, Royal Preston Hospital).

The Plymouth validation cohort comprised patients undergoing major colorectal surgery who participated in the COMPETE-C randomised controlled trial approved by the Cornwall and Plymouth Research Ethics Committee (Ref: 08/H0203/159) and conducted at Derriford Hospital, Plymouth, UK, between March 2009- April 2010 (ISRCTN 14680495) (Challand et al. 2012). A summary of the COMPETE-C protocol as relevant to POM-HR is presented subsequently.

2.2.1 Ethical approval

Overall ethical approval for POM-HR was granted by the NRES Committee London; Camden & Islington (REC: 12/LO/0453). Regulatory and Local Trust R&D approval was granted at all sites.

The Cornwall and Plymouth Research Ethics Committee granted ethical approval for COMPETE-C on 09/09/2008 (ref: 08/H0203/159).

For the retrospective study, written confirmation from the NIGB (Claire Edgeworth, Ethics and Confidentiality Committee) was obtained, confirming that non-identifiable morbidity and preoperative cardiopulmonary exercise testing data could be provided by clinical care teams, thus ensuring no breach of confidentiality. Data from COMPETE-C had already been anonymised as part of the trial protocol. Pre-existing non-identifiable, fully anonymised morbidity, laboratory and preoperative cardiopulmonary test data are collected as part of clinical quality monitoring at UCLH (the UCL CPET database; London - South East REC (12/LO/0192). The UK National Information Governance Board has confirmed that the routine haematology and biochemistry data collected for this study represents clinical audit and service evaluation and therefore did not require direct patient consent for evaluation.

2.2.2 POM-HR Study objectives

The primary objective of the study is to assess whether abnormal heart rate recovery representing parasympathetic autonomic dysfunction following cardiopulmonary exercise testing is related to postoperative complications including mortality and length of hospital stay.

The trial secondary objective is to assess whether abnormal heart rate dynamics following cardiopulmonary exercise testing is related to immune-inflammatory phenotypes.

2.2.3 POM-HR Trial Design and Methods

The full protocol for POM-HR (including prospective data collection, is available online at http://www.ucl.ac.uk/anaesthesia/trials. The summary protocol as relevant to this work is presented below.

2.2.3.1 Retrospective study

Anonymised, individual and cohort laboratory, physiological and clinical data from patients undergoing routine preoperative cardiopulmonary exercise tests were provided by both centres in spreadsheet form. Patients were assigned individual study numbers distinct from hospital number for CPET analysis. At UCLH, individual CPET data was accessed on a non-networked computer using the anonymised database provided. For each individual patient, heart rate dynamic and ECG data was calculated and CPET derived physiological data analysed blinded to outcomes data. Either an experienced exercise physiologist or physician independently verified standard CPET variables.

2.2.3.2 Prospective study

Preoperative patients undergoing major surgery who had been routinely referred for CPET were approached and consented for participation. Clinical Data was prospectively collected. CPET data was collected and analysed as per usual clinical protocol at participating institutions. Blood derived immune cells were collected at the UCLH site only prior to commencement of exercise on the day of CPET. Treatment and analysis of these samples is described subsequently.

Heart rate dynamic data was analysed blinded to outcomes data.

2.2.3.3 Inclusion Criteria

All surgical patients aged 18 or over routinely referred for cardiopulmonary exercise testing preoperatively undergoing major non-cardiac surgery. For patients enrolled in DHP, additional limited inclusion and exclusion criteria were applied which are detailed subsequently.

2.2.3.4 Exclusion Criteria

- Unwillingness to participate
- History of exercise induced angioedema
- Pregnancy
- Any contraindication to cardiopulmonary exercise testing (as outlined by the American Thoracic Society guidelines/ACCP (Weisman et al. 2003)

A summary flow chart representing the study protocol is presented below (Figure 2-1).

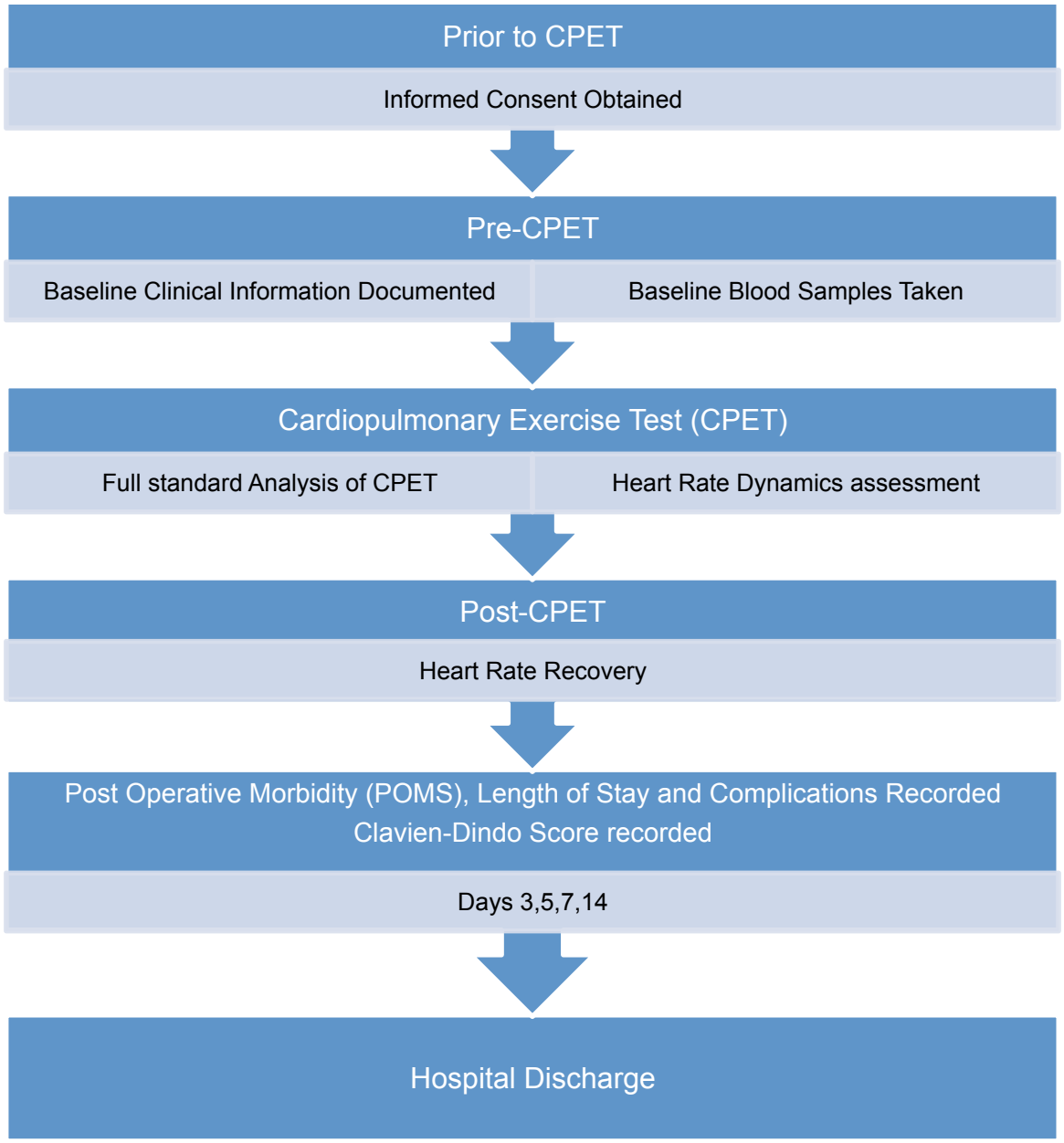

Figure 2-1: Summary flow sheet, POM_HR study protocol

2.2.4 Retrospective Data Collection

Preoperative clinical and demographic data were recorded prospectively as part of the COMPETE-C, and POM-HR clinical trials respectively. These data included age, sex and comorbidities including diabetes, hypertension, ischaemic heart disease, cerebrovascular disease, heart failure and chronic kidney disease. Perioperative risk scores were collected including American Society of Anesthesiologists (ASA) Physical Status Score and the Modified Lee Cardiac Risk Index.

Postoperative data collected included hospital length of stay, readiness for discharge (DHP only), bowel dysfunction data (DHP only) and Clavien-Dindo grade (DHP only).

	Cohort 1: **Derriford Hospital Plymouth** (Derivation) n = 235	Cohort 2: **University College London Hospital** (Validation-Retrospective) n = 814	Cohort 2: **University College London Hospital** (Prospective) n = 46 (POM-O)
Preoperative Parameters:	Demographic Data Medical History Age/Sex/BMI Drug History	Demographic Data Medical History	Pre exercise Heart Rate Variability (POM-O)
Risk Scores:	ASA grade RCRI	RCRI	Not presented here
Laboratory Investigations:	U&E, FBC, CRP	U&E, FBC, CRP, Neutrophil: Lymphocyte ratio	Blood Derived Immune cells (POM-HR)
CPET parameters:	Full standard CPET parameters Continuous 12-lead ECG: ST-segment monitoring, Arrhythmia Heart Rate Dynamic Changes	Full standard CPET parameters Continuous 12-lead ECG: ST-segment monitoring, Arrhythmia Heart Rate Dynamic Changes	Full standard CPET parameters Continuous 12-lead ECG: ST-segment monitoring, Arrhythmia Heart Rate Dynamic Changes
Intraoperative Parameters:	Cardiac Output Vasopressor use Goal directed therapy use Urine output Fluid administered Mode of Analgesia	None	None
Postoperative Parameters	Hospital Length of stay 30 & 90 day mortality Clavien-Dindo Incidence of sepsis Ready for discharge Return of bowel function Return to Critical Care	Hospital Length of stay 30 & 90 day mortality Return to critical care	Heart Rate Variability (POM-O)
Laboratory Investigations		FBC, CRP, U&E	

Table 2-2: Summary Clinical Study Course for all patients presented in this thesis

A summary of data collected at each site is presented in Table 2-2. More detail is provided subsequently on definitions of sepsis, readiness for discharge, return of bowel function and Clavien-Dindo scoring. A table of types of surgery is available in supplemental materials

2.2.5 Lymphocyte Count and Perioperative Inflammation

Perioperative blood samples were taken at UCLH as described previously and white cell count as well as differential were analysed (Sysmex XE2100 analyser, Sysmex, Milton Keynes, UK). Neutrophil to lymphocyte ratio was calculated as a marker of established inflammation, both preoperatively and on the third postoperative day. CRP was similarly measured preoperatively and on the third postoperative day.

2.3 The COMPETE-C trial

The COMPETE-C trial was a prospective randomised controlled trial examining the impact of intraoperative goal directed fluid therapy (GDT) on patients categorised as 'low' or 'high risk' based on preoperative CPET results. The full protocol for COMPETE-C is available in the appendices and at http://www.isrctn.com/ISRCTN14680495. Raw data from the COMPETE-C trial was re-analysed as per the POM-HR protocol.

CPET was carried out and analysed with reference to ATS/ACCP guidelines (Weisman et al. 2003).

Intraoperative oesophageal Doppler monitoring was employed in all patients enrolled in the trial regardless of randomisation to GDT or standard of care.

2.3.1.1 Inclusion criteria in addition to those recorded for POM-HR

- Adult patients undergoing major colorectal surgery on an enhanced recovery after surgery (ERAS) pathway.
- Patients with a measureable anaerobic threshold (AT) above 8ml/O_2/min.

There were no additional exclusion criteria to those recorded for POM-HR

2.3.1.2 COMPETE-C trial protocol as relevant to POM-HR

Written informed consent was obtained from all participants prior to randomisation. Participants were risk stratified according to AT into aerobically unfit (8-10.9 ml/O_2/min) and aerobically fit >11ml/O_2/min) groups. Within these groups, individuals were randomised to receive either standard fluid management or GDT using random block allocation.

Perioperative surgical care was conducted using local ERAS protocols. Perioperative anaesthetic care was carried out using local consensus guidelines. All patients had an Oesophageal Doppler (OD) probe (CardioQ^{TM}, Deltex Medical, Chichester, UK) placed immediately after induction of anaesthesia. Doppler readings were taken and recorded both at pre determined times (pre and post incision, end of surgery) and every 15 minutes until the end of surgery.

Intraoperative fluid therapy depended on randomisation. Patients allocated to standard of care received intraoperative crystalloid or colloid based on estimated fluid requirements, intraoperative losses and measurement of standard haemodynamic variables without reference to oesophageal Doppler readings (HR, BP, CVP etc.). A maintenance fluid rate of 10 ml/kg/h was targeted.

Patients allocated to GDT received supplementary colloid (Voluven^{TM}; Fresenius Kabi Ltd, Cheshire, UK) targeting stroke volume maximisation according to the algorithm provided by the OD manufacturers (Figure 2-2). Group allocation, OD readings and GDT colloid administration was concealed from the surgical team.

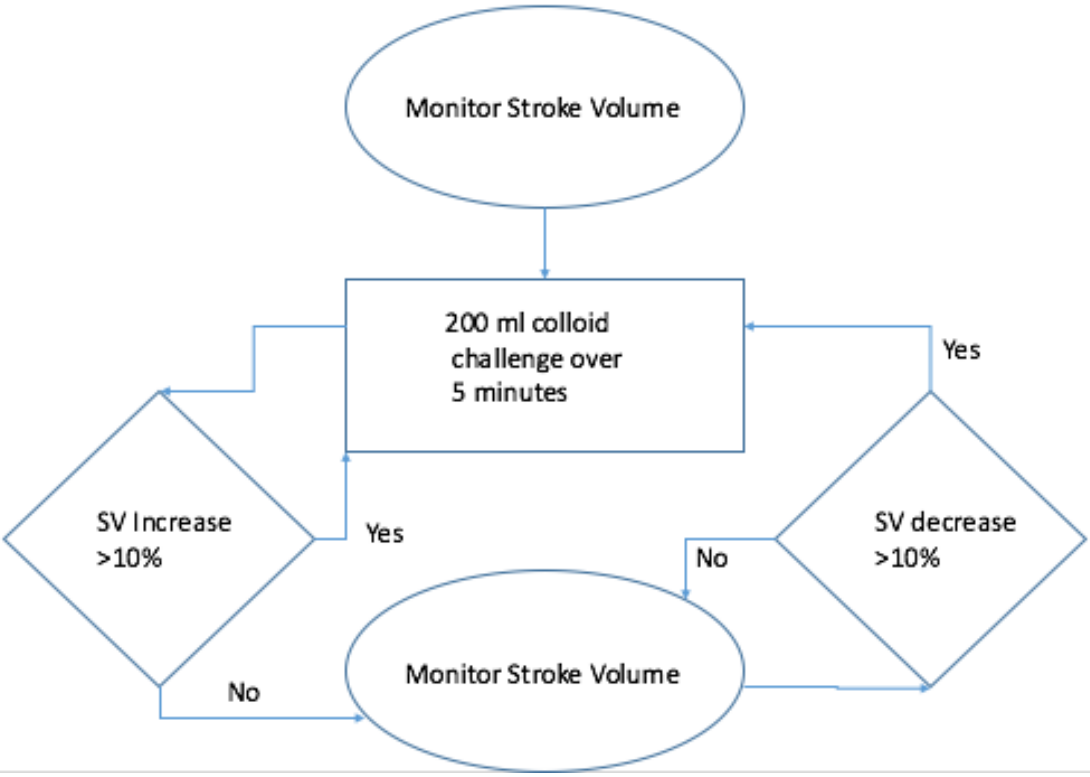

Figure 2-2: Intraoperative goal directed therapy protocol used in the COMPETE-C trial

In patients allocated to the GDT arm of the study, the Stroke Volume (SV) response to an initial colloid bolus, given prior to surgical incision, but after induction of anaesthesia, was monitored. Where fluid responsiveness was present (i.e. a SV increase of >10%), a further fluid bolus was administered. Intraoperatively, a SV decrease of >10% prompted a further challenge.

Intraoperative oesophageal Doppler monitoring provided cardiac output data throughout the case (Figure 2-3). Parameters recorded at the start of surgery, prior to initial skin incision but after induction of anaesthesia and recorded at the end of surgery, after or during skin closure but prior to emergence from anaesthesia are presented in this thesis. These parameters include:

- Peak Velocity (PV)(cm/s): the highest blood velocity detected during systole; used as an indication of ventricular contractility. Typical range 50-80 cm/s for a 70 year old (Singer 1991).
- Stroke Volume (SV) (ml): The volume of blood ejected from the left ventricle per contraction of the heart. Typical range 60 to 100ml.

- Flow Time Corrected (FTc)(ms): the duration of flow during systole corrected for heart rate. Used as an indication of LVEDV and both cardiac afterload and preload. For any given level of inotropy, the time the heart takes to eject depends on SV, which in turn relies on LVEDV giving an indication of preload (a high preload results in a high LVEDV, increased SV and therefore increased ejection time). Similarly, increased resistance to ejection (increased afterload) alters time for ejection. Typically a reduction in both PV and a reduction in FTc suggests increased afterload. Typical range 330-360 ms.
- Cardiac Output (CO)(litres/min): The total volume of blood ejected from the heart per minute. Typical values 4 to 8 l/min.
- Cardiac Index (CI): The cardiac output indexed to body surface area. Typical values 2.5-4 l/min/m^2.

Analysis of Doppler derived cardiac output variables at the pre-specified time-points outlined above were linked to preoperative heart rate dynamic changes measured at CPET, representing parasympathetic or sympathetic autonomic dysfunction.

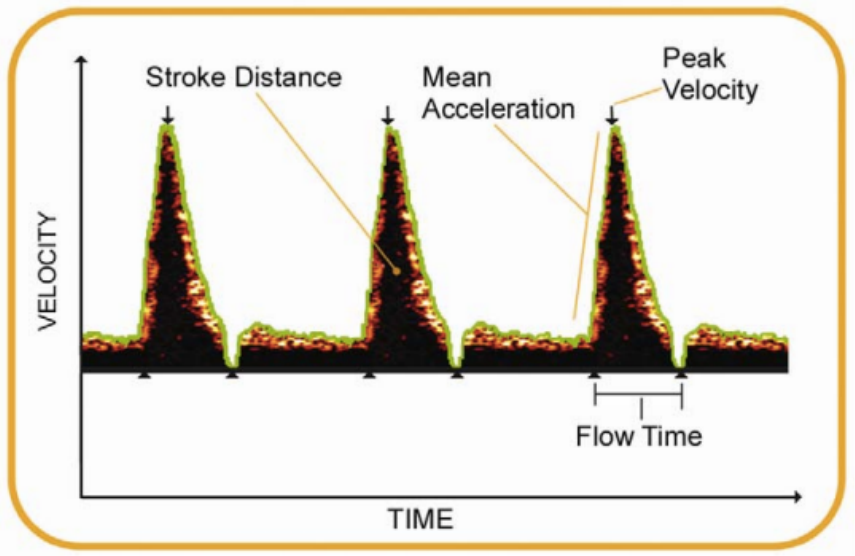

Image of Descending Aortic Waveform

Figure 2-3: A representative oesophageal Doppler trace Stroke Distance (SD) is the distance in cm that a column of blood moves along the aorta with each contraction of the left ventricle of the heart. Values are age and size dependent. Changes in SD are directly related to changes in stroke volume. Flow time corrected (FTc) is representative of left ventricular afterload. Mean acceleration and peak velocity are representative of cardiac contractility. Reproduced from 'Oesophageal Doppler

monitor (ODM) guided individualised goal directed fluid management in surgery a technical review' (Akc et al. 2010)

Postoperative care was as described previously, at the discretion of the treating teams. Enhanced Recovery protocols were specifically targeted at DHP. Postoperative outcomes were recorded prospectively and blinded to group allocation; additional outcome data not available at UCLH is outlined subsequently.

2.3.2 Additional postoperative data derived from the COMPETE-C trial

2.3.2.1 Postoperative Sepsis

Prospective data on the incidence of sepsis was collected at DHP only. Two or more of the following criteria, derived from the surviving sepsis campaign international guidelines 2012, being present defined postoperative sepsis (Dellinger et al. 2013):

- Temperature <36^{o}C or >38^{o}C
- Heart Rate >90 bpm
- Respiratory Rate >20bpm
- Acutely altered mental state
- White cell count <4 or >12
- Suspected infectious cause: origin recorded
- Plasma glucose >14 mMol/L

2.3.2.2 Readiness for Discharge

Readiness for discharge data was available from the Derriford Hospital Plymouth cohort and was assessed using internationally accepted criteria (Fiore et al. 2012) for colorectal surgery by observers blinded to the original intervention or CPET result (Table 2-3).

Criteria	End points
Tolerance of oral intake	Patient can tolerate at least one solid meal without nausea, vomiting, bloating, or worsening abdominal pain. Patient should be actively drinking and not require IV hydration
Recovery of lower gastrointestinal function	Patient should have passed flatus
Adequate pain control with oral analgesia	Patient should be able to rest and mobilise without significant pain (Pain < 4 on a scale of 1 to 10)
Ability to mobilise and self care	Patient should be able to sit up, walk, and perform activities of daily living.
Clinical examination and laboratory tests show no evidence of complications or untreated medical problems	Temperature should be normal Pulse, blood pressure and respiratory rate should be stable and consistent with preoperative levels Haemoglobin should be stable and within acceptable levels Patient should be able to empty the bladder without difficulty or match preoperative level of bladder function

Table 2-3: Criteria for assessment as fit for discharge. When the criteria outlined above have been met, the patient is considered to have reached short-term postoperative recovery and should be considered ready for discharge (Fiore et al. 2012).

2.3.2.3 Postoperative return of normal bowel function

This was assessed at DHP as a component of Readiness for Discharge scoring. Aside from the presence of flatus, patients were expected to have passed stool and be tolerating a light diet normally to be considered as having resumed normal bowel function.

2.3.2.4 Clavien-Dindo grade

Postoperative morbidity scoring was carried out daily at DHP using the Clavien-Dindo scoring system. A Clavien-Dindo grade of >3 at any time in the postoperative period was taken as representative of major postoperative morbidity for POM-HR (Table 2-4).

Grade	Classification
Grade 1	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic or radiological intervention. Allowed therapeutic regimens include: antiemetics, antipyretics, analgesics, diuretics, electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade 2	Requiring pharmacological treatment with drugs other than allowed for grade 1 complications. Blood transfusions and total parenteral nutrition included
Grade 3a	Surgical, endoscopic, or radiological intervention that is not under general anaesthesia
Grade 3b	Surgical, endoscopic, or radiological intervention that is under general anaesthesia
Grade 4a	Life threatening complication requiring intermediate care or intensive care unit management, single organ dysfunction (including dialysis, brain haemorrhage, ischaemic stroke, and subarachnoid bleeding)
Grade 4b	Life threatening complication requiring intensive care unit management, multi-organ dysfunction (including dialysis)
Grade 5	Death of a patient

Table 2-4: The Clavien-Dindo classification of postoperative complications (Clavien et al. 2009)

2.4 Perioperative Morbidity: Oxygen-Delivery (POM-O) Study

Immediate pre- and postoperative Holter monitor data from 46 high risk patients who had undergone preoperative Cardiopulmonary exercise testing and were enrolled in the POM-O study, were examined for association with heart rate recovery data.

The Perioperative Morbidity: Oxygen-Delivery (POM-O) study (Ackland et al. 2015)(UK NIHR CSP 22346, www.ucl.ac.uk/anaesthesia/trials) is a multi centre (University College London Hospital, Royal Free Hospital, Royal Surrey County Hospital, Guildford and St George's Hospital, London) prospective randomised placebo controlled trial of postoperative goal directed therapy, targeting oxygen delivery, in patients undergoing major non-cardiac surgery. The trial was carried out between May 2010 and February 2014. The primary trial hypothesis being that restoring oxygen delivery (DO_2) in the immediate post-operative period to baseline would result in a reduction of patient morbidity. Ethical approval was gained for this trial (Outer South East London REC- South London REC Office, 12/2009, ref: 09/H0805/58).

I used heart rate variability data from 46 patients collected preoperatively (prior to any intervention) at University College London Hospital during this trial to test the association of exercise induced heart rate dynamics with recognised HRV measures of parasympathetic autonomic dysfunction.

My involvement in the execution of this trial was as investigator for the initial three months. Subsequent involvement included data collection and analysis of Holter monitor recordings and CPET test results carried out as part of the trial protocol (this included source recording clean up and editing with subsequent Heart Rate Variability analysis (as detailed later)) as well as the review and analysis of primary data collected during the course of the trial as well as at the time of CPET. Analysis of outcomes relating to goal directed therapy and other related measures has been carried out elsewhere as part of the final analysis of results and do not form part of this thesis.

2.4.1 POM-O Trial protocol

The full trial protocol is available at http://www.ucl.ac.uk/anaesthesia/trials. A flow chart indicating the summary trial protocol as relevant to this thesis is presented below (Figure 2-4).

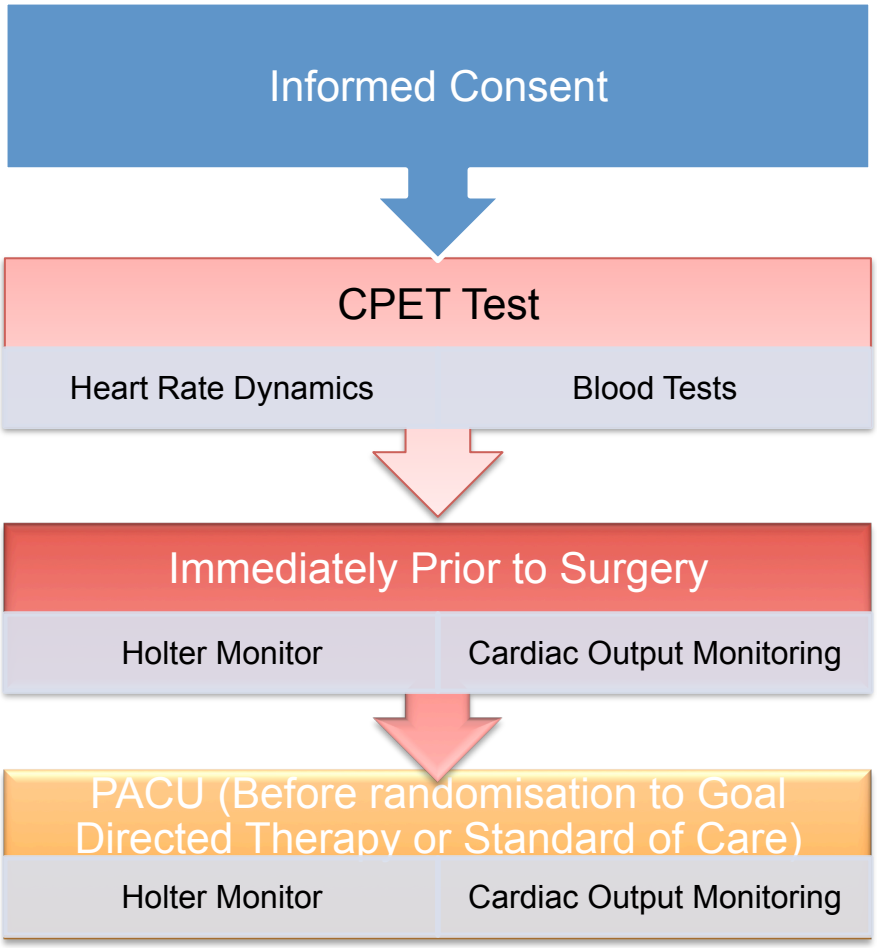

Figure 2-4: POM-O Protocol Flowchart (modified). Data presented in this thesis are derived from recording made up to admission to PACU.

2.4.2 Subject Selection

2.4.2.1 Inclusion Criteria

Adult patients undergoing major elective surgery expected to last for at least 120 minutes were eligible for recruitment provided they satisfied one of the following inclusion criteria for being of high surgical risk:

a) ASA ≥ grade 3.
b) Surgical procedure planned with an estimated/documented risk of postoperative morbidity (as defined by the Post Operative Morbidity Survey) exceeding 5%.
c) A modified Revised Cardiac Risk Score ≥3 (Fleisher et al. 2014), as defined by age ≥70 years, a history of cardiovascular disease (myocardial infarction,

coronary artery disease, cerebrovascular accident, electrocardiographic evidence for established cardiac pathology), cardiac failure, poor exercise capacity (anaerobic threshold <11ml.kg.min^{-1} assessed by cardiopulmonary exercise testing and/or Duke Activity Status Index), renal impairment (serum creatinine ≥130 μmol/l) and/or diabetes mellitus.

2.4.2.2 Exclusion Criteria

a) Refusal of consent.
b) Pregnancy.
c) Lithium therapy or allergy.
d) Recent myocardial ischaemia (within previous 30 days).
e) Acute arrhythmia.
f) Acute bleeding.
g) Patients receiving palliative treatment only.

All patients enrolled in the study at UCLH underwent Holter Monitor recording on the day of surgery (pre- and immediately postoperatively and for the duration of the intervention). On the day of surgery, Holter monitoring was instituted preoperatively in a quiet area in the preoperative assessment unit. Recordings were also taken for two to four hours on postoperative days 2, 5, 7, and on the day of discharge where clinically possible.

A proportion of patients enrolled in the POM-O trial, as dictated by standard clinical protocols at the UCLH, underwent preoperative symptom limited maximal cardiopulmonary exercise testing (CPET). The protocol for CPET exercise testing is described subsequently in this chapter. The CPET test data from these patients was analysed for heart rate dynamics relating to autonomic function as described later in the methods section. It is from these patients that Holter monitor data was analysed for association with heart rate recovery as a measure of parasympathetic autonomic dysfunction.

2.5 Cardiopulmonary Exercise Testing (CPET)

CPET testing was carried out at University College London Hospital and Derriford Hospital, Plymouth. Prior to every test, appropriate calibration measurements were undertaken. These included:

- Ambient pressure and temperature.
- Oxygen cell calibration (Room air (20.95% O_2 and 0.05% CO_2) and Calibration gas (15% O_2, 5% CO_2). Maximum error: ± 0.05% for O_2, ± 0.02% for CO_2.
- Flow-volume sensor calibration: 3 litre syringe, three different computer generated flow rates (maximum error: ± 100 ml).

CPET was carried out on stationary, electronically braked cycle ergometers in both Derriford Hospital (Zan, nSpire, CO, USA) and UCLH (Corival, Lode, Gronigen, Netherlands). In-line breath-by-breath gas analysis, non-invasive blood pressure measurement, Oxygen Saturation (SpO_2) and 12-lead ECG monitoring were recorded continually throughout all tests. All CPET data was freely available to treating clinical teams. American Thoracic Society guidelines were adhered to throughout testing. Contraindications to CPET testing used at both centres are listed in Table 2-5 (Weisman et al. 2003).

Absolute	Relative
Acute Myocardial Infarction (3 to 5 days)	Left coronary stenosis or equivalent
SpO_2 <85% on room air	Moderate stenotic valvular heart disease
Symptomatic, uncontrolled arrhythmias or syncope	Severe arterial hypertension at rest (>200 mmHg systolic, >120 mmHg diastolic)
Thrombosis of lower extremities	Tachyarrhythmias or bradyarrhythmias
Active endocarditis, myocarditis or pericarditis	Orthopaedic impairment that compromises exercise performance
Uncontrolled asthma	Hypertrophic Cardiomyopathy
Symptomatic severe aortic stenosis	Significant pulmonary hypertension
Uncontrolled heart failure	Advanced or complicated pregnancy
Acute Pulmonary Embolism or Infarction	Electrolyte abnormalities
Pulmonary oedema	High degree AV block
Acute non cardiopulmonary disorder affecting or aggravated by exercise	
Mental impairment with the inability to cooperate	

Table 2-5: Contraindications to CPET testing

Prior to CPET, patients were fasted and were asked to refrain from caffeine on the day of testing. A full medical and exercise history was taken prior to each test, as were basic anthropometric measurements including weight, height and blood pressure. All patients underwent spirometry (Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 second (FEV_1) and Maximal Voluntary Ventilation (MVV) prior to testing in order to calculate predicted maximal values for ventilatory capacity amongst other parameters. A haemoglobin value was taken prior to each test in order to calculate oxygen delivery values (HemoCue, Radiometer, Copenhagen, Denmark).

12-lead ECG, blood pressure, breath-by-breath gas analysis and pulse oximetry measurement was continually recorded throughout every test.

Appropriate exercise intensity (incremental wattage ramp test, 5 to 35 Watts/minute) was determined by trained exercise physiologists and physicians and based upon population norms, informed by details from the individual medical histories and reported activity levels of trial participants. Wasserman equations were used to help guide the determination of the work rate increment (Wasserman & Mcilroy 1964):

1. VO_2 (ml/min) = 150 + (6x weight (kg))
2. Peak VO_2 (ml/min) ***Males*** = (height (cm) – age (years)) x 20
3. Peak VO_2 (ml/min) ***Females*** = (height (cm) – age (years)) x 14
4. Work rate increment (W/min) = (Peak VO_2 – VO_2 Unloaded) / 100)

Subjects cycled at a cadence of 60 rpm, the ergometers, which are electronically braked, allowed some freedom around pedal speed. Tests generally lasted 10 to 15 minutes and were symptom limited.

The limit of tolerance (maximum) was defined as the point at which the subject could not maintain a cadence of 60 rpm despite encouragement and/or where symptoms (e.g. chest/leg pain or shortness of breath) precluded continuation of exercise. Full criteria for stopping the tests are based on American Thoracic Society guidelines (Weisman et al. 2003) and are presented in Table 2-6.

Criteria for Cardio Pulmonary Exercise Test termination
- Chest pain suggestive of ischaemia
- Ischaemic ECG changes (Significant ischaemia >1mm ST depression in chest leads or >2mm ST depression in limb leads or ECG pattern consistent with myocardial infarction or evolving ischaemic changes)
- Complex ectopy
- Second or third degree heart block
- Fall in Systolic blood pressure >20 mmHg
- Hypertension (>250 mmHg systolic, >120 mmHg diastolic)
- Severe desaturation (S_pO_2 ≤ 80%) ± signs and symptoms of hypoxia
- Sudden pallor, dizziness or faintness
- Loss of coordination or mental confusion

Table 2-6: ATS/ACCS Criteria for stopping Cardio Pulmonary Exercise Tests

A period of familiarisation and resting baseline measurements were taken for a baseline of 3 minutes sat on the cycle ergometer, followed by 3 minutes of unloaded pedalling with subsequent initiation of incremental loading of the wattage ramp (for approximately ten minutes). Following symptom limited cessation of exercise a three-minute period of unloaded pedalling was undertaken, during which time full physiological monitoring was continued, prior to dismounting from the apparatus (Figure 2-5, Figure 2-6). Heart rate recovery values were measured during this period.

CPET physiological data were recorded continually (and latterly exported to an Excel spread sheet) and also expressed visually as the standard nine-panel plot (Figure 2-7).

The Anaerobic Threshold (AT) was measured as body-mass corrected oxygen consumption ($ml \cdot kg^{-1} \cdot min^{-1}$). AT was determined by modified V-slope technique and confirmed with the ventilatory equivalents method (Figure 2-8; Beaver, Wasserman et al. 1986).

Peak oxygen consumption (VO_2 peak), oxygen pulse (VO_2/HR), and ventilatory equivalents for carbon dioxide (V_E/VCO_2) and Oxygen (V_E/VO_2) were also recorded.

12 lead ECG recordings were continually analysed and assessed for ischaemia and arrhythmias. (Pathfinder SL. Spacelabs Healthcare, Hertford UK). Baseline ECG

tracings were assessed for ST segment neutrality. ST segment depression was assessed in lead II and defined as abnormal when depression of 0.1 mV or more occurred (Weisman et al. 2003). Other leads were available but not specifically assessed.

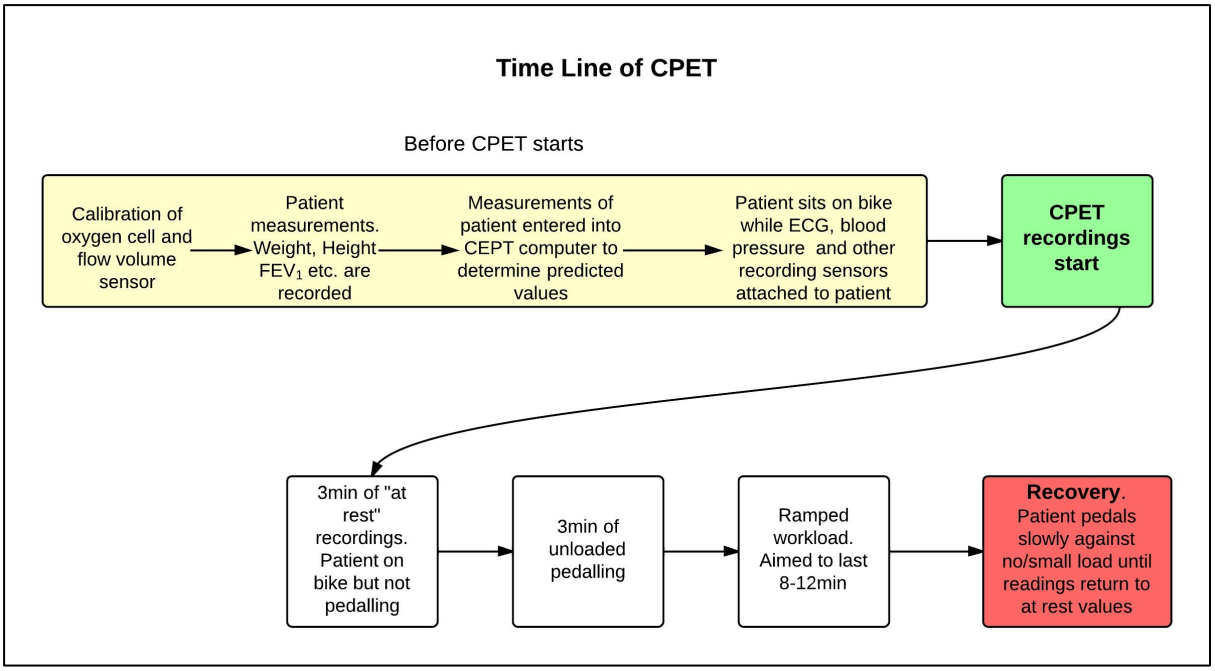

Figure 2-5 Conduct of Cardiopulmonary Exercise Testing

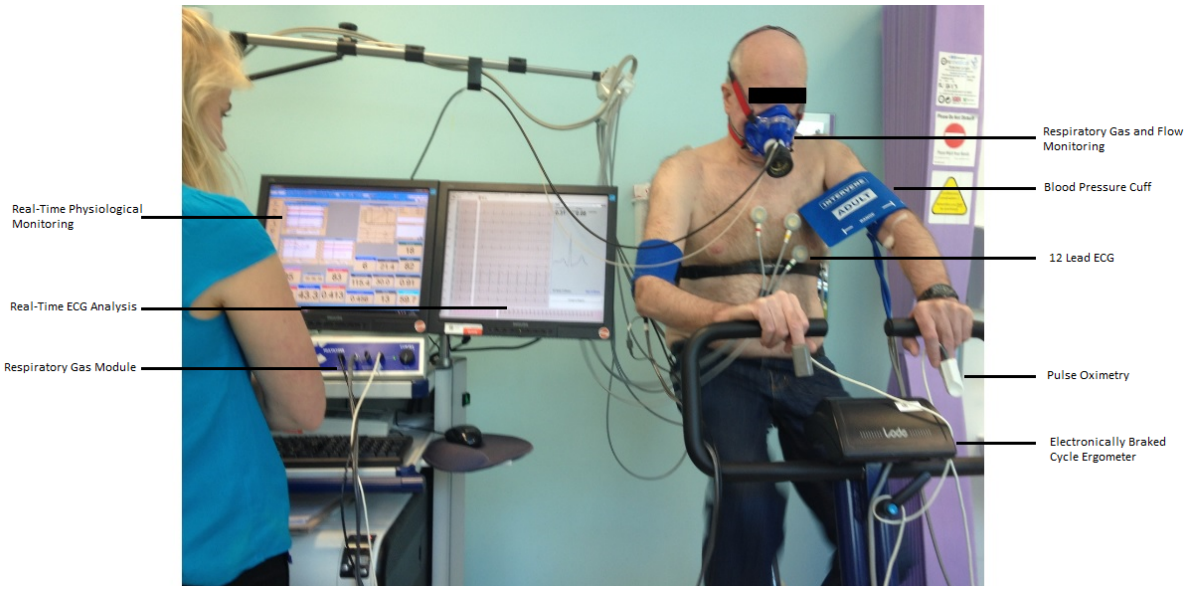

Figure 2-6. Cardiopulmonary exercise test apparatus at UCLH

The test can be categorised as: i) Pre-Test, ii) Acclimatisation, iii) Ramped work, iv) Recovery.

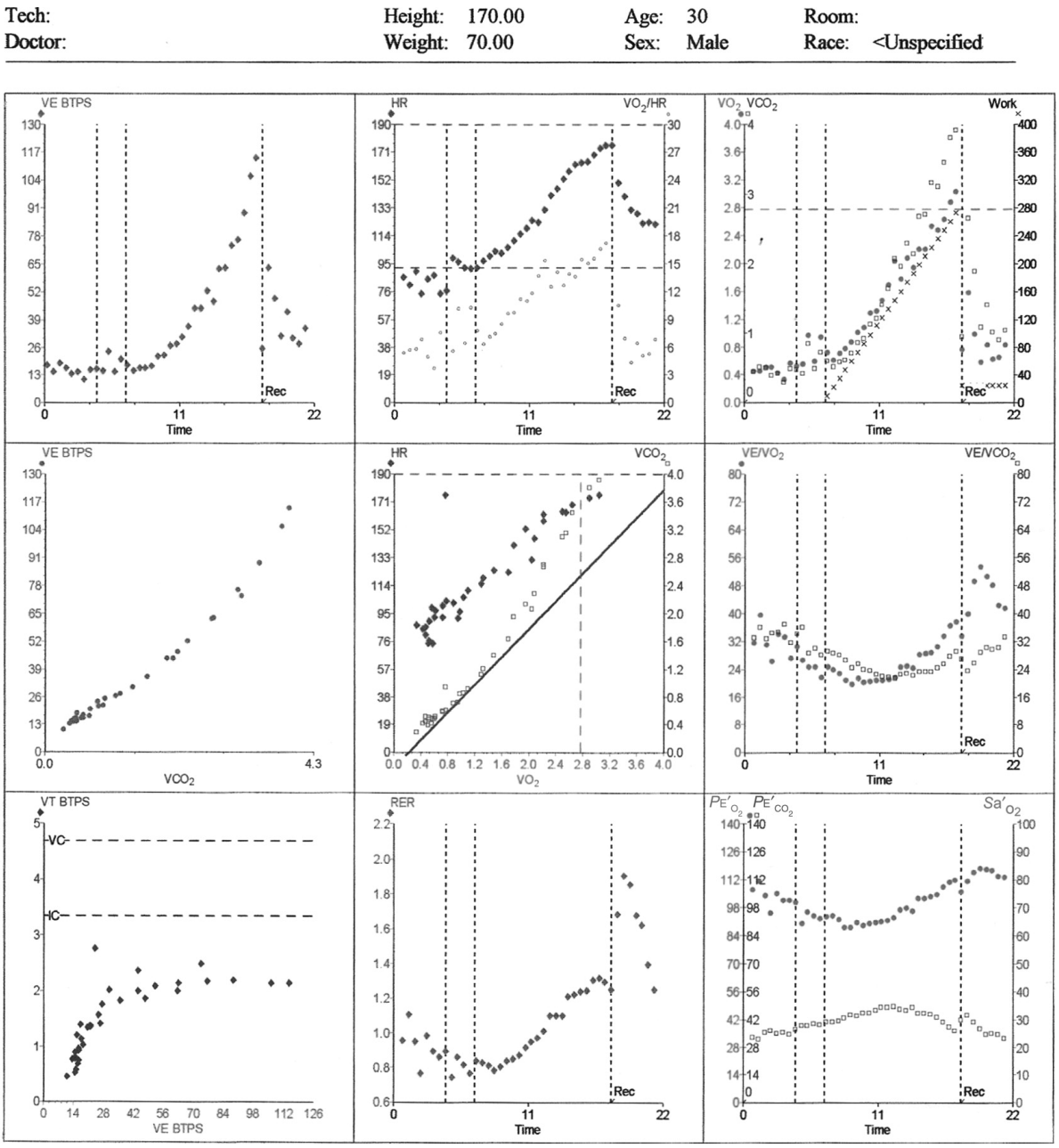

Figure 2-7. The standard nine panel CPET plot

Reproduced from preoperative cardiopulmonary exercise testing (Agnew 2010).
Numbered 1-9 from top left to bottom right. Panels 2,3,5 relate to the cardiovascular system, panels 1,4 and 7 treat ventilation and panels 6, 8 and 9 display ventilation/perfusion relationships.

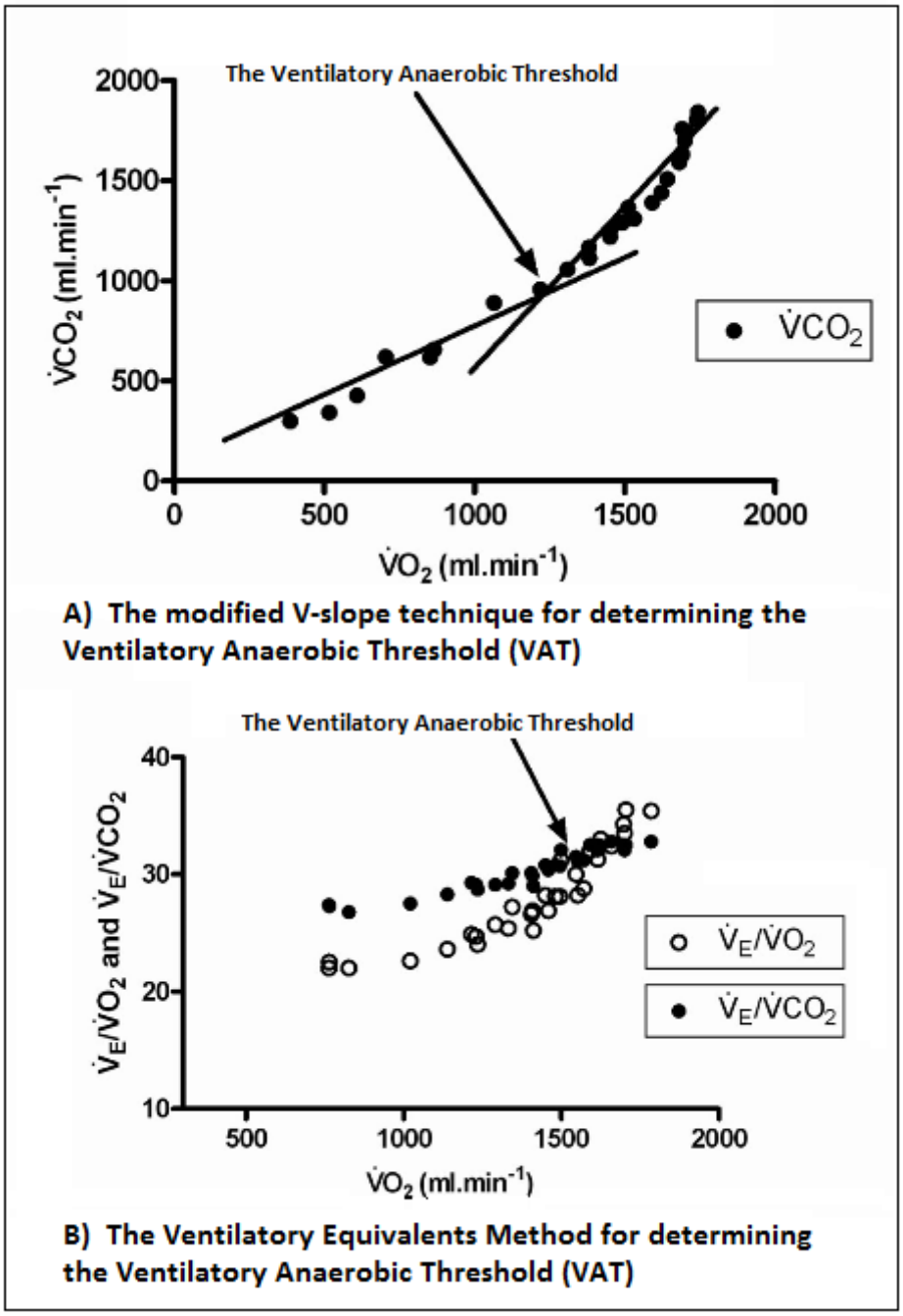

Figure 2-8 Methods for determining the Ventilatory Anaerobic threshold (VAT)

A) The modified V-slope technique: The VAT is the point at which the two regression lines explaining VCO_2 as a function of VO_2 intersect. It is expressed as the VO_2 where the residual sum of squares is minimised.

B) The Ventilatory equivalents method. The VAT is the point at which the V_E/VO_2 begins to increase without an accompanying increase in V_E/VCO_2 and is expressed as the VO_2. Derived from Weissman I. "Clinical Exercise Testing". 4th Ed. Philadelphia: WB Saunders; 1997.

Unless otherwise stated, normal ranges and predicted values for CPET parameters quoted in this thesis are those used routinely at UCLH in the clinical interpretation of Exercise Tests and are outlined in Table 2-7.

Parameter	Predicted Value	Range
VO_2 max (ml/min)	Based on gender, age, height	Lower limit of normal < 80% predicted
Resting VO_2 (ml/min)	150 + (6 X weight in kg)	250 -300 (larger in obese individuals)
Peak Heart Rate (bpm)	220- age	90% predicted ± 15 bpm
Oxygen pulse (ml/beat)	(Predicted VO2 max)÷ (predicted max HR)	80% predicted (~ 15 ml/beat in men; ~ 10 ml/beat in women)
Minute Ventilation (L/min)		Peak Exercise: 70-80% of MVV
Maximum Tidal Volume	60% of the FVC	
V_E/VCO_2 (early exercise)		25-35
V_E/VO_2 (early exercise)		25-35
VD/VT		0.25-0.35 at rest Should decrease with exercise
$P_{ET}CO_2$ (mm Hg)		38-42 (Should decline after ventilatory threshold)
$P_{ET}O2$ (mm Hg)		95-100 (Should rise after ventilatory threshold)
A-a O2 Difference (mm Hg)		Rest: 10 - 20 Peak Exercise: 15-30
SaO_2 (%)		> 95% (Should remain constant with exercise)
Respiratory Exchange Ratio	Rest: 0.8 Peak Exercise: > 1.15	Rest: 0.6-1.0 Peak Exercise: 1.1-1.3

Table 2-7: Normal values and ranges used at University College London Hospital for the interpretation of CPET tests

2.5.1 Dynamic Heart Rate variables collected at time of CPET

The heart rate at rest was determined whilst sitting on the cycle ergometer during the three minutes of rest at the start of the test.

Anticipatory heart rate increase (AHRi) was defined as the difference between the resting heart rate, and the heart rate immediately before the start of loaded exercise.

Peak heart rate was defined as the highest heart rate at the maximum work rate.

This allowed calculation of the heart rate gradient ((HRg): HRg = Peak Heart Rate – Resting Heart Rate).

Chronotropic incompetence was assessed by calculating the adjusted heart rate reserve, which controls for confounding factors including age and resting heart rate. (Brubaker & Kitzman 2011):

$$\frac{\text{(Heart Rate Peak Exercise)} - \text{(Resting Heart Rate)}}{\text{(220-age)} - \text{(Resting Heart Rate)}}$$

Pre-exercise heart rate increase was assessed as the difference between the heart rate at rest and the heart rate measured just before starting ramped loaded pedalling after 3 minutes of 0 Watt (no resistance) exercise (Figure 2-9). Excessive heart rate increase (EHRi) was defined according to population distribution as described in Chapter 6.

Heart rate recovery was defined as the reduction in rate from the heart rate at peak exercise to the heart rate one minute after cessation of loaded exercise (Figure 2-9).

Abnormal heart rate recovery (HRR) was also defined according to population distribution as described in Chapter 3.

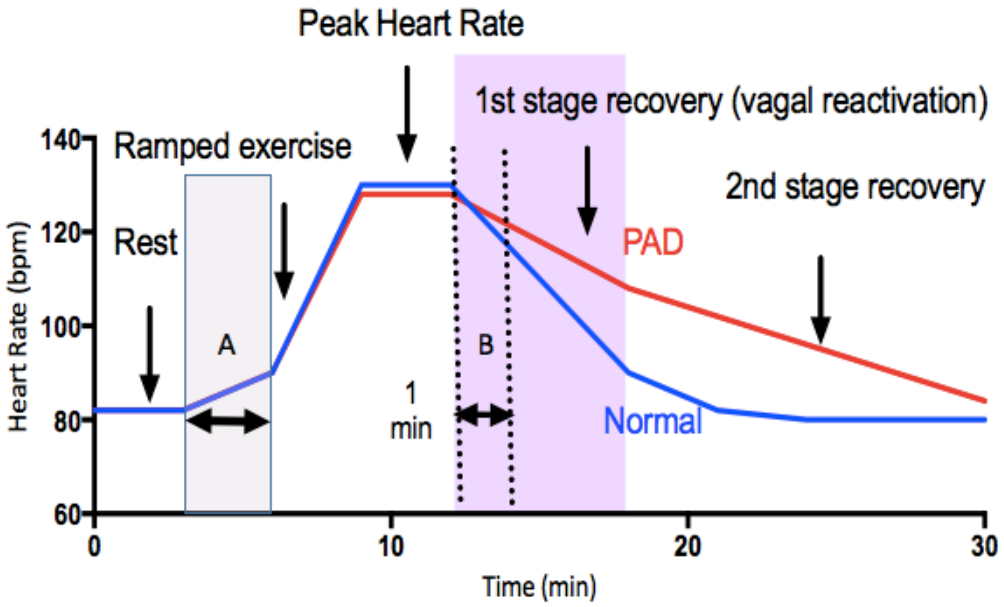

Figure 2-9 Heart Rate dynamics during Cardiopulmonary Exercise Testing

A) Heart Rate increase during unloaded pedalling prior to ramped exercise. Excessive Heart Rate increase (EHRi) was derived from the population distribution of this measurement. B) Heart rate recovery in the first minute after cessation of loaded exercise. Abnormal heart rate recovery (HRR) was derived from the population distribution of this measurement PAD = Parasympathetic Autonomic Dysfunction, bpm= beats per minute.

2.6 Holter Monitoring

Three-lead electrocardiographic recordings were made preoperatively in POM-O trial patients using Lifecard CF digital Holter monitors (Spacelabs Healthcare, Hertford UK) Holter data cleaning and analysis was undertaken blinded to CPET data.

Analysis of Holter monitor data was carried out using Pathfinder Sentinel Software (Spacelabs Healthcare, Hertford UK). Raw ECG recordings were manually reviewed for quality. Patients in Atrial Fibrillation or in other arrhythmias were excluded from analysis (to preserve the validity of heart rate variability analysis). Individual ectopic beats or bursts of ectopy were excluded, as were bursts of arrhythmia (Figure 2-10).

Figure 2-10 Arrhythmias and Ectopic beats were manually edited during Holter analysis

This recording illustrates a ventricular ectopic beat (PVC) captured and edited in an otherwise sinus recording

Following raw data review and editing, files were converted to .RR format (Del Mar™Reynolds™ Holter Binary File) for export to specialised Heart Rate Variability analysis software (Spacelabs HRV Tools Software, Hertford, UK).

2.6.1 Heart Rate Variability Analysis

Heart rate variability (HRV) analysis is routinely used to assess cardiac autonomic regulation through quantification of sinus rhythm variability (hence exclusion of patients in any rhythm other than sinus).

Derivation of the sinus rhythm time series is achieved through the identification of the QRS to QRS interval sequence (RR) and then extracting only normal sinus-to-sinus (NN) interbeat intervals. Whilst the strict definition of the time interval between successive beats would be that interval measured from the start of one QRS complex to the start of the next (QQ), this point can be difficult to measure in noisy or low amplitude ECG recordings. I therefore used the R-wave peak as the reference point for analysis (true R-R; Figure 2-11).

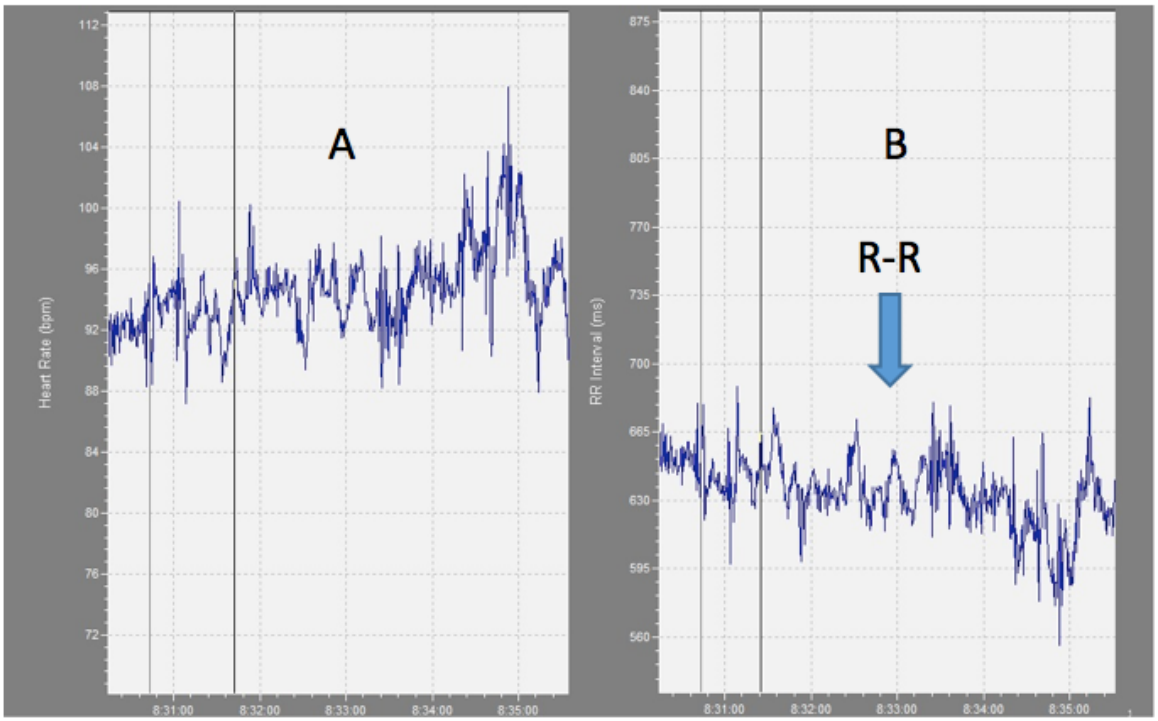

Figure 2-11 R-R interval variation was used as the reference point for heart rate variability

A) Represents heart rate variability (bpm) in an individual over a five-minute reference recording. B) Represents R-R interval (ms) variation over the same five-minute reference recording.

Valid segments of recordings were identified from patients in a quiet environment before the operation. Patients in atrial fibrillation, with frequent ectopy (>1 ectopic beat/minute) and/or other dysrhythmias were excluded. Data quality criteria were in accordance with ACC/ACCS Task Force guidelines (European Society of Cardiology et al. 1996). I assessed time-domain measures from 5-minute recordings, which are preferred to frequency domain methods when only short-term recordings are possible.

All RR files imported from Del Mar^{TM}Reynolds^{TM} Holter analysis had aberrant, HRV inhibited, excluded and paced beat filtration features that had been incorporated into the original Holter edited analysis switched on.

Preoperative and day of surgery Holter data in patients who had been recruited to POM-O and had also undergone routine preoperative CPET testing were selected.

The statistical analysis package incorporated into the Spacelabs software was used to analyse all HRV data. Automatic data editing was enabled to exclude unphysiological or anomalous data. For preoperative variables, data was manually assessed for stability prior to analysis. Five-minute segments of data were analysed for time domain variables.

2.7 Statistical Analysis for Clinical Data

Categorical data were compared using Fisher's exact test or Pearson's Chi squared test and are presented as absolute values (with percentage). Continuous data are presented as median with interquartile range (IQR), or mean with 95% confidence intervals (CI) for normally distributed data. For continuous data, tests for skew were performed to assess normality of distribution. They were compared using the student's t-test for normally distributed data and the Mann-Whitney U test or Kriskall-Wallis test, where appropriate, for non-normally distributed data. Comparisons across grouped variables were made using analysis of variance (ANOVA) with Tukey's post-hoc test for comparisons of pairings within a group. All reported *p* values are two-sided.

Differences in mortality, length of stay and time to become free of morbidity were tested using the log-rank test. Kaplan-Meier curves were plotted for time to hospital discharge, accounting for in-hospital deaths. Adjustment for baseline data was made using a Cox proportional hazards model including age, heart rate recovery, risk factors for cardiopulmonary disease (revised cardiac risk index) and random effect of site. Baseline variables were selected for inclusion in the adjusted analysis according to anticipated relationship with outcome including all variables used in the minimisation algorithm. The Cox model is appropriate for identifying risk factors for prolonged LOS as it does not assume an underlying normal distribution of LOS data (Austin et al. 2002).

The covariates entered into the regression models (age, HRR, AT <11, BMI, RCRI) were chosen either due to association with worsened post operative outcome or due to known influences on cardiac or autonomic performance (Ackland 2009; Khuri et al. 2005; Ackland et al. 2015).

The use of a relatively small number of covariates ensured that the models were adequately powered for the sample populations. Moons recommends that at least 10 subjects are included for each covariate in a regression model (Moons et al. 2009).

Results relating to length of hospital stay and mortality are reported as Hazard Ratios (Mantel-Cox) (HR) with 95% confidence intervals.

Results of the logistic regression model are also reported as adjusted hazard ratios (HR) with 95% CI and Wald test for comparison.

Where and HRR and EHRi were being calculated, the population results were split into quartiles. These are not of even size as heart rate is a whole number. Multiple analyses one-way-analysis-of-variance (ANOVA) was used with post-hoc Tukey test for multiple comparisons of means. Where data was non-Gaussian, a Kruskal-Wallis test was used with Dunn's multiple comparison of quartiles. To compare the

final quartile with the rest of the population an unpaired T-test was used with Welch's correction.

Analyses were performed using SPSS version 20 (IBM, USA). Where appropriate, statistical analysis and graphs were produced using Graphpad Prism (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA) Significance was set at $p<0.05$ (two-tailed).

2.7.1 Sample Size Calculation and Statistical Advice

Dr Steve Harris, Department of Health Services Research and Policy, London School of Hygiene and Tropical Medicine provided statistical advice.

Power calculations for the retrospective analysis of heart rate recovery and excessive heart rate increase are included in the methods sections of the relevant chapters (4 and 6).

2.8 Laboratory work

2.8.1 Miscellaneous Drugs, Chemicals and Reagents

Reagent (batch no. For drugs and antibodies)	Supplier (product no.)	Address
Dulbeccos' Phosphate-Buffered Saline (Calcium and Magnesium-free)(PBS)	Invitrogen Ltd (14190-094)	Paisley, UK
Ficoll-Paque PLUS (Ficoll sodium diatrizoate)	GE Healthcare (17-1440-03)	Buckinghamshire, UK
Dulbecco's Modified Eagle Medium with GlutaMAX, Sodium Pyruvate and 1 $g.L^{-1}$ or 4.5 $g.L^{-1}$ D-glucose (DMEM) Stored at 4^{o}C	Invitrogen Ltd (21885025 & 3196-021)	Paisley, UK
Blood Lysis Buffer: 0.84 g $NaHCO_3$ 7.7g NH_4CL 1000 ml sterilised H_2O (passed through 0.2µm filter) Stored at 4^{o}C	Chemicals from Sigma Aldrich	Dorset, UK
BCA protein assay kit: Reagent A (contains sodium carobonate, sodium bicarbonate, bicinchoninic acid, sodium tartrate in 0.1M sodium hydroxide) Reagent B (contains 4% cupric sulphate) Reagent mixture 50:1	Novagen	Merck, Germany
Heparin sodium 1000$iu.ml^{-1}$ Stored at 4^{o}C in sterile ampoules	Leo laboratories Ltd	Buckinghamshire, UK
Trypan Blue solution (0.4%)	Sigma Aldrich (C2272)	Dorset, UK
Bovine Serum Albumin (lot 099K1447) *Stock Solution 2% (w/v) in sterile PBS* Stored at 4^{o}C	Sigma Aldrich (B4287)	Dorset, UK
Tris base (tris(Hydroxymethyl)Aminomethane)	Chemicals from Sigma-Aldrich	Dorset, UK
Tween	Sigma-Aldrich (P1379)	Dorset, UK
Distilled Water		
Hydrochloric acid (1M and 5M)	Chemicals from Sigma-Aldrich	Dorset, UK
Phosphate buffered Saline (PBS) pH 7.5:	Chemicals from Sigma-Aldrich	Dorset, UK
Tris-buffered saline (TBS) pH 7.6 8g Sodium Chloride 20ml 1M Tris HCl, pH7.6 1000ml distilled water	Chemicals from Sigma-Aldrich	Dorset, UK

Methanol		
Transfer buffer (10x stock), 1L Tris 25mM 30g/L Glycine 192 mM 144 g/L	Chemicals from Sigma-Aldrich	Dorset, UK
Transfer buffer (1x) 400 ml distilled H_20 50ml 10x transfer stock 50ml methanol	Chemicals from Sigma-Aldrich	Dorset, UK
10x Running buffer 1000 ml distilled H_2O 30g TRIS base 144g Glycine 10g SDS	Chemicals from Sigma-Aldrich	Dorset, UK
Laemmli 2x Loading buffer 200 ul 4% SDS 200 ul 10% 2-mee 200 ul 20% glycerol 200 ul 0.125 M Tris HCl	Chemicals from Sigma-Aldrich	Dorset, UK
TEMED	Sigma-Aldrich (T9281)	Dorset, UK
30% Acrylamide	Sigma-Aldrich (A3699)	Dorset, UK
10% SDS	Sigma-Aldrich (L3771)	Dorset, UK
10% ammonium persulphate (APS)	Sigma-Aldrich (A3678)	Dorset, UK
PBS-Tween (PBS-T) 0.1% Tween in PBS	Sigma-Aldrich (P1379)	Dorset, UK
5% Non-fat dried milk (blocking reagent) in PBS-T	Marvel milk powder	Premier foods, St Albans, UK
Cell Lysis buffer (RIPA buffer) 150 mM sodium chloride 1.0% Triton X-100 0.5% sodium deoxycholate 0.1% SDS (sodium dodecyl sulphate) 50 mM Tris, pH 8.0	Chemicals from Sigma-Aldrich	Dorset, UK
Protease inhibitor	GE Healthcare, (80-6501-23) (Sigma)	Dorset, UK
ECL Western Blotting substrate	Pierce	ThermoFIsher Scientific, UK

2.8.2 Antibodies (Western Blot)

Antibody	Clone (product/batch)	Supplier	Utility
GRK2	sc-13143 mouse monoclonal raised against GRK2 of human origin IgG_{2a}	Santa Cruz, Heidelberg, Germany	Immunoblot identification in lysed human lymphocytes
α -tubulin	sc- 53646 mouse monoclonal raised against full length α –tubulin of human origin IgM	Santa Cruz, Heidelberg, Germany	Housekeeping Protein. Reference for Immunoblotting
Peroxidase linked secondary antibody	Rabbit IgG Horeseradish Peroxidase-linked whole antibody	GE healthcare, Amersham, UK	For detection of mouse membrane bound primary antibodies

2.8.3 Miscellaneous equipment and disposables

Equipment	Supplier	Address
Centrifuge: Haraeus Megafuge 1.0R	Kendro Laboratory products	Langenselbold, Germany
Centrifuge: ALS PK 120	ALC	Cologno Monzese Italy
Centrifuge: Eppendorf 5415 C	Eppendorf	Hamburg, Germany
15ml Falcon polypropylene tubes	BD Biosciences	Oxford, UK
50 ml Falcon polypropylene tubes	BD Biosciences	Oxford, UK
250 ml Falcon polypropylene tubes	BD Biosciences	Oxford, UK
1.5 ml microcentrifuge tubes	StarLab GmBH	Ahrensburg, Germany
Costar disposable serological pipettes (10, 25ml)	Corning International	NY, USA
10, 200 and 1000uL Diamond sterilised pipette tips	Gilson Inc	Middleton, USA
96-well plates: Nunclon	Nalge Nunc International	NY, USA
3 ml sterile Pasteur pipettes	Ramboldi Ltd	Limassol Cyprus
Biological Safety Cabinet Class II Holten LaminAir Model 1.2	Thermo Scientific	Waltham, USA
Bench microscope: Zeiss Axiovert 25	Zeiss	Oberkochen, Germany
Neubauer improved haemocytometer	Assisstent	Germany
Synergy Mx monochromator-biased multi-mode microplate reader	BioTek	Winooski, USA
Gel Electrophoresis Apparatus	Mini Protean™ tetra vertical hand	Bio-Rad UK

	cast electrophoresis cell sytem	
Semi-dry blotting apparatus	Transblot™ semi-dry transfer cell	Bio-Rad UK
Orbital shaker		
X-ray film cassette		
X-ray film		
Blotting Membrane	Hybond™ Amersham ECL (nitrocellulose)	GE Healthcare, USA
Full range Rainbow molecular weight marker, natural	RPN800, Amersham	GE Healthcare, USA
Heating Block		
Filter paper for Western Blot	Bio-Rad UK	Bio-Rad UK

2.8.4 Isolation of human mononuclear cells

Twenty ml blood was drawn at rest from patients on the day of CPET, prior to exercise testing. This blood was subsequently heparinised (final concentration 50 $iu.ml^{-1}$) and placed on ice until leucocyte separation (within 1 hour of blood draw).

For separation, the blood was diluted in a 2:1 ratio with PBS at room temperature and carefully pipetted onto Ficoll-Paque in a 50ml centrifuge tube (30ml blood: 15 ml Ficoll-Paque). This blood was centrifuged for 30 minutes at 20^{o}C (1400 rpm) without centrifuge brake (Figure 2-12).

The plasma layer was subsequently aspirated using a sterile Pasteur pipette. The layer containing peripheral blood mononuclear cells (lymphocytes and monocytes) was then carefully aspirated to avoid mixing with Ficoll-Paque and washed twice (mixed with 3x volume of PBS at 4^{o}C and centrifuged at 300 x g for five minutes).

The resulting cell pellet was then resuspended in a set volume of DMEM and a manual cell count established with Trypan Blue dye and a Neubauer-improved haemocytometer. The cells were then resuspended and frozen at a concentration of 1000,000 cells/ml.

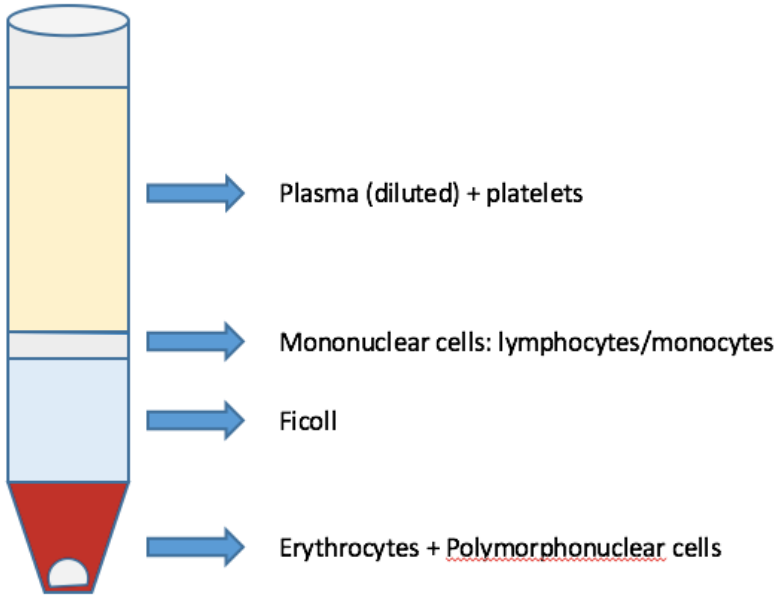

Figure 2-12 Ficoll density gradient separation of mononuclear cells

Lymphocytes were isolated from human whole blood using Ficoll density grade separation. The Mononuclear layer settles above the Ficoll layer and is used for immunoblotting.

2.8.5 Preparation of human mononuclear cells for Immuno- (Western) blot analysis: cell lysis

Isolated human mononuclear cells, pelleted and frozen at 1000,000 cells/ml were brought to room temperature and subsequently mixed with lysis (RIPA) buffer & protease inhibitor (manual disruption of cells was encouraged with vigorous pipetting and continual assessment of pellet viscosity). RIPA buffer was chosen since GRK2 is often membrane-bound (Thermo-Fisher Scientific cell and protein isolation technical handbook 2014). Tris-Triton buffer was also assessed during protocol development and found inferior.

Following room temperature lysis, samples were heated to 95^0C in a heating block for 5 minutes, centrifuged, the supernatant being transferred to clean Eppendorf tubes and then allowed to cool on ice.

Samples protein concentrations were subsequently assessed by BCA protein assay.

2.8.6 Preparation of human lymphocytes for Immuno- (Western) blot analysis: BCA Protein Assay

The BCA Protein Assay is used to determine protein concentrations in the range of 20 to 20,000 ug/ml. The standard manufacturer's assay was used to determine protein concentrations in isolated lymphocyte pellets (as per user protocol TB380 Rev.D0111JN (Novagen, Merck, Germany, 2011)).

2.8.7 Preparation of human mononuclear cells for Immuno- (Western) blot analysis: SDS-PAGE gel preparation and loading

All Immunoblot techniques were carried out in reference to the Amersham ECL Western blotting detection reagents and analysis system protocol handbook (RPN2106/8/9).

10% SDS-Page gels were prepared using standard gel recipes (Sigma-Aldrich/Amersham healthcare). Lysed protein samples were loaded with Laemmli buffer (1:1) into wells produced using 4% stacking gel (Mini Protean^{TM} tetra vertical hand cast electrophoresis cell system). 20 micrograms of protein were loaded per cell. A standard protein ladder was also loaded.

Gels were then run for 45 to 60 minutes at 150v (0.05 Amp maximum).

2.8.8 Western (Immuno-) blotting

SDS-PAGE separated proteins were transferred electrophoretically to a support matrix or membrane. The proteins were then visualized by application of a specific primary antibody (in this case GRK2) after blocking of unoccupied sites (5%-milk-PBS-T), to the membrane. Visualisation was achieved by binding of a secondary antibody to the primary that is conjugated to a chemiluminescent molecule (horseradish peroxidase). Subsequent activation and exposure to X-ray film was followed by development. Semi-quantitative measurement of specific protein abundance could then be carried out, with reference to the molecular weight

indicated by a standard protein ladder, using densitometry normalized to an internal standard (alpha-tubulin).

Following SDS-PAGE gel electrophoresis, samples were transferred onto a nitrocellulose membrane for 45-60 minutes at 15V using SD Semi-dry Transblot Apparatus (Bio-Rad).

Following rinsing and washing in PBS-T (3x 10 minutes), membranes were blocked at room temperature on a rocker for 1 hour in 5% milk-PBS-T.

Membranes were then washed in PBS-T again and incubated overnight at 3^{o}C with 8ul of GRK2 antibody in PBS-T (1:250).

The following morning, membranes were washed and incubated with appropriate secondary-HRP antibodies (1:2000) for one hour on a rocker at room temperature. Membranes were subsequently rinsed and washed as above.

Blotting substrate (Pierce^{TM} chemiluminescent substrate) was then applied to membranes according to manufacturer's specifications. Membranes were developed in a dark room on to X-ray film.

Subsequent re-probing of developed membranes was carried out using alpha-tubulin as an internal control standard. Again, membranes were developed and catalogued as above.

2.8.9 Densitometry

Developed membranes were scanned and catalogued. Densitometry was performed using Image-J (Abràmoff et al. 2004), referenced to alpha-tubulin (internal standard)

3 Exercise physiology in Parasympathetic Autonomic Dysfunction

3.1 Introduction

Heart rate recovery after exercise, which reflects reactivation of parasympathetic tone (Imai et al. 1994), is a robust, readily obtainable marker of parasympathetic autonomic function. Abnormal recovery, representing parasympathetic autonomic dysfunction (PAD), is independently associated with long-term cardiovascular morbidity and all-cause mortality in the general medical population (individuals otherwise asymptomatic) (Cole et al. 2000; Shishehbor et al. 2006; Jouven et al. 2005).

To date, no assessment has been made of the impact of established PAD on surgical outcomes. Impaired preoperative exercise physiology, in particular a reduced Anaerobic Threshold (AT), has been associated with increased postoperative morbidity (Older et al. 1999). Plausible mechanisms, outlined below, exist to suggest that PAD could cause impaired exercise performance.

3.1.1 Heart rate recovery and PAD

Determination of normal ranges for heart rate recovery after exercise in adult surgical patients referred for CPET has not been carried out previously. Previous studies in other contexts have stratified values for heart rate recovery according to quintile or quartile of decrease in heart rate after cessation of activity (Jouven et al. 2005; Cole et al. 1999) or by fitting values for heart rate recovery to first order exponential decay curves (Pierpont et al. 2000; Imai et al. 1994). The value used to represent abnormal heart rate recovery depends very much on the population studied. In otherwise healthy patients, values below 18 bpm and 24 bpm have been defined as abnormal. In athletes, even greater reductions could be considered abnormal (Imai et al. 1994).

The situation is different in patients with known or suspected cardiac morbidity. In 2,428 cardiac patients referred for diagnostic symptom limited exercise testing, a heart rate recovery of less than 12 beats per minute, representing the lowest quartile measured, was independently associated with a two times increase in risk of all cause death (Cole et al. 1999). Similarly, in 9,454 patients referred for exercise ECG testing, using a cut off heart rate recovery of 12 beats per minute, an increase in risk of all cause mortality of 2.1 times was seen, independent of other known risk factors (Nishime et al. 2000).

In general, patients referred for preoperative CPET fall into increased risk categories either by virtue of existing comorbidity and/or high-risk surgery. It might therefore be expected that in this group, the spread of heart rate recovery values could reflect this risk.

In order to determine thresholds for normal and abnormal heart rate recovery in surgical patients referred for preoperative CPET, the same approach, extensively adopted across the wider literature, of stratifying values according to quartile of heart rate recovery was taken in a derivation cohort and a larger validation cohort.

Since abnormal heart rate recovery has not been previously used as a method for determining risk prior to surgery, association of heart rate recovery as a marker of reduced cardiac parasympathetic activity using established values for heart rate variability was carried out in a subset of patients.

3.1.1.1 Impaired heart rate recovery after exercise may signify functional cardiac impairment

Convincing evidence from clinical and laboratory studies conducted in other populations indicate that PAD is strongly associated with, and may be mechanistically responsible for, impaired cardiac performance and therefore reduced exercise performance.

Impaired heart rate recovery after exercise is associated with cardiac failure and reduced exercise capacity (Imai et al. 1994; Ritt et al. 2012). Although the predictive value of reduced heart rate recovery for mortality persists even when impaired left ventricular systolic function, estimated by echocardiography, is controlled for, it remains most strongly associated with functional cardiac impairment (Watanabe et al. 2001).

Formal cardiopulmonary exercise testing allows assessment of the cardiac contractile response to stress (increasing external work done) through measurement of the oxygen pulse, a robust proxy measure of left ventricular stroke volume (Whipp et al. 1996). Oxygen pulse, adjusted for age and sex, at peak VO_2 was therefore used to identify any contractile impairment in response to physiological stress, in individuals with PAD.

3.1.2 Heart Rate Dynamic changes in PAD

Chronotropic incompetence, or the inability of the heart to increase its rate commensurate with increased activity or demand, has frequently been described in both PAD and heart failure (Brubaker & Kitzman 2011) and is in itself associated with increased long term mortality in cardiac patients (Lauer et al. 1999; Dhoble et al. 2014).

Using heart rate dynamic changes recorded during exercise in high-risk surgical patients, the majority without a formal diagnosis of cardiac failure, an

association of chronotropic incompetence with PAD was sought in a novel context.

3.1.3 Cardiac Ischaemia and Parasympathetic Autonomic Dysfunction

Abnormal heart rate recovery is associated with an increased risk of all cause death in cardiac patients (Cole et al. 2000; Nishime et al. 2000). In those with known ischaemic heart disease, abnormal heart rate recovery has been used to predict the severity of coronary artery disease (Ghaffari et al. 2011).

Adequate vagal activity is likely to be protective during myocardial ischaemia (Kakinuma et al. 2014; Frank et al. 2012; Zhang et al. 2014). Vagal withdrawal occurs both before and during cardiac ischaemia (Sroka et al. 1997) and is predictive of impaired outcome (La Rovere et al. 1998). Abnormal heart rate recovery has been associated with myocardial ischaemia as assessed by myocardial perfusion imaging (Georgoulias et al. 2003). Restoration of vagal tone, or markers thereof, in models of cardiac ischaemia results in preservation of the ischaemic myocardium and reduction in the risk of post-reperfusion arrhythmias (Kakinuma et al. 2009; Kakinuma et al. 2005; Ando et al. 2005; Kakinuma et al. 2013; Li et al. 2011).

It is not known whether established vagal withdrawal is related to electrocardiographic or functional markers of cardiac ischaemia during exercise testing in human surgical patients. Perioperative myocardial ischaemia is clearly associated with impaired outcome (Devereaux, Chan, et al. 2014; Landesberg et al. 2009), but not always associated with a formal preoperative diagnosis of ischaemic heart disease (Devereaux et al. 2012; Devereaux, Chan, et al. 2014). Alternative predictors of myocardial ischaemia at times of physiological stress would therefore be of value.

Abnormal heart rate recovery after exercise was therefore investigated as one such marker.

3.1.4 Alterations in G-protein receptor kinase activity underlie functional changes in both myocardial and immune cells

G protein-coupled receptors (GPCRs) are diverse, therapeutically important and play multiple central roles in the physiology of various organs, including the heart. The beta adrenoreceptor and angiotensin II type-1 receptor are key and relevant examples. Signalling initiated by binding of an agonist to the receptor is terminated in part by phosphorylation of the receptor by the family of G-protein coupled receptor kinases (GRKs) (receptor desensitisation) followed by subsequent β-arrestin binding, which uncouples the phosphorylated receptor and G protein and subsequently targets the receptor for internalisation (Lymperopoulos 2011).

In both cardiac failure and critical illness related myocardial dysfunction, beta adrenergic signal transduction is reduced secondary to receptor desensitisation and internalisation, modulated in large part by GRK2 (Lymperopoulos et al. 2013; Lymperopoulos 2011; de Montmollin et al. 2009).

Beta adrenoreceptor expression in circulating lymphocytes mirrors that seen in cardiomyocytes in heart failure. Similarly, GRK2 expression in lymphocytes, which is increased in human cardiac failure, has been demonstrated to not only mirror expression in the cardiomyocyte, but to be related to both the severity of the underlying pathology and its pathogenesis (Iaccarino et al. 2005; Liggett 2005; Maisel et al. 1990).

Vagal withdrawal is strongly associated with and likely mechanistically linked to human cardiac failure. Abnormal heart rate recovery in surgical patients, if representative of impaired cardiac function, would be expected to be associated with neurohormonal activation (including elevations in both adrenal catecholamine release and activation of the renin-angiotensin-aldosterone axis (RAAS) – well described in known heart failure (Anker 1998)), and would be expected to be associated with alterations in the dynamics of beta

adrenoreceptor recycling mediated through GRK2 and its downstream effects (Lymperopoulos 2011).

Preoperative blood samples taken from surgical patients referred for CPET provided an opportunity to assess circulating lymphocytes for evidence of alterations in GPCR modulation thorough a G protein coupled receptor kinase (GRK2) commonly elevated in cardiac failure and link these changes to alterations in vagal activity as assessed by heart rate recovery.

3.1.5 Assessment of the exercise phenotype associated with PAD

Exercise represents an integrated cardiometabolic and respiratory response to physiological stress. To date, the majority of studies that have used heart rate recovery after exercise to define PAD, have used the Bruce protocol, or similar, to investigate heart rate dynamic changes. None have used formal Cardiopulmonary Exercise Testing (CPET). This has not allowed full examination of integrated physiology associated with PAD.

The use of CPET to define PAD allows a thorough examination of the exercise phenotype associated with parasympathetic withdrawal and the opportunity to investigate cardiac performance in a controlled clinical setting.

Using currently accepted physiological parameters, routinely recorded during standard preoperative cardiopulmonary exercise testing, I set out to describe physiological and electrocardiographic patterns of response to graded exercise in patients with heart rate recovery defined PAD.

3.2 Hypothesis

Individuals with Parasympathetic Autonomic Dysfunction demonstrate a distinct physiological phenotype in response to exercise.

3.3 Aims

Primary

- Describe the physiological response to exercise in surgical patients with PAD using standard CPET variables.

Secondary

- Describe the distribution of heart rate recovery values at preoperative exercise testing in a surgical population.
- Associate abnormal heart rate recovery, as a measure of PAD, with heart rate variability measures indicative of cardiac parasympathetic autonomic dysfunction.
- Describe heart rate dynamic changes during exercise testing in a surgical cohort with PAD.
- Identify whether electrocardiographic markers of cardiac ischaemia are more common during exercise in individuals with PAD than in those with normal heart rate recovery.
- Assess circulating lymphocytes from patients with varying degrees of PAD for evidence of alterations in G-protein receptor kinase 2 expression commonly observed in heart failure.

3.4 Methods

3.4.1 Patient Population

Two cohorts at separate centres were studied before planned elective surgery. Patients scheduled to undergo major colorectal surgery were enrolled at Derriford Hospital Plymouth (DHP) and University College London Hospitals (UCLH) having obtained IRB approval (MREC: 11/H0805/58). Informed written consent was obtained from patients undergoing preoperative CPET as routinely requested by their clinical teams prior to major elective surgery. Inclusion criteria were any surgical patient referred for preoperative CPET. Exclusion criteria were according to American Thoracic Society guidelines (Weisman et al. 2003).

All patients enrolled underwent routinely requested maximal, incremental, symptom limited CPET prior to surgery. The full protocol for CPET is in General Methods.

3.4.2 Assessment of Heart rate recovery

The expected heart rate profile during exercise of individuals with and without PAD is illustrated in Figure 3-1. Heart rate recovery was defined as the reduction in rate from the heart rate at peak exercise to the heart rate one minute after cessation of loaded exercise.

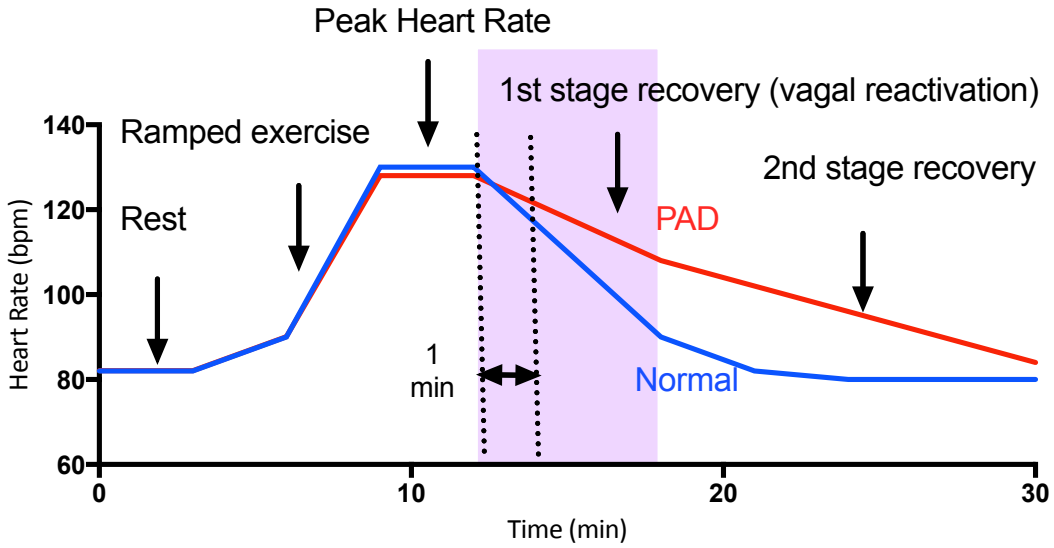

Figure 3-1: Heart rate recovery Profiles in Parasympathetic Autonomic Dysfunction (PAD) and Normal individuals.

Heart rate recovery after peak exercise is normally biphasic with a rapid recovery in the first two minutes after exercise, representing return of vagal tone (pink box), being followed by a slower recovery over subsequent minutes to baseline. Individuals with reduced parasympathetic autonomic activity exhibit delayed heart rate recovery (HRR; red line) as compared to normal individuals (blue line). Heart rate recovery at 1 minute after cessation of exercise was recorded and assessed as measure of return of vagal activity.

3.4.3 Association of Heart rate recovery with heart rate variability measures of Parasympathetic Autonomic Dysfunction in surgical patients

Association of Heart rate recovery with Heart Rate Variability (HRV) measures of parasympathetic autonomic dysfunction was carried out on a subset of 46 patients who had prospectively undergone Holter heart rate variability analysis and preoperative cardiopulmonary exercise testing at University College London Hospital. These patients were enrolled between May 2010 and February 2014 in the POM-O trial of early postoperative goal directed therapy (full protocol and publications related to trial available in general methods and supplemental materials) (Ackland et al. 2015).

Three-lead electrocardiographic recordings were made preoperatively in a quiet environment between 07:00 & 09:00 on the day of surgery using Lifecard CF digital Holter monitors (Spacelabs Healthcare, Hertford UK). Patients in atrial fibrillation, or with frequent ectopy (>5/minute) were

excluded. Data quality criteria were in accordance with task force guidelines (Malik et al. 1996). Holter data cleaning and analysis was undertaken blinded to all other physiological and perioperative outcome data.

Immediate postoperative recordings of heart rate variability on arrival in the post anaesthetic care unit (PACU) were also taken. These recordings were taken at time points free of any other acute medical intervention.

Time domain measures were selected based on signal stability and analysed from at least one stable five minute recorded segment per hour (Spacelabs HRV Tools Software, Hertford, UK). Frequency domain parameters were not assessed, as only short-term recordings were available, potentially reducing the validity of frequency domain measures.

The square root of the mean of the sum of squares of the successive differences between adjacent beat-to-beat intervals (RMSSD) was assessed as being most representative of parasympathetic activity (Nunan, Sandercock, et al. 2010).

Outcome measures were not recorded from these patients since immediate postoperative management was dictated by trial protocol and has been reported elsewhere (Ackland et al. 2015).

3.4.4 Assessment of Heart Rate Dynamic changes in PAD

Chronotropic incompetence was assessed by calculating the adjusted heart rate reserve, which controls for confounding factors including age and resting heart rate (Brubaker & Kitzman 2011).

3.4.5 Electrocardiographic assessment of cardiac ischaemia at peak exercise

Continual three lead ECG recording was carried out throughout exercise testing. Lead II was chosen for investigation as the most easily and reliably obtained during exercise.

The nadir ST value at any time during exercise testing was taken as representing the most significant cardiac ischaemia. ST depression was defined as abnormal when ST depression of 0.1 mV (1mm) or more was recorded (Lurati Buse et al. 2009).

Patients in whom baseline (resting) ST segments were recorded as abnormal were excluded from further analysis. In order to control for the effects of dynamic heart rate changes on the ST segment in ischaemia during exercise, the ST-heart rate (ST/HR) index was calculated (Kligfield 2008; Okin et al. 1996):

$$\frac{\text{ST nadir (mV)}}{\text{Heart Rate Gradient}} * 100$$

Postoperative laboratory blood test data was available from UCLH only. Any cardiac troponin test result requested during the first postoperative week was recorded as evidence of clinical suspicion of myocardial ischaemia in the immediate postoperative period.

3.4.6 Immuno- (Western) blot analysis of circulating mononuclear cells for GRK2

Peripheral blood samples were taken from 20 randomly selected patients prior to cardiopulmonary exercise testing. These samples were placed on ice and transferred immediately to the laboratory for processing.

Mononuclear cells were isolated using Ficoll density gradient centrifugation, as described in general methods.

Immunoblot analysis was carried out as described in general methods.

3.5 Results

3.5.1 Definition of Abnormal Heart rate recovery (HRR)

The distributions of heart rate recovery values recorded at both sites and combined are displayed in Table 3-1 and Figure 3-2. The 25th centile for Heart rate recovery was 12 bpm, corresponding to similar distribution in other medical populations (Cole et al. 2000; Nishime et al. 2000; Shetler et al. 2001). Mean Heart rate recovery for the entire cohort was 18 (17-21) bpm. A heart rate recovery of ≤12 bpm was therefore taken as abnormal when comparisons were made between normal and abnormal parasympathetic autonomic function.

Descriptor	DHP	UCLH	Combined
Total Number of values	235	814	1048
Total Number of excluded values:	0	0	0
Minimum HRR (bpm):	-7	-10	-10
25% Percentile HRR (bpm):	**12**	**12**	**12**
Median HRR (bpm):	17	16	16
75% Percentile HRR (bpm):	27	26	26
Maximum HRR (bpm):	71	92	92
Mean Heart rate recovery (bpm):	19 (17-21)	18 (16-19)	18 (17-19)

Table 3-1: Frequency Distribution Statistics for Heart rate recovery.
bpm= beats per minute.

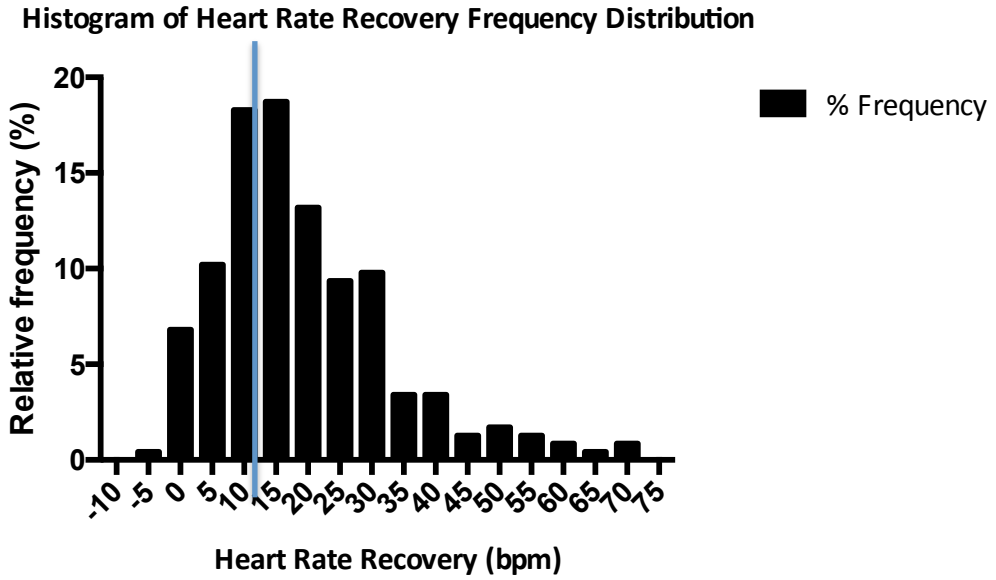

Figure 3-2: Frequency Distribution for Heart rate recovery after cessation of exercise.

The 25% Percentile is marked and is used from now on as the delineator for abnormal heart rate recovery.

3.5.2 Association of abnormal Heart rate recovery with Heart Rate Variability measures of cardiac Parasympathetic Autonomic Dysfunction.

46 individuals who had undergone preoperative CPET testing and Holter ECG recording in the POM-O trial were classified according to heart rate recovery. As described previously, a heart rate recovery of 12 bpm or below was classified as abnormal. Baseline preoperative Holter recordings were analysed in these same individuals for heart rate variability characteristics.

Reduced heart rate recovery after exercise was associated with relative reductions in resting Heart Rate Variability (Table 3-2, Figure 3-3). In the recordings examined, patients classified as having normal heart rate recovery demonstrated values for rMSSD below that of the reported population norm of 42 ms (36.6ms (± 1.9) for HRR >12).

On arrival to PACU, patients without preoperative PAD demonstrated relatively increased heart rate variability as compared with their counterparts with PAD (p=0.07). Neither individuals with PAD nor those with normal preoperative heart rate recovery demonstrated significant drops in heart rate variability on immediate postoperative Holter recordings in PACU (p=>0.1)

Characteristic	Normal HRR (HRR >12)	PAD (HRR ≤12)	P-value
Preoperative rMSSD (ms)	36 ± 2.3	23.4 ± 4.4	0.02
Postoperative rMSSD (ms)	31 ± 3.2	21±3.8	0.07

Table 3-2: Comparison of Heart rate recovery (HRR) and Heart Rate Variability as measures of Parasympathetic Autonomic Dysfunction. MSSD = root mean square of standard deviation of heart rate. PAD = Parasympathetic Autonomic Dysfunction. Values shown are mean (Standard Error of the mean) unless otherwise stated. P-values are for student's unpaired t-test.

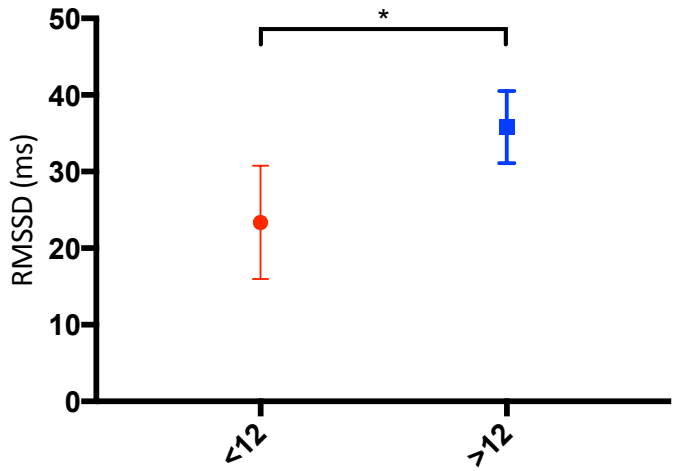

Figure 3-3: Association of Heart rate recovery (HRR) with preoperative Heart Rate Variability as measures of Parasympathetic Autonomic Dysfunction. rMSSD = root mean square of standard deviation of heart rate. Values shown are mean (95% CI). Comparisons were made with Student's un-paired t-test. Asterisk signifies a p <0.05.

Pre and Immediate postoperative values for rMSSD were positively correlated (n=120, Pearson r (95% CI): 0.42 (0.25-0.56); p=<0.001) that is, a low value for heart rate recovery was associated with a low immediate postoperative value and vice-versa (Figure 3-4).

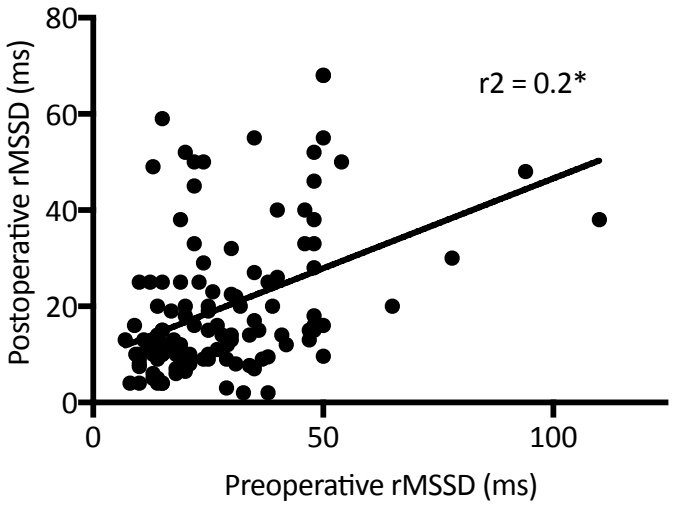

Figure 3-4: Correlation of Preoperative and Postoperative heart rate variability (rMSSD (ms)). rMSSD = root mean square of standard deviation of heart rate. R^2 is for Pearson's correlation coefficient. Asterisk signifies significance (p=<0.05

3.5.3 CPET characteristics in PAD

Baseline CPET characteristics for the two hospital cohorts are displayed in Table 3-3. Full demographics divided according to heart rate recovery are available in Table 4-5.

When combined, the median age across the two cohorts was 66 years, though the Plymouth cohort was older (64 vs. 70 years).

The greater proportion of patients studied were male (62%). Median Anaerobic threshold across both cohorts was low, approaching the prognostically important value of 11 $ml.kg.min^{-1}$. Values for other commonly recorded CPET variables were comparable across cohorts and appropriate for the population studied (Figure 3-10).

CPET Physiology	University College London Hospital (n=817)	Derriford Hospital Plymouth (n=235)	Combined (n= 1052)
Age, years (median, IQR)	64 (55-72)	70 (60-76)	66 (56-73)
Male (n, [%])	573 (65)	133 (56)	706 (62)
Resting Heart Rate (bpm)	81 (72-93)	82 (73-92)	82 (72-93)
Peak Heart Rate (bpm)	137 (121-153)	142 (124-159)	138 (122-154)
Anaerobic Threshold $ml.kg^{-1}min^{-1}$	11.2 (9.3-13.3)	12.2 (10.4-14.4)	11.3 (9.5-13.5)
Peak VO_2 $ml.kg^{-1}min^{-1}$	17 (13 – 20)	19.1 (15.5 – 23)	17.5 (14-21)
Peak VO_2 (% Predicted)	74 (58-88)	76 (60-90)	75 (59-89)
Oxygen Pulse at AT $ml.beat^{-1}$	11 (8.9-13.6)	10.6 (8.2-13.4)	10.9 (9-13.5)
V_E /VCO_2 at AT	29.4 (26.5-33.6)	26.6 (22.2-30.6)	28.3 (25.3-31.4)

Table 3-3: CPET characteristics of the two study populations. Values shown are median and interquartile range (IQR) unless otherwise stated. N=number; bpm, beats per minute; AT, Anaerobic threshold; VO_2, oxygen uptake; V_E/CO_2, Ventilatory Equivalents for Carbon Dioxide.

CPET physiological characteristics for both cohorts combined, divided according to heart rate recovery are presented in Table 3-4 and Figure 3-10.

Proportionately a greater number of patients with PAD had an AT <11 (55% vs. 40% in patients with HRR >12), which has been previously associated with poor postoperative outcomes (RR 1.26 (95% CI 1.1 to 1.6); p=0.02). PAD was also associated with a 10% reduction in peak VO_2 as predicted by age and sex when compared with individuals with normal heart rate recovery (% of predicted VO_2 66% (63.8-69.4) in PAD as compared with 76% (74.8-78.5) in normal HRR).

Oxygen pulse, a validated surrogate for left ventricular stroke volume (Whipp et al. 1996; Crisafulli et al. 2007), was reduced in patients with PAD at peak exercise. Ventilatory equivalents for CO_2 at anaerobic threshold fell within normal range (25-35) for both groups.

CPET Physiology (UCLH and DHP combined):	Normal HRR N=729 (HRR >12)	PAD N=317 (HRR ≤12)	P-value
AT (mean, 95% CI)	13.2 (12-14)	11.1(10.6-11.6)	<0.0001
Peak VO_2 (mean, 95% CI)	18.8 (18.4-19.2)	15.2 (14.8-15.8)	<0.0001
% of Predicted Peak VO_2 (mean, 95% CI)	76 (74.8-78.5)	66 (63.8-69.4)	<0.0001
Oxygen Pulse (mean, 95% CI)	14.6 (13-16)	10.6 (10-11)	<0.0001
V_E /CO_2 at AT (mean, 95% CI)	26 (25-27)	26.3 (24-28)	0.77

Table 3-4: Cardiopulmonary exercise testing (CPET) physiological and autonomic characteristics for individuals with and without Parasympathetic Autonomic Dysfunction (PAD). University College London and Derriford Hospital Plymouth data combined. bpm= beats per minute. AT = Anaerobic threshold (ml O_2 kg^{-1} min^{-1}). VO_2 = Oxygen uptake (ml O_2 kg^{-1} min^{-1}). Oxygen Pulse (ml O_2 $beat^{-1}$). V_E/VCO_2 = ventilatory equivalents for Carbon Dioxide (Normal range 25-35). Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for student's unpaired t-test.

When categorised by quartile of heart rate recovery, peak oxygen pulse increased with each step change in heart rate recovery, plateauing between quartile 3 and 4 (Figure 3-5, Table 3-5).

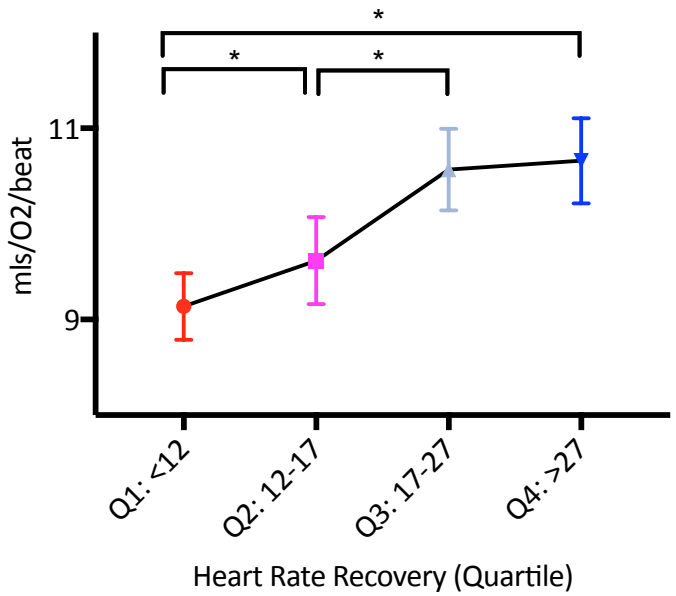

Figure 3-5: Oxygen Pulse (ml.O_2.$beat^{-1}$) classified according to quartile of heart rate recovery. Mean + SD. UCLH data. Heart rate recovery:bpm. * signifies p <0.05 (ANOVA)

Oxygen Pulse ($ml.O_2.beat^{-1}$)	Quartile 1: HRR <12 bpm	Quartile 2: HRR 12-17 bpm	Quartile 3: HRR 17-27 bpm	Quartile 4: HRR >27 bpm
Mean	9.2	9.6	10.6	10.7
95% CI of mean	8.8-9.5	9.2-10	10.1-11	10.2-11.1

Table 3-5: Oxygen Pulse ($ml.O_2.beat^{-1}$) classified according to quartile of heart rate recovery (UCLH data). HRR: Heart rate recovery. **Equivalent data for Anaerobic threshold is available in supplemental materials.**

A reduced AT (<11 $ml.kg.min^{-1}$) was similarly associated with reduced oxygen pulse at peak exercise dropping with each quartile (p=0.02, two-way ANOVA, Supplementary Data), but PAD remained associated with greater reductions in oxygen pulse when compared with individuals with a reduced AT alone (p=0.03, two-way ANOVA). Individuals with both PAD and a low AT demonstrated the lowest values for oxygen pulse (Figure 3-6, Table 3-6).

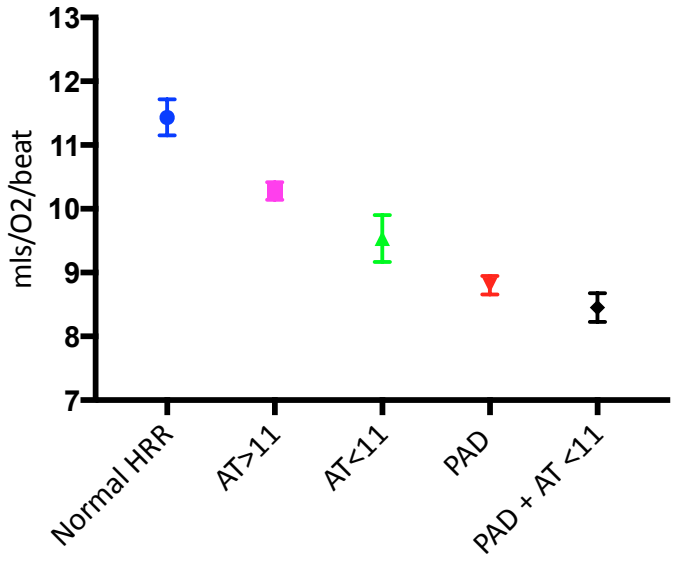

Figure 3-6: Oxygen Pulse ($ml.O_2.beat^{-1}$) classified according to presence or absence of PAD and/or aerobic fitness (Anaerobic threshold > or < 11 $ml.kg.min^{-1}$). Mean + SD. UCLH data. One way classic ANOVA p <0.05.

Oxygen Pulse ($ml.O_2.beat^{-1}$)	Normal Heart rate recovery (>12 bpm)	Aerobically 'Fit': AT > 11 $ml.kg.min^{-1}$	Aerobically 'Unfit': AT <11 $ml.kg.min^{-1}$	PAD (HRR <12 bpm)	PAD + Low AT
Mean	**11.4**	**10.3**	**9.5**	**8.8**	8.4
Median	**10.8**	**9.9**	**8.8**	**8.4**	8.1
95% CI of mean	10.8-11.8	10-10.5	8.8-10.2	8.5-9	8-8.9

Table 3-6: Oxygen Pulse ($ml.O_2.beat^{-1}$) classified according presence of PAD and/or aerobic fitness (Anaerobic threshold > or < 11 $ml.kg.min^{-1}$). (UCLH data). HRR: Heart rate recovery. AT: Anaerobic Threshold. PAD: Parasympathetic Autonomic Dysfunction.

3.5.4 Heart Rate Dynamic Changes in PAD

Individuals with PAD demonstrated chronotropic incompetence, with higher resting heart rates (p=0.03) and blunted peak heart rates (p=<0.001) (Table 3-7).

The majority of published studies have used a failure to attain ≥80% of adjusted heart rate reserve, measured during a graded exercise test, as the primary criterion for chronotropic incompetence (Brubaker & Kitzman 2011). Patients with PAD demonstrated a reduced adjusted heart rate reserve both in absolute terms and when compared to normal HRR (adjusted heart rate reserve: 77 (73-81)%, p=0.009). The cohort without PAD did not demonstrate chronotropic incompetence (achieving 88 (82-94)% of their adjusted Heart Rate Reserve).

CPET Physiology (UCL and DHP combined):	Normal HRR N=729 (HRR >12)	PAD N=317 (HRR ≤12)	P-value
Resting Heart Rate (mean, 95% CI)	81 (78-84)	88 (84-93)	0.003
Peak Heart Rate (mean, 95% CI)	146 (142-150)	133 (128-138)	< 0.0001
Adjusted Heart Rate Reserve (%) (mean, 95% CI)	88 (82-94)	77 (73-81)	0.009

Table 3-7: Heart Rate Dynamic Changes in PAD. University College London and Derriford Hospital Plymouth data combined. Heart Rates are min^{-1}. Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for Student's unpaired t-test.

3.5.5 Electrocardiographic evidence of cardiac ischaemia in PAD

No differences in electrocardiocardiographic markers of cardiac ischaemia during exercise, in terms of nadir ST depression in lead II at peak exercise, were seen between groups (Table 3-8, Figure 3-7). However, an increase in risk for a prognostically significant rise in ST/HR index (>1.6 (Okin et al. 1996)) was seen in the PAD group (RR 1.03 (1.001 to 1.045) p=0.001).

CPET Physiology (UCL and DHP combined):	Normal HRR N=729 (HRR >12)	PAD N=317 (HRR ≤12)	P-value
ST segment Nadir (LEAD II, microV) (mean, 95% CI)	-0.06 (-0.08- -0.03)	-0.06 (-0.07- -0.04)	0.8
ST/HR index (mean, 95% CI)	1.054 (0.7-1.4)	1.473 (1-1.8)	0.12

Table 3-8: Electrocardiographic markers of exercise induced myocardial ischaemia in PAD. Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for student's unpaired t-test.

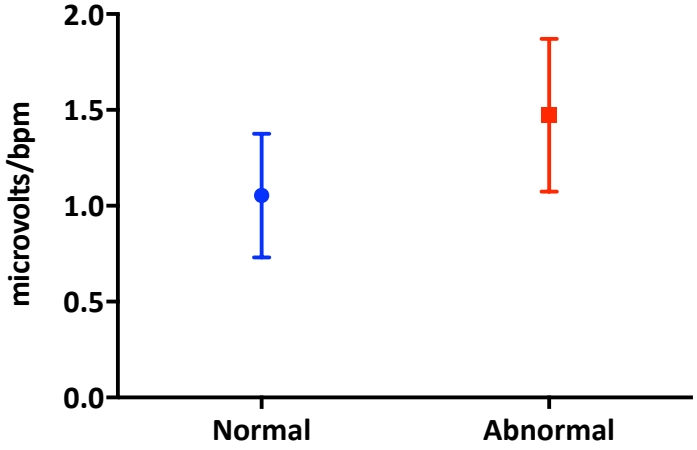

Figure 3-7: ST/HR index at preoperative exercise testing in individuals with (normal heart rate recovery) and without PAD (abnormal heart rate recovery). Values plotted are mean (95% CI).

At UCLH, individuals with PAD were more likely to undergo postoperative troponin testing for suspected perioperative cardiac ischaemia than their counterparts (RR 1.03 (1 to 1.06), p=0.02).

3.5.6 GRK2 and PAN-Arrestin expression in circulating lymphocytes

Heart rate recovery values were assessed in 20 patients. GRK2 expression in whole cell lysate, as measured by immunoblot, was increased in PAD (Figure 3-8).

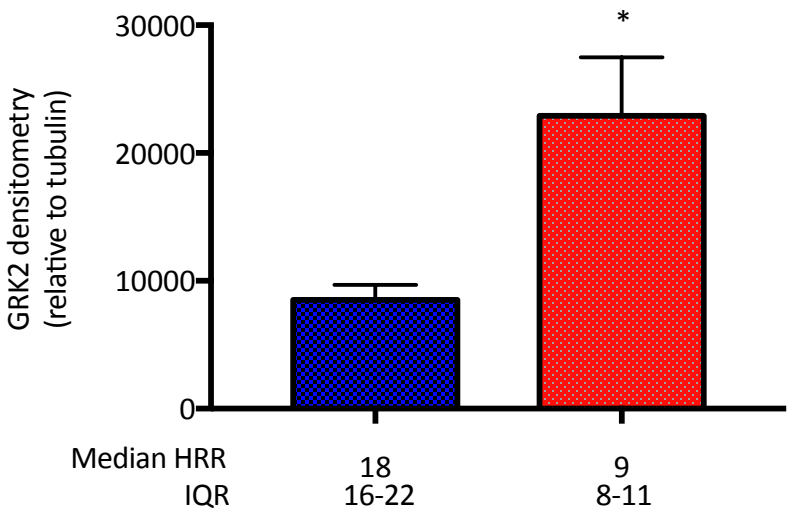

Figure 3-8: Group data for patient immunoblots performed in 20 separate subjects demonstrating each HRR phenotype (10 per group). Median (25th–75th centile) HRR values shown below the bars. *Significant difference ($p < 0.05$). IQR = interquartile range.

GRK2 expression appeared to increase in PAD in a dose dependent manner (Figure 3-9).

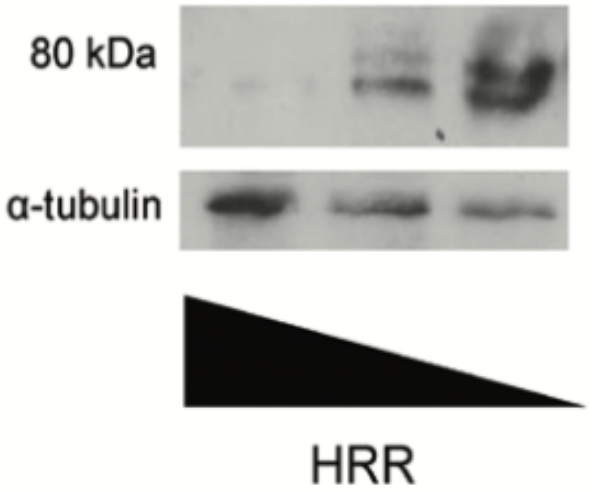

Figure 3-9: Immunoblot for G-protein–coupled receptor- kinase 2 (GRK2) protein expression in isolated mononuclear cells obtained from three consecutively recruited surgical patients undergoing preoperative cardiopulmonary exercise testing

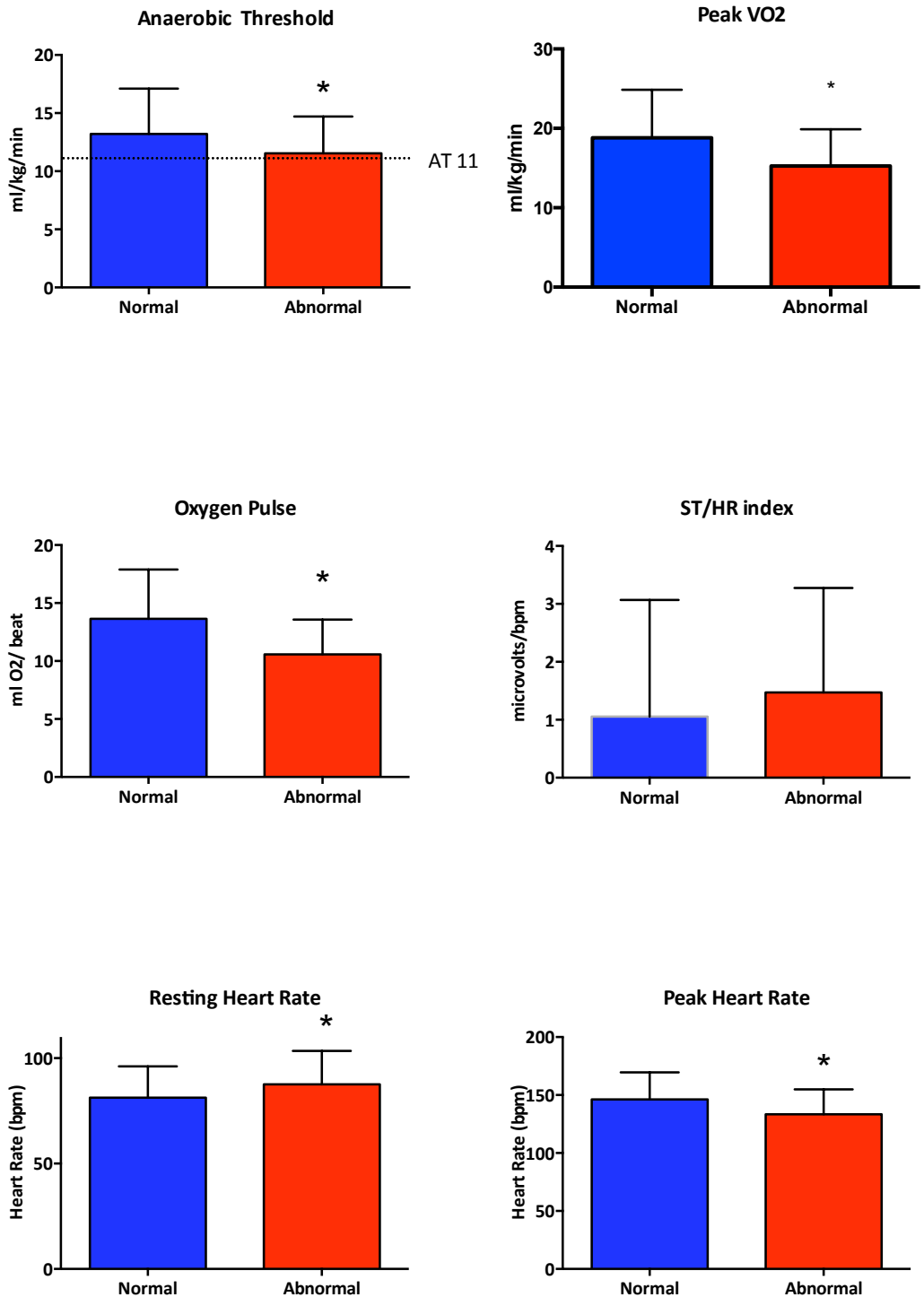

Figure 3-10: Cardiopulmonary exercise performance in patients with parasympathetic autonomic dysfunction (PAD). Abnormal signifies abnormal heart rate recovery after exercise (PAD). Data are presented as mean ± SD. AT = Anaerobic Threshold. The dotted line on the anaerobic threshold figure illustrates the 'high risk' cut off for postoperative complications in common usage. All comparisons made by ANOVA. Asterisk denotes $P \leq 0.05$.

3.6 Discussion

3.6.1 Key findings

1. The distribution of heart rate recovery after exercise in surgical patients corresponds well to that seen in other medical populations.
2. Abnormal heart rate recovery at preoperative exercise testing is associated with reduced heart rate variability in the same individuals, both pre- and immediately post-operatively.
3. Parasympathetic Autonomic Dysfunction as defined by reduced heart rate recovery, was associated with a distinct exercise phenotype, characterised by a reduced stroke volume (as represented by oxygen pulse) at peak exercise, reduced AT and VO_2 peak, and chronotropic incompetence.
4. Parasympathetic Autonomic Dysfunction as defined by reduced heart rate recovery, was associated with evidence for increased risk of electrocardiographically defined ischaemia at preoperative exercise testing, and for clinical suspicion of perioperative myocardial ischaemia.
5. PAD was associated with increased expression of both GRK2 in circulating lymphocytes taken from patients preoperatively. These increases appeared related to the degree of impairment in heart rate recovery after peak exercise or the severity of PAD.

3.6.2 Discussion of Key findings

3.6.2.1 The distribution of heart rate recovery values corresponds well to other medical populations

The distribution of heart rate recovery values recorded in both cohorts correlated well with those recorded in other medical populations (Cole et al. 2000), although, as when comparing otherwise healthy individuals to those with suspected cardiovascular disease, the lowest quartile was reduced when

compared with the general population (Jouven et al. 2005; Cole et al. 1999), perhaps reflecting increased levels of preoperative comorbidity across both cohorts.

The majority of patients undergoing preoperative exercise testing are already suspected as being 'high-risk', either by virtue of comorbidity and/or type of surgery prior to referral. That heart rate recovery values across the population reflect this is perhaps unsurprising.

3.6.2.2 Impaired heart rate recovery after exercise is associated with reduced heart rate variability both pre- and post-operatively

Heart rate recovery after exercise, measured before hospital admission, was associated with a reduction in heart rate variability, commonly associated with parasympathetic autonomic dysfunction, preoperatively on the day of surgery. This has two important implications:

First, abnormal heart rate recovery is associated, in the same individuals, at a later time point, with another widely accepted marker of vagal withdrawal, namely reduced heart rate variability (rMSSD). This supports the assertion that reduced heart rate recovery represents parasympathetic autonomic dysfunction.

Second, the presence of reduced heart rate variability preoperatively on the day of surgery, in the same patients who demonstrated reduced heart rate recovery days to weeks before, implies that vagal withdrawal is established at the time of surgery.

These observations are made in the light of several caveats. Heart rate variability analysis, particularly time domain analysis, despite widespread usage, cannot in itself be considered as the gold standard method for determining vagal activity (Malik et al. 1996). Indeed, evidence from

pharmacological blockade studies indicates that heart rate recovery is possibly a better measure of dynamic vagal activity (Pierpont et al. 2000).

The interaction between heart rate variability and heart rate recovery is complex. All heart rate variability measures reflect both spontaneous sympathetic and parasympathetic activity as well as heart rate at time of measurement (Monfredi et al. 2014), whereas heart rate recovery represents reinstitution of vagal tone from a position of negligible parasympathetic activity at maximal exercise.

Limited evidence comparing athletes and healthy control individuals has shown a variety of relationships between resting HRV, including rMSSD, and heart rate recovery. These range from strong (Danieli et al. 2014; Evrengul et al. 2006) to moderate (Nunan et al. 2010) to none at all (Bosquet et al. 2007).

In one study, where pre exercise HRV values did not correlate well with heart rate recovery data, dynamic changes in heart rate variability after exercise during recovery (i.e. return of complexity and variability) did mirror heart rate recovery (Javorka et al. 2002). Another study showed no relationship between heart rate recovery and resting heart rate variability in the first two minutes after cessation of exercise, but a strong relationship from the third minute of recovery onwards (Antelmi et al. 2008).

Even the mode of exercise testing (treadmill vs. stationary cycle ergometer) can influence the rate of heart rate recovery (Maeder et al. 2009) and heart rate variability (Abrantes et al. 2012).

Evrengul et al. showed a strong correlation between resting measures of heart rate variability and heart rate recovery after exercise in individuals with known coronary artery disease without heart failure, both being reduced (Evrengul et al. 2006). This may better represent the high-risk surgical population than studies on marathon runners and otherwise young healthy

individuals. Simply put, HRV “cannot be used in any simple way to assess autonomic nerve activity to the heart” (Monfredi et al. 2014).

Heart rate variability data are however easily accessible in the clinical setting. Evidence that reductions in HRV represent vagal withdrawal measured by HRR, could prove useful in the continual assessment of the high-risk patient in the perioperative period. In this study, HRV did not significantly change from preoperative values in the immediate postoperative period in either PAD or normal individuals. However, the observations that HRV indices remained low in patients with preoperative PAD and that a positive correlation existed between patients who presented with low preoperative heart rate variability and immediate postoperative heart rate variability may have some bearing on the development of postoperative morbidity. Indeed, in the POM-O trial, reduced parasympathetic activity after goal directed therapy (GDT) was associated with a failure of GDT to improve postoperative outcomes (Ackland et al. 2015).

3.6.2.3 Parasympathetic autonomic dysfunction is associated with a distinct exercise phenotype characterised by impaired cardiac contractility.

Parasympathetic autonomic dysfunction appears to be associated with functional cardiac failure in surgical patients as illustrated by performance of these patients at perioperative cardiopulmonary exercise testing.

i) Reduced VO_2 peak and chronotropic incompetence

Both AT and VO_2 peak were observed to be reduced in PAD, in the context of normal ventilatory equivalents for oxygen and carbon dioxide, likely reflecting impaired cardiac performance. The ability to perform physical work is normally enabled by an up to four-fold increase in VO_2 during exercise in healthy humans. This is achieved by a 2.2 fold increase in heart rate and 0.3 fold increase in stroke volume.

Chronotropic incompetence, a well described characteristic of both parasympathetic autonomic dysfunction and cardiac failure (Brubaker & Kitzman 2011), was demonstrated in this cohort of PAD patients. Whilst a reduced peak heart rate in PAD therefore goes some way to explaining the observed reduction in peak VO_2, it cannot fully be explained by alterations in heart rate dynamics alone, since VO_2 is also strongly influenced by cardiac contractility.

ii) Reduced oxygen pulse at peak exercise

The reduced oxygen pulse noted in PAD at peak exercise is suggestive of reduced stroke volume and therefore cardiac contractility, (the oxygen pulse is equivalent to the slope of the VO_2/Heart Rate relationship; Whipp et al. 1996).

Values for oxygen pulse in patients with normal heart rate recovery agreed well with those reported elsewhere in surgical patients, but those recorded in PAD were equivalent to the lowest quintile recorded in cardiac patients referred for exercise testing (Oliveira et al. 2011; Oliveira et al. 2009). A reduced oxygen pulse in itself has been demonstrated to be a powerful predictor of mortality in patients with cardiovascular diseases (Ritt et al. 2012; Oliveira et al. 2009; Laukkanen et al. 2006).

PAD is particularly common in cardiac failure, where impaired ventricular remodelling and increased levels of inflammation in cardiomyocytes have been noted and associated with vagal withdrawal (Floras & Ponikowski 2015; Li & Olshansky 2011). Strategies to improve cardiac performance through vagal modulation have met with mixed results (Klein & De Ferrari 2010; Zannad et al. 2015), though there is an increasing body of basic science evidence to suggest that vagal activity, acting through ventricular muscarinic receptors, directly increases cardiac contractility (Vatner et al. 1988; Kitazawa et al. 2009; Hussain et al. 2009).

Oxygen pulse appeared related to PAD in a 'dose-dependent' manner. This supports the concept of role for vagal activity in the promotion of positive inotropy. Unfortunately, resting values for oxygen pulse were not available. These would help determine whether intact parasympathetic function is a pre-requisite for an increase in cardiac contractility during exercise.

3.6.3 Evidence for myocardial ischaemia in PAD

There was no clear relationship between PAD and exercise induced myocardial ischaemia in terms of nadir ST segment depression. This supports the findings of previous studies in cardiac populations which showed no interaction between Bruce Protocol elicited ST depression and heart rate recovery (Nishime et al. 2000). An abnormal heart rate recovery has however been associated with inducible impaired left ventricular systolic function during exercise, presumed in part due to myocardial ischaemia, but remaining independently predictive of death (Watanabe et al. 2001).

Patients with PAD were more likely to demonstrate a ST/HR index score suggestive of ischaemic heart disease, putting these individuals at increased risk of positive coronary angiography, cardiac morbidity and mortality (Kligfield 2008; Okin et al. 1996). Indeed, in a non-surgical population, abnormal heart rate recovery has independently been associated with a 59% prevalence of significant coronary artery disease at angiography (Shetler et al. 2001).

Given the prevalence of chronotropic incompetence in PAD, ST/HR index, which allows for ST segment depression due to tachycardia, may be a more appropriate measure of cardiac ischaemia in PAD than nadir ST segment depression.

Further support for inducible cardiac ischaemia in PAD is provided by the observation of a reduced peak oxygen pulse. A reduced oxygen pulse is strongly associated with the presence of myocardial ischaemia during

exercise, possibly due to a flattening of the VO_2/Heart rate curve at the onset of ischaemia due to a reduced stroke volume (Belardinelli et al. 2003).

Taken together, surgical patients with heart rate recovery defined PAD do manifest certain key features suggestive of myocardial ischaemia during exercise testing. This lends support to the concept that PAD is associated with covert cardiac failure in surgical patients, manifest by impaired cardiac performance during CPET.

3.6.4 Parasympathetic Autonomic Dysfunction is associated with increases in GRK2 expression in circulating mononuclear cells

Evidence for increased GRK2 expression in circulating mononuclear cells in PAD has several implications:

First, GRK2 elevation is strongly associated with both adrenergic stimulation and RAAS activation. This implies a degree of neurohormonal activation with consequent alterations in G protein receptor kinase expression in surgical patients with PAD that is similar to that described in other populations where evidence of vagal withdrawal is common (e.g. cardiac failure and critical illness).

Second, when combined with observations of impaired cardiac contractile function and chronotropic incompetence in PAD, elevated GRK2 would be expected to be associated with beta adrenoreceptor and angiotensin II receptor internalization and desensitization. This expected desensitization may partly explain the failure of individuals with PAD to increase both their cardiac contractility and their heart rate in response to endogenous increases in sympathetic activity initiated by exercise.

Third, the combination of elevated GRK2 with impaired exercise performance in PAD suggests that there is a large population of patients with a degree of undiagnosed, functional heart failure routinely presenting for major surgery.

Evidence for increases in GRK2 expression in circulating mononuclear cells alongside increasing degrees of PAD mirror similar severity related increases observed in individuals with known heart failure (Iaccarino et al. 2005). In heart failure, elevations in lymphocyte GRK2 have a prognostic value (Rengo et al. 2016) that could be explored in PAD in the perioperative context.

Use of CPET elicited heart rate dynamic variables may therefore be of some use in identifying these patients prospectively and initiating medical optimization. This raises questions with regard to targeted beta blockade and the introduction of other drugs, such as angiotensin II antagonists and GRK2 blockers, shown to be associated with amelioration of cardiac dysfunction in heart failure. Similarly, improvements in GRK2 expression in circulating lymphocytes after exercise training in patients with heart failure appear to predict survival (Rengo et al. 2014) and may therefore show potential for quantifying response to exercise based prehabiliation strategies.

Fourth, evidence of increased, or at least unopposed sympathetic activity associated with vagal withdrawal has not been specifically investigated here, though it is highly likely. Abnormal heart rate recovery is very specifically associated with delayed restoration of vagal tone, however, it does not exclude concomitant sympathetic activation, and reduced heart rate variability is also associated with increased sympathetic activity. The high prevalence of increased sympathetic activity in heart failure would further suggest the presence of the same in individuals with PAD.

Finally, vagal withdrawal is associated with altered immune function, specifically mediated through obtundation of the vagal anti-inflammatory reflex, with clear negative implications for the perioperative period.

Additional observations of elevated GRK2 expression in mononuclear cells in PAD may carry further implications for the immune response to surgery. Elevated GRK2 is strongly implicated in the modulation of pro-inflammatory toll like receptor signalling, as well as in impaired monocyte chemotaxis

(Vroon et al. 2006). The observation of elevated GRK2 expression in PAD could provide a supplementary explanation for the pro-inflammatory phenotype noted in vagal withdrawal, and carry implications for the development of postoperative morbidity.

3.6.5 Conclusions

PAD is known to be an independent predictor of morbidity and mortality across several patient populations. PAD is also known to be both associated with and mechanistically contribute to heart failure and the response to myocardial ischaemia. This study has supported these latter observations in a new (surgical) population. Addition of heart rate recovery to preoperative CPET testing could add further prognostic value and aid targeted, personalized preoperative medical optimisation.

3.6.6 Further work

Focused further study should investigate the temporal relationships between time and frequency domain measures of vagal activity at baseline and heart rate recovery before, during and after exercise, as well as in the perioperative period, in greater numbers of surgical patients. This could help distinguish static and dynamic components of alterations in autonomic activity, including vagal withdrawal. The rate of recovery of heart rate variability measures in the perioperative period, particularly related to therapeutic interventions, may also provide useful information about the influence of vagal withdrawal on postoperative outcome (Ackland et al. 2015).

Correlation with baseline oxygen pulse (rest) and the oxygen pulse curve would be useful in determining whether parasympathetic withdrawal results in dynamic contractile failure, and in describing the contribution of heart rate to the reduction in peak oxygen pulse. Similarly, echocardiography would add value in determining dynamic alterations in left ventricular systolic function in PAD.

Use of the non-standard CM5 ECG lead may be more sensitive in detecting myocardial ischaemia and should be considered in future studies examining electrocardiographic evidence of myocardial ischaemia. Global measurement of cardiac biomarkers such as troponin and BNP, both at baseline, after exercise and in the post-surgical period would be more sensitive at determining the effect of PAD on myocardial ischaemia.

Further exploration of evidence for both sympathetic and RAAS activation in PAD may help target patients who would benefit from perioperative beta blockade and/or Angiotensin II inhibition. Mechanistic animal studies could help differentiate the role of GRK2 in the development of the cardiac phenotype described here. Detailed immunophenotyping would be of benefit in the assessment of the immune implications of vagal withdrawal in these surgical patients.

4. Parasympathetic Autonomic Dysfunction and Surgical Outcome

4.1 Introduction

Vagal withdrawal has been associated with a pro-inflammatory state, metabolic dysregulation (Pavlov & Tracey 2012) impaired cardiac performance (Olshansky et al. 2008), gastrointestinal dysfunction (Karmali et al. 2015) and impaired outcomes across various inflammatory insults, including during the perioperative period (Lankhorst et al. 2014), trauma (Colombo et al. 2008; Ryan et al. 2011) and sepsis (Schmidt et al. 2005).

Studies in these contexts to date have not directly examined the influence of established, or pre-existing Parasympathetic Autonomic Dysfunction (PAD) on outcome.

Whether established PAD is detrimental to outcome following surgery, an inflammatory insult where the timing of onset can be predicted, in human patients is unknown. Higher risk individuals, more susceptible to developing significant perioperative morbidity and mortality (Khuri et al. 2005) are of particular interest.

In order to explore any association between preoperative PAD and clinically relevant adverse outcomes to surgical trauma, high-risk patients, in two well-characterised populations undergoing major surgery were studied. Heart rate recovery immediately after routine preoperative Cardiopulmonary Exercise Testing (CPET) was used as an indicator of parasympathetic autonomic function.

4.1.2 PAD and outcome after major surgery

PAD is strongly associated with several plausible mechanisms, outlined previously, for the development of postoperative complications.

Data from preoperative exercising testing suggest that individuals with PAD display a distinct physiological phenotype that could additionally affect perioperative outcome in several ways:

i) Reduced anaerobic threshold. In itself, in similar populations, an anaerobic threshold of <11 ml.kg.min^{-1} is strongly predictive of impaired postoperative outcomes (Older 2013; Older et al. 1999; Forman et al. 2010). Individuals with PAD defined by reduced heart rate recovery after exercise were more likely to demonstrate an AT of < 11.

ii) Reduced cardiac contractility at peak exercise. As a component of oxygen delivery, should this reduced contractility translate to reduced cardiac output at times of increased metabolic demand, then perioperative tissue hypoperfusion with resultant consequences may be more likely (Rhodes et al. 2010; Lees et al. 2009a).

iii) In itself, reduced exercise capacity, in the absence of a diagnosis of heart failure, is associated with increases in markers of inflammation (Sultan et al. 2014). Vagal withdrawal, a pro-inflammatory stimulus, may support or even augment this process.

iv) Increased GRK2 expression, noted previously in circulating immune cells in patients with PAD, is also associated with a dysregulated, pro-inflammatory immune response; potentially further increasing the risk of postoperative immune complications.

PAD was associated with cardiac impairment at the time of preoperative exercise testing. In heart failure, parasympathetic withdrawal is strongly associated with myocardial inflammation (Li & Olshansky 2011).

Lymphopaenia, likely secondary to sympathetic activation, is prevalent in heart failure and strongly linked to outcome. Reduced circulating lymphocyte counts, which when expressed as a neutrophil to lymphocyte ratio (NLR) reflect systemic inflammation (Zahorec 2001; Venkatraghavan et al. 2015), are also associated with poor prognosis in cardiac failure (Benites-Zapata et al. 2015), and in other inflammatory conditions such as cancer (Jung et al. 2011; De Martino et al. 2013; Walsh et al. 2005), sepsis and critical illness (Salciccioli et al. 2015; Akilli et al. 2014).

Preoperative changes in the neutrophil to lymphocyte ratio have also been shown to be predictive of postoperative outcome in colorectal surgery (Malietzis et al. 2013; Walsh et al. 2005; Cook et al. 2007). Since established vagal withdrawal is hypothesised to produce an inflammatory state, resultant changes in NLR could be predicted and would, if present, be expected to be associated with impaired postoperative outcome.

Postoperative complications are strongly associated with increased length of stay and resource utilisation (Khan et al. 2006; Odermatt et al. 2015; Clavien et al. 2009).

In particular, postoperative gastrointestinal dysfunction (PGID; most commonly manifest as ileus) is strongly associated with systemic inflammation, sympathetic activation and vagal withdrawal in both gastrointestinal and remote site surgery (Karmali et al. 2015; Holte & Kehlet 2000a). Indeed, several current strategies to treat ileus focus on restoration or augmentation of vagal activity (Van Den Heijkant et al. 2015; The et al. 2011). Prolonged postoperative ileus is common and is associated with discomfort, patient dissatisfaction and prolonged hospitalization (Artinyan et al. 2008). The contribution of preoperative or existing PAD to the development of this important postoperative complication is not known.

I set out, therefore, to determine whether preoperative parasympathetic autonomic dysfunction as represented by impaired heart rate recovery after

exercise was associated with impaired postoperative outcomes in the same populations of patients investigated previously with preoperative cardiopulmonary exercise testing.

4.2 Hypothesis

Preoperative Parasympathetic Autonomic Dysfunction is associated with increased postoperative morbidity after major surgery.

4.3 Aims

This hypothesis was addressed by exploring the effect of preoperative parasympathetic autonomic dysfunction on the following primary and secondary outcomes:

Primary:

- Delayed postoperative recovery, represented by length of hospital stay.

Secondary:

- Major postoperative morbidity as defined by Clavien-Dindo Score.
- Return of bowel function.
- Episodes of sepsis and changes in Neutrophil to Lymphocyte ratio.
- Mortality.

4.4 Methods

4.4.1 Patient Population

One thousand and fifty two patients scheduled to undergo major colorectal surgery, as described previously, were enrolled at University College London and Derriford Hospital Plymouth.

The Derriford Hospital cohort of patients comprised patients undergoing major colorectal surgery who also participated in the COMPETE-C randomized controlled trial (ISRCTN 14680495). Preoperative CPET data, intraoperative physiological data and extensive postoperative outcome data was available from this group, which acted as a derivation cohort.

Patients recruited at UCLH acted as a validation cohort. Less complete postoperative morbidity data was available from these patients. A larger number of patients, more information on preoperative medication and medical history and blood results, not available from Plymouth, were however available.

The protocol used for CPET is described in General Methods. The full protocol and primary analysis for the COMPETE-C trial is included in the appendix (C Challand et al. 2012).

CPET data from both sites was combined and classified according to heart rate recovery in the first minute after cessation of maximal symptom limited exercise. A heart rate recovery of 12 bpm or below (the lowest quartile of the tested population) was classed as abnormal based on population distribution statistics and published literature as described previously. Primary and Secondary outcomes were examined after dividing the patients into normal (N) and abnormal (HRR) heart rate recovery groups.

4.4.2 Primary Outcome: Hospital Length of stay

Hospital Length of stay, available from both cohorts, is frequently used as a marker of delayed recovery (Librero et al. 2004). Since hospital length of stay may be affected by non-complication related factors such as availability of social services, or rehabilitation facilities, readiness for discharge (RfD) has been used in many studies assessing post operative morbidity and may be a better measure of postoperative complications (Challand et al. 2012; Noblett et al. 2006; Gan et al. 2002).

Readiness for discharge data was available from the Derriford Hospital Plymouth cohort and was assessed using internationally accepted criteria (Fiore et al. 2012) for colorectal surgery by observers blinded to the original intervention or CPET result (Table 4-1).

Criteria	End points
Tolerance of oral intake	Patient can tolerate at least one solid meal without nausea, vomiting, bloating, or worsening abdominal pain. Patient should be actively drinking and not require IV hydration
Recovery of lower gastrointestinal function	Patient should have passed flatus
Adequate pain control with oral analgesia	Patient should be able to rest and mobilise without significant pain (Pain < 4 on a scale of 1 to 10)
Ability to mobilise and self care	Patient should be able to sit up, walk, and perform activities of daily living.
Clinical examination and laboratory tests show no evidence of complications or untreated medical problems	Temperature should be normal Pulse, blood pressure and respiratory rate should be stable and consistent with preoperative levels Haemoglobin should be stable and within acceptable levels Patient should be able to empty the bladder without difficulty or match preoperative level of bladder function

Table 4-1: Criteria for assessment as fit for discharge. When the criteria outlined above have been met, the patient is considered to have reached short-term postoperative recovery and should be considered ready for discharge (Fiore et al. 2012).

4.4.3 Secondary Outcome measures

i) Clavien-Dindo Grade

Data on major postoperative morbidity at any time in the postoperative course, as classified by a Clavien-Dindo grade ≥3 were available from the DHP cohort.

A Clavien-Dindo grade of ≥ 3 was chosen as being most representative of major postoperative morbidity (including life threatening complications requiring intensive care management, single organ dysfunction or multi-organ dysfunction) (Clavien et al. 2009).

ii) Return of bowel function

This was assessed at DHP as a component of ready for discharge criteria. Aside from the presence of flatus, patients were expected to have passed stool and be tolerating a light diet normally to be considered as having resumed normal bowel function.

iii) Postoperative sepsis and neutrophil to lymphocyte ratios

Prospective data on the incidence of sepsis was collected at DHP only. Two or more of the following criteria, derived from the surviving sepsis campaign international guidelines 2012, being present defined postoperative sepsis (Dellinger et al. 2013):

- Temperature <36^{o}C or >38^{o}C
- Heart Rate >90 bpm
- Respiratory Rate >20bpm
- Acutely altered mental state
- White cell count <4 or >12
- Suspected infectious cause: origin recorded
- Plasma glucose >14 mMol/L

iv) White Cell Count, Neutrophil to Lymphocyte ratio

Full blood counts were analysed at our institution's haematology laboratory on a Sysmex XE2100 analyser (Sysmex, Milton Keynes, UK).

v) Mortality

Ninety day mortality was recorded, since recent data demonstrate that morbidity at this time point has the greatest impact on long term outcome after surgery for colorectal malignancy (Visser et al. 2009).

4.4.4 Sample Size and Statistical Analysis

Abnormal heart rate recovery was defined as described previously by categorizing the distribution of values for heart rate recovery into quartiles and choosing the 25th centile or lower quartile as the cut off for abnormality.

The assumption was made that a heart rate recovery in the lowest quartile (<12 bpm) would confer around a two-fold relative risk of adverse outcomes (Cole et al. 2000; Cole et al. 1999; Jouven et al. 2005). Given that the serious morbidity prevalence was 15% in COMPETE-C, a total of at least 59 patients would be required to detect a 2-fold increase in major morbidity in PAD patients, with 90% power at a two-sided 5.0% significance level.

A Cox proportional hazards regression model was constructed for length of stay at UCLH. Age, heart rate recovery, a Revised Cardiac Risk Index (RCRI) >3 (representing significant heart disease and/or diabetes) (Lee et al. 1999) and low AT (<11 ml.kg.min^{-1}) were incorporated. Length of stay data was censored from 50 days (based on ROUT analysis of length statistical outliers (Q=1%), mortality data (15 deaths at 90 days) was included and treated as a competing risk (Brock et al. 2011).

Log-rank analysis of length of stay under various conditions was also carried out. Patients who died in this analysis were right-censored as longest length of stay, which was again censored at 50 days.

General statistical analysis of data was carried out as described in General Methods.

4.5 Results

4.5.1 Patient clinical and demographic variables

Patient clinical and demographic variables for both cohorts are displayed in Table 4-2. A total of 1,052 patients were studied with a median age of 67 years. 50% of DHP and 46% of UCLH patients had a primary diagnosis of carcinoma. Proportionately, the UCLH cohort was composed of more male patients (65%) and was younger (mean age 64 years at UCLH vs. 70 years at DHP).

Both cohorts had low median anaerobic threshold values, (UCLH median AT 11 $ml.kg^{-1}.min^{-1}$, DHP median AT 12.2 $ml.kg^{-1}.min^{-1}$) and had median BMI values that are classified as overweight.

Baseline Patient Characteristics	Derriford Hospital Plymouth	University College London Hospital
Total Number	235	817
Age, years (median, IQR)	70 (60-76)	64 (55-72)
Male (n, [%])	133 (56)	573 (65)
BMI (median, IQR) $kg.m^2$	27.4 (25-31)	27.2 (23-30)
RCRI (mode, range)	1 (1-4)	1 (1-4)
ASA grade (mode, range)	2 (1-3)	1 (1-3)
Carcinoma (%)	50	46
AT (median, IQR) $ml.kg^{-1}min^{-1}$	12.2 (10.4-14.4)	11 (9-13)
Peak VO_2 (median, IQR) $ml.kg^{-1}min^{-1}$	19.1 (15.5 – 23)	17 (13 – 20)
Oxygen Pulse (median, IQR) $ml.beat^{-1}$	10.6 (8.2-13.4)	11 (8.9-13.6)
V_E /VCO_2 at AT (median, IQR)	26.6 (22.2-30.6)	29.4 (26.5-33.6)

Table 4-2: Clinical and demographic features of the whole study cohort (University College London Hospital – UCLH; Derriford Hospital Plymouth- DHP). Values shown are median and interquartile range (IQR) unless otherwise stated. N=number; BMI, Body Mass Index; RCRI, Revised Cardiac Risk Index; ASA, American Society of Anesthesiologists grade; bpm, beats per minute; AT, Anaerobic threshold; VO_2, oxygen uptake; V_E/CO_2, Ventilatory Equivalents for Carbon Dioxide.

4.5.2 Global length of stay day data

Unadjusted length of stay data and 30- and 90-day mortality for both sites is presented in Table 4-3. Mortality data in both cohorts was reduced as compared with population based studies (Byrne et al. 2013).

Postoperative Outcome	Derriford Hospital Plymouth	University College London Hospital
Hospital LOS, days (median, IQR)	7.8 (5.75-9.9)	9 (6-16)
Clavien-Dindo ≥3 (%)	9.8	Not collected
30-day mortality (%)	1.28	1.4
90-day mortality (%)	3.4	2.4

Table 4-3: **Postoperative outcome data for the whole study cohort (University College London Hospital; Derriford Hospital Plymouth).** LOS; Length of stay.

Length of stay data was significantly skewed by the presence of a small number of outliers, potentially affecting the validity of survival analysis (Figure 4-1)

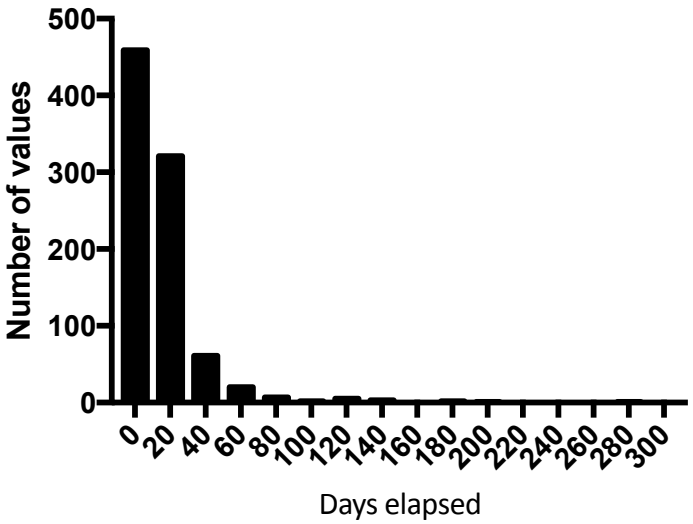

Figure 4-1: Raw length of stay data for both study cohorts. Length of stay was significantly skewed by outlying values.

When outliers were excluded as described in the methods section (ROUT; Q=1%), length of stay data reflected nationally reported data (Figure 4-2, Table 4-4).

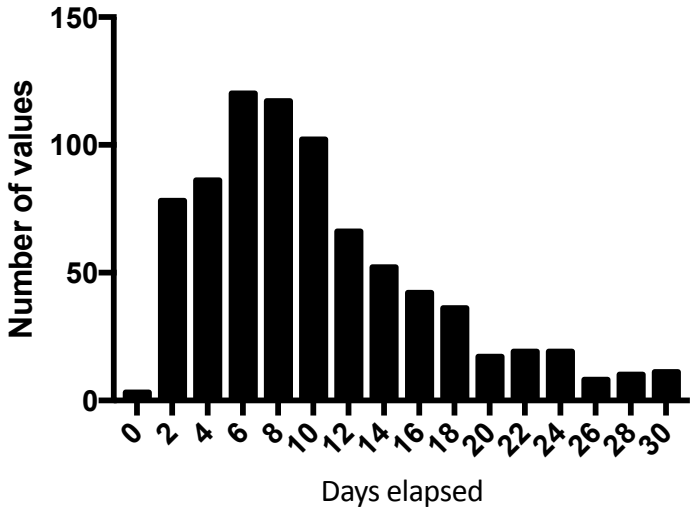

Figure 4-2: Length of stay distribution after exclusion of significant outliers for both study cohorts.

Postoperative Outcome	Derriford Hospital Plymouth	University College London Hospital
Hospital Length of stay, days (median, IQR)	7 (5-9)	8 (5-13)
Mean LOS (SD)	8 (7-8)	10 (9-10)

Table 4-4: Postoperative outcome data, outliers excluded (University College London Hospital; Derriford Hospital Plymouth). LOS; Length of stay.

4.5.3 Patient Clinical and demographic variables. PAD vs. Normal

30% of all patients studied exhibited PAD. Patients with PAD were, in general, older (p=<0.001), more likely to be hypertensive (RR 1.38 (95% CI 1.06-1.79); p=0.03), have a diagnosis of cardiac failure (RR 1.02 (95% CI 0.9 to 1.043); p=0.04) or to be Diabetic (RR 1.1 (95% CI 1-1.185); p=0.03). Aside from age, perioperative demographics, types of surgery and analgesic techniques were similar between normal and PAD groups (Table 4-5).

In the UCLH cohort, similar numbers of patients with and without PAD were taking Beta blocking medication (12% vs. 10%), however more were taking ACE inhibitors (16% vs. 23%), reflecting the increased likelihood of a diagnosis of hypertension. Data was not available for other antihypertensive medications.

	DHP Normal HRR (HRR>12)	PAD (HRR≤12)	P	UCLH Normal HRR (HRR >12)	PAD (HRR≤12)	P
Number (%)	153 (65.1)	82 (34.9%)	-	576 (60)	235 (40%)	-
Age (years)	63.3 (61-65)	71.4 (68-74)	<0.001	59.8 (58-61)	66 (64-68)	<0.001
Male (n,[%])	91 (61%)	42 (51%)	-	160 (68%)	235 (64%)	-
BMI	28.2 (27.5-28.9)	29 .3 (28.6-29.7)	0.06	26.6 (26.2-27.1)	27.3 (26.5-28.1)	0.3
RCRI (mode, range)	1 (1-4)	1 (1-3)	-	1 (1-4)	1 (1-4)	-
ASA grade (mode range)	2 (1-3)	2 (1-3)	-	n/a	n/a	-
Carcinoma (%)	51	49	-	46	46	-
Pack Year History Smoking	n/a	n/a	-	15.5 (13.7-16)	25 (23-27)	<0.001
Epidural (%)	45	55	-	n/a	n/a	-
Beta Blockers (%)	n/a	n/a	-	12	10	-
ACE inhibitors (%)	n/a	n/a	-	16	23	-
Nitrates (%)	n/a	n/a	-	4	4	
Hypertension (%)	n/a	n/a	-	32	45	-
Angina (%)	n/a	n/a	-	5	6	-
Cardiac Failure (%)	n/a	n/a	-	<1	2.5	-
Diabetes (%)	n/a	n/a	-	8	18	-

Table 4-5: Baseline demographic Characteristics for patients with and without Parasympathetic Autonomic Dysfunction (PAD). Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for two-way ANOVA , ASA = American Society of Anesthesiologists grade. BMI = Body Mass Index. RCRI = Revised Cardiac Risk Index. n/a= not available.

4.5.4 Primary Outcome – Hospital Length of stay

PAD was associated with prolonged hospital length of stay at both sites in those patients who subsequently underwent major surgery (Table 4-6). The finding of prolonged length of stay in PAD at DHP was supported by an increased time until ready for discharge (PAD was associated with a five-day delay until assessment of readiness for discharge (p=0.001).

	Derriford Hospital Plymouth (n=235)			University College London (n=811)		
Characteristic:	**Normal HRR (HRR >12)**	**PAD (HRR ≤12)**	**P value**	**Normal HRR (HRR >12)**	**PAD (HRR ≤12)**	**P value**
Length of stay (days) (mean, 95% CI)	10 (8-12)	19 (10-27)	0.018	14 (12-15)	20 (16-23)	0.0004
Ready for Discharge (days) (mean, 95% CI)	7 (6.9-8)	12 (10-14)	0.001	n/a	n/a	n/a

Table 4-6: Primary Outcome: Hospital Length of stay, divided by cohort. Values shown are mean (95% Confidence Interval) or median (range). P-values are for ANOVA for means.

PAD remained associated with prolonged hospital length of stay when data from both sites were combined (combined 12 ± 11 days (9-16) vs. 8 ± 5 days (6-8.5), p=0.01) in those patients who subsequently underwent major surgery (unadjusted Hazard Ratio 1.48 (95% CI: 1.15-1.95); p=0.0027) (Table 4-7, Figure 4-3).

	Normal HRR (HRR >12)	PAD (HRR ≤12)	P value
Length of stay (days) (mean, SD, 95% CI)	8 ± 5 (6-8.5)	12 ± 11 (9-16)	0.001

Table 4-7: Primary Outcome: Hospital Length of stay, both cohorts combined. Values shown are mean (95% Confidence Interval) or median (range). P-values are for ANOVA for means, Mann-Whitney for Medians.

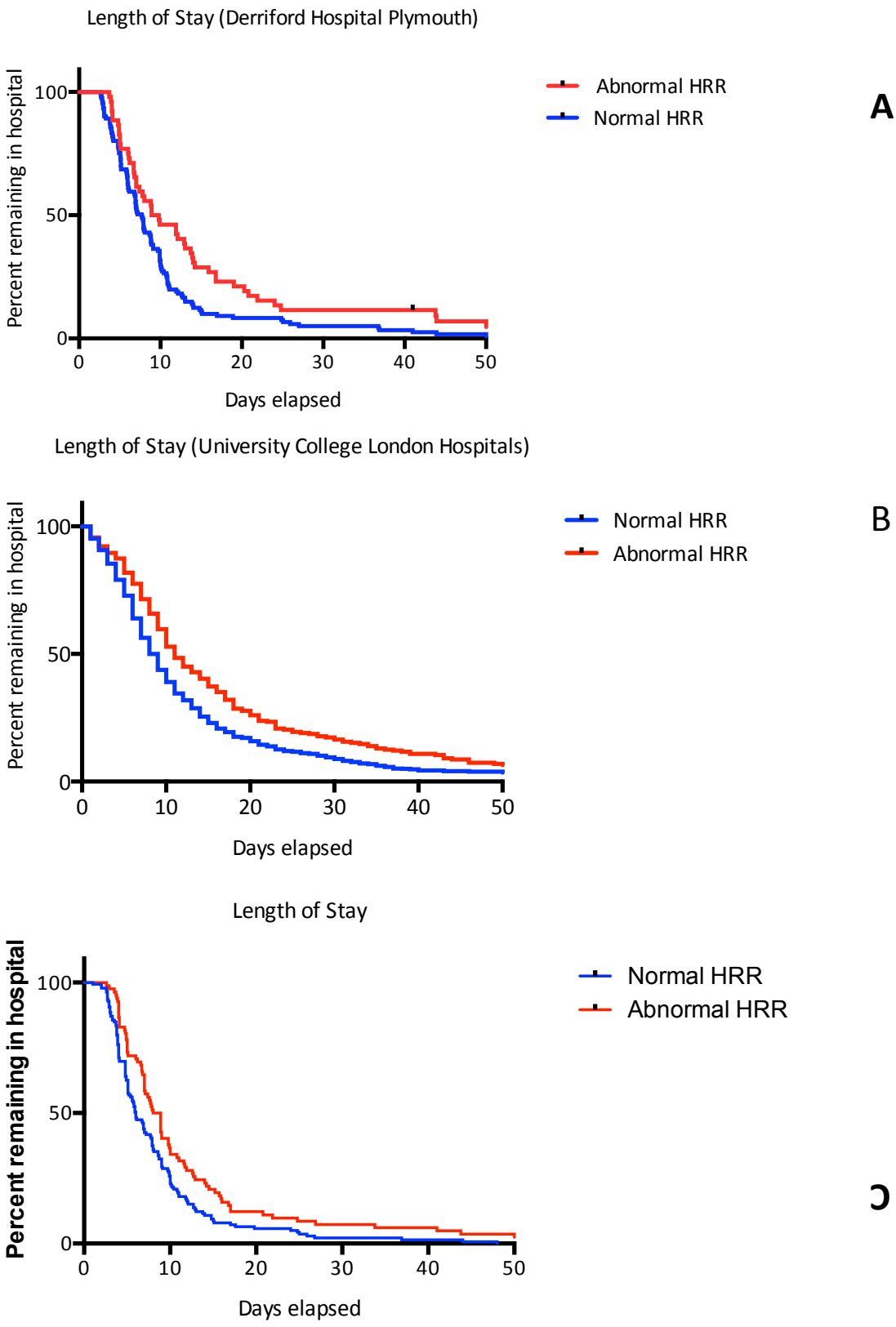

Figure 4-3: Increased postoperative length of stay in Parasympathetic Autonomic Dysfunction (A: DHP Only; B: UCLH Only; C: DHP and UCLH cohorts combined). Log-rank (Mantel-Cox) test: DHP p=0.018, UCLH p=<0.001, combined p=0.0027. DHP= Derriford Hospital Plymouth, UCLH – University College London Hospitals.

4.5.5 Length of hospital stay by quartile of heart rate recovery

In order to assess whether a heart rate recovery of <12 bpm best reflects increased risk of postoperative morbidity, hospital length of stay data from UCLH was categorised according to quartile of heart rate recovery.

A heart rate recovery of <12 bpm remained the best predictor of increased hospital length of stay (ANOVA p=0.005, Bartlett's test for homogeneity of variances p=< 0.001).

Length of stay (days)	Quartile 1: HRR <12 bpm	Quartile 2: HRR 12-17 bpm	Quartile 3: HRR 17-27 bpm	Quartile 4: HRR >27 bpm
Mean	20	14	15	13
Median	11 (7-21)	9 (5-16)	8 (5-14)	8 (5-14)
95% CI of mean	16-23	12-16	12-18	10-15

Table 4-8: Primary Outcome: Hospital Length of stay (days), UCLH classified by quartile of heart rate recovery. Values shown are mean (95% Confidence Interval) or median (interquartile range). P-values are for ANOVA for means, Mann-Whitney for Medians.

There was no reduction in hospital length of stay between the second and fourth quartiles of heart rate recovery after exercise (p=0.5). Between the first and fourth quartile there was a one-week difference in mean length of stay and a three-day difference in median length of stay (p=< 0.01) (Table 4-8, Figures 4-4 & 4-5).

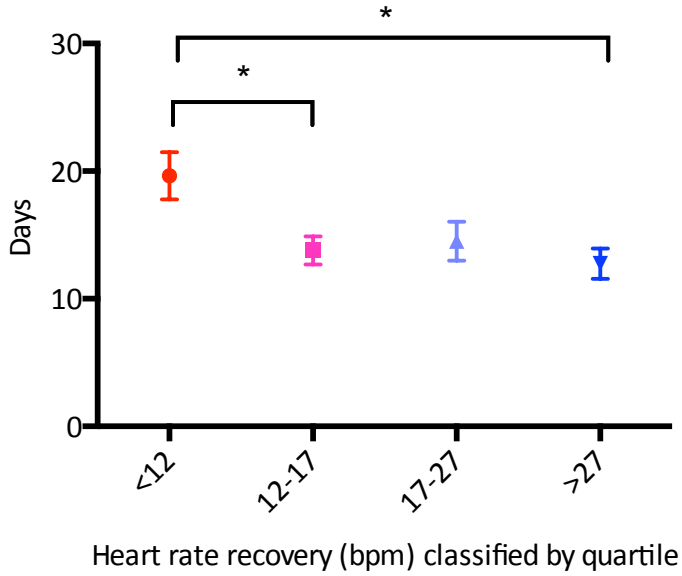

Figure 4-4: Mean (SD) length of stay (days) classified by quartile of heart rate recovery.
* represents a P-value of <0.001 (ANOVA).

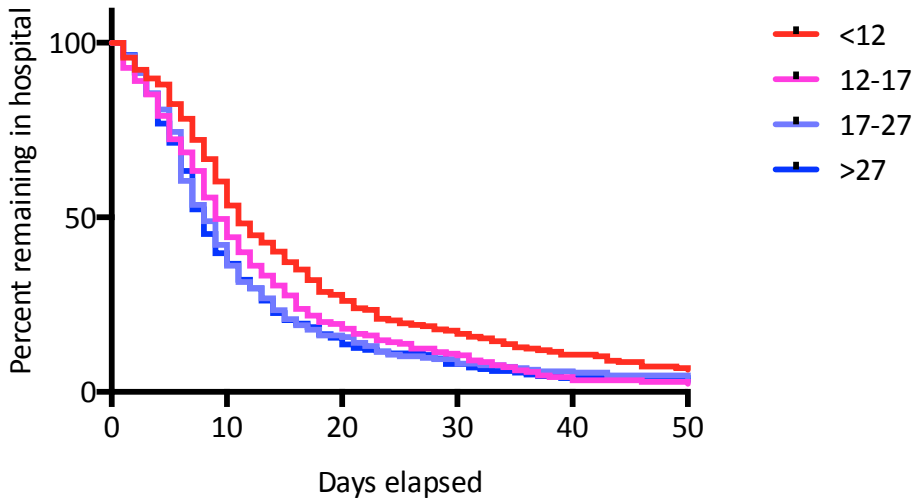

Figure 4-5: Length of hospital stay classified according to quartile of preoperative heart rate recovery. A heart rate recovery of <12 remains the best predictor of increased hospital length of stay (Log-rank (Mantel-Cox) test for trend: p=<0.001).

4.5.6 Does anaerobic threshold independently predict an increased hospital length of stay?

For these analyses, the UCLH cohort was chosen. This was in order to avoid the possible effects of inclusion in the COMPETE-C study (DHP). The inclusion criteria of COMPETE-C precluded inclusion of patients with a range of relevant AT's.

An anaerobic threshold of <11 was also predictive of an increased hospital length of stay (Hazard Ratio (Mantel-Cox): 1.28 (95% CI: 1.14-1.49); p=0.0002) (Figure 4-6, Figure 4-7).

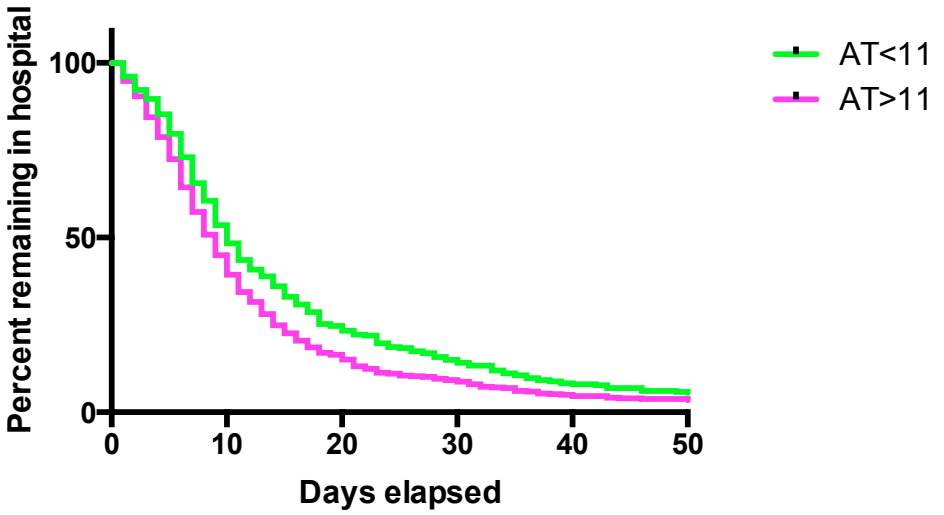

Figure 4-6: Increased postoperative length of stay with a preoperative anaerobic threshold (AT) of <11 ml.kg.min (University College London Hospitals). Log-rank (Mantel-Cox) test p=0.0002).

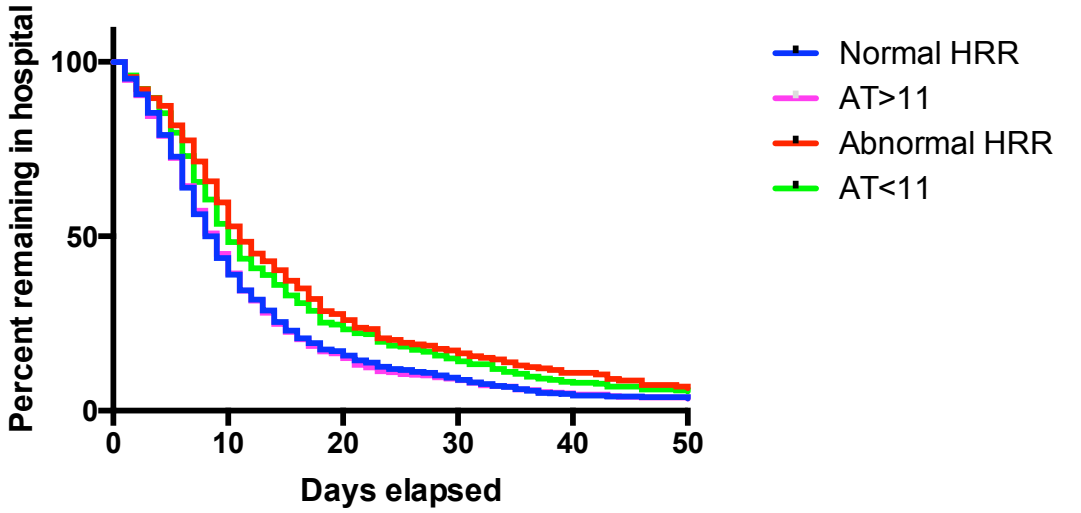

Figure 4-7: Both an abnormal heart rate recovery and an anaerobic threshold (AT) of <11 ml.kg.min are predictive of increased hospital length of stay (data from University College London Hospitals cohort).

After analysing only patients with an anaerobic threshold of <11, PAD remained predictive of a prolonged length of hospital stay (Hazard Ratio (Mantel-Cox): 1.3 (95% CI: 1.1-1.7); p=0.013) (Figure 4-8).

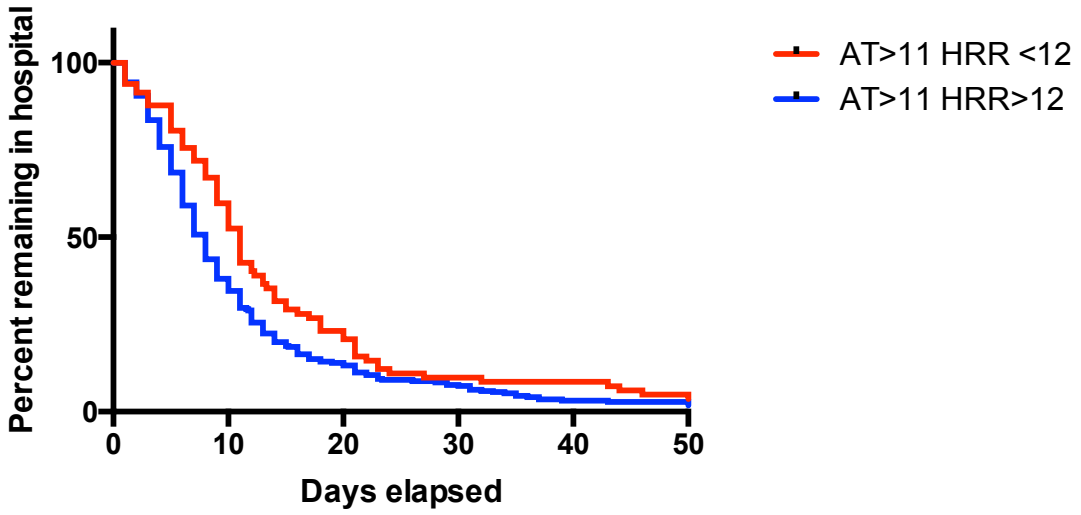

Figure 4-8: Postoperative length of stay remains increased in Parasympathetic Autonomic Dysfunction when anaerobic threshold (AT) is controlled for (University College London Hospitals cohort). Log-rank (Mantel-Cox) test: p=0.013.

The presence of both PAD and an AT of <11 were most strongly predictive of a prolonged hospital length of stay when compared with patients with normal HRR (HR 1.5 (Mantel-Cox) (95% CI: 1.3-1.8; p=<0.0001) (Figure 4-9) (supplemental materials).

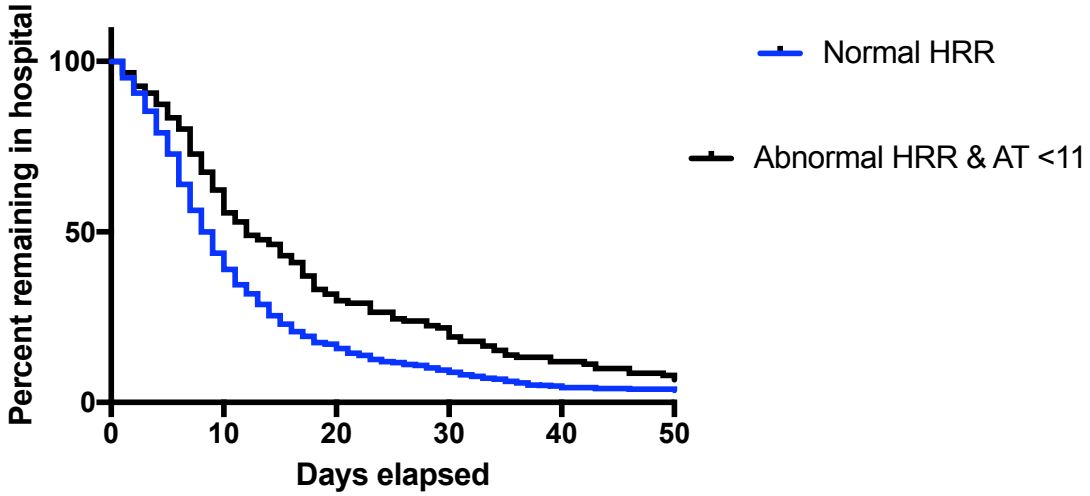

Figure 4-9: An increased postoperative length of stay is most strongly predicted by an anaerobic threshold of <11 ml.kg.min^{-1} and PAD (University College London Hospital cohort). Log-rank (Mantel-Cox) test: p= <0.0001.

4.5.7 Cox Proportional Hazards Model

Cox proportional hazards analysis indicated that PAD was independently associated with delayed hospital discharge (HR: 1.22 (95% CI 1.19-1.55); p=0.002). Age and an anaerobic threshold <11 ml.kg.min^{-1} were also independently associated with delayed discharge (p=0.003 and 0.004 respectively; Table 4-9)

	Hazard Ratio	95% Confidence Interval	Wald Test	p-value
Heart rate recovery <12 bpm	1.22	1.19-1.55	10	0.02
Age	1.07	1.01-1.13	9	0.03
Anaerobic Threshold <11 ml.kg.min^{-1}	1.19	1.12-1.26	8.3	0.04
BMI	1.01	0.99-1.03	0.57	0.45
RCRI ≥3	1.13	0.97-1.29	0.64	0.43

Table 4-9: Cox Proportional Hazards Model. BMI = body mass index, RCRI= revised cardiac risk index.

4.5.8 Secondary Outcomes – Morbidity and Mortality

Clavien-Dindo grades and bowel function data were collected at DHP only.

4.5.8.1 Clavien-Dindo grade

Prospectively gathered morbidity data demonstrated a greater likelihood of major complications in the PAD group (Clavien-Dindo Score >3) in the postoperative period (RR: 1.75 (95% CI: 1.16-2.66; p=0.044). Postoperative complications were associated with a failure to be fit for discharge and therefore prolonged hospital stay (Log-rank (Mantel-Cox) test p=0.0001). Patients with preoperative PAD were more likely to require further critical care following initial postoperative discharge (OR 2.9 (95% CI: 1.1 -8.8; p=0.05)). This was not attributable to reoperation, rates of which were similar between groups (p=0.78).

4.5.8.2 Return of bowel function

Postoperative ileus (no return of bowel function at 5 days) was also more likely in PAD (HR 1.4 (1.03-2.1), Mantel-Cox log rank p=0.0492). The median time for return of bowel function after major colorectal surgery in patients with PAD was 3 days (2.3-4.1). In those with normal heart rate recovery, median time for return of bowel function was 1.9 days (1.9-2.6) p=0.003) (Figure 4-10).

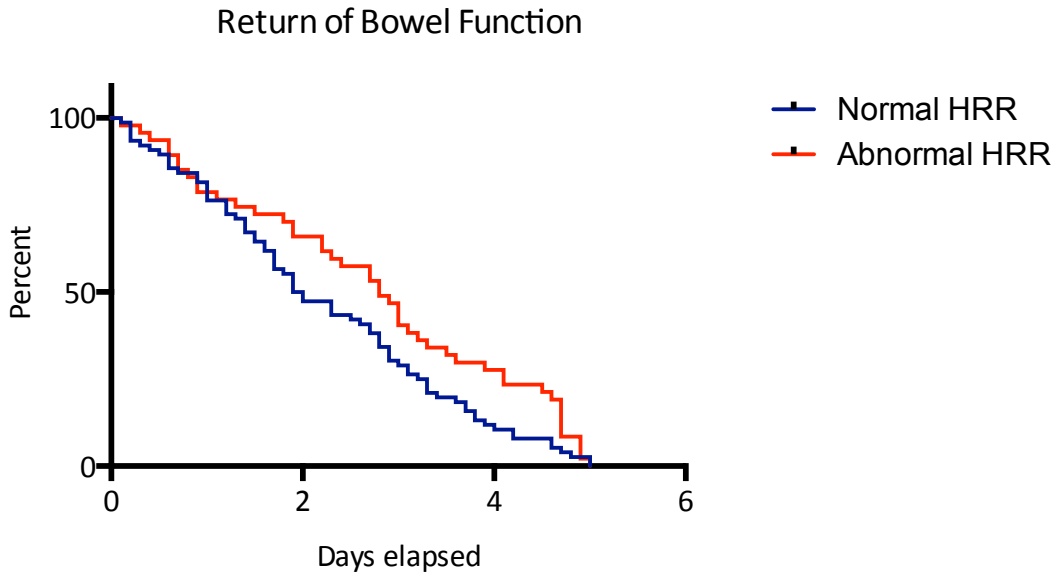

Figure 4-10: Delayed Postoperative Return of bowel function in patients with Parasympathetic Autonomic Dysfunction. Log-rank (Mantel-Cox) test: p=0.003.

4.5.8.3 Postoperative sepsis and perioperative white cell counts in PAD

Episodes of sepsis (sources: chest, urine, wound) were more common in patients with PAD (RR 1.3 (1.02-2.4), p=0.049).

Postoperative white cell counts were higher at day 3 in PAD (15.9 (14.7-16.3)) as compared with patients with normal heart rate recovery (12.2 (10.5-13.9); p=0.02). Similarly, Neutrophil to Lymphocyte ratio on day 2, was raised in PAD (14.1 (12.5-15.4) when compared with patients with normal heart rate recovery (11.2 (10.3-12.2), p=0.05.

Preoperative Neutrophil to Lymphocyte ratios were not different between groups (Table 4-10).

	Normal HRR N=576 (HRR >12)	PAD N=235 (HRR ≤12)	P-value
Preoperative White Cell count (x10^9/L)	10.76 (10.5-11.1)	11.09 (10.5-11.7)	0.3
Day 3 Postoperative White Cell Count (x10^9/L)	12.2 (10.5-13.9)	15.9 (14.7-16.3)	0.02
Preoperative Neutrophil:Lymphocyte Ratio	3 (2.8 – 1.3)	3.4 (3 – 3.7)	0.16
Day 3 postoperative Neutrophil:Lymphocyte Ratio	11.2 (10.3-12.2)	14.1 (12.5-15.4)	0.05

Table 4-10: Preoperative white cell counts and neutrophil:lymphocyte ratios for individuals with and without Parasympathetic Autonomic Dysfunction (PAD). University College London Hospital data. Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for ANOVA.

CRP was not different between PAD and normal groups preoperatively or on day 3 postoperatively (p=0.55 for day 3 CRP).

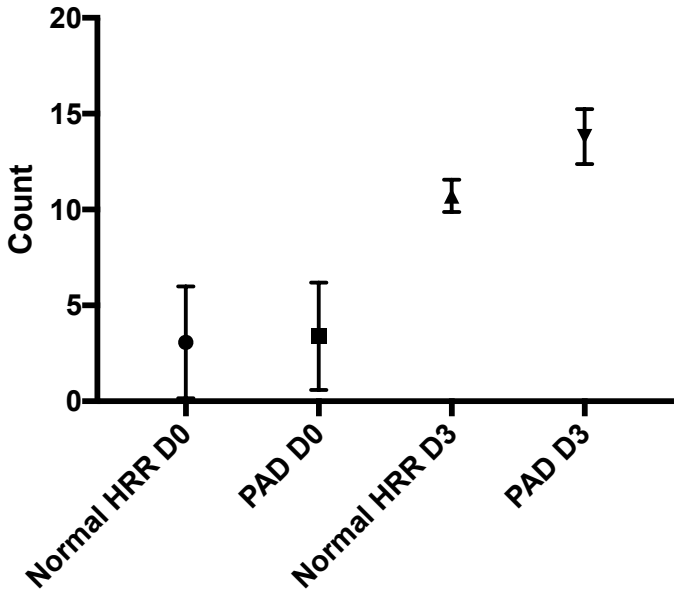

Figure 4-11: Preoperative neutrophil: lymphocyte ratio (NLR) was not different between PAD and normal heart rate recovery groups. Data shown as mean + SD Postoperative day 3 NLR was increased in PAD (p=0.05), as was total white cell count (p=0.02). D0 = day 0, D3 = postoperative day 3. Count = Number x 10^9 cells/ml.

4.5.8.4 90 day mortality

90-day mortality was increased in patients with PAD as defined by abnormal heart rate recovery when compared with individuals with normal heart rate recovery (RR 1.1 (1.01 to 1.41), p=0.008) (Table 4-11).

Postoperative outcome: Parasympathetic Autonomic Dysfunction (PAD) compared with normal heart rate recovery.	
90 day Mortality:	Overall Mortality: 3% Normal HRR: 1% Abnormal HRR: 8% Relative Risk 1.1 (1.04 to 1.41) p = 0.008
Any morbidity (Clavien-Dindo >3)	Relative Risk 1.75 (1.16 – 2.66) p=0.044
Sepsis	Relative Risk 1.3 (1.02-2.4) p = 0.049
Delayed return of bowel function (5 days)	Hazard Ratio 1.4 (1.03-2.1) p=0.0492
Median return of bowel function	3 days (0 to 9) (vs. 1.9 days (0 to 8) in normal HRR)
Return to Critical Care	Relative Risk 3.11 (1.6-6.07) p= 0.0003

Table 4-11: Summary of postoperative outcome in Parasympathetic Autonomic Dysfunction. Values shown are median (range) unless otherwise stated. P-values are for Mann-Whitney test for Medians or Fisher's exact test for proportions (90 day mortality, Clavien-Dindo defined morbidity, return to critical care), alpha <0.05 taken as significantly significant. The Log-rank (Mantel-Cox) test was used to calculate a Hazard Ratio for return of bowel function within 5 days.

4.6 Discussion

4.6.1 Key findings

1. Parasympathetic Autonomic Dysfunction is common in the high-risk surgical population.
2. Parasympathetic Autonomic Dysfunction is independently associated with increased postoperative morbidity, reflected in increased incidence of major complications, episodes of sepsis, delayed return of bowel function, increased hospital length of stay and increased mortality.

4.6.2 Parasympathetic Autonomic Dysfunction is associated with morbidity across a variety of domains

4.6.2.1 Parasympathetic Autonomic Dysfunction is common

These data indicate that established parasympathetic dysfunction, as defined by impaired heart rate recovery after exercise testing, is common and associated with increased postoperative morbidity and mortality in individuals undergoing major colorectal surgery.

The association of certain key co-morbidities with PAD is perhaps unsurprising. Impaired heart rate recovery and autonomic dysfunction have been described in diabetes (Turker et al. 2013; Cheng et al. 2003; Sacre et al. 2012), heart failure (Imai et al. 1994b; Nanas et al. 2006; Arena & Sietsema 2011), hypertension (Best et al. 2014) and with increasing age (Shetler et al. 2001; Trevizani et al. 2012), though there is some question as to whether impairment in the elderly is due to the increased likelihood of other confounding comorbidities (Darr et al. 1988; Dawson et al. 1999; Thayer et al. 2010). In all of these conditions, reduced heart rate recovery is independently associated with impaired outcomes.

Although impaired heart rate recovery after exercise has not frequently been described in hypertension; autonomic dysfunction, particularly baroreceptor dysfunction, is however common (Schroeder et al. 2003; Singh et al. 1998; Thayer et al. 2010; Thayer & Lane 2007).

4.6.2.2 Parasympathetic autonomic dysfunction is associated with increased postoperative morbidity

Hospital length of stay data was comparable with other similar surgical populations (Cohen et al. 2009) (Odermatt et al. 2015; Ma et al. 2011).

PAD was associated with increased postoperative morbidity as reflected by an increased hospital length of stay in both cohorts studied. In the DHP cohort, indirect support for an increase in morbidity causing an increased length of stay was provided by an observed delay in readiness for discharge in PAD.

A heart rate recovery of <12 bpm appears to best predict impaired postoperative outcome. Length of stay data did not reduce in a 'dose dependent' manner with more rapid reductions in heart rate.

4.6.2.3 Is the increased length of stay noted in PAD due to association with other common comorbidities?

The preoperative comorbidities associated with PAD, both in this study and in the general literature, (diabetes, congestive heart failure, hypertension) have all previously been associated with an increased incidence of postoperative complications (Khuri et al. 2005).

However, a relative risk reduction of 36% for a prolonged hospital length of stay was noted in the absence of PAD in patients with a Revised Cardiac Risk Index of >3 (which accounts for age, diabetes mellitus and known ischaemic heart disease), when compared with patients with PAD, suggesting an independent role for PAD in the development of postoperative morbidity.

Indeed, when other known major contributors to increased postoperative length of stay were either excluded from analysis (Anaerobic Threshold), or included in a proportional hazards model (AT, RCRI, BMI, increased age), PAD remained the strongest independent predictor or impaired postoperative outcome. When combined with a reduced AT (<11 ml.kg.min^{-1}), the risk of adverse postoperative outcomes with PAD increased.

4.6.2.4 Are the impaired outcomes seen in PAD secondary to reduced aerobic fitness?

Reduced heart rate recovery and reduced heart rate variability are both associated with reduced aerobic fitness in otherwise healthy individuals. Both can be improved with aerobic training (De Meersman, 1993; Jin et al. 2005; Streuber, Amsterdam, & Stebbins, 2006; Trevizani et al., 2012). The reduced anaerobic threshold noted in this study in individuals with PAD, at first assessment, supports these observations.

However, the mean AT of 12.2 ml.kg^{-1}min^{-1} recorded across all patients enrolled, regardless of heart rate recovery after exercise, is low and corresponds with a NYHA cardiac failure class of 1 (Hansen et al. 2012), suggesting that the entire population studied was aerobically deconditioned (The mean AT of 11.5 ml.kg^{-1}min^{-1} seen in the PAD cohort corresponds to an NYHA class of 2, corresponding only with slight limitation of ordinary physical activity, and not falling below the commonly accepted prognositically important value in the perioperative period of 11 ml.kg^{-1}min^{-1} (Older 2013)).

On the other hand, the peak VO_2 of 19.1 ml.kg^{-1}min^{-1} recorded across all patients is age appropriate (the population median age being 67 years); Wasserman equation predicted VO_2 max for this age group being: 19.7 ml.kg^{-1}min^{-1}. Peak VO_2, described previously, was statistically different across between PAD patients and those with normal heart rate recovery, but practically the difference measured (2.2 ml.kg^{-1}min^{-1}) is likely to be

unimportant and could represent the age difference between groups (peak VO_2 reducing with age and the PAD group being older).

When combined with the observation that impaired heart rate recovery continues to be predictive of postoperative morbidity where a reduced AT (<11) is absent, and that the combination of a low AT with a low heart rate recovery further increases risk, PAD remains an independent risk factor for post-operative complications instead of simply mirroring reduced aerobic fitness.

4.6.2.5 Delayed return of bowel function as a consequence of PAD

Postoperative gastrointestinal dysfunction (PGID) has repeatedly been shown to increase hospital length of stay (Doorly & Senagore 2012; Iyer et al. 2009; Van Acht et al. 2010; Holte & Kehlet 2000b; Karmali et al. 2015) .

The role of intact vagal activity in normal gastrointestinal function is well established (Hall & Guyton 2006). Even under normal conditions, gastrointestinal vagal activity may be impaired in the perioperative period by circulating inflammatory mediators, commonly administered drugs (such as Propofol and morphine) and prolonged perioperative starvation (X. Wang et al. 2004; Schwartz 1983; Havel et al. 1992). Reduced vagal tone in the postoperative period may delay restoration of normal gastrointestinal function (Lubbers et al. 2010). Delayed return of gastrointestinal function secondary to vagal withdrawal occurs even in surgeries distant from the gastrointestinal tract (Karmali et al. 2015).

Strategies to treat PGID through augmentation of vagal activity have included direct vagal stimulation and early administration of lipid rich feed (Lubbers et al. 2010) as well as sham feeding (which has been shown to beneficially modulate autonomic activity as measured by heart rate variability) (Karmali et al. 2015) and avoidance of morphine (Tu et al. 2014).

To date no study has been made of the influence of established PAD on postoperative gastrointestinal function, though a plausible role in the pathogenesis of PGID could be hypothesised. Confirmation of this role is proffered by the recorded delay in resumption of bowel function in this study.

4.6.2.6 The incidence of sepsis is increased in PAD, alongside alterations in neutrophil to lymphocyte ratio

Many plausible mechanisms for immune dysregulation in PAD have already been put forward. These appear to be supported by evidence for an increased likelihood of developing septic complications in the postoperative period in PAD. Whilst no specific assays of white cell function were carried out in this study, circumstantial evidence derived both from the observations of increased GRK2 expression in circulating lymphocytes in PAD and from alterations in perioperative NLR, support the concept of immune dysregulation in surgical patients presenting with established vagal withdrawal.

Though preoperative neutrophil to lymphocyte ratios were not different between cohorts, they were elevated in both patients with normal heart rate recovery and PAD as compared with population norms (Azab et al. 2014; Venkatraghavan et al. 2015).

A low anaerobic threshold at preoperative CPET has been associated with a raised preoperative NLR, and down regulation of monocyte $CD14^{+}$ expression, representing chronic inflammation. Values for preoperative NLR seen here are equivalent to those seen in high risk patients as defined by low AT in a similar surgical context (Sultan et al. 2014).

Postoperatively however, greater elevations in NLR were noted in individuals with PAD, reflecting acquired lymphopenia in these patients. Perioperative acquired lymphopenia has been associated with global bioenergetic impairment as well as NLRP1 inflammasome activation and increased risk of postoperative infectious complications (Edwards et al. 2015). Whilst no

difference in CRP was noted between groups in the postoperative period, more subtle interrogation of perioperative inflammatory parameters are warranted by these findings.

4.6.3 General Discussion

Perioperative studies to date have been hampered by differences in measurement modality; focus on the severe autonomic dysfunction noted in diabetic neuropathy and lack of adequate preoperative characterization and baseline measurements of function prior to the onset of an acute stressor (surgery). Specific study has not been made of the effects of established preoperative parasympathetic autonomic dysfunction on the development of postoperative complications. Neither have any attempts been made to develop mechanistic explanations for negative outcomes that have been described beyond haemodynamic imbalances (Lankhorst et al. 2014).

Mechanisms by which vagal withdrawal could result in immune and cardiac dysfunction are yet to be tested in the perioperative context. Experimental studies in other contexts where vagal activity has been modulated have shown improved outcomes in various disease processes, including myocardial ischaemia, sepsis and ileus. (Pavlov & Tracey 2005; Saeed et al. 2005; Kakinuma et al. 2005; Thayer et al. 2011).

Vagal modulation in the perioperative period could help determine the contribution of PAD to the development of postoperative morbidity. Preoperative interventions such as exercise-based prehabilitation could be expected to improve baseline vagal tone. Perioperative use of pharmacological vagal modulation and/or direct nerve stimulation may prove problematic due to the potential for off target effects and would need to be assessed carefully prior to use in a randomised controlled trial.

4.6.4 Significance of the findings

These data provide an alternative explanation for the development of postoperative morbidity in certain high-risk patients undergoing major surgery. The presence of parasympathetic autonomic dysfunction in other medical populations has provided several potential therapeutic targets to reduce the risk of morbidity and mortality, which could translate to the perioperative period.

Second, these data highlight a population of high-risk patients who may not be identified by current conventional methods of testing, but who are at risk of perioperative morbidity. Identification of these individuals in the preoperative period could help identify those who warrant increased vigilance in the perioperative period.

4.6.5 Study Strengths and limitations

4.6.5.1 Strengths:

The study was designed, powered and executed based on an *a priori* hypothesis generated by reference to published clinical and translational literature. The use of two cohorts from different centres adds strength to the results.

Primary and Secondary outcomes were defined before analysis. Individuals blinded to the eventual outcomes carried out data collection. Similarly, analysis of physiological data was also undertaken prior to analysis of outcomes data.

The demographics of patients included in this study, as well as the postoperative course of the study population suggest generalizability of the results may be reasonable.

4.6.5.2 Limitations:

This was an observational study where much of the data was retrospectively collated.

The inclusion of patients enrolled in a clinical trial (COMPETE-C) may limit the strength of conclusions drawn. However, steps were taken to minimise the impact of this on outcome data analysis where known confounders were excluded from analysis. Furthermore, both patients who did and did not receive GDT were equally represented in both cohorts and the final outcome of COMPETE-C showed no benefit for GDT.

Different management (e.g. use of critical care services) in patients who presented with a low Anaerobic Threshold at UCLH cannot be excluded.

Full Morbidity data was not available from the UCLH site (Clavien-Dindo). Further useful information could be gleaned from prospective data collection of other risk and morbidity scores and analysis of morbidity data as time to become morbidity free. This would avoid the 'snap-shot' nature of recording single worst morbidity scores.

Length of stay, bowel function and mortality data do however seem to support the Clavien-Dindo findings.

Full laboratory and medication history data was not available from the DHP site. This was mitigated by subsequent collection and analysis of the UCLH data, after it had been established that length of stay was increased at DHP.

No specific immune function assays were carried out to support the hypothesis that PAD is associated with immune dysregulation in surgical patients.

4.7 Conclusion and further work

Established Parasympathetic Autonomic Dysfunction (PAD) is common in high-risk surgical patients and is associated with increased postoperative morbidity and mortality.

Further work will focus on description of the physiological phenotype associated with high-risk surgical patients with PAD. Interventional studies where parasympathetic withdrawal is targeted will determine both whether PAD is a mechanism and/or a modifiable risk factor for the development of a suite of postoperative complications.

5 Intraoperative cardiac function in Parasympathetic Autonomic dysfunction

5.1 Introduction

Preoperative parasympathetic autonomic dysfunction (PAD), as defined by reduced heart rate recovery after exercise, is associated with reduced cardiac function at preoperative CPET and impaired postoperative outcomes.

5.1.1 Global Oxygen Delivery and postoperative outcome

Reduced cardiac output and therefore DO_2 with resultant end organ hypoperfusion in the perioperative period are strongly implicated in the subsequent development of multi organ dysfunction (MODS) and consequent morbidity and mortality. This has resulted in widespread adoption of goal directed therapy (GDT) protocols that specifically target increased cardiac output and DO_2 with some success in reducing postoperative complications (Aya et al. 2013; Grocott et al. 2013; Lees et al. 2009b; Shoemaker 1988).

However, in the COMPETE-C trial where intraoperative GDT (stroke volume optimisation) was targeted at aerobically fit (AT >10.9 $ml.kg.min^{-1}$) and unfit (AT <11 $ml.kg.min^{-1}$) individuals, no additional benefit was conferred by GDT and indeed, patients in the 'fit' cohort suffered detrimental outcomes (Challand et al. 2012).

These findings are relevant since cardiac output measures in COMPETE-C improved from the start to the end of the case in both GDT and standard fluid therapy (control) groups (more so in aerobically fit individuals). Given that a large proportion of patients with a low AT in COMPETE-C also manifest PAD (47% vs.25% in the aerobically 'fit' group), it is possible that PAD could underlie the additionally impaired response to fluid resuscitation seen in the aerobically 'unfit' cohort. When combined with other potential mechanisms for

impaired postoperative outcomes in PAD discussed in previous chapters, it could be hypothesised that PAD confounded any potential outcome benefit for GDT in aerobically unfit patients. Similarly, given that 1 in 4 aerobically 'fit' patients in COMPETE-C demonstrated PAD, a potential influence on response to fluid resuscitation and outcome could be postulated in these individuals.

The preoperative exercise phenotype in PAD results in reduced global oxygen delivery at peak exercise. What cannot be determined from these findings, however, is whether this translates into impaired intraoperative cardiac performance.

In order to explore the relationship between preoperative PAD and intraoperative cardiac performance, the intraoperative cardiac output data of the Derriford Hospital Plymouth (COMPETE-C) cohort was reanalysed stratified for heart rate recovery.

5.1.2 Hypothesis

Parasympathetic autonomic dysfunction identified at preoperative exercise testing is associated with impaired intraoperative cardiac performance.

5.1.3 Aims

This hypothesis was addressed by exploring the effect of preoperative parasympathetic autonomic dysfunction on the following primary and secondary outcomes:

Primary:

- Intraoperative cardiac output as measured by non-invasive cardiac output monitoring (the oesophageal Doppler).

Secondary:

- Use of intraoperative vasopressor medication.
- Intraoperative fluid administration.

5.2 Methods

5.2.1 Patient Population

Patients scheduled to undergo major colorectal surgery were enrolled in the COMPETE-C study at Derriford Hospital Plymouth (DHP) as previously described (Chapter 3).

Data available from this study was reanalysed based on preoperative heart rate recovery parameters. Abnormal heart rate recovery was defined as previously as a heart rate recovery of <12 bpm at 1 minute after cessation of loaded exercise.

Data derived from the COMPETE-C trial provided information on intraoperative haemodynamic changes in patients randomised to intraoperative oesophageal Doppler guided stroke volume optimisation or current standard of care fluid therapy. Due to the confounding nature of a pneumoperitoneum on intraoperative haemodynamics and autonomic function (Odeberg et al. 1994; Gannedahl et al. 1996), laparoscopic procedures were excluded from analysis and only open colorectal surgery examined.

5.3 Results

5.3.1 Group demographics

Global demographic data for the DHP cohort are presented in Chapter 3.

There were no differences in intraoperative fluid administration between groups. Neither was there any difference in epidural allocation. Allocation to GDT or standard of care fluid therapy was even between groups (Table 5-1).

Intraoperative Physiology:	Normal HRR (HRR >12) n=153	PAD (HRR ≤12) n=82	P-value
GDT allocation (%),	48	52	---
Total volume of Intraoperative fluid administered (ml), (mean, SEM)	2684 (2393-2974)	2706 (2276-3135)	0.9
Urine output (ml kg^{-1} h^{-1})	1.97 (0.82-3.5)	1.93 (0.94-3.35)	0.8
Epidural (%)	50%	49%	-

Table 5-1: Global characteristics of Normal Heart rate recovery (HRR) and Parasympathetic Autonomic Dysfunction groups (PAD). Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for two-way ANOVA. GDT = Goal Directed Therapy.

5.3.2 Intraoperative Haemodynamic Parameters

PAD was associated with reduced cardiac contractility as defined by peak velocity and reflected in stroke volume at both the start and the end of the operative case (Table 5-2).

In both the PAD and the normal heart rate recovery groups, peak velocity had increased by the end of the case (Normal: p=<0.01, PAD: p=<0.01; two way ANOVA). Peak velocity increased proportionately less in PAD than in individuals with normal parasympathetic autonomic function (20% increase in peak velocity in the normal heart rate recovery group at the end of the case vs. 13% increase in PAD, p=<0.05).

These changes in peak velocity were reflected in reduced stroke volume in PAD patients, both at the start and end of the case (25% increase in the control group vs. 9% in PAD, p=<0.05).

No difference was seen in heart rate between the groups at any point. At the end of the operation, both PAD and control groups had lower heart rates as compared with the start of the operation.

Mean Arterial Pressure (MAP) was similar across groups at the start of surgery. Though Flow Time Corrected (FTc: a measure of total peripheral resistance), whilst in the normal range for both at the start of surgery, was reduced in the PAD group suggesting increased afterload. MAP dropped after induction of anaesthesia in both groups, but was more likely to drop a prognostically significant >20% in individuals with PAD (RR 1.4, (1.2-1.6) p=0.08) (Bijker et al. 2007).

MAP at the end of surgery was reduced in both cohorts. In PAD, MAP at the end of the operation was more likely to be a prognostically important <55 mmHg than in normal patients (RR: 1.3 (1.09-1.4); p=0.03) (Walsh et al. 2013).

Cardiac index was globally preserved across both cohorts at both the start and the end of surgery, independent of the presence or absence of parasympathetic dysfunction (p=0.35). However, patients with PAD were more likely to exhibit a drop in cardiac index at the end of surgery, despite fluid resuscitation than their comparators without PAD ((30% of patients with PAD exhibited a drop in CI vs. 15% of those with normal HRR) Fisher's exact test: RR 1.26 (95% CI 1.05 to 1.5); p=0.006).

PAD patients received an increased total intraoperative metaraminol dose (vasopressor) as compared to those with normal heart rate recovery (4.8 mg vs. 7.7 mg, p=0.001), though they were no more likely to receive metaraminol than those with normal HRR (76% vs. 78%, P=0.76 (Fisher's exact test)).

18% of patients with PAD received a dose of metaraminol in excess of 10mg vs. 9% of those with normal HRR (OR 2.2 (95% CI 1.1 to 5.1); p=0.05).

Intraoperative Physiology:	Normal HRR (HRR >12) n=153	PAD (HRR ≤12) n=82	P-value
Heart Rate at start of case (bpm),	75 (72-77)	78 (74-82)	0.22
Heart Rate at end of case (bpm),	68 (66-70)	71 (67-74)	0.22
MAP at start of case (mmHg)	106 (102-109)	106 (102-110)	0.8
MAP at end of case (mmHg),	79 (76-80)	74 (71-76)	0.02
Peak Velocity start of case cm/s	66.1 (61.4-71)	55.6 (52-59)	0.003
Peak Velocity end of case cm/s	80.5 (75-85)	63.8 (59-68)	<0.01
Stroke Volume (ml) Start of case	85.8 (81-91)	76.2 (71-81)	0.01
Stroke Volume (ml) End of case	113.6 (107-120)	86.4 (81-91)	< 0.01
FTc, (s) Start of Case	356 (348-364)	342 (330-354)	0.05
FTc (s) End of Case	380 (373-386)	370 (360-381)	0.08
Cardiac Index Start of Case	3 (2.5-3.2)	2.9 (2.5-3.1)	0.28
Cardiac Index End of Case	3.9 (3.7-4)	3.7 (3.4-4)	0.35
Intraoperative Metaraminol (mg, (mean, SEM)	4.8 (4-5.5)	7.7 (5.7-9.7)	0.01

Table 5-2: Intraoperative Haemodynamic parameters in patients undergoing major colorectal surgery with and without Parasympathetic Autonomic Dysfunction (PAD). Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for two-way ANOVA. MAP = Mean Arterial Pressure FTc = Flow Time Corrected.

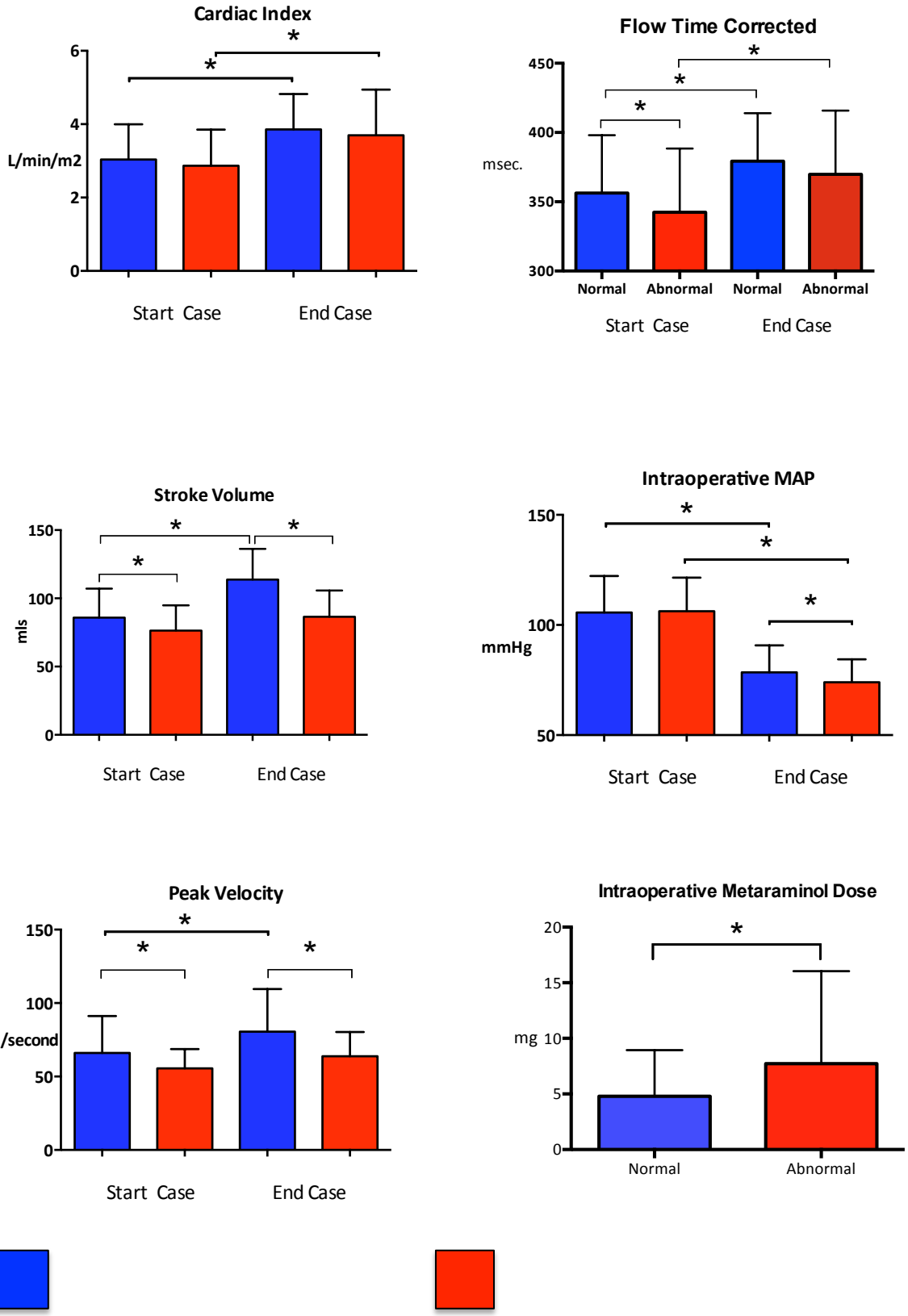

Figure 5-1: Impact of PAD on intraoperative cardiac performance. All comparisons made by two-way ANOVA. Data are presented as mean ± SD. Asterisk= P ≤0.05

5.3.3 The relationship between reduced anaerobic threshold (<11 ml.kg.min^{-1}) and intraoperative haemodynamic parameters

Given that the results presented above indicate that PAD is associated with impaired intraoperative cardiac performance, and that PAD is associated with a reduced anaerobic threshold, I examined whether a prognositically significant anaerobic threshold of 11 ml.kg.min^{-1} alone would reproduce the same haemodynamic profile seen in PAD.

No difference was seen in stroke volume, heart rate, blood pressure or total peripheral resistance between groups at any point intraoperatively (Table 5-3). At the end of surgery, cardiac contractility as represented by peak velocity appeared to be reduced in individuals with a low AT, though not to statistical significance.

Intraoperative Physiology:	AT >11 ml.kg.min^{-1}	AT <11 ml.kg.min^{-1}	P-value
Heart Rate at start of case (bpm),	76 (75-80)	78 (74-80)	0.5
Heart Rate at end of case (bpm),	69 (67-72)	68 (66-70)	0.4
MAP at start of case (mmHg)	106 (103-109)	105 (103-108)	0.8
MAP at end of case (mmHg),	77 (75-79)	79 (77-81)	0.3
Peak Velocity start of case cm/s	64 (57-65)	61 (57-65)	0.3
Peak Velocity end of case cm/s	78 (72-83)	72 (66.5-76)	0.07
Stroke Volume (ml) Start of case	77 (72-81)	74 (71-76.6)	0.1
Stroke Volume (ml) End of case	93 (87-99)	91 (89-96)	0.9
FTc, (s) Start of Case	355 (347-364)	350 (343-358)	0.36
FTc (s) End of Case	379 (372-386)	375 (370-382)	0.44
Cardiac Index Start of Case	**3.6 (3.4-3.8)**	**2.9(2.8-3.1)**	**<0.001**
Cardiac Index End of Case	**3.9 (3.6-4)**	**3 (2.8-3.2)**	**<0.001**

Table 5-3: Intraoperative Haemodynamic parameters in patients undergoing major colorectal surgery with and without an anaerobic threshold <11. Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for two way ANOVA. GDT = Goal Directed Therapy. MAP = Mean Arterial Pressure FTc = Flow Time Corrected.

5.3.4 Intraoperative haemodynamic parameters in aerobically 'fit' and 'unfit' patients with and without PAD

Since a low anaerobic threshold alone did not reliably reproduce the haemodynamic profile noted globally in PAD, but was associated with reduced cardiac contractility at the end of surgery, I sought to confirm the association of impaired cardiac contractility with vagal withdrawal by examining the influence of PAD on intraoperative haemodynamics in individuals who were aerobically 'fit' (AT >11) and 'unfit' (AT 11).

In ‘unfit’ individuals, cardiac contractility, as represented by peak velocity and reflected in stroke volume, was again reduced in PAD as compared with normal HRR. (Table 5-4).

Intraoperative	Normal HRR	PAD	P-value

Physiology:	(HRR >12) (n=48)	(HRR ≤12) (n=31)	
All data are from patients with a preoperative Anaerobic Threshold of < 11 ml.kg.min^{-1} **(Aerobically unfit)**			
Heart Rate at start of case (bpm),	75 (70-79)	78 (72-84)	0.4
Heart Rate at end of case (bpm),	65 (62-67)	70 (65-77)	0.06
MAP at start of case (mmHg)	106 (101-111)	104 (98-110)	0.6
MAP at end of case (mmHg),	80 (75-84)	83 (76-89)	0.5
Peak Velocity start of case cm/s	62.4 (53.6-71.2)	55 (49-59.7)	0.15
Peak Velocity end of case cm/s	73.5 (65.3-81.6)	60.5 (54.8-67.1)	0.03
Stroke Volume (ml) Start of case	73.8 (64-83.7)	63.3 (54.6-71.9)	0.04
Stroke Volume (ml) End of case	87.2 (78-96.2)	70 (60-80)	0.02
FTc, (s) Start of Case	349 (335.6-363)	338.3 (319-357)	0.4
FTc (s) End of Case	378 (366-389)	357.6 (339-376.5)	0.05
Cardiac Index Start of Case	2.9 (2.6-3.2)	2.8 (2.4-3.3)	0.8
Cardiac Index End of Case	3.7 (3.3-4)	3.4 (3-4)	0.4

Table 5-4: Intraoperative Haemodynamic parameters in patients undergoing major colorectal surgery with and without Parasympathetic Autonomic Dysfunction (PAD). All patients in this analysis have an anaerobic threshold of <11 ml.kg.min^{-1}. Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for student's unpaired t-test or ANOVA. GDT = Goal Directed Therapy. MAP = Mean Arterial Pressure FTc = Flow Time Corrected.

Similarly, when only haemodynamic parameters in aerobically 'fit' individuals with an AT >11 were examined, a similar haemodynamic profile was produced in individuals with PAD of relatively reduced cardiac contractility at both the start and end of surgery (Table 5-5).

Intraoperative Physiology:	Normal HRR (HRR >12) (n=80)	PAD (HRR ≤12) (n=33)	P-value
All data are from patients with a preoperative Anaerobic Threshold of > 11 ml.kg.min-1 **(Aerobically fit)**			
Heart Rate at start of case (bpm),	75 (72-80)	79 (73-85)	0.33
Heart Rate at end of case (bpm),	70 (67-72)	70 (66-74)	0.9
MAP at start of case (mmHg)	105 (101-109)	107 (102-113)	0.6
MAP at end of case (mmHg),	78 (75-80)	76 (71-81)	0.5
Peak Velocity start of case cm/s	66.9 (61.3-72.2)	56.3 (51.7-61)	0.02
Peak Velocity end of case cm/s	82.3 (75.6-89.14)	65.9 (59.8-72)	0.005
Stroke Volume (ml) Start of case	80.3 (74-86.7)	67.6 (62-73)	0.02
Stroke Volume (ml) End of case	98.9 (90.8-107)	79.1 (71.8-86.4)	0.005
FTc, (s) Start of Case	360.3 (351.1-369.5)	343.1 (325.6-360.6)	0.06
FTc (s) End of Case	379.6 (372-387.1)	378.4 (362.7-394.1)	0.9
Cardiac Index Start of Case	3 (2.8-3.3)	2.8 (2.5-3.2)	0.2
Cardiac Index End of Case	3.9 (3.6-4)	3.9 (3.4-4.3)	0.97

Table 5-5: Intraoperative Haemodynamic parameters in patients undergoing major colorectal surgery with and without Parasympathetic Autonomic Dysfunction (PAD). All patients in this analysis have an anaerobic threshold of >11 ml.kg.min^{-1}. Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for student's unpaired t-test or ANOVA. GDT = Goal Directed Therapy. MAP = Mean Arterial Pressure FTc = Flow Time Corrected.

Both PAD and a low AT were associated with reduced cardiac contractility, as represented by peak velocity, at the end of surgery. However, the presence or absence of PAD had a greater influence on peak velocity than AT alone (Figure 5-2, Table 5-6). PAD with a reduced AT was associated with the most severely impaired cardiac contractility (One way ANOVA p=<0.001).

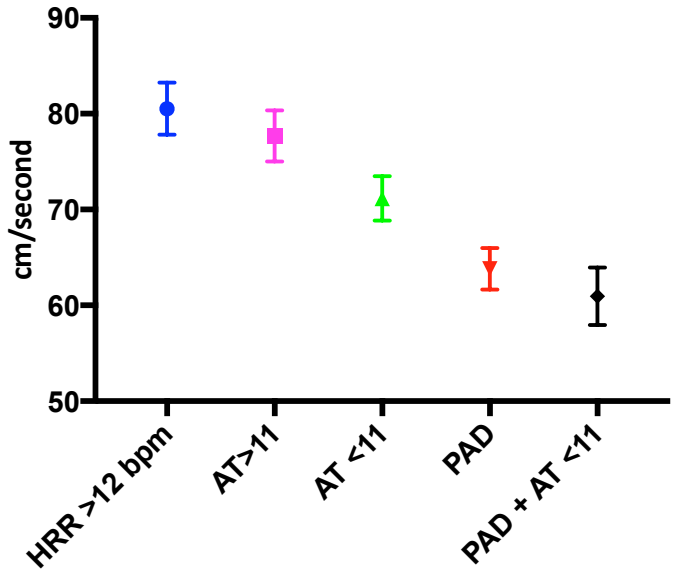

Figure 5-2: Peak velocity (mean +/- sd) at the end of surgery classified according to presence or absence of PAD and/or aerobic fitness (Anaerobic threshold > or < 11 ml.kg.min^{-1}). HRR: Heart rate recovery, AT: Anaerobic threshold (ml.kg.min^{-1}), PAD: Parasympathetic Autonomic Dysfunction. One way classical ANOVA, p=<0.001)

Peak Velocity (cm/ond)	Normal Heart rate recovery (>12 bpm)	Aerobically 'Fit': AT > 11 ml.kg.min-1	Aerobically 'Unfit': AT ><11 ml.kg.min-1	PAD (HRR <12 bpm)	PAD + Low AT
Mean	80.5	77.7	71.1	63.8	61
95% CI of mean	75-86	72.4-82.9	66.5-75.8	59.5-68.1	55-67

Table 5-6: Peak velocity (mean +/- sd) at the end of surgery classified according to presence or absence of PAD and/or aerobic fitness (Anaerobic threshold > or < 11 ml.kg.min^{-1}). HRR: Heart rate recovery, AT: Anaerobic threshold (ml.kg.min^{-1}), PAD: Parasympathetic Autonomic Dysfunction.

5.4 Discussion

5.4.1 Key findings

1. Cardiac Contractility and Stroke Volume were reduced in patients with PAD at start and finish of surgery.
2. Mean Blood Pressure was reduced in PAD at the start and finish of surgery.
3. Intraoperative heart rate was not affected by PAD at any time point intraoperatively.
4. An Anaerobic Threshold of <11 alone was not sufficient to produce the same haemodynamic profile as PAD.
5. An Anaerobic Threshold of <11 and PAD were associated with the greatest impairments in cardiac contractility.

5.4.2 Discussion of Key findings

Patients with PAD demonstrate a distinct intraoperative haemodynamic profile characterised by reduced cardiac contractility, increased total peripheral resistance as represented by a reduced FT_c and intraoperative hypotension requiring increased vasopressor usage.

This profile complements the findings of reduced oxygen pulse, reduced peak VO_2 and hypertension noted in individuals with PAD at preoperative exercise testing.

5.4.2.1 Cardiac Contractility

As discussed previously, an increasing body of evidence is accumulating to support a role for intact parasympathetic function in mediating increased cardiac contractility.

PAD was associated with reduced Stroke Volume (SV) and peak velocity (representing cardiac contractility) at both the start and end of the operative

case. Of note, whilst both groups demonstrated an increase in SV and contractility at the end of the case (perhaps reflecting both fluid resuscitation and increased sympathetic drive as a result of surgical trauma), individuals with PAD failed to significantly increase contractility when compared with individuals with intact parasympathetic function. For most GDT protocols, a sustained increase in stroke volume of 10% is taken as marker of successful fluid resuscitation. As a cohort, patients with PAD failed to increase stroke volume in response to fluid therapy by 10% from baseline implying that this group of patients might not benefit from standard stroke volume guided GDT protocols, but in fact might be at risk of fluid overload.

5.4.2.2 Heart Rate

Significantly, intraoperative heart rate at any time point intraoperatively was not different between groups. Using conventional monitoring (Heart Rate alone), it would not be possible to distinguish between individuals with and without PAD, emphasizing the utility of flow based intraoperative monitoring. Similarly, heart rate alone would not distinguish reduced cardiac output in aerobically ‘fit’ and ‘unfit’ individuals.

5.4.2.3 Blood Pressure

Intraoperative hypotension is associated with PAD. Patients with PAD received higher doses of intraoperative vasopressor to maintain MAP than their comparators, despite similar fluid doses.

This finding of increased systemic vascular resistance is supported by observations in Sino Aortic Denervation (SAD), an animal model of neurogenic hypertension characterised by impaired afferent parasympathetic function, of increased total peripheral resistance (Rodrigues et al. 2011).

Similarly, parasympathetic withdrawal and increased systemic vascular resistance are key features of human hypertension (Julius 1991; Berg & Jensen 2011; Shepherd 1990; Davis et al. 2012).

Sustained, or even increased afterload, represented by a reduced corrected flow time on oesophageal Doppler monitoring, suggest that reductions in intraoperative blood pressure in PAD are due to reductions in Stroke Volume rather than vasoplegia.

Intraoperative hypotension has been described previously in global autonomic dysfunction (Mazzeo et al. 2011; Knuttgen et al. 1990; Latson et al. 1994; Huang et al. 2006) and is associated with impaired postoperative outcomes, including stroke, acute kidney injury, myocardial ischaemia and infarction, increased length of stay and 1-year mortality (Walsh et al. 2013; Bijker et al. 2012; Tassoudis et al. 2011; Bijker et al. 2009).

In routine clinical practice, where cardiac output monitoring is infrequently undertaken (Pearse et al. 2014), hypotensive episodes are treated with fluid or further vasopressors (both of which have been associated with adverse clinical outcomes). In the PAD patients examined here, such interventions may not be rational and could even be detrimental by causing fluid overload and/or further impairments in cardiac output. Preoperative evidence of PAD should trigger increased intraoperative vigilance for hypotension and potentially the use of invasive blood pressure monitoring as well as flow directed cardiac output monitoring.

5.4.2.4 Reduced Anaerobic Threshold and intraoperative haemodynamic parameters

Low anaerobic threshold may be associated with impaired perioperative outcomes through mechanisms other than impaired cardiac output. It is possible that PAD, a common association with a low AT is one of these mechanisms.

At preoperative exercise testing, PAD was associated with a reduced anaerobic threshold. Indeed, a greater proportion of patients with PAD

demonstrated a prognostically important AT of <11 ml.kg.min^{-1} than those without (RR 1.26 (95% CI 1.1 to 1.6); p=0.02).

In this cohort of surgical patients, an AT of <11 ml.kg.min^{-1} alone did not reproduce the same intraoperative haemodynamic findings as PAD. Furthermore, PAD produced the same distinctive haemodynamic profile in patients with an AT >11 ml.kg.min^{-1}.

PAD was associated with both a reduced oxygen pulse at preoperative CPET and reduced intraoperative contractility. The greater reductions in preoperative oxygen pulse seen in PAD when compared with low aerobic threshold preoperatively appeared to translate into impaired contractility at the end of surgery, again suggesting that impaired heart rate recovery represents established vagal withdrawal rather simply global deconditioning.

Unlike in PAD, individuals with a low AT exhibited a reduced cardiac index at both the start and end of the operative case as compared with controls. This comparatively low cardiac index despite no difference in SV, MAP or heart rate may be explained by an increased BMI in the AT <11 cohort as cardiac output not indexed to body weight was similarly unchanged between groups. Importantly, the cohort with a low AT was able to significantly increase stroke volume from the start to the end of the case (by 20%), demonstrating fluid responsiveness not seen in PAD.

Low cardiac output is thought to fuel the development of perioperative organ dysfunction through tissue hypoperfusion. Augmentation of DO_2 using GDT in patients with a low AT did not however reduce morbidity in this cohort of patients (Challand et al. 2012) or others (Lai et al. 2015). Indeed, the POM-O trial (Ackland et al. 2015) reported that patients with a low AT were more likely to achieve a DO_2 (100 ml.min^{-1}) associated with reduced postoperative morbidity. This finding is reflected in this patient cohort by an increase in SV from start to end case of 17% in patients with an AT <11 ml.kg.min^{-1}, 3% less than those with an AT >11.

5.4.2.5 The implications of PAD on intraoperative cardiac output management

Multiple studies have demonstrated the deleterious effects of impaired global oxygen delivery on post-operative outcome (Aya et al. 2013). Reduced cardiac performance caused by PAD, manifested in a preoperative reduction in oxygen pulse, chronotropic incompetence, a reduced stroke volume and cardiac contractility associated with hypotension, intraoperatively could result in reduced tissue oxygen delivery, causing impaired tissue oxygenation and the triggering of the neuroimmune cascade that results in multiorgan dysfunction.

Given that the individuals studied were enrolled in a negative goal directed therapy trial, PAD presents an alternative explanation for failure to improve outcomes.

Vagal withdrawal in itself has been associated with deleterious effects on cellular REDOX status and microcirculatory function (Wei & Kaul 2004; Pavlov & Tracey 2012) which may further be compounded by impaired cardiac function.

Neither microcirculatory parameters, nor markers of tissue hypoperfusion were available in this data set to test the assumption of tissue hypoxia secondary to reduced oxygen delivery. These would be best examined in a prospective trial.

Vagal innervation is cardioprotective during cardiac stress (Mastitskaya et al, 2012), withdrawal of which may be one of several potential mechanisms explaining why cardiac contractility was impaired intraoperatively. However, no evidence was recorded for overt cardiac ischaemic pathophysiology in the perioperative period. It is possible, however, that subclinical ischaemic episodes, associated with intraoperative hypotension, could have contributed to the negative postoperative outcomes recorded (Devereaux et al. 2012).

Cardiac Troponin or other markers of cardiac ischaemia were not measured in the DHP cohort. Patients with PAD in the UCLH cohort did undergo cardiac troponin testing as part of clinical care in the first postoperative day more frequently than those without, perhaps suggesting an increased clinical suspicion of perioperative myocardial ischaemia (though for both groups troponin testing was a rare occurrence. RR 1.03 (1 to 1.05), p=0.02). Future work may benefit from detailed recording of perioperative markers of cardiac ischaemia including pre and postoperative troponin and BNP.

5.4.3 Significance of the findings

Established parasympathetic autonomic dysfunction is associated with impaired intraoperative cardiac performance, characterised by a low stroke volume and cardiac contractility associated with a reduced blood pressure.

The identification of PAD in the preoperative period, aside from providing pertinent information for assessment of preoperative risk, should prompt more rigorous monitoring or management of intraoperative haemodynamic parameters. Consideration should also be given to increased vigilance in these individuals for markers of perioperative cardiac ischaemia.

PAD may predict patients who will not respond in a conventional manner to goal directed stroke volume optimisation. Flow based haemodynamic monitoring should be employed in these patients to minimise the impact of excessive fluid therapy and vasopressor usage.

5.4.4 Study Strengths and limitations

1) **Strengths:**

- The study was designed and executed based on an *a priori* hypothesis generated by reference to published clinical and translational literature and evidence gained from prior experiments.
- The study is the first to examine a relationship between preoperative established PAD and intraoperative cardiac performance

- The study is the first to examine a relationship between preoperative CPET derived measures of aerobic fitness and intraoperative cardiac performance.
- The study is linked to robust clinical outcome measures.

2) Limitations:

- The retrospective nature of the study may limit the strength of the conclusions drawn.
- The inclusion of patients from a trial studying intraoperative GDT may confound the results.
- The relatively small numbers of patients enrolled in the study may limit the wider applicability of findings.

5.4.5 Conclusion and further work

Established Parasympathetic Autonomic Dysfunction (PAD) is associated with a distinct intraoperative haemodynamic phenotype consisting of reduced cardiac contractility and is associated with intraoperative hypotension.

Future studies should be prospective and examine the effects of established PAD on intraoperative cardiac performance under controlled uniform conditions. Attention should be paid to microcirculatory and cellular REDOX parameters, as well as perioperative markers of myocardial ischaemia. Specific attention should be paid to stroke volume optimisation based Goal Directed Therapy to identify whether PAD is detrimental to outcome. In terms of the wider anaesthetic literature, attention should be paid to addressing the lack of direct correlation between preoperative CPET markers of aerobic performance and intraoperative cardiac performance.

Therapeutic studies, where vagal activity is modulated in the perioperative period, could establish whether the cardiac phenotype noted in PAD is reversible and whether restoration of parasympathetic autonomic function in this context is protective.

6 Sympathetic Autonomic Hyperactivity

6.1 Introduction

6.1.1 Sympathetic autonomic hyperactivity is associated with impaired outcomes

Sympathetic activation is central to an adaptive, coordinated cardiorespiratory, metabolic and immune response to infection and tissue injury. Acute hyperadrenergic stimulation, such as that seen during acute mental stress (Priyadarshini & Aich 2012), exercise (Fattor et al. 2005) and surgery (Desborough 2000), is characterised by several fold increases in the levels of circulating catecholamines, an increase in heart rate and blood pressure, endothelial activation and rapid, parallel decreases in heart rate variability (Mazzeo et al. 2011; Reyes del Paso et al. 2013; Nunan, Jakovljevic, et al. 2010; Thayer et al. 2010).

Excessive or inappropriate sympathetic activation can lead to pathological derangement and is a ubiquitous feature at the onset of critical illness. During the development of critical illness, persistent sympathetic autonomic activation, characteristically associated with cardiac dysfunction and immune paralysis, is a potent driver for the development of multi-organ dysfunction (Ackland et al. 2015).

Acute psychological stress, associated with increased sympathetic activity, alone can in susceptible individuals provoke excessive sympathetic outflow with resultant deleterious effects. Excessive sympathetic activation due to mental stress directly causes impairment of cardiac regulatory mechanisms, contributing to the development of cardiac ischaemia and endothelial dysfunction (Yeung et al. 1991; Ghiadoni et al. 2000; Deanfield et al. 1984; Rozanski et al. 1988; Ramachandruni et al. 2006).

In a minority of apparently otherwise healthy individuals, the thought of vigorous exercise alone results in excess increases in heart rate (EHRi), representing sympathetic autonomic hyperactivity. These individuals are at increased risk of sudden cardiac, and all-cause death (Jouven et al., 2010).

This excessive heart rate increase prior to exercise is easy to measure, but has not been used to define perioperative sympathetic autonomic hyperactivity in surgical patients previously.

In surgical patients, reduced heart rate variability, associated with sympathetic autonomic hyperactivity, has been associated with perioperative haemodynamic instability and prolonged postoperative myocardial ischaemia (Huang et al. 2006; Laitio et al. 2004). It could therefore be hypothesised that individuals exhibiting preoperative sympathetic autonomic hyperactivity defined by excessive heart rate increase before exercise might be at greater cardiac risk in the perioperative period.

Given the utility of heart rate dynamic measures recorded at the time of preoperative CPET in predicting PAD and its sequelae, I set out to investigate whether a similar approach could be used to identify established sympathetic autonomic hyperactivity in high-risk surgical patients. I also attempted to identify whether individuals with sympathetic autonomic hyperactivity as defined by excessive heart rate increase prior to commencement of loaded exercise at preoperative CPET, demonstrated altered cardiac function and increased risk of myocardial ischaemia during exercise and in the perioperative period.

6.1.2 Is sympathetic autonomic hyperactivity present in parasympathetic autonomic dysfunction?

Parasympathetic autonomic dysfunction, acting through several mechanisms outlined previously, is associated with a distinct physiological phenotype and

impaired postoperative outcomes in higher risk patients presenting for major surgery.

What is not clear, however, is the role, if any, of 'unopposed' sympathetic autonomic activity in PAD. A raised resting heart rate and raised blood pressure, seen in PAD, are traditionally associated with increased sympathetic outflow. Heart rate variability analysis in patients with abnormal heart rate recovery after exercise demonstrated reduced global heart rate variability, also associated with sympathetic autonomic hyperactivity. Similarly, evidence for raised GRK2 and Pan-Arrestin in circulating lymphocytes was suggestive of increased sympathetic autonomic activity and renal-angiotensin-aldosterone system activation. Reduced heart rate recovery itself may not always represent reduced parasympathetic activity alone, especially where 'severe' exercise has been undertaken (Buchheit, Laursen, et al. 2007).

Autonomic activity is often described as a balance between sympathetic and parasympathetic function; both limbs of the ANS having unique yet complementary roles in homeostasis. Whilst the two systems clearly interact, it does not automatically follow that parasympathetic withdrawal results in sympathetic autonomic hyperactivity, or that excessive sympathetic activation is always associated with chronic parasympathetic withdrawal.

In animal models of baroreceptor dysfunction, where PAD is produced by surgical baroreceptor deafferentation (sinoaortic denervation or SAD, analogous to the baroreceptor dysfunction described in PAD), after an initial increase in blood pressure and heart rate, accompanied by increased circulating levels of catecholamines, (Cornish & Gilmore 1985; Schreihofer & Sved 1994) blood pressure and heart rate tend to normalise in the long term, to be replaced by blood pressure debuffering at times of stress (Norman et al. 1980), accompanied by spiked increases in catecholamine production and sympathetic autonomic outflow (Cornish & Gilmore 1985; Schreihofer & Sved 1994).

Chronic SAD is associated with reduced heart rate variability, diastolic dysfunction and reductions in the high frequency and very low frequency components of frequency domain measures of HRV, with an increase in the low frequency component. These changes represent sympathetic hyperactivity associated with vagal withdrawal (Moraes-Silva et al. 2010). It is highly possible that this phenotype would be reflected in patients with PAD.

6.2 Primary Hypothesis

Exaggerated heart rate rise prior to the onset of vigorous exercise (Cardiopulmonary Exercise Testing) is associated with electrocardiographic evidence of myocardial ischaemia.

6.3 Aims

This hypothesis was addressed by exploring the effect of preoperative sympathetic autonomic dysfunction on the following primary and secondary outcomes:

Primary:

- Electrocardiographic evidence of myocardial ischaemia in lead II during Cardiopulmonary Exercise Testing.

Secondary:

- Preoperative Exercise Physiology.
- Intraoperative Cardiac Physiology.
- Post operative outcome as defined by length of hospital stay (LOS).

6.3.1 Secondary Hypothesis:

Parasympathetic Autonomic Dysfunction as defined by abnormal heart rate recovery after exercise is associated with sympathetic autonomic hyperactivity.

6.4 Methods

6.4.1 Patient Population

Two cohorts at separate centres were studied before planned elective surgery. Patients scheduled to undergo major colorectal surgery were enrolled as previously described at Derriford Hospital Plymouth (DHP) and University College London Hospitals (UCLH) having obtained IRB approval (MREC: 11/H0805/58). Informed written consent was obtained from patients undergoing preoperative CPET as routinely requested by their clinical teams prior to major elective surgery. Inclusion criteria were any surgical patient referred for preoperative CPET. Exclusion criteria were according to American Thoracic Society guidelines (Weisman et al. 2003).

6.4.2 Cardiopulmonary Exercise Testing (CPET)

All patients enrolled underwent routinely requested maximal, incremental, symptom limited CPET prior to surgery. The full protocol for CPET is in General Methods. Figure 6-1 illustrates the phases of the CPET protocol. As before, patients were acclimatised by sitting on the cycle for 3 minutes prior to the start of exercise. During this period, baseline readings were recorded. Patients then undertook 3 minutes of zero load pedalling, prior to the initiation of ramped exercise. Non-invasive blood pressure was taken at the start and end of the exercise test.

Heart rate increase was assessed as the difference between the heart rate at rest and the heart rate measured just before starting ramped loaded pedalling after 3 minutes of 0 Watt exercise.

6.4.3 Intraoperative Haemodynamics

As previously described, patients enrolled in the COMPETE-C trial at DHP underwent intraoperative cardiac output monitoring using the oesophageal Doppler monitor (see Chapter 11).

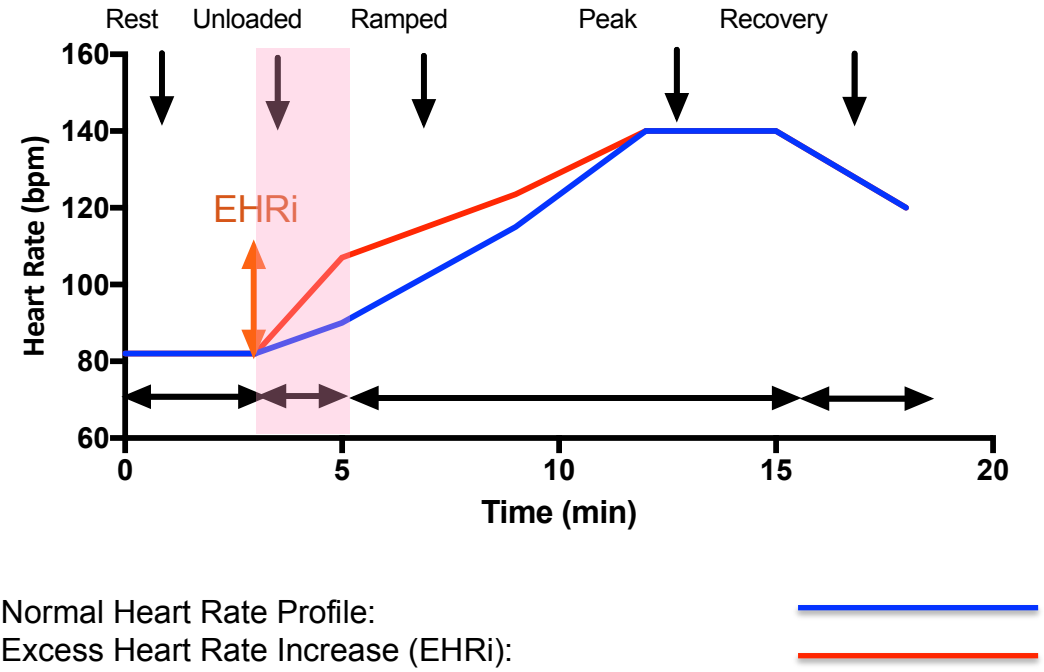

Figure 6-1: EHRi was calculated during unloaded pedalling prior to ramped exercise

Anaerobic Threshold and other physiological markers of exercise performance were measured as previously described.

6.4.4 Exercise evoked ischaemia

ST-segment depression was captured in lead II, as this lead is most easily obtained with conventional monitors and reflects the majority of ischaemic

events in the general population. The nadir value at any time during exercise testing was taken to represent the most significant cardiac ischaemia.

ST changes in this lead were defined as abnormal when ST depression of 0.1 mV (1mm) or more occurred (Lurati Buse et al. 2009). In order to control for the effects of dynamic heart rate changes on the ST segment during exercise, the ST-heart rate (ST/HR) index was calculated:

$$\frac{\text{ST nadir (mV)}}{\text{Heart Rate Gradient}}$$

Atrial and/or ventricular dysrhythmias, including ectopic beats, were also noted.

6.4.5 Sample Size and Statistical Analysis

Using the definition of myocardial injury reported in the VISION study (Botto et al. 2014), where around 8% of patients who had undergone non-cardiac surgery developed myocardial injury, it was estimated that significant ST depression would occur in around twice the number of patients with EHRi. Having established a prevalence of 43% in the Plymouth derivation cohort, at least 900 patients would be required to sufficiently power the study (alpha 0.05; power 80%).

6.5 Results

6.5.1 Patient Clinical and demographic variables

Clinical and demographic variables were as described for the entire study population and are described in Chapters 9 and 10. 1,052 patients were recruited across both centres. The Heart Rate data was not recorded in three patients where technical error resulted in lost data.

6.5.2 Frequency Distribution Statistics

Heart rate increase was distributed into quartiles. Excess Heart Rate Rise was defined as an exaggerated heart rate rise of ≥15 bpm based on the cut off for the upper quartile for combined UCLH and DHP data (Table 5-1, Figure 5-2). This value was slightly higher than previous data showing an association between stress-evoked heart rate changes before the onset of exercise (≥12)(Jouven et al. 2009), but may reflect the effects of even the minimal physical activity caused by zero load cycling on heart rate (a small minority of patients achieved anaerobic threshold even during this light exercise). Changes in heart rate during acclimatisation to the cycle ergometer were similar between centres.

Descriptor	**UCLH**	**DHP**	**Combined**
Total Number of values	817	232	1049
Total Number of excluded values:	0	0	0
75% Percentile HRi (bpm):	**15**	**16**	**15**
Median HRi (bpm):	9 (8-9)	10 (9-11)	9 (8-10)
25% Percentile HRi (bpm):	5	5	5
Maximum HRi (bpm):	81	63	81
Mean HRi (95% CI) (bpm):	11 (10 – 11.5)	12 (10.5 – 13)	11 (10.4-11.7)
D'Agostino & Pearson omnibus normality test (alpha 0.05)	No	No	No

Table 6-1: Frequency Distribution Statistics for Excessive Heart Rate Increase (EHRi), cohorts 1 & 2 (University College London Hospital: UCLH, Derriford Hospital Plymouth: DHP). HRi= Heart Rate increase prior to loaded exercise during unloaded pedalling. BPM= Beats per minute.

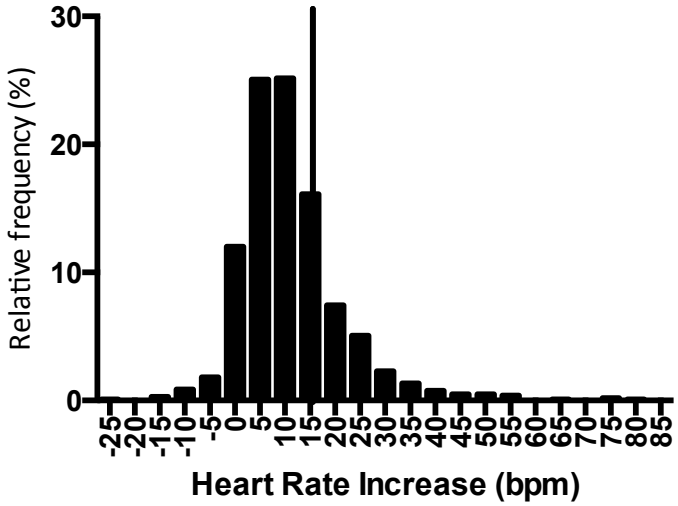

Figure 6-2: Distribution of changes in heart rate prior to loaded exercise. The 75% Percentile is marked and taken as representing an excessive heart rate increase (EHRi).

6.5.3 Baseline Patient Clinical and demographic variables Stratified by Heart Rate Increase prior to exercise

	UCLH			DHP		
	Normal (HRi <15)	**EHRi (HRi ≥15)**	**P**	**Normal (HRi <15)**	**EHRi (HRi ≥15)**	**P**
Number (%)	626 (77%)	192 (23%)	-	133 (57.3%)	99 (42.7%)	-
Age (years)	60 (59-61)	65 (63-67)	<0.001	65 (63-68)	67 (65-70)	0.68
Male (n,[%])	452 (73%)	83 (43%)	-	87 (65%)	44 (46%)	-
BMI (kg/m^2)	27 (26-28)	28 (27-29)	-	27 (26.7-28.2)	29 (28.2-30)	-
RCRI >2 (%)	5%	6%	-	2%	2%	-
ASA grade	n/a	n/a		2 (1-3)	2 (1-3)	
Malignancy (%)	46%	46%	-	77.4%	75%	-
Beta Blockers (%)	12.5%	12.6%	-			
Calcium Channel Blocker (%)	5%	6%	-			
Hypertension (%)	33%	54%	-			
Diabetes (%)	10%	10%				

Table 6-2: demographics for both cohorts, stratified by heart rate change during unloaded cycling (zero workload) of patients acclimatised to the exercise bike conditions. Values shown are median (95% CIs) unless otherwise indicated. Data analysed by ANOVA or Fisher's exact test. ANOVA = analysis of variance; EHRi= Excess Heart Rate Increase; HRi= Heart Rate increase; N=number; bpm= beats per minute; BMI= Body Mass Index (kg/m^2); RCRI= Revised Cardiac Risk Index; ASA= American Society of Anesthesiologists grade; bpm= beats per minute.

In the UCLH cohort, patients exhibiting EHRi were slightly older. This was not the case in the DHP cohort. In both cohorts, proportionately more females exhibited EHRi than males. Body Mass Index, use of cardiovascular drug therapy, and ASA were similar between and within groups.

There was no relationship found between EHRi and the Revised Cardiac Risk index (relative risk of RCRI >2 1.00 (95% CI 0.77 to 1.29); p=0.98) or in the DHP cohort an ASA grade >2 (RR 0.9 (95% CI 0.68 to 1.35; p=0.95).

6.5.4 Primary Outcome: Exercise Evoked Ischaemia

Continuous ECG recordings revealed an increased relative risk of developing significant ST depression (<1 mm) at any time during exercise in individuals with EHRi (RR: 1.7 (95% CI 1.3 to 2.1); p=,0.001). Similarly, EHRi was associated with a greater risk of demonstrating a prognostically significant ST/HR index of >1.6 (RR 1.3 (95 % CI 1.1 to 1.4); p= <0.01).

EHRi was not associated with exercise-evoked atrial and/or ventricular dysrhythmias (0 episodes of new atrial fibrillation in either group; 4 (1.4%) episodes of non-sustained VT in the EHRi group; 10 (1.5%) in the ‘normal’ group).

EHRi was further associated with an increased risk of undergoing troponin testing at any point in the first 7 postoperative days (RR 1.03 (95% CI 1 to 1.06); p=<0.03).

	Normal HRi	EHRi	p-value
ST change (mm)	-0.5 (-0.6 to -0.46)	-0.7 (-0.8 to -0.55)	0.04
ST/HR index	0.7 (0.6 to 0.8)	1.3 (1 to 1.5)	<0.001
Nadir ST segment > 1mm		(RR: 1.7 (1.3 to 2.1)	<0.001
ST/HR index >1.6		RR 1.3 (1.1 to 1.4)	<0.001

Table 6-3: Exercise Evoked Ischaemia stratified by heart rate change during unloaded cycling. Data are shown as mean (95% CI) for both cohorts. Data are analysed by one-way ANOVA (Analysis of Variance). Fisher's exact test used to calculate Relative Risk (RR). HRi = Heart Rate increase; EHRi = Excess Heart Rate Increase.

6.5.5 CPET physiological and autonomic characteristics

Neither resting, nor peak heart rates during exercise were associated with EHRi. Consistent with hyper-adrenergic stimulation, blood pressure at rest was higher in patients with EHRi. Blood pressure recordings at peak exercise appeared unrelated to EHRi, though consequently the blood pressure rise recorded during exercise was lower in EHRi.

Given the finding of an association between EHRi and ST-segment depression and an increased ST/HR index, compatible with myocardial ischaemia, an impaired cardiopulmonary reserve was expected.

	Normal	EHRi	p Value
Pre-exercise			
Resting heart rate (bpm)	84 (82-85)	82 (80-84)	0.09
Zero workload heart rate (bpm)	90 (89-91)	107 (104-110)	<0.001
Heart rate change (bpm)	7 (7-9)	27 (24-26)	<0.001
Exercise			
Peak heart rate during exercise (bpm)	138 (135-139)	139 (134-142)	0.45
Heart rate gradient during exercise (bpm)	54 (51-57)	56 (53-57)	0.6

Table 6-4: CPET Heart Rate Data, stratified by heart rate change during unloaded cycling (zero workload) of patients acclimatised to the exercise bike conditions. N=number; bpm= beats per minute; Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for student's unpaired t-test for means with Welch's correction where standard deviations cannot be assumed. Relative Risk and odds ratios calculated by Fisher's exact test, Two-tailed, alpha <0.05 taken as statistically significant.

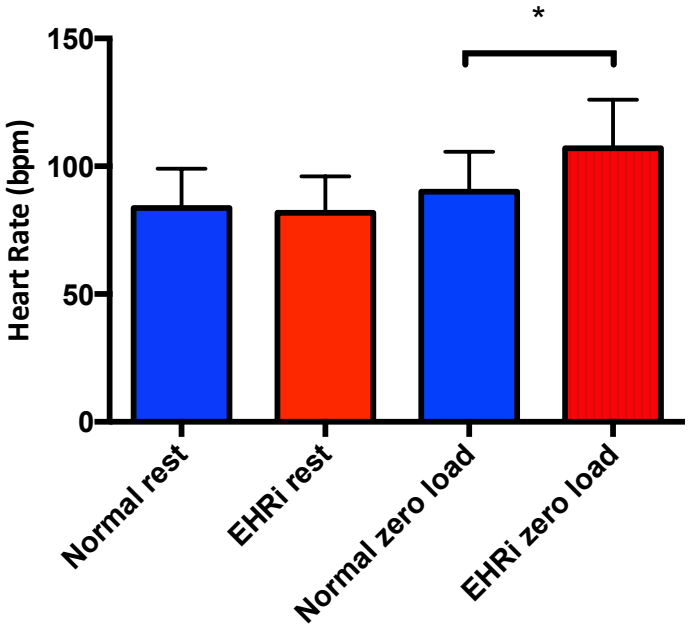

Figure 6-3: CPET Heart Rate Data, stratified by heart rate change during unloaded cycling (zero workload) of patients acclimatised to the exercise bike conditions. CPET = Cardiopulmonary Exercise Test; EHRi = Excess Heart Rate increase. BPM= beats per minute. Data analysed by one-way ANOVA. Asterisk indicates statistical significance ($p<0.05$).

Values for AT were not different between groups. Peak VO_2 and Oxygen pulse were both reduced in individuals with EHRi (oxygen pulse was reduced by around 6%). Indeed, oxygen pulse, a robust surrogate for left ventricular performance, was less likely to meet age, gender and weight predicted norms in patients with EHRi (relative risk 1.26 (95% CI 1.14 to 1.39); p,0.001).

CPET Physiology:	Normal HRi	EHRi	p-value
AT (mean, 95% CI)	11.6 (11.38-11.85)	11.5(11-11.8)	0.5
% of Predicted Peak VO_2 (mean, 95% CI)	76% (74-77)	73% (70-75)	0.06
Oxygen Pulse At AT ml.beat^{-1} (mean, 95% CI)	10.9 (10.16-11.1)	8.7 (8.3-9)	<0.0001
% of predicted Oxygen Pulse (mean, 95% CI)	91% (89-94)	85% (82-88)	0.002
V_E /CO_2 at AT (mean, 95% CI)	30 (29-31)	31 (30-31_	0.16
Baseline SBP (mmHg):	144 (142 – 147)	157 (153- 161)	0.02
Baseline DBP (mmHg):	83 (81 – 84)	86 (84 – 88)	0.02
Peak SBP (Exercise, mmHg)	190 (186 -193)	192 (187 - 195)	0.22
Peak DBP (Exercise, mmHg)	89 (86-92)	95 (88 – 102)	0.11
Increase from baseline (SBP, mmHg)	46 (43 – 49)	35 (31 – 38)	<0.001

Table 6-5: Cardiopulmonary exercise test (CPET) Physiological Data, stratified by heart rate change during unloaded cycling (zero workload) of patients acclimatised to the exercise bike conditions.) HRi = Heart Rate increase; EHRi = Excess Heart Rate Increase; bpm= beats per minute. AT = Anaerobic threshold (ml O_2 kg^{-1} min^{-1}). VO_2 = Oxygen uptake (ml O_2 kg^{-1} min^{-1}). Oxygen Pulse (, ml O_2 beat^{-1}). V_E/VCO_2 = ventilatory equivalents for Carbon Dioxide (Normal range 25-35). SBP = Systolic Blood Pressure (mmHg); DBP = Diastolic Bood Pressure (mmHg). Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for student's unpaired t-test.

6.5.6 Intraoperative Haemodynamic Parameters

Patients with normal heart rate profiles and EHRi were distributed evenly across the two trial arms. Both groups received equivalent volumes of fluid and neither demonstrated an increased vasopressor requirement.

	Normal (HRi <15)	EHRi (HRi ≥15)	P -value
GDT allocation (%),	49	51	---
Intraoperative Fluid (ml), (mean,95% CI)	2675 (2385-2966)	2732 (2303-3160)	0.8
Intraoperative Metaraminol (mgs), (mean, 95% CI)	5.5 (4.7 – 6.3)	5.5 (4.3-6.7)	0.9

Table 6-6: Fluid and vasopressor therapy in patients undergoing major colorectal surgery stratified by heart rate change during unloaded cycling. Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for one-way ANOVA. GDT = Goal Directed Therapy.

Heart rate after induction of anaesthesia was similar between groups. There were no differences recorded in cardiac contractility (as measured by peak

velocity), stroke volume, baseline MAP or FTc after induction of anaesthesia between groups.

By the end of surgery, mean MAP had reduced 5% more in patients exhibiting EHRi. This was accompanied by both a relative increase in FTc, representing peripheral vasodilation, and a smaller increase in stroke volume, perhaps reflecting the reduced afterload (given that cardiac contractility was not measured as different between the cohorts). Heart rate was relatively increased in the EHRi (Table 6-7).

Cardiac Index was preserved throughout the operative period in both groups.

	Normal (HRi <15)	EHRi (HRi ≥15)	P -value
Heart Rate at start of case (bpm), (mean, 95%CI)	75 (72-77)	78 (74-82)	0.22
Heart Rate at end of case (bpm), (mean, 95% CI)	68 (66-70)	80 (77-84)	< 0.001
MAP at start of case (mmHg), (mean, 95% CI)	106 (102-109)	106 (102-110)	0.8
MAP at end of case (mmHg), (mean, 95% CI)	79 (75-83)	74 (69-79)	0.07
FTc at start of case	349 (342-357)	358 (344-372)	0.24
FTc at end of case	373 (366-379)	385 (373-400)	**0.05**
Peak Velocity start of case cm/s (mean, 95% CI)	62.3 (58.5-67)	62.2 (56.7-68)	0.2
Peak Velocity end of case cm/s (mean, 95% CI)	76.5 (71.6-81.3)	70.5 (63.5-78.2)	0.09
Stroke Volume (ml) Start of case (mean, 95% CI)	85.8 (81-91)	80 (74.5-85.5)	0.1
Stroke Volume (ml) End of case (mean, 95% CI)	110.6 (105.4-117.6)	88.1 (82.6-94)	**< 0.001**
Cardiac Index Start of Case	3 (2.7-3.05)	2.9 (2.8-3.3)	0.3
Cardiac Index End of Case	4.0 (3.6-4.3)	3.7 (3.6 – 3.9)	0.3

Table 6-7: Intraoperative Haemodynamic parameters in patients undergoing major colorectal surgery stratified by heart rate change during unloaded cycling. Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for student's unpaired t-test or ANOVA. GDT = Goal Directed Therapy. MAP = Mean Arterial Pressure, FTc – Flow Time corrected (seconds).

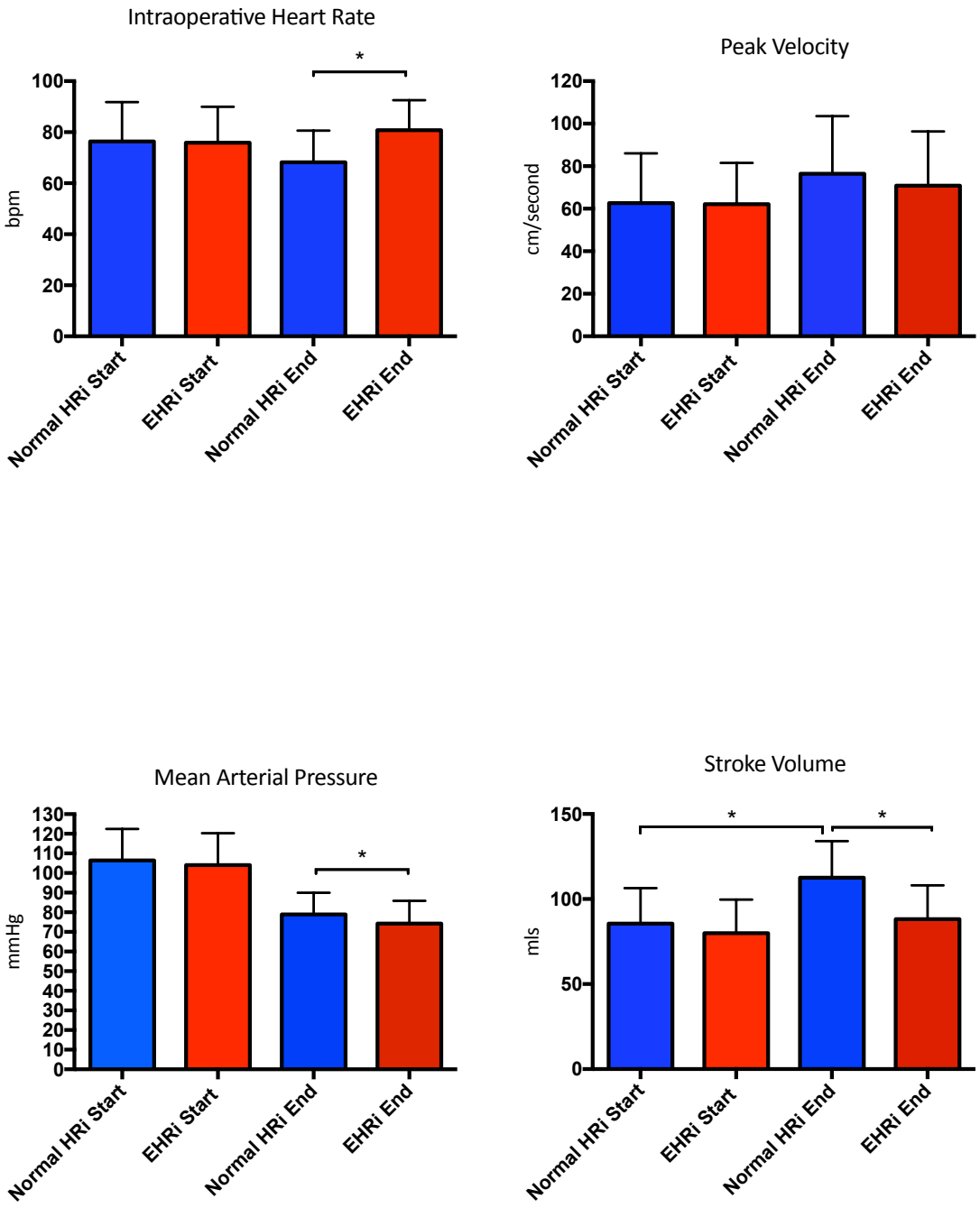

Figure 6-4: Intraoperative Physiological Parameters stratified by heart rate change during unloaded cycling at time of preoperative exercise testing. All comparisons made by ANOVA. Asterisk denotes P ≤0.05.

6.5.7 Secondary Outcomes

6.5.7.1 Hospital Length of stay

EHRi was associated with an increased risk of a prolonged hospital stay (Table 6-8).

	Normal (HRi <15)	EHRi (HRi ≥15)	P -value
Hospital LOS (days)	8 (7-8)	9 (8.1-10)	0.0023
Hazard Ratio: Hospital LOS N= 818	HR 0.8 (0.6 to 0.9)	HR 1.3 (1.1 to 1.5)	0.0003

Table 6-8: Hospital Length of stay (mean, SD) stratified by heart rate change during unloaded cycling (zero workload) at time of preoperative exercise testing Values shown are median (95% Confidence Interval) unless otherwise stated. P-values are for student's unpaired t-test. The Mantel-Cox (Log-rank) test was used for calculation of Hazard Ratios.

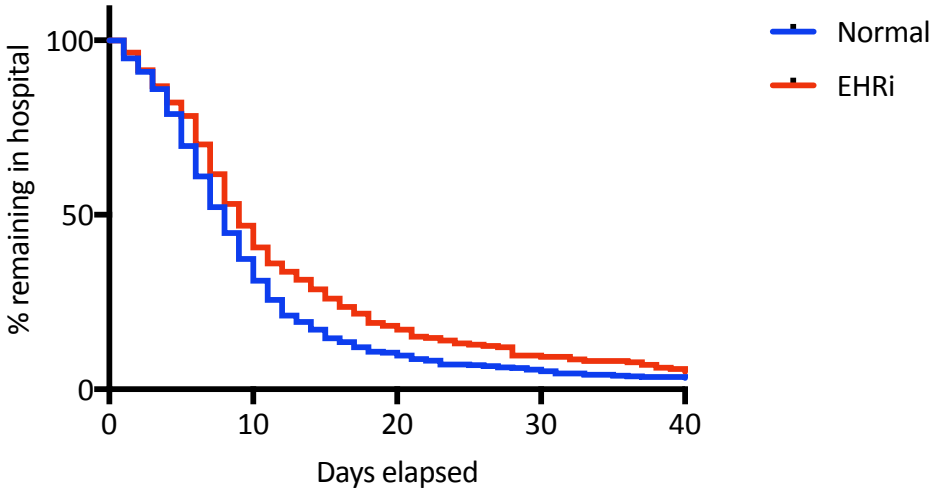

Figure 6-5: Hospital Length of stay stratified by heart rate change during unloaded cycling (zero workload) at time of preoperative exercise testing. Gehan-Breslow-Wilcoxon log-rank test: n=818, p=0.0012

6.5.7.2 Relationship between PAD and Sympathetic autonomic hyperactivity (EHRi)

There was no relationship between the presence of PAD and the likelihood of demonstrating EHRi (RR 1 (95% CI 0.92 to 1.1), p>0.9).

The exercise and intraoperative phenotypes described in EHRi alone are not equivalent to those seen in PAD (Table 6-9).

Individuals who had both PAD and EHRi at preoperative exercise testing demonstrated significantly impaired oxygen pulse and aerobic performance when compared to PAD or EHRi alone and the entire study population.

CPET Physiology	Whole Study Cohort N=1052	EHRi N= 315 (HRi >15)	PAD N=317 (HRR ≤12)	PAD + EHRi N=62	P-value (ANOVA)
AT (mean, 95% CI)	11.5 (9.5-13.5)	11.5 (11-11.8)	11.1 (10.6-11.6)	10.39 (9.8-10.9)	<0.0001
Peak VO_2 (mean, 95% CI)	17.5 (14-21)	16.6 (14.3-17.9)	15.2 (14.8-15.8)	10.5 (8.8-12.1)	<0.0001
Oxygen Pulse (mean, 95% CI)	10.9 (9-13.5)	8.7 (8.3-9)	10.6 (10-11)	6.3 (5.2-7.4)	<0.0001

Table 6-9: Cardiopulmonary exercise testing (CPET) physiological and autonomic characteristics for individuals with and without Parasympathetic Autonomic Dysfunction (PAD) and/or Excessive Heart Rate increase (EHRi) before exercise.
University College London and Derriford Hospital Plymouth data combined. AT = Anaerobic threshold (ml O_2 kg^{-1} min^{-1}). VO_2 = Oxygen uptake (ml O_2 kg^{-1} min^{-1}). Oxygen Pulse (ml O_2 $beat^{-1}$). Values shown are mean (95% Confidence Interval) unless otherwise stated. P-values are for one-way classic ANOVA.

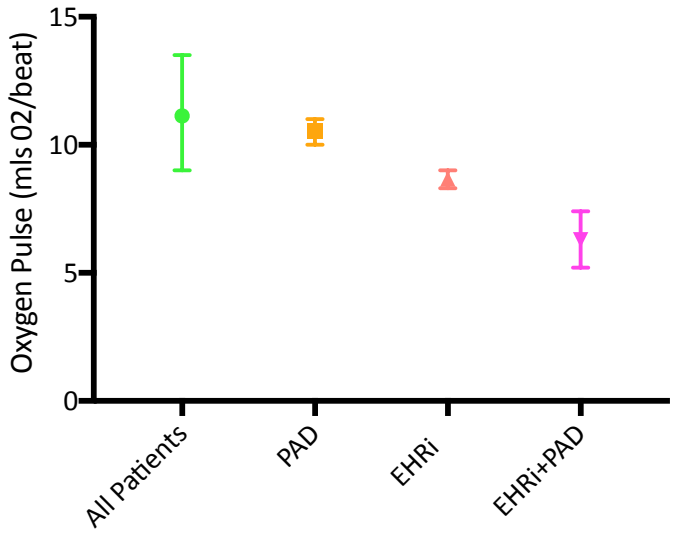

Figure 6-7: Oxygen Pulse (ml.O_2.beat $^{-1}$) classified according to presence or absence of PAD and/or EHRi. Mean + SD. UCLH data + DHP data combined. One-way classic ANOVA $p < 0.05$

	All patients	EHRi	PAD	PAD + EHRi	P value
Length of stay (days) (mean, SD)	8 (6-16)	9 (7-10)	12 (9-16)	15 (12-19)	0.001

Table 6-10: Hospital Length of stay stratified according the presence/absence of Sympathetic Autonomic Hyperactivity (EHRi), Parasympathetic Autonomic Dysfunction (PAD) or both. P-value is for one-way classic ANOVA.

6.6 Discussion

6.6.1 Key findings

1. Excessive Heart Rate increase (EHRi) prior to the commencement of loaded exercise was associated with electrocardiographic evidence of myocardial ischaemia.
2. EHRi was associated with hypertension before exercise testing.
3. EHRi was associated with impaired cardiac performance during exercise.
4. EHRi was associated with reduced stroke volume and relative tachycardia at the end of surgery.
5. EHRi was associated with an increased hospital length of stay.
6. EHRi was not associated with parasympathetic autonomic dysfunction.

6.6.2 Significance of the findings and discussion of results

These data indicate that in surgical patients, the sympathetic autonomic response to unloaded pedalling during CPET is associated with an increased risk of myocardial ischaemia as well as impaired cardiac performance during exercise.

6.6.2.1 Does EHRi represent mental stress?

The excessive increases in heart rate noted in individuals with EHRi are likely predominantly due to mental stress and not due to physical exertion. Despite the global lack of aerobic conditioning in the patient cohorts described, only a very small minority of patients achieved anaerobic threshold during the ‘warm up’ period of the test. Similarly, anaerobic threshold itself was not different across cohorts, suggesting that aerobic conditioning did not play a major part in observed differences in CPET physiology. Most pertinent, perhaps is the observation that peak heart rate did not differ between individuals with and without EHRi.

Data from other clinical circumstances have quite clearly demonstrated similar excessive heart rate increases with the thought of exercise alone and linked these to impaired cardiac outcome (Jouven et al. 2009). A certain degree of anticipatory heart rate rise is expected prior to starting exercise, and is associated with physiological 'preparation' for the impending physical challenge (Hall & Guyton 2006).

Mental stress related increases in heart rate and blood pressure have been associated with increased sympathetic activity. Similarly, mental stress has been associated, in a variety of contexts, with reduced heart rate variability, increased low frequency power and reduced high frequency power (Dishman et al. 2000; Taelman et al. 2009; Boonnithi & Phongsuphap 2011).

It is reasonably common for individuals to express anxiety about exercise testing, this anxiety being manifest in both trained and untrained individuals (O'Connor et al. 1995). Further evidence for an excessive sympathetic response, likely due to mental stress, underlying the excess heart rate increase seen prior to loaded exercise comes from the observation of increased blood pressure in patients exhibiting EHRi prior to exercise. This is despite no difference in the prevalence of hypertension in this group. This anticipatory blood pressure rise has been linked to increased risk for the subsequent development of hypertension in middle aged men (Everson et al. 1996).

The population of surgical patients examined here consists overwhelmingly of older adults. Late-life anxiety disorders and situational anxiety are common and rank amongst the 'geriatric-giant' disorders, being two times as prevalent as dementia among older adults and up to eight times more prevalent than major depressive disorders, resulting in significant impact on quality of life, morbidity, and mortality in this population (Hybels & Blazer 2003).

It appears likely then, that mental stress plays a key part in the excessive pre exercise heart rate rise investigated here.

6.6.2.2 Is the high prevalence of females among the cohort with EHRi relevant?

In this cohort of surgical patients, it is striking that the greater proportion of patients exhibiting EHRi were female. There are two interacting reasons why this might be the case.

Firstly, females demonstrate increased and different physiological responses to stress when compared with males from puberty onwards, possibly due to underlying differences in hypothalamic-pituitary-adrenal axis and autonomic nervous system function, influenced by gonadal hormones (Ordaz & Luna 2012). This translates to greater 'cardiac' or heart rate reactivity than males (who tend towards greater 'vascular' or blood pressure responses) when presented with a stressful situation (Allen et al. 1993).

Second, excessive anticipatory heart rate rises are likely to reflect both increased anxiety and lack of experience of physical exercise in this cohort. Anxiety and anxiety disorders are, additionally, more common in females than males (Kessler et al. 2010).

The greatest prevalence of physical inactivity reported is in adults over the age of 65 (66%), representing the same age group as these high-risk surgical patients. In older adults, only 30% of older men and 15% of older women participate in any form of sustained regular physical activity (Schutzer & Graves 2004), potentially further increasing the likelihood of anxiety about exercising in females prior to formal exercise testing.

In patients with chest pain referred for exercise testing, females are more likely to demonstrate higher anxiety scores at pre-test questionnaire (Rohani et al. 2011). In the context of exercise testing in the diagnosis of chest pain, despite the lower likelihood of a test positive for myocardial ischaemia when compared with men, it is more likely for females referred for testing to demonstrate anxiety traits (Channer et al. 1985).

Despite the lower global likelihood of a positive diagnosis of ischaemic heart disease, amongst women referred with signs and symptoms of cardiac ischaemia, anxiety is predictive of increased cardiac symptom severity and greater healthcare utilisation costs (Rutledge et al. 2013). Importantly, anxiety in women appears strongly predictive of the development of subsequent cardiac events (Rutledge et al. 2009).

6.6.2.3 Mental stress and myocardial ischaemia

Mental stress at rest has been strongly associated with evidence for myocardial ischaemia at the time of exercise testing. Mental stress alone frequently elicits ECG changes associated with myocardial ischaemia (Wong & Freedman 1997; Deanfield et al. 1984), but not in all patients with known stable angina.

Cardiac PET imaging studies in patients referred for exercise testing with known stable angina, however, demonstrate altered myocardial perfusion, in the absence of ECG changes (silent myocardial ischaemia), at times of mental stress in the majority of patients (Soufer et al. 2009; Deanfield et al. 1984). Patients known stable angina who develop silent myocardial ischaemia in response to mental stress exhibit abnormal myocardial perfusion and accompanying ST depression and angina after exercise testing (Deanfield et al. 1984).

In the REMIT trial, reduced ventricular ejection fraction was recorded in at risk individuals susceptible to mentally evoked stress (W Jiang et al. 2013; Wei Jiang et al. 2013). These changes have been paired with the observation of an increased peripheral vascular response where left ventricular afterload is increased during mental stress, mirrored by an increase in diastolic blood pressure in particular. (W Jiang et al. 2013 a; W Jiang et al. 2013 b). This peripheral vascular response is particularly predictive of the development of myocardial ischaemia and appears to be present in the individuals examined in this study.

Mental stress induced cardiac ischaemia is not limited to patients with demonstrable coronary artery occlusion. Myocardial ischaemia provoked by mental stress appears to occur predominantly in coronary distributions in the absence of a flow limiting plaque; leading to the suggestion that mental stress may cause dynamic coronary obstruction (Legault et al. 1995). This may be particularly relevant to the results from the Coronary CTA VISION study, where coronary occlusive load on CT angiography was poorly correlated with postoperative troponin leak. This observation led the authors to conclude that several pathophysiological mechanisms for myocardial infarction could exist in the perioperative period (Sheth et al. 2015).

Furthermore, aspects of the ischaemic cascade noted in individuals with mental stress induced cardiac ischaemia appear to occur at lower myocardial oxygen demand, relevant of course to the perioperative period (Burg & Soufer 2014; Soufer et al. 2009; Krantz et al. 1996).

Sympathetic hyperactivity secondary to mental stress can result in endothelial activation up to 90 minutes after the initiation of the stressor. Excessive sympathetic stimulation can also cause extra-cardiac cellular injury (Barnes & Ackland 2010). This would appear to be particularly relevant to the perioperative context, where pre-operative stress has previously been associated with impaired postoperative outcomes including pain (Heller et al. 1984; Vaughn et al. 2007), intraoperative anaesthetic requirements (Kil et al. 2012), postoperative nausea and vomiting (Van Den Bosch et al. 2005), cardiac complications including ischaemia and arrhythmias (Tully et al. 2011) and even mortality after cardiac surgery (Székely et al. 2001).

In these high-risk surgical patients, a greater risk for exercise induced ST depression of >1 mm was noted. In other, asymptomatic populations, ST depression to this degree has been identified as predictive of future cardiac events (Rywik et al. 2002).

6.6.2.4 Preoperative exercise testing and cardiac risk

Although ST segment depression during exercise is well established as a marker of cardiac ischaemia, ST segment depression alone at preoperative exercise testing does not always correlate well with perioperative cardiac complications (Fleisher et al. 2014).

The majority of studies examining preoperative exercise testing for prediction of cardiac risk have occurred in vascular patients as these patients are generally viewed as being at highest risk of postoperative ischaemic complications. Study quality and study protocols have varied greatly, and patient outcomes in these studies are not thought to reflect current event rates (Fleisher et al. 2014).

Data from some of these studies have suggested that the achievement of less than 75% to 85% of predicted maximal heart rate combined with ST segment depression of >1mm appears to be most predictive of post operative cardiac complications (Cutler et al. 1981). However, in theses studies, ST/HR index was not used, the implications being that those individuals who did develop significant ST depression may have done so at lower heart rates.

Exercise capacity remains the current greatest predictor of perioperative cardiac events, patients who can achieve 7 to 10 METs appearing at lowest risk, those performing <4-5 METs being at highest (Kertai et al. 2003). As a discriminator in this study, this is unhelpful as the equivalent METs achieved by both groups (1 MET = 3.5 ml.O_2.kg^{-1}) are low ('Normal'=4 METs, EHRi =3 METs).

Current ACC/AHA guidelines (Fleisher et al. 2014) suggest that it would be reasonable and useful for patients with poor exercise capacity (<4 METs) to undergo preoperative pharmacological stress testing. In these patients, the presence of myocardial ischaemia is predictive of postoperative cardiac complications. Stress echocardiography may be superior to both thallium imaging and electrocardiography at both identifying myocardial ischaemia and

predicting outcome. Mental stress can be conceived as autologous stress testing. Future work may focus on these more sensitive discriminators for myocardial ischaemia.

6.6.2.5 Perioperative cardiac parameters and myocardial ischaemia

In addition to electrocardiographic evidence of myocardial ischaemia, patients exhibiting EHRi demonstrated impaired cardiac performance at times of physiological stress. A blunted oxygen pulse is a well-established marker of cardiac ischaemia (Belardinelli et al., 2003).

Patients with EHRi demonstrated a significantly impaired oxygen pulse, not only when compared those without EHRi, but also when compared with the oxygen pulse recorded in the same population in PAD (10.9 vs. 10.6 (PAD) vs 8.7 (EHRi).

Similarly, despite comparable stroke volume and peak velocity after induction of anaesthesia, patients with EHRi had reduced values for these indices at the end of surgery. It is possible that some of this deficit could be explained by undetected myocardial ischaemia.

6.6.2.6 Potential implications for EHRi

There are many clinical implications arising from this study. Published literature, particularly the POISE trials (Devereaux 2008, 2012), supports the notion that a cohort of patients exists who may benefit from perioperative sympatholysis. Currently, identification of precisely who will benefit the most is imperfect, and since the risks of post operative hypotension and CVA appear to outweigh the benefits of universal beta-blockade, it is possible that a population of patients who may benefit most from targeted, titrated beta-blockade are being missed.

Patients exhibiting EHRi who demonstrate apparently excessive sympathetic activity in response to stress could be postulated to be at greatest risk from

surgical stressors in the perioperative period. Certainly in this cohort, an increased length of hospital stay was described, alongside a greater risk of being tested for myocardial ischaemia.

6.6.2.7 Parasympathetic Autonomic Dysfunction and Sympathetic Autonomic Hyperactivity.

The presence or absence or PAD appears to make no difference to the likelihood of exhibiting EHRi.

Work presented in Chapter 9 of this thesis suggests that individuals with PAD are subject to sympathetic stress (raised GRK2 and Pan-Arrestin was described in these individuals). These observations are combined with translational evidence for angiotensin II activation and impaired cardiac contractility in both animal and human models of PAD (Ackland et al. 2016).

The exercise phenotype of EHRi alone was markedly different from that described in PAD. Markers of cardiac ischaemia during exercise were more prominent in EHRi, as was impairment in oxygen pulse at peak exercise. Classical markers of aerobic fitness were not however significantly impaired when compared with control in EHRi, unlike in PAD.

It is therefore likely that EHRi and PAD are describing different phenomena. EHRi appears to describe a group of individuals who respond to endogenous and exogenous stressors with an exaggerated sympathetic response. Sympathetic activity in PAD may be unopposed, elevated to compensate for cardiometabolic consequences of vagal withdrawal, or even possibly reduced. More subtle interrogation of autonomic parameters in patients with PAD defined by heart rate recovery is needed to differentiate between these states.

These suppositions are supported by the description of a sub-group of patients with PAD who also demonstrate EHRi. These individuals demonstrate markedly impaired performance at preoperative CPET, most strongly represented by a severely reduced oxygen pulse at peak VO_2.

These patients appear also to be at an even greater risk of postoperative morbidity, represented by increased length of stay.

It is conceivable that this at-risk group comprise individuals with PAD who also have a tendency towards sympathetic autonomic hyperactivity at times of stress. Strong evidence supports the concept of a genetic influence on sympathetic activity, both in terms of heritable predisposition to anxiety disorders (Gordon & Hen 2004; Zavos et al. 2012) and on baseline sympathetic function (Zhu et al. 2005; Snieder et al. 2002). The concept of genetic variation in adrenergic activity has been raised previously, and cited as a potential reason for variable effectiveness of perioperative beta blockade (Nagele & Liggett 2011). These individuals comprise a population of patients who warrant further investigation.

6.6.3 Study strengths and limitations

Strengths of these data are that all analyses were performed blinded to both primary and secondary outcomes. The description of EHRi in the context of perioperative CPET allowed thorough investigation and correlation with comprehensive physiological data that was not previously available in these individuals.

Established biological plausibility for the phenomenon of EHRi and supporting observations in other medical contexts lends support to findings described in this chapter.

The observational nature of the study, lack of intervention and lack of through autonomic and genetic phenotyping may limit more robust conclusions being drawn from this work. Lack of granular information on the precise nature of postoperative complications in these patients is also missing.

6.6.4 Further Work

Further work should focus on more thorough autonomic phenotyping. This should include a thorough description of potential subtypes of autonomic dysfunction through a variety of alternate methodologies, including genetic. Translational work should focus on interventions to modify autonomic phenotype, looking for impact on cardiac, metabolic and immune outcomes in response to cardiac ischaemia, surgical stress and in models of sepsis. These interventions may include pharmacological therapy, including beta blockade and where appropriate anxiolysis, as well as prehabilitation.

Important outcomes in further studies of EHRi should include perioperative biomarkers of cardiac injury including BNP and troponin, as well as dynamic perfusion imaging +/- angiography in affected patients to further elucidate the link between EHRi and coronary artery disease. Further exercise testing should focus on types and timing of ST depression.

Anxiety scoring, incidence of depressive and affective illness and investigation of the cardiac responses to ‘pure’ mental stress (such as mental arithmetic) linked to perioperative outcome in targeted patients may help to non-invasively identify patients at greater risk of adverse perioperative cardiac outcomes, even in the absence of known cardiac disease.

6.6.5 Conclusion

This study has identified a significant cohort of patients who exhibit cardiovascular changes associated with excess sympatho-adrenal activity in the perioperative setting. These patients develop exercise induced ST depression (ECG defined ischaemia) and suffer a prolonged hospital length of stay.

These patients may represent a cohort who could benefit from targeted intervention, such as perioperative sympatholysis, in order to avoid the

deleterious effects of sympathetic autonomic hyperactivity on physiological and cellular function.

Prior to the initiation of further perioperative beta blockade trials, a mechanistic re-evaluation of the appropriate clinical indications and timing for perioperative sympatholysis is warranted.

Data presented in this chapter has also been published in an accompanying paper (see appendices) (Whittle et al. 2015)

7 Discussion

7.1 Introduction

Convincing clinical and translational data predict a mechanistic role for autonomic dysfunction in the pathogenesis of perioperative morbidity. Data presented in this thesis indicate that preoperative parasympathetic autonomic dysfunction (PAD) as defined by impaired heart rate recovery in the first minute after maximal symptom limited exercise is common (>35% of patients studied) and is associated with increased postoperative morbidity and mortality in high risk patients undergoing major surgery. Similarly, sympathetic autonomic hyperactivity as defined by excessive heart rate rise prior to exercise (EHRi) is common (>40% of patients studied), distinct from PAD and associated with increased perioperative morbidity in the same group of surgical patients.

Physiological data derived from preoperative cardiopulmonary exercise testing (CPET) revealed that impaired post exercise heart rate recovery is associated with a distinct exercise phenotype consisting of impaired cardiac contractile and chronotropic performance associated with a reduced aerobic exercise capacity. Impairments in cardiac contractile performance were further reported intraoperatively.

Lymphocytes isolated preoperatively from individuals with PAD exhibited raised levels of a key mediator of G-protein receptor function, G-protein receptor kinase 2 (GRK2). Raised GRK2 levels have not only important implications for both beta adrenoreceptor and angiotensin receptor expression, with downstream consequences on the cardiac function, but also on innate immunity; likely reflected in the increased septic complications reported in patients with PAD.

These data imply that PAD represents a common, yet previously under-recognised pathological mechanism, with potentially important implications for preoperative risk prediction, perioperative management and postoperative outcomes.

Data presented in this thesis derived from the same cohorts of patients, but examining sympathetic autonomic hyperactivity as defined by excessive heart rate rise (EHRi) prior to the commencement of loaded exercise at the time of cardiopulmonary exercise testing, described a group of patients with an exercise phenotype distinct from those with PAD, characterised by cardiac functional impairment and an increased propensity to myocardial ischaemia during exercise.

Intraoperatively, patients with preoperative EHRi were more likely to develop significant hypotension; which has elsewhere been associated with impaired perioperative outcome (Bijker et al. 2009).

These data again describe a distinct cohort of patients who warrant further study, particularly in terms of whether targeted sympatholysis may or may not be beneficial in these individuals, in the light of on going efforts to personalise medical therapies.

7.2 Summary of key findings

7.2.1 Parasympathetic Autonomic Dysfunction

7.2.1.1 Heart rate recovery is a measure of preoperative parasympathetic autonomic dysfunction

Delayed heart rate recovery after cessation of exercise represents delayed recovery of vagal tone or parasympathetic autonomic dysfunction (Pierpont et al. 2000; Imai et al. 1994a; Savin et al. 1982; Cole et al. 2000). In an analogous population to the one examined in this thesis, delayed heart rate

recovery has further been associated with baroreceptor dysfunction, another correlate of PAD (Ackland et al., 2016). Heart rate recovery values in the high risk surgical population presented in this thesis, correlate well with those seen in at-risk cardiac patient populations, where slower recovery has previously been associated with vagal withdrawal.

Heart rate variability, a modality commonly used to assess all aspects of autonomic function, also appears related to heart rate recovery in the surgical population examined in this thesis. Reduced heart rate recovery was associated with reduced RMSSD heart rate variability (representing vagal withdrawal) values both pre- and immediately postoperatively. RMSSD is widely accepted, amongst time domain measures of heart rate variability, as being the most representative of parasympathetic withdrawal.

7.2.1.2 Reduced heart rate recovery after exercise is associated with a distinct exercise phenotype characterised by impaired cardiac contractility at times of physiological demand

Reduced heart rate recovery after exercise is associated with physical deconditioning, illustrated by reductions in commonly accepted markers of aerobic fitness, including VO_2 peak and anaerobic threshold.

Reduced heart rate recovery after exercise is further associated with chronotropic incompetence, reflected in a raised resting heart rate and reduced peak heart rate at time of CPET, which may influence the observed reductions in peak VO_2 in patients with PAD.

Impaired cardiac contractility in PAD, particularly at times of increased physiological demand, was demonstrated by a reduced oxygen pulse at peak exercise, which is a surrogate for reduced stroke volume.

7.2.1.3 Parasympathetic Autonomic Dysfunction is associated with an increased risk of myocardial ischaemia as defined by heart rate associated ECG changes during exercise

Patients exhibiting impaired heart rate recovery after exercise are more likely to demonstrate a ST/HR index score suggestive of cardiac ischaemia during exercise testing. Furthermore, a reduced oxygen pulse, also common in these individuals is strongly associated with myocardial ischaemia.

7.2.1.4 G-protein receptor kinase 2 (GRK2), a key regulator of beta adrenoreceptor activity, is elevated in circulating lymphocytes isolated from individuals with PAD

GRK2 was elevated in circulating lymphocytes derived from patients with impaired heart rate recovery. These increases appear to reflect the degree of PAD in each individual. Alterations in GRK2 levels in circulating white cells are likely to mirror myocardial expression of the beta adrenoreceptor and be associated with alterations in innate immune function.

7.2.1.5 Abnormal heart rate recovery is common in high risk surgical patients presenting for major surgery

More than 30% of the patients in the study population exhibited reduced heart rate recovery after exercise. Parasympathetic autonomic dysfunction has not previously been described in the surgical context. Patients with PAD presenting for surgery may benefit from targeted interventions to reduce perioperative risk.

7.2.1.6 PAD is associated with hypertension

Hypertension is strongly associated with baroreceptor dysfunction, itself associated with afferent vagal dysfunction (Thrasher 2005). PAD is also associated with diabetes and cardiac failure, conditions where autonomic dysfunction is strongly related to prognosis.

7.2.1.7 Preoperative parasympathetic autonomic dysfunction predicts postoperative morbidity and mortality independently of other common risk factors

Whilst an anaerobic threshold of less than 11 ml/kg/min, commonly used to predict increased perioperative risk, did predict an increased hospital length of stay in the study population, reduced heart rate recovery (PAD) was the strongest predictor of a prolonged hospital admission when included in a Cox's proportional hazards model.

In terms of postoperative morbidity, PAD was strongly associated with delayed return of normal bowel function, a process dependent on vagal activity.

7.2.1.8 Parasympathetic Autonomic Dysfunction is associated with an increased risk of postoperative infectious complications

White cell count and neutrophil to lymphocyte ratio were both increased in PAD on postoperative day 2 as compared with controls. Postoperative sepsis was also more common in patients with PAD.

7.2.1.9 Parasympathetic Autonomic Dysfunction is associated with reduced intraoperative cardiac contractility

Relatively reduced cardiac contractility, as defined by peak velocity, was present in PAD at the start of the operative case prior to skin incision. PAD was further associated with a failure to increase cardiac contractility significantly in response to fluid resuscitation, resulting in relatively reduced cardiac performance at the end of the operative case. The probability of success of fluid optimisation algorithms may be unsure in individuals with PAD, warranting further study.

7.2.1.10 Parasympathetic Autonomic Dysfunction is associated with intraoperative hypotension

The observation of significantly reduced mean arterial pressure intraoperatively in patients with PAD was accompanied by an exaggerated decrease in cardiac afterload, measured as an increase in corrected flow time on the oesophageal Doppler. Patients with PAD received greater doses of vasopressor drugs to maintain blood pressure during surgery. Cardiac autonomic dysfunction has previously been associated with intraoperative haemodynamic instability, which in itself is associated with adverse perioperative outcomes.

7.2.2 Sympathetic Autonomic Hyperactivity

7.2.2.1 Sympathetic autonomic hyperactivity is associated with exercise induced cardiac ischaemia

Excess sympathetic drive has previously been shown to be strongly associated with increased myocardial oxygen demand, coronary arterial spasm, and the promotion of myocardial ischaemia through mechanical processes. Patients with sympathetic autonomic hyperactivity in this study were more likely to demonstrate significant ST depression during exercise testing. A reduced oxygen pulse at peak exercise was also demonstrated which may also be related to ischaemia related impairments in cardiac function. The degree of electrocardiographically defined cardiac ischaemia described was greater in the EHRi cohort than the PAD cohort.

7.2.2.2 EHRi represents a physiological phenotype distinct from the one described in PAD

Aside from EHRi, heart rate dynamics were not different before, during and after exercise testing in patients with sympathetic autonomic hyperactivity. Neither was anaerobic threshold.

Abnormal heart rate recovery after exercise (PAD) was not associated with EHRi. Cardiac contractile impairment and ischaemia during exercise were indicated through reductions in oxygen pulse at peak exercise, as well as Peak VO_2. These findings were mirrored by relative reductions in cardiac performance at the end of surgery (represented by stroke volume and peak velocity measured with the oesophageal Doppler), despite values for these measurements being equivalent after induction of anaesthesia, unlike in PAD where relative cardiac contractile impairment was present after induction of anaesthesia.

7.2.2.3 EHRi is associated with a prolonged hospital length of stay

Specific morbidity domains were not explored in this thesis, however, the increased likelihood of inducible ischaemia during CPET may translate to an increased risk of perioperative myocardial injury (Botto et al. 2014; Devereaux et al. 2012), itself associated with impaired postoperative outcome.

Global impairments in cardiac function are associated with postoperative morbidity in many domains, as well as prolonged hospital length of stay and increased mortality.

7.3 Interpretation of results

7.3.1 Parasympathetic Autonomic Dysfunction

7.3.1.1 Parasympathetic Autonomic Dysfunction is common in surgical patients and is related to a range of environmental, physiological and pathophysiological influences

Parasympathetic autonomic dysfunction has previously been associated with impaired outcome in both the short and long-term in both cardiac and general medical populations (Cole et al. 2000; Shishehbor et al. 2006; Jouven et al. 2005). Moreover, PAD may not immediately be apparent from history or clinical examination alone, requiring specialist testing to reveal any deficits.

Abnormal heart rate recovery after exercise is common in both the general and the wider cardiac population, with the incidence ranging from 20 to 40% (Cole et al. 2000; Nishime et al. 2000; Dhoble et al. 2014; Jouven et al. 2005). The incidence of >35% in the high-risk surgical population in this study corresponds well with that described in cohorts with known cardiac failure.

Heart rate recovery thresholds vary across several large prospective studies. The threshold of 12 beats per minute in the first minute after exercise identified in this study population corresponded well with that identified in high risk cardiac patients (Cole et al. 1999; Nishime et al. 2000). In other study populations however, a range of measurement techniques, time points and values for abnormality have been identified (Imai et al. 1994; Jouven et al. 2005; Pierpont et al. 2000). What is consistent across these studies is that abnormal heart rate recovery values identify patients at highest risk of mortality regardless of which specific definition is used. Similarly, it is well established that early heart rate recovery after exercise is representative of return of vagal activity (Imai et al. 1994; Sugawara et al. 2001). The influence of vagal withdrawal on both physiological performance and postoperative outcome appears to be graded, and patients with the lowest parasympathetic activity are at the greatest risk.

The causes of PAD in the surgical population are likely to be varied and not linked to a single pathology, but rather a combination of genetic, age related, environmental, therapeutic and disease related processes (Trevizani et al. 2012; Ghaffari et al. 2011; Nishime et al. 2000; Yilmaz et al. 2013; Bilsel et al. 2006; Sacre et al. 2012).

Impaired heart rate recovery, in this study, did not appear to be influenced by whether the patient was taking beta-blocking medication. This finding is supported by those of other studies that also failed to demonstrate any influence of continued beta-blockade on heart rate recovery (Pal et al. 2013; Crouse et al. 1989). The lack of influence of sympatholytic medication on the

recovery of heart rate after exercise further reinforces the role of return of vagal tone over withdrawal of sympathetic activity (Imai et al. 1994).

The PAD phenotype was not solely ascribable to the most commonly understood cause of autonomic dysfunction, namely diabetes mellitus, since a diagnosis of diabetes was present in less than 20% of the population with PAD. In this study population, hypertension was the diagnosis most commonly associated with PAD. This finding is supported by previous reports describing hypertension as the most common pathology associated with autonomic impairment (Pal et al. 2013).

7.3.1.2 Heart rate recovery after exercise as a measure of parasympathetic autonomic dysfunction.

Heart rate recovery after exercise is well established as a measure of return of vagal activity (Pierpont et al. 2000; Lauer 2009; Imai et al. 1994). Whilst CPET was used in this study, in practice, more simple apparatus could be used to determine heart rate recovery. Use of exercise to determine vagal withdrawal has the advantage of being non-invasive and also of not requiring sophisticated software to determine heart rate dynamics.

In this study, heart rate recovery was measured in the seated position following symptom limited maximal exercise. This technique is both internally and externally reproducible and is also compatible with currently used exercise testing techniques in the surgical population (e.g. CPET and Bruce protocol).

Delayed heart rate recovery, in the patient population studied, was associated with reductions both pre and postoperatively with another widely accepted marker of vagal withdrawal, namely reduced heart rate variability (rMSSD).

The interaction between heart rate variability and heart rate recovery is complex. Indeed, heart rate variability alone has been questioned as a

reliable indicator of sympatho-vagal balance (Billman 2013; Milicević 2005). Certainly, measures of parasympathetic tone derived from analysis of heart rate variability are confounded by differences in basal heart rate (O Monfredi et al. 2014).

It is for this reason that heart rate variability measures were not used to primarily identify either parasympathetic or sympathetic autonomic dysfunction in this thesis. It is unhelpful to conceive of autonomic function as a pendulum swinging from enhanced vagal to sympathetic activity dependent on the individual and environmental conditions. Both sympathetic and parasympathetic activities interact, but either system may independently demonstrate impairments in function. This concept is clearly illustrated by the lack of interaction between heart rate recovery and pre-exercise heart rate increase in the study population described in this thesis.

Where rMSSD is decreased preoperatively in patients with impaired heart rate recovery, heart rate variability measures could conceivably be used to predict or track perioperative autonomic function, in particular responses to physiological challenges and therapeutic strategies. This would particularly be the case where heart rate dynamics at preoperative CPET support a diagnosis of PAD or otherwise. The observation in the POM-O study that reduced parasympathetic activity, measured using heart rate variability, after goal directed therapy (GDT) was associated with a failure of GDT to improve postoperative outcomes is supportive of this concept (Ackland et al. 2015).

Further support for the notion that delayed heart rate recovery is representative of parasympathetic dysfunction is provided by observations of low spontaneous baroreceptor sensitivity (BRS) in a comparable cohort of patients with impaired heart rate recovery to the one described in this thesis (Ackland et al., 2016). BRS, measurable contemporaneously, also proffers a potential monitoring modality for perioperative parasympathetic autonomic function.

7.3.1.3 Clinical and experimental translational research supports a mechanistic role for vagal withdrawal in the promotion of perioperative morbidity

Both clinical and experimental translational studies have demonstrated in other contexts that loss of parasympathetic efferent activity promotes the development of cardiovascular and immune impairment, in part through the promotion of systemic inflammation (Ackland et al., 2016; Czura & Tracey, 2005; Tang, Dewland, Wencker, & Katz, 2009).

Conversely, parasympathetic neuromodulation has been used to limit tissue injury and morbidity in a range of pathological and experimental conditions (Kox et al. 2011; Cheyuo et al. 2011; Dos Santos et al. 2011).

Both established PAD and further reductions in vagal activity in the perioperative period might therefore directly contribute to the development of postoperative morbidity and mortality.

Data presented in this thesis supports this hypothesis, since both all cause and discrete morbidities, linked in other populations with PAD, are associated in the surgical population with delayed heart rate recovery. In particular, morbidities mechanistically linked with parasympathetic modulation of tissue injury, such as infection, impaired gastrointestinal function and myocardial dysfunction, were more common in patients with PAD.

More general markers of perioperative morbidity were also increased in PAD, with hospital length of stay, intraoperative hypotension and elevated or severe overall morbidity (Clavien-Dindo) scores being more common in this population. Importantly, mortality was also increased in the PAD population. These findings are supported by the established pathological role of parasympathetic autonomic dysfunction in cardiac failure, where failure to improve autonomic function despite maximal medical therapy is also associated with increased mortality (Nolan et al. 1998). A further small study in thoracic surgical patients supports morbidity findings presented in this

thesis, where a threshold HRR of <12 beats/minute following a six-minute walk test was associated with postoperative cardiopulmonary complications (Ha et al. 2015).

Excess morbidity in specific domains associated with PAD (infectious/inflammatory and gastrointestinal complications) lends further support to the hypothesis that vagal withdrawal is the motor driving the development of perioperative organ dysfunction.

7.3.1.4 Immune dysfunction secondary to vagal withdrawal may underlie the development of perioperative inflammatory and infectious complications

The vagal anti-inflammatory reflex (Tracey 2002) is well established as a key modulator of innate immunity. Specific influences on the inflammatory response have been well described in both clinical and translational models of vagal withdrawal (Czura & Tracey 2005; Desborough 2000b; Rosas-Ballina & Tracey 2009; Rosas-Ballina et al. 2011; Thayer et al. 2011; Jankowska et al. 2006).

Neutrophil to lymphocyte ratio (NLR), a marker of inflammation associated with impaired immune function, was not significantly different at baseline in patients with PAD, in the postoperative period. However, NLR values in PAD were raised relative to those in patients without PAD.

NLR has been shown in several studies to be a useful biomarker for both postoperative morbidity and cancer recurrence (Sultan et al. 2014). The observation that NLR values at baseline were elevated in both patients with and without PAD as compared with population norms, may reflect the high prevalence of carcinoma (50%) as well as aerobic deconditioning (Sultan et al. 2014) in the study population. Certainly, a low anaerobic threshold, very common in the studied cohorts, has been associated with a raised baseline NLR as well as down regulation of monocyte $CD14^{+}$ expression, representing chronic inflammation. Values for preoperative NLR seen here are equivalent

to those seen in high risk patients as defined by low AT in a similar surgical context (Sultan et al. 2014).

A 641 patient multi-centre prospective observational study (Ackland et al 2016, manuscript under review), carried out after collection of the data presented in this study and with the same inclusion criteria showed graded increases in total leucocyte count as well as progressive decreases in lymphocyte fraction associated with progressively lower heart rate recovery values at baseline. A heart rate recovery of <12 bpm was associated with an NLR of >2.8 and a reduced lymphocyte: monocyte ratio. Evidence from both studies would therefore warrant closer and more detailed examination of the role of chronic inflammation in patients with PAD in the development of postoperative inflammatory and infectious complications.

CRP, a commonly used marker of inflammation in the clinical setting, was not different in individuals with PAD in this study. However, single CRP values alone, which are expected to rise in the postoperative period in all patients, do not tell the full story of the perioperative inflammatory response. Full profiling of CRP ebb and flow was not carried out, neither was high sensitivity CRP, a potentially more suitable test, assessed at baseline (Edwards et al. 2011).

Further evidence for a potential role for PAD in altered immunity is proffered by the observation of raised GRK2 in circulating lymphocytes derived from individuals with impaired heart rate recovery at preoperative CPET.

Translational work, where sinoaortic denervation in rodents (SAD) was used as a model of parasympathetic autonomic dysfunction carried out in the same laboratory (Ackland et al., 2016) supports this hypothesis.

Chronic SAD is associated with elevated angiotensin II levels, which are thought to lie at the centre of the physiological and cellular consequences of sinoaortic denervation. Chronic elevations in Angiotensin II, common in cardiac failure and baroreceptor dysfunction, mediate end organ damage,

hypertension, endothelial, cellular and immune dysfunction (Shan et al. 2004). In our SAD model, elevations in angiotensin II were implied through observed increases in angiotensin-II receptor 1a expression in conditions of baroreceptor dysfunction (Ackland et al., 2016).

One mechanism through which angiotensin II generates cellular dysfunction is through mediation of mitochondrial Reactive Oxygen Species (ROS) production. Angiotensin II stimulation induces mitochondrial K_{ATP} channel opening resulting in mitochondrial membrane depolarisation and consequent reversal of electron transfer (Dikalov & Nazarewicz 2013).

The GRK2 up-regulation described in circulating immune cells in this study might be attributable in part to increased sympatho-adrenal activity and angiotensin II levels, mediated by the beta adrenergic receptor and the angiotensin II type 1a receptor (Rockman et al. 1996) (Ackland et al., 2016; Lymperopoulos, 2011), however alternative pathways for the modulation of this highly conserved mediator, including the generation of mitochondrial ROS, have been described and appear increasingly important (Ciccarelli et al. 2013).

Inflammation, associated with vagal withdrawal, produces mitochondrial damage in host cells, compromising cell survival and organ function (Singer 2007). The observation that GRK2 localizes at the mitochondrion where it plays a protective role during hypoxic/ischaemic conditions by promoting mitochondrial biogenesis and increasing ATP production, has led to an interest in the role of mitochondrial reactive oxygen species (ROS) in the modulation of GRK2 expression (Ciccarelli et al. 2013). GRK2 levels in mitochondria are noted to be elevated in inflammatory states, this elevation is felt to be adaptive and protective; in models where GRK2 is deleted, exaggerated inflammatory cytokine/chemokine production and end-organ damage has been described (Sorriento et al. 2013). Raised GRK2 in leucocytes in PAD might therefore be expected in response to an inflammatory phenotype generated by increased mitochondrial reactive

oxygen species production in response to increased circulating levels of angiotensin II.

Mitochondrial destabilisation with subsequent ROS production is the final common pathway of NLRP3 inflammasome activation (Zhou et al. 2010). The mitochondrial destabilisation caused by elevated angiotensin II levels in SAD, would therefore be predicted to result in activation of the NLRP3 inflammasome, further promoting a pro-inflammatory state.

In the SAD model described previously, macrophage mitochondrial ROS production at baseline was elevated (Figure 7-1). GRK2 levels were raised in both cardiomyocytes and peritoneal lymphocytes derived from animals with experimental baroreceptor dysfunction. Inflammasome activation with consequent increases in caspase-1 was also evident at immunoblot. These observations support the hypothesis that elevated angiotensin II levels in response to baroreceptor dysfunction promote mitochondrial ROS release, which in turn promotes NLRP3 inflammasome activation and compensatory increases in GRK2.

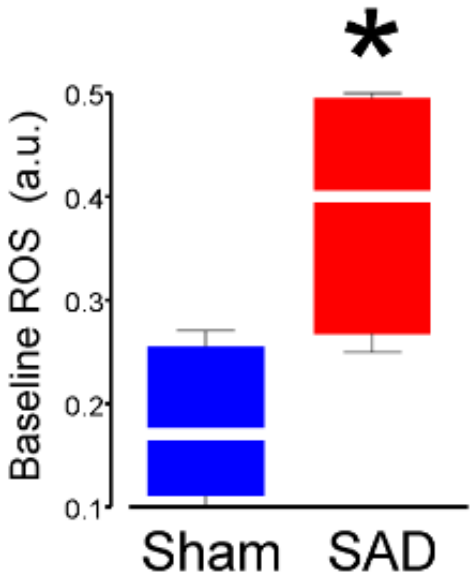

Figure 7-1: Constitutive ROS generation in peritoneal macrophages is increased in SAD animals as compared with sham operated litter mates (a.u. = arbitrary units, p=<0.05). (Unpublished work – Ackland, Del-Arroyo, Whittle)

Critical to the function of monocytes as effector cells is their ability to protect the host by migrating to inflammatory sites. This chemotactic role is mediated by MCP-1, which binds the G protein-coupled receptor CCR2. Increases in GRK2 expression desensitize the CCR2 receptor, regulating the intensity and duration of chemokine stimulation resulting in reduced migration of cells in response to chemotactic stimuli (Liu et al. 2013; Aragay et al. 1998). Raised GRK2 levels secondary to increased levels of both adrenocortical and RAAS stimulation, resulting in increased mitochondrial ROS production, would be expected to result in impaired monocyte chemotaxis, further contributing to immune dysregulation.

Elevated GRK2 levels in circulating lymphocytes derived from patients with parasympathetic autonomic dysfunction may therefore signal a distinct inflammatory immunophenotype. Full characterization of ROS production, mitochondrial function and inflammasome activation is therefore warranted in patients with PAD.

7.3.1.5 Parasympathetic autonomic dysfunction results in postoperative gastrointestinal dysfunction (PGID)

Not only is PGID linked with increased hospital length of stay (Doorly & Senagore 2012), it is also strongly linked with postoperative vagal withdrawal (Lubbers et al. 2010; Karmali et al. 2015) and inflammation (itself associated with PAD) (Havel et al. 1992).

In this study population, PAD was strongly associated with delay in resumption of normal bowel function as well as increased hospital length of stay.

Strategies to treat PGID through augmentation of vagal activity have included direct vagal stimulation and early administration of lipid rich feed (Lubbers et al. 2010) as well as sham feeding (which appears to beneficially modulate

autonomic activity as measured by heart rate variability) (Karmali et al. 2015) and avoidance of morphine (Tu et al. 2014). These approaches have met with mixed success.

To date no study has been made of the influence of established PAD on postoperative gastrointestinal function, though a plausible role in the pathogenesis of PGID could be hypothesised. Confirmation of this role is proffered by the recorded delay in resumption of bowel function in this study. The PAD population may proffer a well-defined cohort in whom strategies to augment vagal activity in an attempt to limit PGID could be of benefit.

7.3.1.6 The distinct cardiac phenotype described in surgical patients with PAD is supported by both translational laboratory work and findings in other medical populations

PAD was associated with both reduced anaerobic threshold and peak VO_2. Chronotropic incompetence, well described in autonomic dysfunction (Brubaker & Kitzman 2011), is likely to influence reduced peak VO_2, although does not fully explain the observed reductions in physical performance.

Reduced cardiac contractility at peak exercise and at the end of surgery was evident in patients with PAD. These reductions appeared ‘dose-dependent’, supporting a role for vagal activity in the promotion of positive inotropy. In this study, patients with PAD were unable to increase cardiac output in response to inotropic influences in the same way as those with normal parasympathetic function, despite baseline cardiac contractility not being different between groups.

Reduced vagal activity and autonomic dysfunction are common in, and contribute to, the pathogenesis of critical illness (Thayer et al. 2011). At the same time, in critically ill patients, an inability to increase cardiac output in response to beta adrenoreceptor stimulation is a negative prognostic factor (Collin et al. 2008). It would appear that patients presenting for major surgery

with established parasympathetic autonomic dysfunction, who are at risk of developing postoperative morbidity, including critical illness, share this aspect of the critical illness phenotype even before the onset of critical illness.

Data from a wide canon of published work, particularly in the cardiac patient population, demonstrate that PAD is mechanistically responsible for impaired cardiac and therefore reduced exercise performance (Imai et al. 1994b; Ritt et al. 2012; Watanabe et al. 2001). Increasing degrees of autonomic dysfunction have previously been linked to increasing impairments in left ventricular function (Compostella et al. 2014; Nolan et al. 1998). Strong supportive evidence is accumulating in the wider literature for a mechanistic role for sustained vagal activity in the promotion of cardiac contractility.

The M3 subtype of the muscarinic acetylcholine receptor may underlie some of the deficits in cardiac function noted in PAD. Intact M3 activity appears central to the modulation of inotropy, especially in pathological conditions. Additionally, M3 activity appears to support cytoprotection and adaptive ventricular remodelling in cardiac ischaemia. In models of both cardiac failure and cardiac ischaemia, ventricular cardiomyocyte expression of the M3 receptor appears to increase (Wang et al. 2007; Kakinuma et al. 2009; Kakinuma et al. 2005; Kakinuma et al. 2013). This may reflect an adaptive response to the progressive vagal withdrawal that is both prognostic and pathognomonic in these conditions. Further exploration of this relationship in human PAD may provide a therapeutic target for the maintenance of cardiac function in critical illness and the perioperative period.

A series of experiments carried out with a partner laboratory at UCL (Machada et al 2016) support the notion that the strength of vagal tone determines exercise capacity. In this series of experiments, vagal preganglionic neurons in the dorsal vagal motor nucleus (DVMN) were genetically targeted. Silencing of these neurons dramatically impaired exercise capacity, whereas optogenetic recruitment of the same neurons enhanced cardiac contractility and prolonged exercise endurance.

These results are supported by the human data presented in this thesis (and in the larger surgical population reported in the accompanying manuscript). Stroke volume, as represented by oxygen pulse at CPET appears to be related in a graded manner to heart rate recovery (or vagal activity).

Parasympathetic autonomic dysfunction represents a disease state where appropriate biological variability is impaired, with consequent neurohormonal dysregulation. RAAS and adrenal activation are common, with resultant alterations in G-protein coupled receptor (GPCR) trafficking through phosphorylation dependent and independent mechanisms (Ackland et al., 2016). These changes result in impaired signalling due to homologous receptor desensitisation and down regulation (particularly beta adrenergic) and are in large part responsible for the loss of inotropy noted in cardiac failure.

Cardiac oxidative stress as a result of angiotensin induced mitochondrial ROS release, generated though NADPH-oxidase-2 (NOX2) and subsequent mitochondrial dysfunction may also underlie decrements in cardiac contractile function (Ackland et al., 2016). NOX2 activation also contributes to cardiac hypertrophy and may further impair contractility through impairment of optimal calcium release in response to stretch (Murdoch et al. 2011; Bendall et al. 2007; Choi et al. 2008).

The elevated GRK2 levels noted in circulating lymphocytes derived from patients with PAD are likely to reflect expression in ventricular cardiomyocytes from the same individuals (Ackland et al., 2016; Iaccarino et al., 2005). This observation has been confirmed in a series of translational experiments carried out with other investigators in the same laboratory at UCL (Ackland et al., 2016)(see appendix for manuscript). SAD was again used as an experimental model of PAD. Molecular and physiological changes invoked by sinoaortic denervation were compared with those noted in the same patient population described in this thesis.

As in critical illness, and likely through shared mechanisms, PAD was associated with an inability to mount an appropriate contractile response to beta adrenergic stimulation (both during exercise and surgery). SAD, similarly, was associated with a failure to respond to inotropy (a dobutamine challenge test) with increases in cardiac contractility.

In a parallel manner, as in established cardiac failure, GRK2 elevation was noted in both circulating lymphocytes in PAD and in both peritoneal leucocytes and ventricular cardiomyocytes in SAD. Beta-arrestin expression, a downstream mediator from GRK2 of beta adrenoreceptor recycling and of cell survival signalling, was also increased in the rat model of SAD.

As previously described, NOX2 induced ROS release, acting in part to increase GRK2 expression, may underlie some of the observed changes in cardiac contractility as with alterations in immune function. In a murine NOX2 knockout model, GRK2 levels did not rise after SAD in ventricular cardiomyocytes derived from knockout animals. Ejection fraction was, moreover preserved in the same NOX2 $^{-/-}$ animals after SAD. These findings strongly support a role for oxidative stress underlying the cardiac dysfunction noted in both SAD and PAD (Ackland et al. 2016).

Taken together, a series of mechanisms have been described in the laboratory whereby intact vagal function is necessary to support demand related increases in cardiac contractile function. These laboratory studies are supported both by the observation of impaired cardiac contractility in surgical patients with PAD and by evidence of altered G-protein coupled receptor regulation in the same patients. A series of potential therapeutic targets for the support and preservation of cardiac inotropic function are implied by the influence of PAD on cardiac contractility.

7.3.1.7 Parasympathetic Autonomic Dysfunction is associated with an increased risk of inducible myocardial ischaemia

PAD was associated with an increased likelihood of exhibiting an ST/HR index score suggestive of myocardial ischaemia and ischaemic heart disease. Individuals with an elevated ST/HR score are at increased risk of positive coronary angiography, cardiac morbidity and mortality (Kligfield 2008; Okin et al. 1996). Furthermore, an abnormal heart rate recovery has, in cardiac patients, been associated with inducible impairments in left ventricular systolic function during exercise, presumed in part due to myocardial ischaemia, but remaining independently predictive of death (Watanabe et al. 2001).

Further support for inducible cardiac ischaemia in PAD is provided by the observation of a reduced peak oxygen pulse in this study, which perhaps reflects inducible impairments in left ventricular systolic function described by Watanabe. A reduced oxygen pulse is strongly associated with the presence of myocardial ischaemia during exercise, possibly due to a flattening of the VO_2/Heart rate curve at the onset of ischaemia due to a reduced stroke volume (Belardinelli et al. 2003).

Surgical patients with heart rate recovery defined PAD manifest certain key features suggestive of myocardial ischaemia during exercise testing. Coronary artery disease is increasingly recognized as a disease of chronic inflammation. This lends support to the observations that PAD is associated with ischaemic heart disease and cardiac failure in surgical patients, manifest by impaired cardiac performance during CPET.

These observations have important clinical implications for the high-risk surgical patient with PAD. Not only is increased vigilance for perioperative myocardial ischaemia mandated by these findings, but also support of vagal function is suggested as a potential therapeutic target in the prevention of perioperative myocardial injury.

7.3.1.8 Prospective data supports a role for parasympathetic autonomic dysfunction in the generation of postoperative morbidity

In a multi-centre prospective study (POM-HR) of 641 patients with the same inclusion criteria as described in this thesis (Ackland 2016 – under review brain immunity and behaviour), post operative morbidity was measured using the Postoperative morbidity score (POMS) on days 3 and 5. Abnormal heart rate recovery, present in 44% of the studied population, was defined, as here, as being ≤12 bpm.

Delayed heart rate recovery was more common in patients with a reduced anaerobic threshold (<11.1 ml.kg.min^{-1}), as reflected in the data presented in this thesis. Again, beta blockade was not associated with reduced heart rate recovery. However, as in this study, the likelihood of receiving cardiac or diabetic medications was associated with PAD. Hypertension was again affirmed as the most commonly linked pathology with autonomic impairment.

PAD was associated with increased postoperative cardiovascular dysfunction, specifically hypotension, and renal morbidity by postoperative day 5. Similarly, time to become morbidity free was delayed across all patients and operation types included in the study. This was reflected in prolonged hospitalisation; PAD being independently associated with delayed hospital discharge in Cox regression analysis.

As described previously, patients with impaired heart rate recovery enrolled in POM-HR were more likely to demonstrate leucocyte subset measures associated with chronic inflammation and postoperative morbidity/cancer occurrence at baseline. Postoperative changes in NLR were not described.

These data, suggesting not only a chronic inflammatory phenotype in PAD, but organ system specific morbidity that can plausibly be linked to parasympathetic autonomic dysfunction, further support a role for established vagal withdrawal in impaired perioperative outcomes.

7.3.2 Sympathetic Autonomic Dysfunction

7.3.2.1 Sympathetic Autonomic Dysfunction is associated with a distinct cardiac phenotype and impaired perioperative outcomes

Both sympathetic and parasympathetic autonomic dysfunction are, through complementary, sometimes overlapping, but ultimately different, mechanisms, predicted to result in impaired perioperative outcomes. Data presented in this thesis demonstrate that an abnormal sympathetic autonomic response provoked by unloaded pedalling prior to loaded exercise at CPET is associated with a specific physiological phenotype, comprising ECG defined cardiac ischaemia, impaired cardiac performance and resulting in a prolonged hospital stay postoperatively.

Importantly, the cohort exhibiting an excessive heart rate increase (EHRi) prior to exercise, whilst overlapping with, was not the same as that exhibiting PAD. The presence of both forms of autonomic dysfunction was associated with an especially poor perioperative prognosis.

Excessive heart rate increase prior to exercise is more common in females than males and is related to mental stress (Jouven et al. 2009). Despite similar levels of deconditioning across groups (anaerobic threshold was not different in between cohorts with and without EHRi), peak VO_2 was impaired in patients with sympathetic hyperactivity.

Regardless of a similar prevalence of hypertension across groups, blood pressure was also raised at the start of exercise in the EHRi cohort, further suggesting that sympathetic activation due to anxiety underlies the excessive heart rate increase noted. Population data supporting an increased prevalence of anxiety and an increased likelihood of heart rate reactivity in females further support mental stress underlying heart rate dynamic changes described in this thesis.

In the wider literature, several studies using different experimental models have also demonstrated that mental stress alone can trigger myocardial ischaemia (Deanfield et al. 1984; Yeung et al. 1991). It is therefore conceivable that mental stressors such as task-orientated tests (e.g. mental arithmetic) could similarly identify individuals at preoperative assessment who are at risk of sympathetic autonomic hyperactivity. Both this possibility and a potential role of preoperative anxiolysis in these higher risk individuals should form the basis of future studies.

Systemically, sympathetic autonomic hyperactivity has been implicated in immune and cellular dysfunction, bacterial overgrowth, infection, intraoperative haemodynamic embarrassment, hepatic dysfunction and the pathogenesis of acute lung injury (Toner et al. 2013). Sympathetically mediated intraoperative hypertension and tachycardia have been associated elsewhere with postoperative morbidity and prolonged hospital stay after major non-cardiac surgery. Again, inter-individual genetic differences (previously described in the perioperative population in the context of beta-blocker responsiveness and perioperative myocardial infarction) in adrenergic response, may underlie the heart rate response to mental stress and should form the basis of future studies.

The preoperative identification of individuals at highest risk of excessive sympathetic activation could form the basis for targeted sympatholysis. This is important, since to date, strategies where all higher risk patients are submitted to beta-blockade have demonstrated an excessive incidence of off-target effects (POISE Group, 2008).

These data suggest a need to re-evaluate perioperative modulation of sympathetic outflow in the light of this description of a potential ‘at-risk’ cohort. Mechanistic re-evaluation of indications and timing of perioperative beta and alpha blockade may prove fruitful in maximising benefit and minimising harm resultant from these potentially powerful interventions.

7.4 Importance of results

Data presented in this thesis represent the first time that preoperative heart rate dynamics measured at time of CPET have been used to evaluate the impact of autonomic dysfunction on relevant perioperative outcomes.

Both parasympathetic and sympathetic autonomic dysfunction are revealed as common in the high risk surgical population and are associated with cardiopulmonary dysfunction and increased risk of impaired perioperative outcomes. The demographics of the populations presented in this thesis would suggest that results presented here are generalizable to the wider high-risk non-cardiac surgical population.

Autonomic dysfunction, through a variety of mechanisms, is highly likely to mechanistically drive multi-organ dysfunction. In particular, important perioperative morbidities including hypotension, infection and cardiac dysfunction were more common in patients with PAD, results that are consistent with laboratory data exploring the pathophysiological role of parasympathetic autonomic dysfunction.

Aside from reinforcing the need to continue relevant translational mechanistic research into pathways by which autonomic dysfunction drives cardiac, metabolic and immune dysfunction, several potentially powerful therapeutic interventions are indicated for further investigation.

7.5 Further work

The exploratory work presented in this thesis suggests several promising potential avenues for further investigation.

7.5.1 Clinical studies

7.5.1.1 Confirmation of autonomic dysfunction as an independent risk factor for perioperative morbidity and improvement of surgical risk prediction tools

The results of the first multi centre prospective study in high-risk surgical patients using the same criteria as described in this study for defining PAD have now been collated (see Appendices). These data confirm an independent association between PAD and increased perioperative morbidity.

Further study should now confirm the association in different specific surgical populations (e.g. cancer patients, especially those who have received chemotherapy which is strongly associated with oxidative stress and decrements in autonomic function (Adams et al. 2015; Morrow et al. 1992; Tjeerdsma et al. 1999; Nuver et al. 2005)). Similarly, a role for chronic inflammation associated with autonomic dysfunction in the development of postoperative morbidity should be explored. Particular areas of focus should include pre-defined morbidity (specifically gastrointestinal and cardiac dysfunction), as well as examining in more detail other complementary readouts of autonomic function including heart rate variability and baroreceptor sensitivity.

These data should then be used both to refine existing risk prediction models and in the development of new risk prediction tools.

7.5.1.2 Detailed profiling of perioperative autonomic activity in relation to outcome, cardiac and immune function

Profiling of a 'normal' autonomic response to a pre-defined surgical/anaesthetic insult would help define abnormality and allow identification of an abnormal perioperative autonomic trajectory. A potential role for autonomic monitoring in the perioperative period (baroreceptor sensitivity or heart rate variability) to guide therapeutic interventions could then be investigated. Similarly, description of baseline levels and perioperative profiles of serum angiotensin II and catecholamine levels in patients with and without autonomic dysfunction may aid in the development of specific therapeutic strategies. In particular, relation of catecholamine and angiotensin levels to markers of cardiac and immune dysfunction as well as continuous measures of autonomic dysfunction could tailor therapeutic interventions to time points of greatest efficacy.

Biomarkers of cardiac dysfunction and/or damage should be examined throughout the operative period in patients with and without autonomic dysfunction and associated with outcome. These should include troponin, HsCRP and BNP. Association with markers of severity of autonomic dysfunction should be sought. These observations could inform therapeutic trials of 'tailored' interventions, such as beta blockade or anxiolysis, through identification of those at highest risk of complications.

7.5.1.3 Risk profiling and proactive management of specific morbidities associated with autonomic dysfunction

Proactive management of other specific postoperative morbidities associated with autonomic dysfunction should be explored; this could include sham feeding for PGID.

The use of heart rate dynamics during exercise alone, without formal Bruce protocol testing or CPET could be examined. Other modes of assessment of

autonomic function should also be explored, including heart rate responses to mental stressors (e.g. mental arithmetic).

7.5.1.4 Interventions to enhance vagal activity

Strategies to optimise vagal tone throughout the perioperative period may be explored. Aerobic training may improve autonomic function in addition to aerobic fitness (Collier et al. 2009; Jin et al. 2013), though sedentary individuals with low vagal activity at the start of an exercise programme appear to benefit least (Hautala et al. 2003).

Similarly, relaxation techniques such as guided meditation may be explored in the preoperative period for any impact on parasympathetic activity and postoperative outcome.

Therapeutic interventions common in the perioperative period such as neuraxial anaesthesia, analgesic choice (e.g. use of remifentanil), avoidance of anticholinergic agents, early feeding etc. could be bundled to produce a 'vagus friendly' strategy which would be hypothesised to reduce perioperative morbidity in patients with PAD. This would be best tested in a randomised controlled trial.

Finally, non-invasive vagal stimulation throughout the perioperative period should be evaluated as a potential therapeutic modality for individuals with PAD. Non invasive vagal stimulation has been used with success in several conditions such as rheumatoid arthritis, depression, epilepsy and in cardiac failure (Kraus et al. 2007; Clancy et al. 2014; Bonaz, Bruno; Sinniger, Valerie; Pellissier 2011; Krahl & Clark 2012; De Ferrari 2014). It has not, however, been used in the acute setting, its efficacy in this context is therefore not known.

7.6 Conclusion

Maintenance of biological variability is central to health and is coordinated by the autonomic nervous system. Loss of biological variability, manifest in both parasympathetic and sympathetic autonomic dysfunction, promotes a multitude of pathophysiological processes resulting in morbidity and mortality.

In high-risk patients undergoing major surgery, autonomic dysfunction is associated with several pathophysiological mechanisms that have been previously demonstrated to negatively influence postoperative outcome. Data presented in this thesis demonstrate that both parasympathetic and sympathetic autonomic dysfunction is associated with increased perioperative morbidity and hospital length of stay.

Parasympathetic Autonomic Dysfunction is common in the high-risk surgical population and is associated with markers of systemic inflammation and immune dysfunction as well as impairments in cardiac contractile function at times of increased physiological demand.

Sympathetic Autonomic Dysfunction, also common in the study population, was present in a population distinct from that presenting with PAD and was associated in particular with an increased risk of inducible myocardial ischaemia accompanied by decrements in myocardial contractile performance.

Several implications arise as a consequence of these data. First, further work to establish population norms for this particular group of patients may provide a useful preoperative screening test. Second, these data provide an alternative, novel explanation for the continuing debate over conflicting data in the perioperative haemodynamic literature (Doherty & Buggy 2012). Third, controversies over various perioperative pharmacological interventions including beta-blockade (Devereaux 2008) and clonidine (Devereaux, Sessler, et al. 2014) may be re-addressed by targeted, personalized drug

administration guided by autonomic phenotype. The precedent of sympathetic (beta adrenoreceptor) blockade improving autonomic function in heart failure (Floras 2009) is an important example of how uncovering otherwise clinically concealed information can refine management significantly.

These data offer further support to the concept that autonomic dysfunction, far from being merely associated with pathology, underlies and mechanistically drives the development of multi-organ dysfunction.

Further investigation is warranted, particularly in the context of the high risk surgical patient, of both mechanisms through which autonomic dysfunction might drive the development of morbidity and therapeutic interventions and strategies to minimise the unwanted impact of this previously under recognised disease process.

2) COMPETE-C Study protocol

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Cardiac Output Monitoring and Pre-operative Exercise Testing for Colorectal surgery

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Study information

Scientific title

Amongst patients having major elective colorectal surgery does intraoperative goal directed fluid therapy particularly decrease postoperative hospital length of stay in patients who are categorised "high risk" on the basis of a preoperative cardiopulmonary exercise test result? - a double-blind parallel-arm randomised controlled single-centre trial

Acronym

COMPETE-C

Study hypothesis

Major colorectal surgery has traditionally been associated with a prolonged hospital stay and a moderate risk (1-5%) of mortality or serious cardiopulmonary morbidity.

Improved outcomes have been reported by international units and in NHS hospitals using a specific care bundle for major colorectal surgery: "Enhanced Recovery after Surgery" (ERAS). Such patient care pathways employ a multimodal approach to promote a rapid return to normal function. The Danish group around Kehlet, the originator of ERAS, claim that a hospital stay of 3 days or less for major colorectal surgery is now customary in their institutions whilst median stays using an ERAS approach of around 6 days have been

produced elsewhere

Controversies exist regarding intraoperative fluid management for major colorectal surgery. In recent decades fluid restriction has been advocated, however such an approach uniformly applied risks bowel ischaemia and other complications.

Goal Directed Fluid therapy (GDT) refers to fluid management targeted toward improving the cardiac output to set levels, hence improving end organ perfusion. Minimally invasive devices are now available to measure cardiac stroke volume. Intra-operative GDT has been shown to reduce median hospital length of stay after major colorectal surgery from 9 to 7 days.

This study aims to determine whether goal directed fluid therapy gives additional benefit to patients undergoing surgery within an ERAS programme, and aims to address the controversies regarding fluid management.

As of 2007, Plymouth Hospitals NHS Trust provides a service to risk stratify patients undergoing elective surgery by Cardiopulmonary Exercise Testing (CPET), which can identify patients with a low cardiorespiratory reserve. These patients are at increased risk of complications after surgery. We have the capacity to perform CPET pre-operatively for all patients booked for elective major colonic or colorectal resection surgery. It may be that a subset of less fit patients may benefit more from GDT than a fitter cohort.

Ethics approval

Cornwall and Plymouth Research Ethics Committee, approved on 09/09/2008 (ref: 08/H0203/159)

Study design

Double-blind parallel-arm randomised controlled single-centre trial

Primary study design

Interventional

Secondary study design

Randomised controlled trial

Trial setting

Hospitals

Trial type

Treatment

Condition

Elective major colorectal surgery

Intervention

1. Cardiopulmonary Exercise Test (CPET)
The CPET test will be done in accordance with the consensus protocol from UK centre with reference to American Thoracic Society (ATS)/ American College of Chest Physicians (ACCP) recommendations. Anaerobic threshold will be determined by the V slope method with correlation with ventilatory equivalents.

2.Oesophageal Doppler monitoring (and Goal Directed Fluid Therapy)
All patients will receive oesophageal Doppler monitoring throughout the operation. This monitoring will be carried out by a trained investigator. Patients in the intervention group will be given warmed colloid (6% Hydroxyethyl starch) before surgical incision to reach GDT goals as per a predetermined protocol and have additional fluid boli administered as dictated by this algorithm until cessation of surgery.

The disposable oesophageal probe will be removed at the conclusion of surgery. The anaesthetist responsible for care will be blinded to the Doppler readings. The researcher carrying out the optimisation will be aware of all anaesthetic activity.

Intervention type

Procedure/Surgery

Primary outcome measures

Amongst patients having major elective colorectal surgery under an Enhanced Recovery after Surgery (ERAS) pathway:
1. Does intraoperative goal directed fluid therapy decrease postoperative hospital length of stay when compared with standard therapy alone in patients who are categorised “high risk” on the basis of a preoperative cardiopulmonary exercise test result?
2. Does intraoperative goal directed fluid therapy decrease postoperative hospital length of stay when compared with standard therapy alone in patients who are categorised “standard risk” on the basis of a preoperative cardiopulmonary exercise test result?
3. Does intraoperative goal directed fluid therapy decrease postoperative hospital length of stay when compared with standard therapy alone in all patients?

Secondary outcome measures

Amongst patients having major elective colorectal surgery under an ERAS pathway, does intraoperative goal directed fluid therapy make a difference to the following when compared with standard therapy alone?:
1. Peri-operative mortality (30 days)
2. Postoperative hypotension
3. Postoperative fluid requirement
4. Return of gastrointestinal function
5. Tolerance of diet

Secondary outcomes 2-5 will be assessed every post-op day until the participants meet discharge criteria (median length of stay for this surgery is currently 9 days [as of 17/02/2009]).

Eligibility

Participant inclusion criteria

1. Both males and females, age 18 and older
2. Elective colorectal surgical patients scheduled for major surgery
3. Patients with a measurable anaerobic threshold (AT) above 8 ml O2/kg/min

Participant type

Patient

Age group

Adult

Gender

Both

Target number of participants

168

Participant exclusion criteria

Absolute:
1. Unwillingness to participate
2. Inability to perform the tests and consent within the timetable for elective surgery
3. Measured Anaerobic Threshold below 8 ml O2/kg/min. Such patients will be considered to be extremely unfit and as such will not be suitable for inclusion in the trial.
4. Withdrawn by anaesthetist or surgeon
5. Acute myocardial infarction (3–5 days)
6. Unstable angina

7. Uncontrolled arrhythmias causing symptoms or haemodynamic compromise
8. Syncope
9. Active endocarditis
10. Acute myocarditis or pericarditis
11. Symptomatic severe aortic stenosis
12. Uncontrolled heart failure
13. Acute pulmonary embolus or pulmonary infarction
14. Thrombosis of lower extremities
15. Suspected dissecting aneurysm
16. Uncontrolled asthma
17. Pulmonary edema
18. Room air desaturation at rest <85%
19. Respiratory failure
20. Acute noncardiopulmonary disorder that may affect exercise performance or be aggravated by exercise (i.e. infection, renal failure, thyrotoxicosis)
21. Severe oesophageal disease
22. Recent oesophageal or upper airway surgery
23. Systemic steroid medication
24. Bleeding diathesis

Relative:
25. Left main coronary stenosis or its equivalent
26. Moderate stenotic valvular heart disease
27. Severe untreated arterial hypertension at rest (200 mm Hg systolic, 120 mm Hg diastolic)
28. Tachyarrhythmias or bradyarrhythmias
29. High-degree atrioventricular block
30. Hypertrophic cardiomyopathy
31. Significant pulmonary hypertension
32. Advanced or complicated pregnancy
33. Electrolyte abnormalities
34. Orthopaedic impairment that compromises exercise performance

Recruitment start date

10/03/2009

Recruitment end date

31/12/2010

Publication summary

2011 results in http://www.ncbi.nlm.nih.gov/pubmed/21873370

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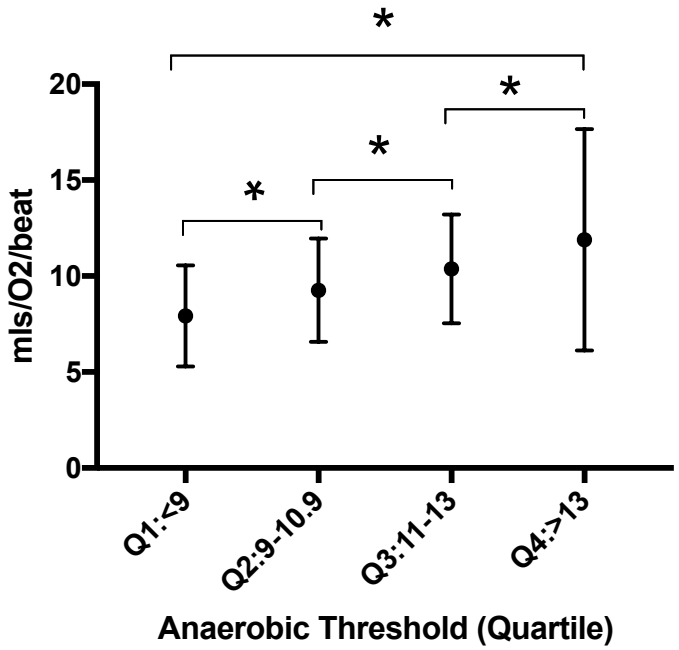
Supplemental Materials:

1) **Summary Table of all types of surgery included in this thesis**

Surgical Specialty	**Type of surgery:**	**Total number per specialty**
Urology:	Prostatectomy	170
	Nephrectomy	
	Cystectomy	
	Cystourethoscopy	
	Neobladder formation	
	Mitrofannoff formation	
General Surgery	Laparotomy	406
	Reversal colostomy	
	Colectomy	
	Formation ileostomy	
	Reversal ileostomy	
	Anterior Resection	
	Incisional Hernia Repair	
	Hiatus Hernia repair	
	Proctectomy	
	Division of Adhesions	
	Exanteration pelvis	
	Inguinal Hernia repair	
	Enterocutaneous fistula repair	
	Rectopexy	
	Pouch formation	
	Panproctocolectomy	
	Small bowel resection	
	Reversal of Hartmann's	
	Rectopexy	
Upper GI/HPB surgery	Oesophagectomy	81
	Gastrectomy	
	Liver resection	
	Whipple's procedure	
Plastics:	Abdominal Wall reconstruction	2
Vascular Surgery	Open repair AAA	57
	Endovascular repair AAA	

	(EVAR & FEVAR)	
	Repair Thoracoabdominal aneurysm	
	Popliteal aneurysm repair	
	Aortic arch repair	
Gynaecological surgery	Hysterectomy, Salpingo-oophorectomy	4
	Rectal-Vaginal fistula repair	
	Transvaginal rectocele repair	
	Myomectomy	
Maxillo-Facial Surgery	Free-Flap	90
	Neck Dissection	
	Surgical Resection of oral tumour	
	Resection parotid tumour	
Orthopaedic	Sacrum fixation	4
	Hip Replacement	
	Knee Replacement	
	Other Orthopaedic Procedure	

2) Oxygen Pulse classified by quartile of preoperative anaerobic threshold



Anaerobic threshold quartile is ml.O_2.kg

Oxygen Pulse ($ml.O_2.beat^{-1}$)	Quartile 1: AT<9	Quartile 2: AT 9 -10.9	Quartile 3: AT 11-13	Quartile 4: AT >13
Mean	9	10.2	11.4	12.9
95% CI of mean	8.5-9.4	9.9-10.6	11-11.7	12.1-136

3) Full Hazard Ratios for hospital length of stay

Likelihood of remaining in hospital		
Condition:	Hazard Ratio (Mantel-Cox) (95% CI)	P value
Normal Heart Rate Recovery vs. Abnormal Heart Rate Recovery	1.45 (1.14-1.9)	0.0027
Anaerobic Threshold <11 vs. Anaerobic Threshold >11	1.3 (1.13-1.5)	0.0004
Normal Heart Rate Recovery vs. Anaerobic Threshold <11 & Abnormal Heart Rate Recovery	1.5 (1.3-1.8)	<0.001