The effects of SARS-CoV2 on exercise capacity, respiratory and cardiovascular systems in UK military personnel.

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Declaration

The work presented in this thesis is my own unless otherwise indicated. It has not been submitted, either wholly or substantially, for another degree at this university, or for a degree at any other institution. I have acknowledged assistance from collaborators where relevant.

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Abstract

Background. At the end of 2019 the novel SARS-CoV-2 virus spread globally. Early research indicated similarities with previous coronavirus epidemics, with acute respiratory distress syndrome in severe cases, and lung function abnormalities common post hospital discharge. Furthermore, techniques such as cardiac magnetic resonance imaging identified myocarditis as a potentially common finding. Patients of working age were likely to develop a mild illness, and the consequences of mild or moderate infection in this group were unknown as many self-managed at home. Nor were the effects of SARS-CoV-2 infection on the ability of such a population to undertake physical exercise understood. This knowledge gap was of concern to organisations such as the military who require their personnel to safely endure arduous physical activity in austere environments.

Aims. To close this gap, I assessed the exercise capacity of UK military personnel following COVID-19 infection, with further characterisation of cardiac and pulmonary function. I wanted to detect any reduction in exercise capacity post infection, identify potential causes, and measure any resolution following convalescence.

Methods. A comprehensive assessment of cardiopulmonary function was made of military personnel post COVID-19 infection and a matched control population. This included cardiopulmonary exercise testing, pulmonary function testing, cardiac MRI and exercise echocardiography. Exercise capacity and lung function was retested after 6 months.

Results. At baseline assessment, patients post COVID-19 had reduced exercise capacity in comparison to controls, with those who required hospital treatment demonstrating the largest deficits. However, despite the changes, absolute values remained in the normal clinical range, which is most likely a reflection of a higher baseline level of cardiovascular fitness compared to the general population. After 6 months, there remained differences, but the efficiency of exercise had improved in the post COVID groups. Cardiac assessment demonstrated some sub-clinical myocardial

inflammation, but no significant fibrosis or deterioration in function. Differences in chamber size were consistent with deconditioning. Post infection, lung volumes were smaller and a deficit in gas transfer was seen which is likely related to reduced alveolar volume. Although some improvement was seen at 6 months, deficits in gas transfer remained. Again, absolute values failed to reach the clinical cut-off for diagnostic disease.

Conclusion. Deficits in exercise capacity and lung function are seen following SARS-CoV-2 in military personnel, however, due to low incidence of pre-morbid chronic conditions and higher levels of cardiovascular fitness, changes remain in the sub-clinical range. Furthermore, myocardial fibrosis and impairment of cardiac function is not seen. After 6 months there is some recovery in exercise capacity via increased exercise efficiency. A plausible mechanism for the findings is a combination of cardiovascular deconditioning and loss of alveolar volume, with subsequent reduction in pulmonary gas diffusion.

Abbreviations and definitions

^{31}P	stable phosphorus isotope
6MWD	6-minute walk distance
A-a	Alveolar – arterial
ACE	Angiotensin converting enzyme
ARDS	Acute respiratory distress syndrome
ATP	Adenosine triphosphate
BMI	Body mass index
BNP	B-type natriuretic peptide
BSA	Body surface area
CD4+	CD4 T lymphocytes
CK	Creatine kinase
CMR	Cardiac magnetic resonance
COVID-19	Coronavirus disease 2019
CO_2	Carbon dioxide
CPET	Cardiopulmonary exercise test
Cr	Creatinine
CRP	C-reactive peptide
CTPA	Computed tomography pulmonary angiography
DCRS	Defence COVID Recovery Service

DE	Dual Energy
DL_{CO}	Diffusing capacity of the lungs for carbon monoxide
DMRC	Defence Medical Rehabilitation Centre
ECG	Electrocardiogram
ECMO	Extra corporeal membrane oxygenation
ECV	Extracellular volume
EDV	End-diastolic volume
EF	Ejection fraction
ESV	End-systolic volume
FA	Fatty acid
FAO	Fatty acid oxidation
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
H1N1	Haemagglutinin 1 neuraminidases 1
Hct	Haematocrit
HCV	Hepatitis C virus
HF	Heart failure
HFrEF	Heart failure with a reduced ejection fraction
HIV	Human immunodeficiency virus
HR	Heart rate

HRCT	High resolution computed tomography
ICU	Intensive care unit
LFT	Lung function test
LV	Left ventricle/ventricular
M-COVID	Military COVID-19 Observational Case Outcome in a Viral Infectious Disease
MERS-CoV	Middle eastern respiratory syndrome- coronavirus
MR(I)	Magnetic resonance (imaging)
MR(S)	Magnetic resonance (spectroscopy)
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health and Care Research
NHS	National Health Service
NYHA	New York Heart Association
O_2	Oxygen
OCMR	Oxford Centre for Magnetic Resonance Research
OUES	Oxygen uptake efficiency slope
OUH	Oxford University Hospitals
PCr	Phosphocreatine
$petCO_2$	End-tidal partial pressures of CO_2
$petO_2$	End-tidal partial pressures of O_2
PDH	Pyruvate dehydrogenase

PPARα	peroxisome proliferator-activated receptor alpha
PROMS	Patient reported outcome measures
Q	perfusion
RNA	Ribonucleic acid
RV	Right ventricle (ventricular)
SARS	Severe acute respiratory syndrome
SARS-CoV-1/2	SARS associated coronavirus 1/2
SV	Stroke volume
TLC	Total lung capacity
Va	Alveolar volume
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E$	Pulmonary Ventilation
$\dot{V}E/\dot{V}CO_2$	Ventilatory equivalent for carbon dioxide
$\dot{V}O_2$	Oxygen consumption
WHO	World Health Organisation

Chapter 1- Introduction

1.1 The SARS-CoV-2 Virus

Severe acute respiratory syndrome (SARS) is a viral respiratory illness caused by a coronavirus, called SARS-associated coronavirus (SARS-CoV). SARS-CoV-2 belongs to the Coronaviridae family, specifically to the genus Betacoronavirus[1]. It shares similarities with other coronaviruses that have caused epidemics in humans, namely SARS-CoV (responsible for the 2002-2003 SARS epidemic in Asia) and Middle Eastern Respiratory Syndrome- coronavirus (MERS-CoV) responsible for MERS outbreaks primarily in the Middle East[2]. Structurally, the virus is enveloped and possesses a single-stranded positive-sense RNA genome. The genome is approximately 30 kb in length, making it one of the largest RNA viral genomes.

1.1.1 SARS-CoV-2 Virus Infection

One of the defining features of SARS-CoV-2 is the presence of spike (S) proteins protruding from its surface, which give it the characteristic "crown" appearance (hence the name "corona", Figure 1.1A and B)[3]. These S proteins bind to the human ACE2 receptor to enter human cells. Once inside the host cell, the virus RNA is then processed by the host cell to produce its structural proteins (Spike, Membrane, Envelope, and Nucleocapsid) and then assembles and buds off from the host cell, cloaked in a piece of the host's membrane, allowing it to evade the immune response and infect other cells. The primary mode of transmission of SARS-CoV-2 is respiratory droplets disseminated through coughing, sneezing, talking, or breathing. It can also survive on surfaces for varying amounts of time, depending on the material, leading to the potential for fomite transmission (Figure 1.1.C)[4].

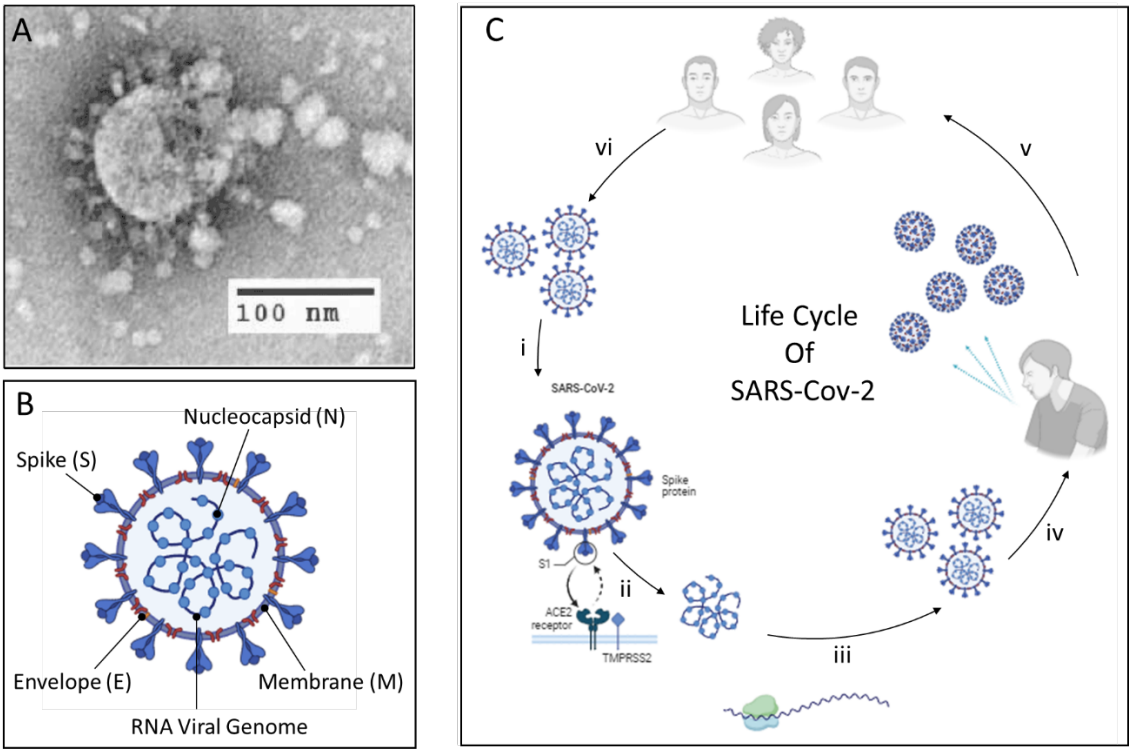


Figure 1.1 (A) Electron micrograph of SARS-CoV-2, (B) A diagram of the structure of SARS-CoV-2, and (C) the lifecycle of SARS-CoV-2 showing i) endocytosis membrane entry, ii) cell entry, iii) RNA replication, iv) fusion and release with viraemia, v) droplet spread, vi) infection and transmission

1.2 The COVID-19 Pandemic

COVID-19, caused by the SARS-CoV-2 virus, was first identified in December 2019 in Wuhan, Hubei province of China. The disease spread rapidly, overwhelming hospitals in Wuhan and leading to a rigorous citywide lockdown in January 2020. Despite this, the virus crossed borders, with countries Italy, South Korea and Iran facing outbreaks from February 2020. By March 2020, the virus had spread globally, and the World Health Organization (WHO) declared it a pandemic[5].

The SARS-CoV-2 virus responsible for COVID-19 has undergone several mutations, leading to the emergence of various variants. Some of these have shown increased transmissibility, altered disease severity, or resistance to neutralisation by antibodies. The Alpha variant, first identified in the UK in September 2020, spread quickly to other countries due to its heightened transmissibility. By early 2021, the Beta variant, detected initially in South Africa, raised concerns due to mutations potentially

affecting vaccine efficacy. Brazil witnessed the rise of the Gamma variant around the same period, with similar concerns as Beta. The Delta variant, first detected in India in late 2020, rapidly became the dominant strain worldwide by mid-2021 because of its high transmission rate. Toward the end of 2021, the Omicron variant emerged, initially identified in South Africa. Characterized by a large number of mutations, especially in its spike protein, Omicron quickly spread globally, reigniting concerns about vaccine effectiveness, transmission rates, and disease severity.

1.2.1 SARS-CoV-2 and its Effect in the UK

The COVID-19 pandemic will be remembered as a defining moment of the 21st century. The UK was one of the worst-hit nations and experienced major health and socio-economic consequences. As of September 2023, there have been greater than 20,8 million infections in the UK. Figure 1. 2 shows the number of cases since the start of the pandemic, by age and sex. From this, it is clear that whilst the virus affected all age groups, the working age cohort between 18 and 65 years bore the brunt of the pandemic by absolute numbers infected, although the clinical impact was felt predominantly by older age groups, who had more severe disease.

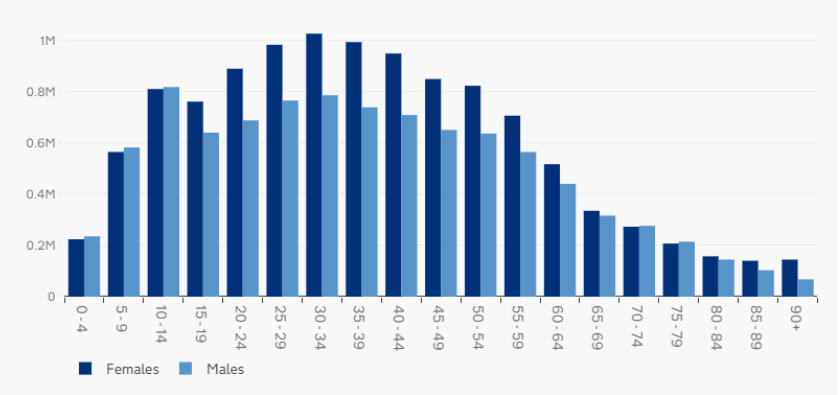


Figure 1.2 Total UK number of cases since the start of the pandemic, by age and sex (source https://coronavirus.data.gov.uk/).

The UK's COVID-19 response (Figure 1.3A) saw a series of national lockdowns and societal restrictions aimed at limiting the viral spread. The first national lockdown began in March 2020, restricting movement and closing non-essential venues. A regional tier system was introduced mid-2020 to

address local outbreaks, leading to a second national lockdown in November. December saw a revised tier system, including a new Tier 4, and changed Christmas plans due to a more transmissible virus variant. January 2021 marked the third national lockdown, with schools closed and strict stay-at-home orders. A phased roadmap out of lockdown was introduced from April 2021, with full reopening in England delayed until 19 July 2021 because of the Delta variant. Notably, the UK government also had a "Plan B" in their arsenal, which encompassed potential restrictions such as mandating face coverings in certain settings, advising people to work from home, and introducing vaccine passports for large events. Throughout, Scotland, Wales, and Northern Ireland set their own policies, occasionally differing from England's approach. Despite this, the UK experienced substantial COVID-19 impacts.

As of September 2021, >150,000 deaths have been linked to COVID-19 were reported (Figure 1.3B). The NHS faced huge pressures, particularly during the pandemic peaks. The introduction of vaccines dramatically changed the trajectory of the pandemic in the UK. The UK vaccination program was launched in December 2020, with rapid roll out and majority of the adult population were vaccinated by September 2021. This led to a large decrease in severe COVID-19 cases, hospitalizations, and deaths (Figure 1.2B). While some variants still pose challenges to vaccine efficacy, when this thesis was written, they remain effective at limiting the number of severe outcomes.

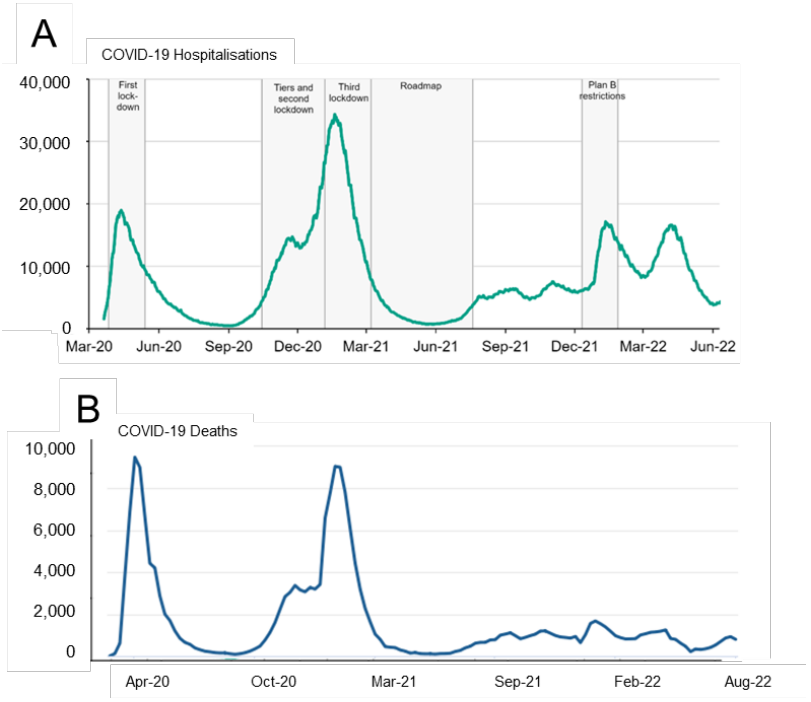


Figure 1.3 – (A) Hospitalisation Rates in the UK during the COVID 19 Pandemic, (B) COVID-19 Deaths in the UK (adapted from the Office of National Statistics).

1.3 Health Effects of Coronavirus Infection

The novel SARS-CoV-2 in late 2019 was recognised as a primarily respiratory illness, with hypoxia and acute respiratory distress syndrome (ARDS) in the most serious cases. To evaluate the medium- and long-term effects on the lungs it is first worthwhile considering the end organ sequalae of other viral infections which have previously been studied. The H1N1 influenza pandemic in 2009 and the SARS-CoV-1 epidemic in 2004 are both recent examples of large-scale outbreaks of viruses which have been studied with contemporary scientific methods.

1.3.1 Influenza and other viral illnesses

The H1N1 pandemic in 2009 affected 70 countries worldwide and amounted to over 30,000 cases, with a high requirement for intensive care treatment with mechanical ventilation[6]. A research group in Taiwan assessed the pulmonary function of patients one-month post discharge from hospital after H1N1 infection and compared the changes at 3 and 6 months. The sample size was small, with only 9 patients, who were found to have spirometry, Forced Vital Capacity (FVC) and diffusion capacity of

carbon monoxide (DLCO) deficits which improved from month 1 to month 3. There was no significant improvement in the measurements from 3-6 months post-acute infection[7]. A study in Spain reviewed a mixed group of patients who were discharged from intensive care during the H1N1 2009 pandemic. Seven had polymerase chain reaction confirmed influenza A (H1N1) and 4 had tested negative for influenza. In this study, patients were reviewed at 6 months only and there were no differences in spirometry or gas transfer between the two groups. Furthermore, the values for these measurements were within the normal range. Their conclusion was that H1N1 does not lead to persistent lung involvement, even in a population who have required respiratory support any more than other conditions causing ARDS might[8].

The most severe cases of H1N1 influenza infection required extra corporeal membrane oxygenation (ECMO) in addition to traditional mechanical ventilation. A group in Paris reviewed a group of their H1N1 patients 1 year post discharge from ICU. Those who had received ECMO and those who had not were compared. At the 12-month point, there were persistent minor deficits seen in the DLCO, but otherwise overall lung function had returned to nearly the normal range for a non-infected population in both groups[9].

Chronic viral infections which have not traditionally been associated as primarily affecting the lungs have also been found to affect pulmonary mechanics and diffusing capacity of carbon monoxide (DLCO). A large cohort of patients living with Human Immunodeficiency Virus (HIV) in New York underwent pulmonary function testing as part of registry surveillance. Those with advanced infection, and particularly in cases with a CD4+ counts of $<200/mm^3$ had a reduced DLCO, which was independent of other co-existing lung disease or smoking history[10]. A separate, female only study population of 463 patients and a HIV negative comparator group of 208 also demonstrated that DLCO was lower in the HIV positive population. Again, those with a lower CD4+ count had more significant gas transfer deficits, as did those with co-infection with Hepatitis C Virus (HCV)[11]. Furthermore, a small study in Turkey evaluating patients with HCV alone also found abnormal gas transfer and spirometry

abnormalities in up to 76%, some of which also had interstitial lung changes present on cross sectional imaging[12].

1.4. Post COVID Syndrome

Post-COVID illness, (colloquially known as long-COVID) is now a common and disabling long term complication of SARS-CoV-2 infection. Given the large number of UK infections, the current and longer-term health and social / economic impact of post-COVID illnesses for the UK general population are considerable. Whilst the focus of management was initially around the acute illness, with the pandemic declared by the WHO as finished, the longer-term effects are now more important to the UK pandemic recovery.

The National Institute for Clinical Excellence (NICE)/Scottish Intercollegiate Guidelines Network/Royal College of General Practitioners guidance on managing long-term effects of COVID-19 (updated November 2021) gives the following clinical definitions below:

- Ongoing symptomatic COVID-19: signs and symptoms of COVID-19 from four to 12 weeks
- Post COVID-19 syndrome: signs and symptoms that develop during or after COVID-19 and continue for more than 12 weeks and are not explained by an alternative diagnosis.

In the literature, Post COVID syndrome incidence following COVID ranges from 2.3-10%[13,14]. By March 2023, the UK Office for National Statistics (ONS) estimated that 1.9 million people living in private households in the UK (2.9% of the population) were experiencing self-reported symptoms greater than four weeks following symptoms. Self-reported Long COVID symptoms adversely affected the day-to-day activities of 1.5 million people (79% of those with self-reported long COVID), with 381,000 (20%) reporting that their ability to undertake their day-to-day activities had been “limited a lot”. Fatigue continued to be the most common symptom reported as part of individuals’ experience of long COVID (72% of those with self-reported long COVID), followed by difficulty concentrating (51%), muscle ache (49%) and shortness of breath (48%). As a proportion of the UK population, the prevalence of self-

reported long COVID was greatest in people aged 35 to 69 years, females, people living in more deprived areas, those working in social care, those aged 16 years and over who were not working and not looking for work, and those with another activity-limiting health condition or disability.

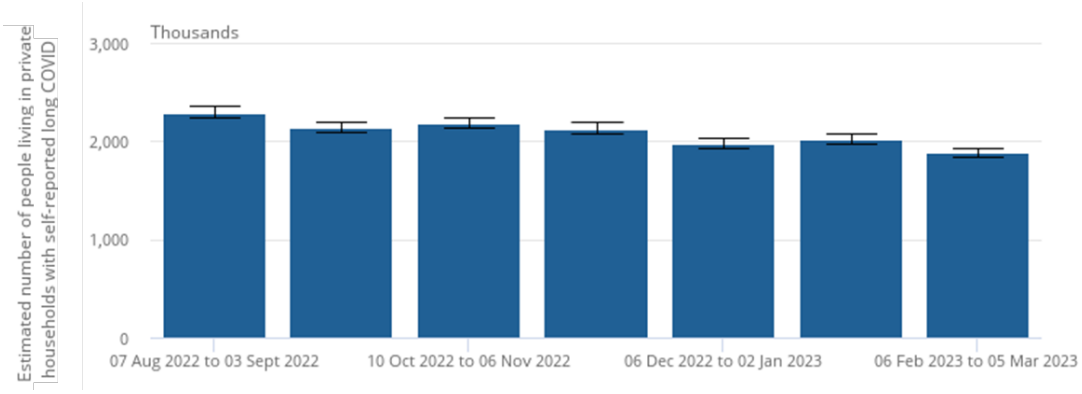


Figure 1.4 – Figure from COVID-19 Infection Survey from the Office of National Statistics (as of March 2023) showing the estimated number of people living in private households with self-reported long COVID of any duration, UK, four-week periods ending 3 September 2022 to 5 March 2023

The prevalence in the UK for those with symptoms persisting more than a year from diagnosis is thought to be greater than 70,000 (Figure 1.4)[14]. Whilst data on confirmation of infection make the interpretation of these data challenging, that they are self-reported symptoms implies that the impact on work and family life, in a working-age population, many of whom have no pre-morbid health conditions will be substantial. In line with this, a National Institute for Health Research (NIHR) survey of 3,286 respondents with long-COVID, shows 80% reported their symptoms were interfering with work [15].

1.4.1 SARS-CoV 2 and the Military Population

However, in contrast to the general public, military personnel are exposed to high volume/intensity exercise as a core component of their role. As such, any residual exercise limitation following COVID 19 infection may have significant occupational implications.

Service personnel represent a relatively healthy adult group engaged in full-time work, with minimal pre-existing medical conditions. Their service mandates an annual fitness assessment, and they might

be tasked with high-intensity exercises and handling safety-critical duties in potentially hazardous or isolated settings. Therefore, it is essential from an occupational health perspective to detect and document any significant residual organ abnormalities and/or to robustly confirm their absence before allowing a return to strenuous activities or high-risk environments.

As a direct result of this, early in the global COVID-19 pandemic, the UK Defence Medical Services (DMS) identified a need to provide thorough, standardised clinical assessment of serving personnel who had recovered from severe (hospitalised) COVID-19 illness, or who were experiencing prolonged (>12 weeks) symptoms (i.e. post COVID syndrome)[16]. Service personnel with hospitalised COVID-19 illness or post COVID syndrome were occupationally restricted because of their illness and were unable to undertake their usual work.

1.4.2 The Defence COVID Recovery Service (DCRS)

The DCRS is a UK initiative designed to support these Armed Forces personnel. It aims to ensure that military personnel receive the necessary medical assessments, rehabilitation, and support to return to full health and military duty. As the most common symptoms described in post-COVID syndromes, such as breathlessness, fatigue, 'brain-fog' and poor memory, are non-specific [17-19], they could be as a result of direct viral injury for example to the brain, heart, skeletal muscle; or from the any health anxiety and distress resulting from a global pandemic, or indeed from a combination of both.

As such, the approach of DCRS was set up to be holistic, assessing both the physical and psychological impacts of COVID-19. It was comprised of a comprehensive clinical pathway for affected personnel; included data on subjective symptoms, patient-reported outcome measures (PROMs) for anxiety, depression, stress, wellbeing, and fatigue, and also a physician-supervised objective measure of cognitive performance in every patient. These measurements were paired with objective measurements of functional capacity, and cardiopulmonary performance.

The aim was to exclude clinically significant pathology, such as lung fibrosis, pulmonary embolus, or myocarditis with LV impairment, and then to either direct appropriate clinical management for any disease identified, or to provide reassurance and direct attention towards rehabilitation and recovery. Diagnostic and prognostic evaluation of workers with post-COVID illness was conducted in concert with a parallel rehabilitation pathway delivering remote (initial video tele-consultation) consultation with a rehabilitation consultant, followed by two week tailored residential rehabilitation programme where indicated.

1.4.3 Military COVID-19 Observational Case Outcome in a Viral Infectious Disease (M-COVID) study

Nested within the DCRS was a 113-person, case-controlled cohort study designed to assess the effects of SARS-CoV-2 on the UK military population. M-COVID provides detailed characterisation of cardiopulmonary pathology and functional, neurocognitive capacity and mental health status and across three cohorts: hospitalised severe illness, non-severe community illness, and an age, sex and occupational role matched military control population without prior infection. Clinical assessments occurred over three days. Two days at the Defence Medical Rehabilitation Centre (DMRC) Stanford Hall where cardiopulmonary exercise testing (CPET), functional tests, cognitive assessment, spirometry, electrocardiogram (ECG), blood samples and patient reported outcome measures were taken and the third day at Oxford University Hospital (OUH) NHS Foundation Trust and the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) for cardiac magnetic resonance imaging (CMR); ^{31}P spectroscopy, rest and stress echocardiography, high-resolution computed tomography, (HRCT); dual-energy computed tomography pulmonary angiogram (DE CTPA), and full lung function testing (Figure 1.5, relevant methods expanded in Chapter 2). This thesis documents the pulmonary, cardiovascular and cardiopulmonary exercise testing results from the M-COVID study.

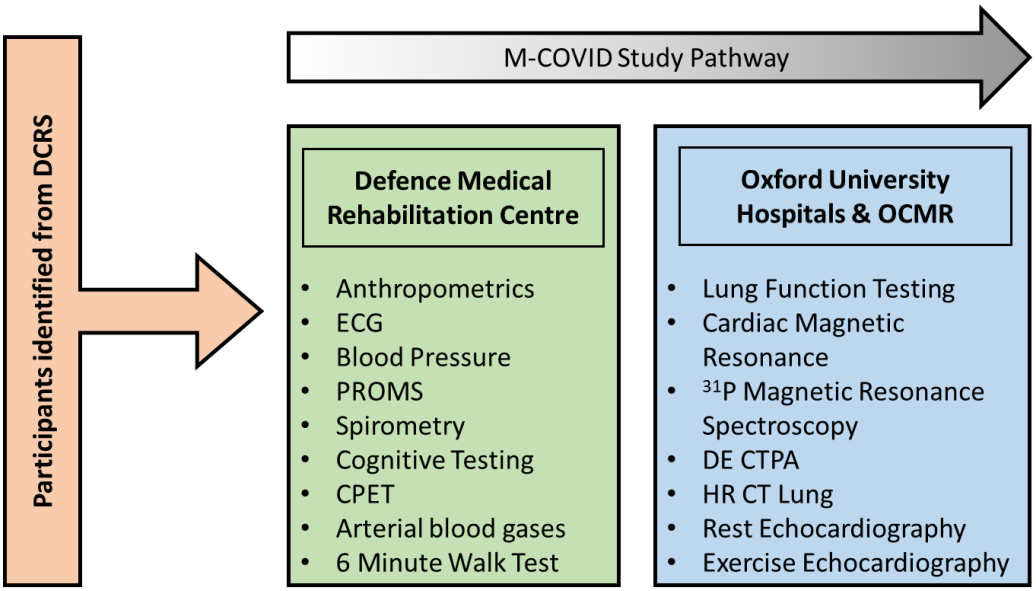


Figure 1.5 A high level overview of the M-COVID study

1.5 Determinants of Exercise Capacity

1.5.1 Exercise intolerance as a multisystem process

To maximise exercise capacity, the cardiovascular and respiratory systems must work together to provide both a delivery system of oxygen (O_2) and a removal system of carbon dioxide (CO_2) to the working muscles. As such, the ability to perform physical exercise is directly related to:

1. The cardiovascular capacity to supply oxygen (O_2) to the muscles
2. Pulmonary ventilation and diffusion capacity to move O_2 and CO_2 between the lungs and the blood
3. Transport of O_2 and CO_2 in the blood
4. Capillary gas exchange, of O_2 and CO_2 between the capillary blood and the working skeletal and cardiac muscle
5. Skeletal and cardiac muscle mitochondrial electron transport chain (ETC) capacity to use the O_2 to facilitate ATP synthesis

1.5.2 The Oxygen Cascade

The oxygen transport cascade describes the path of oxygen from the atmosphere to the tissues. In general, it is an anatomical map starting with the extra and intrathoracic large airways and ending in the mitochondria. Beyond the lungs and alveolar pulmonary capillary membranes, the heart pumps blood across the interface of the pulmonary capillaries and alveoli to exchange O_2 (and CO_2). Oxygenated blood travels the systemic circulation oxygen to the microcirculation in the peripheral organs for use by muscle. The venous system then returns the deoxygenated blood back to the right heart and the cycle continues. Figure 1.6 is a schematic of the cascade and shows the major steps. The overall behaviour of the cascade can also be described via the Fick equation which simply states oxygen consumption ($\dot{V}O_2$) = cardiac output ($\dot{Q}$) × AV difference (a- v oxygen content difference).

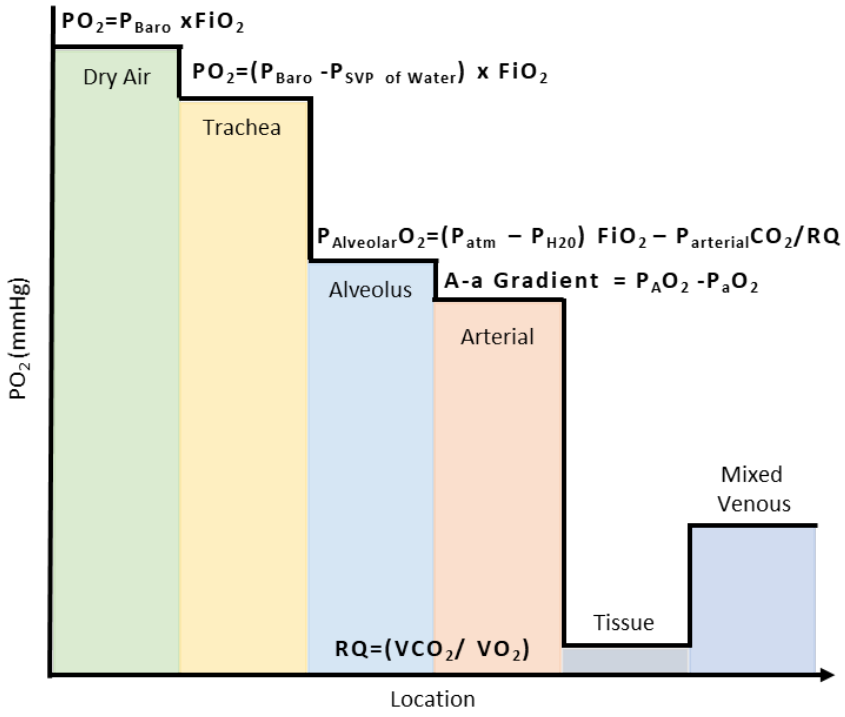


Figure 1.6 – The Oxygen Cascade

1.5.3 Respiratory, cardiac and skeletal muscle responses to exercise

In order to maintain O_2 delivery and CO_2 removal respiratory, cardiac and skeletal muscle physiology changes during exercise as shown below. Reduced exercise capacity is a common feature of post

COVID syndrome[20], and a failure of any one or more of these several responses to exercise could be the cause of exercise intolerance. As a result, optimal treatments for exercise intolerance in post COVID syndrome are very likely to involve treatments that are targeted to these organ systems. In order to identify the source of exercise limitation a suite of tests is needed that can identify the source of limitation and allow targeting of treatments.

1.5.4 Cardiac and Skeletal Muscle ATP metabolism

Regardless of the aetiology, the majority of cardiac diseases eventually manifest in one final common phenotype of LV systolic dysfunction. As delivery of ATP to the myocardium is essential for normal contractile function it follows that a reduction in ATP delivery could serve as the final common pathway that allows many different aetiologies to manifest as the same end phenotype i.e., systolic failure. Given this, and that the delivery of ATP is essential for skeletal muscle contraction, alterations in ATP metabolism may play a role in the exercise intolerance in post COVID syndrome.

1.5.4.1 Cardiac Metabolism in the Normal Heart

In the healthy heart at rest, the majority of adenosine triphosphate (ATP) is generated by fatty acid oxidation (FAO, 60-90%) with the remainder mainly from glucose oxidation (10-40%, Figure 1.7A). The advantage of FAO is that it yields 3.5 times more ATP per mole than oxidising glucose. When oxygen is not limited, this overcomes the disadvantage that FAO is inherently less oxygen efficient (oxidising palmitate requires 23 molecules of O_2 to produce 105 ATP (phosphate/oxygen, P/O ~2.33) whereas glucose requires only 6 O_2 molecules to produce 31 ATP (P/O ratio 2.58)[21,22]. Hence, when oxygen and fats are freely available, oxidising fats is advantageous, as more ATP is generated, but when oxygen becomes limited e.g., during ischaemia, metabolising glucose becomes beneficial. The normal healthy heart displays remarkable metabolic flexibility, altering its substrate use depending on prevailing supply, and metabolic conditions.

1.5.4.2 Cardiac Metabolism in Heart Failure

In heart failure (HF) FAO rates are lower [23]across animal models of infarction [24], pacing-induced HF [25], and also in human patients with heart failure[26,27]. This results from reduced PPARα signalling, reduced FA transport and downregulation of FAO enzymes [28]. In animal models, this reduction is not accompanied by decreased FA uptake [29] leading to triglyceride storage, non-oxidative metabolism and lipotoxic species which may contribute to HFrEF progression [30]. In addition, myocardial insulin resistance reduces glucose uptake, pyruvate dehydrogenase (PDH) flux (10), and therefore reduced glucose oxidation (Figure 1.7B). Consequently, in heart failure both glucose oxidation and FAO are decreased. [31-35] As FAO is reduced relatively more there is a relative 'substrate switch' towards glucose metabolism [36]. This is postulated to reduce ATP production and downstream ATP delivery and contractile function.

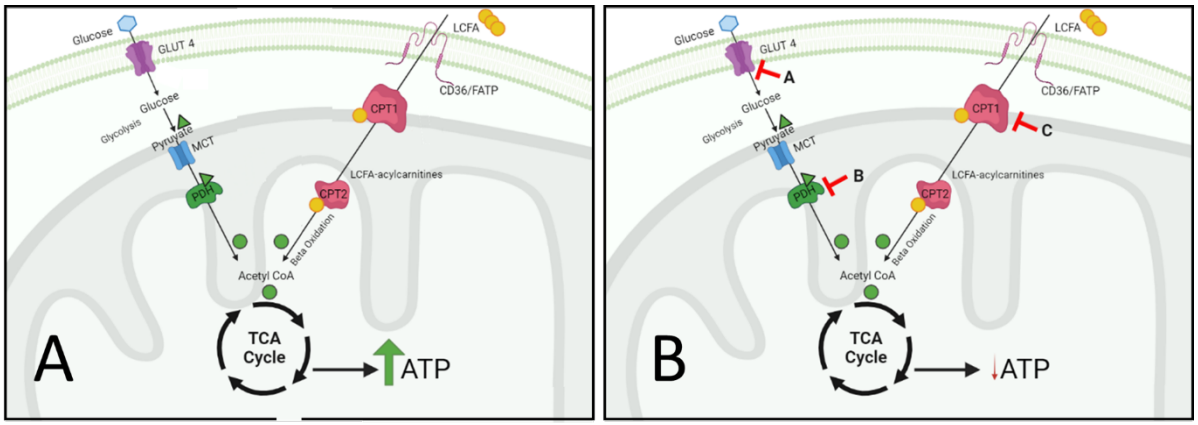


Figure 1.7 A schematic of cardiac substrate metabolism in A) the normal heart and B) the failing heart

1.5.5 Cardiopulmonary Exercise Testing for Exercise Capacity

In clinical practice, quantitative assessment of exercise capacity is usually achieved through measurement of peak oxygen consumption ($\dot{V}O_2$) during cardiopulmonary exercise testing (CPET). Whilst many patients with cardiovascular disease will have reduced peak $\dot{V}O_2$ primarily due to limited augmentation of cardiac output, an important secondary cause of exercise limitation is reduced

exercising skeletal muscle oxygen extraction[37]. The traditional CPET approach, however, cannot differentiate between these two causes of poor exercise capacity. One solution to this is simultaneously measuring $\dot{V}O_2$ and cardiac output during exercise and calculating arteriovenous oxygen difference (a proxy for peripheral oxygen extraction) using the Fick equation[38]

1.5.5.1 ^{31}P-MRS for assessing high-energy phosphate metabolism

Phosphorus ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) is the only noninvasive way to quantify high-energy phosphate metabolites in the human heart. ^{31}P-MRS allows the *in vivo* quantification of phosphorus (^{31}P)-containing metabolites involved in energy metabolism, such as phosphocreatine (PCr) and adenosine triphosphate (ATP). Using ^{31}P MRS, various indices of the CK system have been measured to assess mitochondrial energetics, including the PCr/ATP ratio[172]. Cardiac ^{31}P-MRS in humans is typically performed at higher magnetic field strengths of 1.5–3T and takes 10-30 minutes to acquire. Studies have shown that PCr/ATP is related to cardiac function, and is a better predictor of long-term survival than NYHA functional class or LV EF in dilated cardiomyopathy,[173] hypertrophic cardiomyopathy,[174] HF with preserved ejection fraction,[175] and ischemic heart disease[176].

1.5.5.2 Assessing pulmonary function

It is possible to determine if exercise limitation is due to a ventilatory defect using cardiopulmonary exercise testing, however, for a complete characterisation of lung volumes, flow dynamics and diffusive capacity, full formal lung function testing is necessary. In routine clinical and research practice, three tests are used, and together they can measure lung volumes, gas diffusion properties across the alveolar-capillary membranes, and dynamic breath manoeuvres which inform the nature of any pathologies identified.

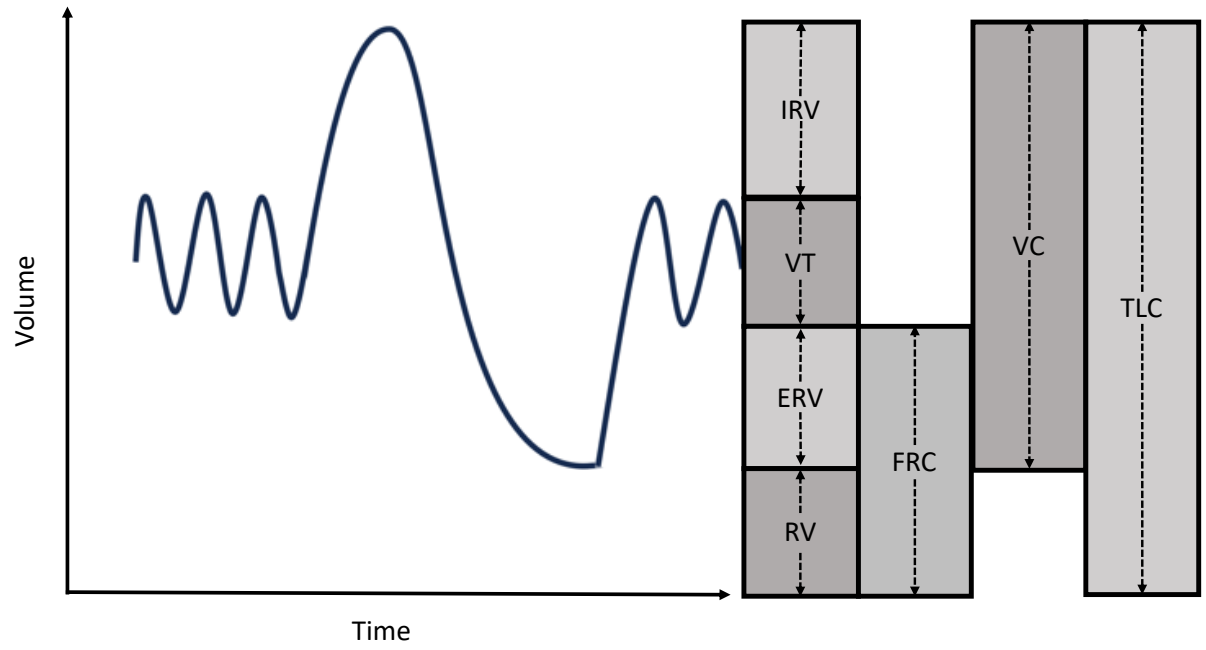


Figure 1.8: Lung volumes. RV- residual volume, ERV- expiratory reserve volume, VT- tidal volume, IRV- inspiratory reserve volume, FRC- functional residual capacity, VC- vital capacity, TLC- total lung capacity.

1.5.5.3 Dynamic lung function and volumes by spirometry

Different components of lung function can be measured in different ways. Tidal volumes, inspiratory and expiratory reserve volumes (which are measured during resting-relaxed breathing, deep inspiration and full expiration respectively) are measurable using flow volume measurements taken from a standard spirogram (see fig 1.8). By adding in dynamic manoeuvres, such as forced expiration, it is possible to determine how much of the vital capacity (VC) can be forcibly expelled in one second (FEV1), and how this volume compares to the forced vital capacity (FEV1/FVC). This ratio is important because a low value is suggestive of obstructive airways disease.[39]

A standard spirogram, or spirometer, consists of a mouthpiece attached to a flow meter. The participant is asked to form a tight seal around the mouthpiece and take regular quiet breaths which are measured to determine the tidal volume. The participant is then asked to take a maximal breath in, followed by a rapid, forced, maximal breath out. The velocity (in litres per minute) inspired and

expired is plotted against time to produce a flow-volume loop, the shape of which can be used to detect obstructive or restrictive lung physiology.

Abnormal values in spirometry can direct the investigator to the pathological process underlying the abnormality. In airflow obstruction, the amount of air which can be expelled quickly from the lung (in 1 second, or FEV_1) is reduced in comparison with the total functional lung volume (usually taken to be the maximal expired volume e.g., FVC) as a result of relatively narrow airways compared to the size of the lung. This metric is expressed as FEV_1/FVC and values below the 5th percentile that is predicted are diagnostic[40]. Predicted values are adjusted for height, weight, age, sex and ethnicity. In restrictive lung physiology, the FEV_1/FVC ratio will remain within normal limits or may be above predicted levels, but the FVC will be reduced. To confirm abnormal lung physiology on spirometry, spirometry should be repeated with further explanation and support to control for errors in respiratory effort which can cause a false positive. [40]

1.5.5.4 Static Lung volumes by N_2 washout method

The component of the lung volumes which is not measurable by dynamic assessment is the residual volume and therefore also the vital capacity (VC) and total lung capacity (TLC) (Fig 1.8). The residual volume is never expelled during breath manoeuvres, and indeed if part or all of a lung completely deflates, it is mechanically difficult to reinflate. To estimate residual volume, techniques such as whole body plethysmography or gas dilution must be employed[41].

Given the fractional concentration of atmospheric N_2 (F_{iN2}) is known to be 0.78 altering the inhaled concentration of N_2 with a washout phase and then measuring the expired volume of N2 allows calculation of the total lung volume, according to the relationship below:[42]

$$V_{lung} = V_{eN2}/F_{iN2}$$

Subjects breathe pure (100%) oxygen for 7minutes, until the expired N_2 concentration is not detectable, indicating that N_2 washout from the tissues is complete. Spirometry is then performed

using atmospheric air, and the concentration of N_2 in the VC can be used to determine the volume of the residual volume, and thus the TLC[43].

In obstructive lung conditions such as emphysema, the TLC and residual volume are expected to be elevated in comparison to age/sex/height/weight corrected reference values. Restrictive defects are confirmed by measuring a TLC which is more than 5% below the percent predicted minimum normal value, with a normal FEV_1/FVC [40].

1.5.5.5 Diffusing capacity

Lung mechanics are responsible for gas delivery from the atmosphere to the alveolar-capillary membrane, but to determine the ability for gases to cross the alveolar capillary membrane into the circulation, measurements of diffusing capacity are undertaken. Although oxygen is the principal gas transferred across the membrane for use by the tissues in cellular respiration, it is not measured in lung function testing, as its ability to diffuse is affected by the amount of blood flow through the pulmonary circulation, in addition to the diffusing capacity of the membrane. For lung function testing then, a separate gas, carbon monoxide is used, as its absorption is determined only by the diffusing capacity of the membrane[44].

Using a single breath diffusion method, a gas mix including a known concentration of CO is inhaled and held at end inspiration for 10 seconds. During this time, the gas mix partially diffuses into the circulation and waste gases from the circulation diffuse into the alveoli. Following the breath hold, the concentration of CO is measured during a slow expiration. The diffusing capacity of the lungs for CO can be determined using the following equation:[45]

$$DLCO = \frac{VA}{\left[\left(\frac{BHT}{60}\right) \times (Pb - P_{H2O})\right]} \times \ln\left[\frac{FA_{trace} \times F_{ICO}}{FI_{trace} \times F_{ACO}}\right]$$

The composite parts that affect diffusing capacity (DL_{CO}) are the alveolar volume (Va) available to support diffusion, and the efficiency of gas transfer across the membrane (K_{CO}, or Krogh factor) as

described below. When DL_{CO} is reduced, the cause may be identified by determining the relative extent of Va or K_{CO} is impaired[45].

$$DL_{CO} = Va \times K_{CO}$$

DCLO can be impaired in several pathological processes, which are broadly: parenchymal lung disease, pulmonary vascular disease or anaemia[45,46]. Values are expressed after correction for Hb. Impaired DLCO with both Va and KCO components reduced requires destruction of either the alveoli or reduced accessible alveoli in the case of air trapping. Conditions resulting in these findings include emphysema and diffuse interstitial lung disease and fibrosis [47,48]. KCO can be impaired with a preserved VA in pathologies which result in microvascular damage such as pulmonary vasculitis and idiopathic pulmonary hypertension[49,50], or with microvascular remodelling as in the case of hepatopulmonary syndrome [51]. Elevated KCO measurements can also be seen in cases of relative increased pulmonary blood flow such as post pneumonectomy [52], microvascular congestion in obesity [53] or left to right cardiac shunts[54].

1.6 SARS-CoV-1 and the effect on pulmonary function

The SARS-CoV-1 epidemic in 2003, which affected multiple countries worldwide, allows insight into the potential consequences of SARS-CoV-2, presuming there may be similarities in the clinical presentation. Research published from regional hospitals in affected countries with high volumes of cases have described pulmonary involvement which persists after the acute illness.

A regional centre in Hong Kong reviewed 57 adult patients 6 months after SARS-CoV-1 and performed lung function testing[55]. Using spirometry, gas diffusion and static lung volume testing, 20 subjects were found to have reduced DLCO (median value 63% of predicted), abnormal Total Lung Capacity was seen in 17 and 4 had abnormal Forced Vital Capacity (FVC). A smaller number of subjects had either obstructive, restrictive or mixed defects in spirometry testing[55].

A group in Singapore made similar observations after a median of 3 months post-acute infection[56]. 46 patients were studied. All had been admitted to hospital during their illness and 22% had been admitted to intensive care. Subjects underwent pulmonary function testing and cardiopulmonary exercise testing. Of the 46, 50% were found to have some abnormality on their pulmonary function test. Impaired gas transfer was most commonly seen, in 39%, with abnormal FEV1 in 26% and abnormal FVC in 15%. The authors noted that the measured exercise capacity deficit was not matched by the abnormal pulmonary function results, and concluded that it was likely due to an alternative cause[56].

Serial analyses of pulmonary function deficits post SARS-CoV-1 with time have also been conducted. A group in Beijing performed lung function testing at two consecutive points within a three-month period post hospital discharge in a study population of 258. Study visits were scheduled at approximately one- and three-months post discharge from hospital, and the investigators found that the recorded abnormalities in diffusion capacity had improved from 15.8 ± 2.8 ml/min/mmHg to 17.7 ± 2.9 ml/min/mmHg, which was a highly significant improvement[57].

Another group took a smaller number of SARS-CoV-1 survivors and recorded lung function tests every three months for a total of 17 months. One third of the subjects had restrictive ventilatory defects and/or abnormal diffusion function at baseline. These abnormalities remained unchanged at 3 months, but demonstrated a trend for improvement at and after the 6 month point[58].

Persistent deficits have also been recorded by a Hong Kong research group, who performed serial measurements of lung function and exercise capacity in 97 survivors. At 12 months, 23% had a persistent reduction in their gas diffusion function, 5.2% had abnormal TLC and 4.1% had abnormal FVC. Those with the largest persistent defect were noted to be patients who had required ICU support[59].

1.6.1 SARS-CoV 2 and the effect on pulmonary function - General population

In the general patient population, there are a number of small observational studies which describe the post infection pulmonary findings after SARS-CoV-2. These studies are extremely heterogenous and use a variety of exclusion criteria and time points post-acute infection at which to evaluate persistent lung deficits. The majority use combinations of measuring static lung volumes, spirometry and gas transfer metrics with or without radiographic techniques to evaluate persistent post-infective effects. A Chinese research group investigated a cohort of 110 adult survivors who specifically had non-severe disease (all those requiring ICU care or mechanical ventilation were excluded) and performed pulmonary function tests on the day of hospital discharge[60]. The mean age of the studied cohort was 49 years, and 50% were female. Underlying comorbidities or cardiopulmonary risk factors were present in 40%, and abnormal gas transfer of carbon monoxide (DLCO) was present in 47%. Abnormally low total lung capacity (TLC) was identified in 25%, and abnormal FEV1 in 13.6%. Additionally, DLCO deficit was more common as the severity of the disease increased.

In Mexico, a Hispanic population were investigated 1 month after diagnosis with SARS-CoV-2. A total of 146 participants underwent pulmonary function testing after a mild case of the disease (defined as the absence of radiographic evidence of pneumonia and hypoxia) and found impaired gas transfer of carbon monoxide (DLCO) of <80% of the predicted figure in 43% of the study population. A restrictive spirometry pattern was also noted in 20%[61].

At 4 months post-acute infection, an Italian group reviewed 379 patients who were admitted to hospital and compared findings by severity of the acute illness. 60% required oxygen supplementation and 15% were admitted to ICU. The median age of the cohort was 56 and 45% were male. Those who developed respiratory failure were older and had a higher body mass index (BMI). They also had more pre-existing comorbidities. Patients who were classed as having severe disease had a lower forced vital capacity (FVC) and lower total lung capacity (TLC) than those without. The group who were admitted to ICU had a lower DLCO/VA, a lower TLC and FVC than those with pneumonia who did not

require intensive care[62]. Other researchers compared male and female survivors 4 months after acute infection in a 'Long COVID' clinic. By definition, the participants are subject to selection bias and are all persistently symptomatic in some way. The cohort was 58% male, but the groups were matched for disease severity. Abnormal gas transfer was seen more commonly in females than in male subjects (defined as DLCO < 80% predicted, 53% vs. 31%) and more females had a severe reduction in gas transfer than males (defined as DLCO <70% predicted, 40% vs. 20%)[63].

A meta-analysis of studies which look at early post infection sequalae (<4 months) also identified altered diffusion capacity as a principle finding. A total of 6 studies (from an initial 1971) were amalgamated and found DLCO abnormalities in 39% of cases, a restrictive spirometry pattern in 15% and an obstructive pattern in 7% of cases[64]. The authors were critical of the decision to measure pulmonary function earlier than 3 months when looking for persistent changes as a period of recovery time is expected after a severe respiratory infection.

There are also reports in the literature which look at pulmonary effects post SARS-CoV-2 over longer periods. Comparisons from measurements at 3 and 6-months post infection were made at a Canadian centre. The study population were a mixture of hospitalised patients and those managed in the community (n=51, 30 hospitalised and 21 community cases). The mean age was 51 years, and the population was 39% female. Abnormal DLCO values were noted, as in the previous studies, but there was no significant difference seen when the tests were repeated at 6 months[65].

A multicentre study with even longer follow up was conducted in Switzerland. This group compared severe SARS-CoV-2 cases who received ICU care or had ARDS, with non-severe cases who were admitted to hospital. Participants were reviewed at 3, 6 and 12 months post-acute infection. The mean age was 58 years and in total 584 participants were studied, with 214 still enrolled at 12 months. Overall, the authors demonstrated an improvement in pulmonary function over time. By the 12-month point, there was no difference in FVC between groups, TLC was 12% lower in the severe group, and DLCO was 10% lower. Despite improvement in all values with time, the severe group continued

to have a substantially larger number of participants with impaired DLCO in comparison to the non-severe group (68% vs.37%)[66].

Studies observing the respiratory outcomes post COVID-19 between 6-12 months post infection have been reviewed in meta-analysis. A total of 10,945 cases across 40 studies were evaluated. Persistently impaired pulmonary function was observed consistently. DLCO measurements of <80% predicted were seen in 49% of participants, which improved to 32% beyond the 12-month point. Reduced FVC to <80% predicted was seen in 14% at the 6-12 month point, and there was no significant change at the >12 month point[67].

The common findings across SARS-CoV-1 and SARS-CoV-2 post-acute infection appear to be a reduction in pulmonary function, including both static lung volumes and of diffusion capacity. During the SARS-CoV-1 outbreaks, a disproportionate number of affected persons were healthcare workers, and were of working age with a heavy female bias. The SARS-CoV-2 data collected in the general public highlights that older people are particularly susceptible. Obesity and being overweight also predisposed to severe illness. Neither of these groups are directly comparable to the UK military population, who are majority male, and tend to be younger, with fewer comorbidities and a higher baseline level of cardiovascular fitness. It is therefore helpful to consider other groups, such as athletes or military populations from other countries when considering what might be expected in this population.

1.6.2 Limitations of current literature on the pulmonary effects of SARS-CoV 2

It is known that viral infections can have pulmonary effects whether they are respiratory viruses or not, and both the SARS-CoV-1 and 2 outbreaks have recognised alterations in pulmonary function. These are measurable months after recovery in members of the general public who had a severe infection. The athlete populations who have been investigated post SARS-CoV-2 tended to have asymptomatic or mild infection, either as a result of having overall lower risk of developing severe infection, or because their infections were picked up as part of squad screening, rather than as a

function of them needing to seek medical care. Studies to date suggest that although a small number do demonstrate residual impairment, it is less common than in an older, more comorbid population. The military population has not previously been investigated in its entirety for persistent abnormalities in lung volumes or gas transfer. A naval medicine research group in the Netherlands has reported the results of a small study concerning military divers. In a retrospective observational study, divers who had asymptomatic or mild illness underwent spirometry and gas transfer analysis 1-month post infection and results were compared with a SARS-CoV-2 negative matched control group. No differences were found between groups[68]. Larger studies, and those which look at gas transfer in military populations who developed severe disease have not been performed.

1.7 Assessing Cardiac Structure and Function

In clinical practice, detailed assessment of the heart structure and function is usually achieved via cardiovascular imaging, the modality of choice depends on the aspect of cardiac function that is of interest. Chamber size, wall thickness, systolic, diastolic function and valve function can be assessed using transthoracic ultrasound, but for characterisation of the myocardial tissues, assessment of the presence and magnitude of myocardial scar and fibrosis, cardiac magnetic resonance (CMR) imaging is required. CMR with additional protocols to characterise tissue is recommended in clinical guidelines for the assessment of heart failure, cardiomyopathies and myocarditis[69 70,71]. It is easily reproducible, is not subject to inaccuracies due to poor echo windows and has lower intra-observer variability.

In addition to standard 2D cine imaging, the magnetic properties of the tissues during magnetic resonance imaging (T1, T2 and T2*) can be used to give further information regarding fibrosis and oedema. These parameters represent the time taken for recovery or decay of directional magnetization. The properties can be visualised in weighted images, where the myocardium is imaged according to each magnetic property, or with parametric maps[72]. Parametric maps are secondary images which are formed using individual pixels representing different values of the property (T1, T2 or T2*) that are superimposed onto magnetic resonance images. In this way, both

focal and diffuse pathology in the myocardium can be visualised, but also quantitatively assessed[72]. With the use of weighted image techniques alone, focal areas of pathology are identifiable due to the clear demarcation of diseased vs. healthy myocardium, whereas subtle global pathology may not be identified.

Late gadolinium enhancement images are acquired following the injection of a gadolinium based IV contrast. Images are then taken of the ventricle in different orientations, at the point when the contrast has cleared the blood pool and myocardium, with the exception of residual contrast which remains in the extra cellular space in areas of fibrosis or scar. This can be qualitatively assessed and the underlying pathology determined by the scar pattern (e.g. mid-wall bands are consistent with a non-ischaemic cardiomyopathy, partial thickness infarction is sub-endocardial, whereas full thickness infarction is transmural, myocarditis is patchy and subendocardial, whereas cardiac sarcoid is often a mixture of subepicardial and subendocardial)[73,74]. Fibrosis can also be assessed quantitatively after IV gadolinium injection, by performing a post contrast T1 map in addition to a pre-contrast native T1 map. By measuring the different T1 times in the blood pool and the myocardium pre and post contrast, and by knowing the patient's haematocrit, the extracellular volume fraction can be calculated, to determine whether there is widespread extracellular space as a result of scar or fibrosis[72]. A known cause of cardiac involvement post viral infection is myocarditis, and the European Society of Cardiology recommends the use of CMR with a combination of cine imaging, late gadolinium enhancement and T1 and T2 weighted imaging sequences to make a diagnosis and to guide management[71,75,76].

1.7.1 Cardiovascular Effects of SARS-CoV 2

Both non-infectious inflammatory chronic diseases such as SLE and chronic viral infections such as HIV can display a spectrum of cardiac involvement, which can range from the overt to the subclinical[77-79]. At the start of the COVID-19 pandemic, acute cardiac injury was highlighted as a potentially common

clinical feature and increasingly, the extent of mild or asymptomatic cardiac disease was uncovered in various populations.

In addition to features of myocardial fibrosis or inflammation, that can be identified using Cardiac Magnetic Resonance (CMR) imaging, it is helpful to remember that COVID-19 is often a prostrating illness, even in its milder forms. This can also affect cardiac function. Multiple bedrest studies in both men and women have found changes after the equivalent of 2-3 weeks of bedrest including reduction in ventricular volumes, stroke volume and ventricular filling parameters[80-82].

Research to date has resulted in the Society for Cardiovascular Magnetic Resonance Imaging recommendations to perform CMR with chamber volume and function measurements, and parametric tissue mapping as a minimum standard to detect subtle myocardial involvement in cases of concern post COVID-19 infection[83].

1.7.2 SARS-CoV 2 and the effect on cardiovascular function - General population

Most general population studies which look at the effect of COVID-19 on the heart in the post-acute period consider cohorts of patients reviewed after discharge from hospital[84,85]. Such patients therefore required inpatient care for their acute illness, and many required mechanical ventilatory support on intensive care. The most detailed studies used CMR to assess ventricular function, tissue characterisation with native T1 and T2 mapping, and with late gadolinium enhancement to look for patterns of fibrosis. Median ages of the patients studied ranged from 49-52 years old and found cardiac involvement in between 30-78% of cases[85-87].

Common findings included impaired left ventricular function, increased left ventricular volumes and late gadolinium enhancement of either the myocardium or the pericardium[85-87]. Elevated native T1 and T2 values, which are measures of tissue oedema and inflammation were also elevated in as many as 60% of cases, although group averages were below the clinical cut-offs for abnormal values[86,88].

Many of the patients investigated were asymptomatic at the time of testing or had not presented with cardiac symptoms[86,87,89].

These studies suggest that there is potentially a large burden of cardiac involvement as a consequence of COVID-19 infection, which is not necessarily identifiable from symptoms or clinical severity. However, the studies are heterogenous in both the way subjects are selected and in the length of time between acute infection and investigation. Additionally, the population is not an ideal comparator with the UK Armed Forces, owing to increased age, higher background level of clinical co-morbidities and reduced level of baseline cardiovascular fitness.

1.7.3 SARS-CoV 2 and the effect on cardiovascular function in the military population

Specific research into the effects of SARS-CoV-2 on military personnel is limited. This group of patients does not consistently fit the training criteria to be considered elite athletes, and yet they are on average younger and more physically active than the general population. As a result, there is value in considering this group separately, particularly as some of the intensely physical jobs in the military could contribute to sudden cardiac death in the presence of an undiagnosed cardiac condition, such as myocarditis[90].

A research group in the US have looked at the extent of cardiac pathology in a military population who had persistent cardiorespiratory symptoms following COVID-19 infection[91]. Cardiac MRI with parametric mapping was undertaken, in addition to standard cardiovascular examination and ECG recording. A retrospectively selected age, gender and employment type matched control group was used to compare. Myocarditis was found in 8% of the post COVID-19 soldiers, Takotsubo cardiomyopathy was found in 2% and biventricular systolic dysfunction was found in 2%. In comparison with the control group, the patient group had reduced RV systolic function and reduced indexed LV mass. Other metrics such as LV and RV end diastolic volumes, and subepicardial late gadolinium enhancement appeared close to reaching significance but may be limited by the small sample sizes. No differences were seen in segmental or global mapping values.

Although the authors identified the 'subjects' pre-infection cardiorespiratory fitness based on established employment requirements, the study did not characterise the exercise capacity with a detailed objective measure such as CPET, nor did they use stress to evaluate the changes with exercise or catecholamines to uncover subtle impairment which may not be seen at rest. It is interesting to note that 10% of their patient cohort received inpatient care, either on a ward or on ICU, a clinical characteristic which was not seen in the athlete groups.

1.7.4 SARS-CoV 2 and the effect on myocardial metabolic function

Many of the research articles into cardiac involvement post COVID-19 have demonstrated sub-clinical findings, with either a largely asymptomatic acute course or relatively mild features. It is possible to use other metrics to assess subtle changes in myocardial function in addition to standard advanced imaging techniques. ^{31}P Magnetic Resonance Spectroscopy (31-P MRS) allows the relative quantification of phosphorus containing molecules in a set voxel of tissue [92]. Such molecules in the myocardium are responsible for the final part of energy metabolism, that of ATP transfer from mitochondria to cells via the creatine kinase shuttle[92]. Measurements of the ratio between phosphocreatine (PCr) and ATP are a sensitive marker of myocardial efficiency[93]. In disease states, there is a reduction in PCr relative to ATP which is thought to represent a reduction in the energy reserve available to constantly active muscle fibres. PCr/ATP ratio is a proven highly sensitive marker of severe heart failure of multiple aetiologies but has not hitherto been used to look for changes post viral infections[94,95]. In conditions where the myocardial involvement in a disease process is subtle, such as gene positive but phenotype negative inherited cardiomyopathy, differences in the myocardial energetics were best appreciated during exercise or catecholamine stress[95,96]. It is plausible that such a technique could reveal changes at the metabolic level in military personnel who have had COVID-19.

1.7.5 Limitations of current literature on cardiovascular effects of SARS-CoV 2

It is evident that COVID-19, whilst primarily being a respiratory illness, has multisystem effects, including cardiac involvement in some. Current studies vary widely in their reporting of the incidence and prevalence of cardiac sequelae, but residual fibrosis and inflammation as a result of myocarditis seems to be the predominant finding. The majority of the literature pertains to hospitalised patients, who are not representative of the UK military population - they tend to be older, with more females and a higher level of baseline co-morbidities. As a result, it is possible that these studies overrepresent the presence of cardiac involvement that one might anticipate in the military. In the athlete studies, the amount of cardiac involvement was generally low. Conversely however, this group are mostly younger than our cohort, and will, as a group have a higher degree of baseline fitness in comparison to the military population.

Many of the studies did not have a contemporaneously recruited and matched control group to compare against, and not all studies performed all investigations on all subjects. It is possible in these examples, that selection bias has artificially inflated the amount of detected cardiac involvement in the investigated cohort. Considering the single study that specifically investigated a military population, it seems likely that the frequency of cardiac involvement in the military may be closer to that of the athlete population than the patient population.

1.8 SARS-CoV 2 and the effect on Exercise Capacity

1.8.1 The use of exercise testing in health and disease states

Exercise has many benefits in characterising the effect of a specific disease on a human population. First, by analysing the components of a cardiopulmonary exercise test (CPET), one can identify the adequacy of the exercise attempt, the overall cardiorespiratory fitness, and which point of the oxygen and energy delivery pathway is responsible for any deficit (be that circulatory, ventilatory or peripheral)[97]. Second, lower exercise capacity has been demonstrated in multiple studies to be an

independent risk factor for all-cause mortality and death due to cardiovascular disease[98]. This is seen across a range of ages, sex and ethnicity, with the reduction in exercise capacity by 1 metabolic equivalent (MET) conferring an increased mortality risk in the region of 10-25%[99,100].

1.8.2 Exercise capacity and episodes of prolonged bed rest

The novel SARS-CoV-2 virus, which emerged in late 2019, appears to be a primarily respiratory virus with some influenza like features. The course of the illness is asymptomatic or mild for some, whilst leading to long periods of bed rest or hospital admission for others[101,102]. In addition to the catabolic effects of an acute infectious disease, there are additional changes that are noted simply from spending multiple days lying down in bed. The effects of gravity on the cardiovascular system contribute to homeostatic mechanisms which maintain plasma volume, and cardiac function[103]. Since studies in the 1960s, it has been known that periods of bed rest also cause significant deconditioning. The Dallas Bed Rest study investigated 5 healthy men in their 20s and compared a CPET at baseline with one following a period of bed rest which lasted 3 weeks. Cardiac output, stroke volume and total exercise capacity, measured by $\dot{V}O_2$ max, all dropped significantly. A rehabilitation training period of 8 weeks was required to recover from the deconditioning and return exercise variables back to their pre-bed rest levels[104]. The same cohort of participants was followed up serially over a 40-year period, and their baseline exercise capacity at the 40-year follow up point was similar to their post bed rest exercise capacity measured during the first study. The authors suggest therefore that a period of 3 weeks bed rest is equivalent to 40 years of normal aging[104].

1.8.3 Exercise capacity and previous coronavirus outbreaks

Both 6 Minute Walk Distance (6MWD) and CPET have previously been used to describe the effects of coronavirus outbreaks. In the 2003 SARS coronavirus epidemic, which affected several countries in Asia, exercise capacity was found to be reduced in survivors of the disease. A group of 138 patients admitted to a hospital in Hong Kong with a mean age of 35 years demonstrated lower than expected 6MWD, with an average of 464m (±83) at 3 months post infection, which improved to 502m (±95) by

6 months[105]. By 12 months, there was no additional improvement in exercise capacity for the same group[106]. Additionally, the investigators saw no difference in exercise capacity between those who were admitted to ICU vs those who were nursed on regular wards.

In a different study, a group of hospitalised patients (n=46, mean age 37 years ±10) performed a CPET after their hospital discharge [107]. 59% had 'normal' exercise capacity ($\dot{V}O_2$ max ≥ reference value minus 1.65SD). Of the 18 individuals with an abnormal test result, the mean $\dot{V}O_2$max was 63% predicted. 11 of these were deemed to have a circulatory limitation as the cause of the impairment, one of whom also had a ventilatory component. The remaining 8 had a limitation due to deconditioning.

1.8.4 Exercise capacity and SARS-CoV-2

Since the SARS-CoV-2 pandemic, CPET has been used in a number of studies looking at the effect of the disease on patients. A study on patients admitted to hospital with COVID-19 in Switzerland examined exercise capacity and pulmonary function at 3 months post discharge. A total of 35 patients with a mean age of 58 and a median hospital stay length of 14 days had a CPET. An abnormally low $\dot{V}O_2$ max was seen in 54% of patients. Of those 19, 9 were due to deconditioning, 5 had circulatory limitations and 5 had ventilatory limitations. Some of the non-deconditioning limitations were ascribed to pre-existing medical conditions[108].

A multicentre study on a Norwegian population investigated a larger group (n=156, mean age 56, median length of hospital stay 6 days) and performed CPET at 3 months post discharge, which was compared with a pre-existing reference dataset. Reduced $\dot{V}O_2$ max (≤80% predicted) was seen in 31%, and deconditioning was the main component of causes of limitation found (seen in 63% of those with an abnormal test)[109].

A UK study of 58 hospital patients (mean age 55, median length of hospital stay 9 days) was compared with a prospectively recruited control population, and had multiorgan investigations, including CPET[88]. $\dot{V}O_2$ max was significantly reduced in the post-COVID-19 population in comparison to the controls,

with 55% achieving <80% predicted. Differences were also seen in the anaerobic threshold, the $\dot{V}E/\dot{V}CO_2$ slope and the Oxygen Uptake Efficiency Slope. Deconditioning was a prominent feature in the hospital group. The patient group in this study had repeat assessments at 6 months post discharge, and found that the number who had a persistently low peak $\dot{V}O_2$ (<80% predicted), had reduced to 31%[89]. The control arm of this study was not re-tested.

Deconditioning was also found to be a prominent component of the CPET in a study from Italy. 75 patients (mean age 57 years) underwent CPET between 2-4 months post discharge. The mean $\dot{V}O_2$ max was 83% predicted, and 41 patients (55%) had a $\dot{V}O_2$max of <85%. None had a ventilatory limitation, 13 had circulatory limitations and the remainder had deconditioning. Other notable features included a reduced peak workload, early anaerobic threshold, lower O_2 pulse (which is a surrogate marker of stroke volume and/or peripheral oxygen extraction), longer heart rate recovery and a larger % breathing reserve. Ventilatory efficiency was noted to be within the normal range[110].

Finally, a different group in Italy investigated 18 patients (mean age 66) and compared them with an age matched control group[111]. This study added echocardiography, which, through its ability to estimate stroke volume, permitted estimation of peripheral oxygen uptake (A-V difference), from the CPET $\dot{V}O_2$. These patients had a longer hospital stay (median length of stay 30 days) but were also noted to have reduced $\dot{V}O_2$ max and peak workload achieved. The peak $\dot{V}O_2$ was less than 70% in 61 cases. They did not find circulatory or ventilatory limitation but did identify a reduction in calculated arteriovenous oxygen difference. They therefore concluded that this was the source of the limitation, as measured stroke volume differences were smaller than expected for such prolonged periods of bed rest. They hypothesised that this could be a result of reduced oxidative energy metabolism at the muscle level and might be due to steroid use in the treatment of the acute infection.

1.8.5 Limitations of current exercise testing literature in SARS CoV-2

During the SARS CoV-2 pandemic, government mandated lockdowns resulted in some of the population undertaking less physical activity than was usual for them pre-pandemic[112]. As a result, it

is possible that any exercise study taken later in the pandemic might show globally reduced exercise capacity, as a result of societal changes rather than disease pathology. This potential confounder can only be controlled for using a prospectively recruited control population. Some, but not all of the current studies have used a pre-existing reference population for their comparison. Notably, these studies recruited patients in their 50s. This is to be expected the higher risk of severe disease requiring hospitalisation in older patients.

By contrast, the SARS epidemic in 2003 affected primarily healthcare workers in localised outbreaks in several countries, and the lower ages of patients in the 2004-2005 studies reflects this. There are no comprehensive COVID-19 studies which look at a younger population, who also contracted SARS-CoV-2 infections but were less likely to be hospitalised.

There are also no studies which examine exercise capacity in the patient population who self-managed at home. This group is much larger in number than those who were admitted to hospital and includes the majority of working adults. Younger adults are also more likely to have an active lifestyle and/or job which requires physical activity, for example the emergency services or Armed Forces.

Only one group has performed serial measurements of exercise capacity following the SARS-CoV-2 pandemic. This was in hospitalised patients and the longitudinal follow up was not extended to the controls.

1.9 Effect of SARS-CoV-2 in athletes.

Whilst M-COVID is not study a study of athletes, and should not be taken as such, the level of exercise taken is typically higher than the general population studies, and as such draws some parallels with athletes. It is therefore salient to consider the M-COVID study in light of studies of the post Covid effects in athletes. To date, the published athlete research predominantly concerns sport teams or squads where individuals were screened and found to have an asymptomatic infection with SARS-CoV-2, or who had mild symptoms only. It is possible that having a high baseline level of cardiovascular

fitness is protective and as a result the clinical course is less severe. Alternatively, due to the physical nature of an athlete's job, having a high level of routine clinical testing over and above what another young person may expect might have selected a population of particularly mild cases which would not typically be identified in the clinical research recruitment environment. Testing in these cases also tended to be for the purposes of risk assessment before returning an athlete to intensive training or to competition, and so in comparison with the general population studies, testing occurs very soon after the acute infection.

1.9.1 Pulmonary Effects of SARS-CoV-2 in athletes

A Danish study has examined the pulmonary function 10-16 days post infection in a mixed athlete population, who were predominantly footballers. The mean age was 25 and 28% were female. The population measurements of static lung volumes and gas transfer were within the normal range. 11 patients (from a total of 120) had an obstructive pattern on spirometry[113]. A case control study in a population of handball players compared participants 20 days post infection with their uninfected teammates. Full lung function testing was not performed, but there were no differences seen in either FVC or FEV1 between the groups[114]. By contrast, a Turkish study investigated Olympian judo team members and compared those with confirmed SARS-CoV-2 with age and experience matched controls. At baseline, there was a significant reduction in FEV1 and FVC in the post infection group, which had begun to improve by 52 days post infection[115]. When considering a group who undertake high volume exercise training, having a matched control group confers additional benefit as the standardised data which is used to calculate predicted values is based on non-trained individuals, and the 'normal for athlete' range is generally not known.

As such, it is likely that the respiratory consequences of SARS-CoV-2 in military personnel are intermediate between the civilian experience and the athlete experience, but this is not known. Although the military population tend to have an increased cardiorespiratory fitness level and fewer

comorbidities than the general population, it would not be accurate to consider them the same as professional athletes in their 20-30s.

1.9.2 Cardiovascular effects of SARS-CoV 2 in athletes

Several studies have been published on the post COVID cardiac effects on athletes, which collectively may suggest which effects could be expected in the post-COVID UK military. However, most assess student athletes, and as such, are younger than would be expected in our population. Particularly, the risk of subclinical viral myocarditis, and the subsequent possible detrimental effects of strenuous exercise during the recovery period (that of adverse remodelling and sudden cardiac death) has resulted in intense interest in rates of possible myocarditis in patients who undertake high levels of exercise [116].

A small cohort of Polish Olympian athletes have undergone asymptomatic cardiac screening[117]. This population of 26 had mild or moderate COVID-19 infection and were managed in the community. They were all asymptomatic post infection and underwent a CMR. Abnormal findings which could not be attributed to changes consistent with adaptation to exercise were found in 19% of subjects. These were predominantly elevated T2 values, late gadolinium enhancement, pericardial or pleural effusion. The study lacked a control group but suggests that there are sub-clinical cardiac findings in an asymptomatic, usually fit population post COVID-19.

The US college sports system and pre-pandemic cardiac screening programs allow researchers the opportunity to extensively characterise student athletes post COVID-19. Three such attempts have been made at the time of writing (September 2023). A small study of 26 athletes were investigated at Ohio University, all of whom had either mild or asymptomatic disease. At follow up, all subjects had a normal echo and ECG, but 15% had CMR findings which were consistent with myocarditis. Findings included late gadolinium enhancement (46% in total), elevated T2 values (15%) and pericardial effusion (8%)[118].

A cohort of 54 student athletes who had recovered from mild or asymptomatic COVID-19 infection underwent testing including echocardiography and CMR at West Virginia University[119]. Abnormal findings were found in 56% of subjects. However, it is important to note that many of the abnormalities identified by the authors, such as borderline RV dilatation or dysfunction, can be considered athletic adaptation in the sports cardiology literature, so it is unclear to what extent these 'abnormalities' represent pathology. Certainly, abnormalities of tissue characterisation, such as late gadolinium enhancement, elevated native T1 and pericardial effusion were seen, as with the Ohio study.

A larger, survey-based study of US collegiate athletes who had tested positive for COVID-19 was carried out, which included 1597 athletes[120]. It looked at both clinically indicated and screening investigations, symptom derived diagnoses and findings of local clinicians. The prevalence of myocarditis by symptoms was determined to be 0.31%, whereas by CMR this increased to 2.3%, suggesting that the subclinical burden of cardiac involvement is difficult to identify without the additional diagnostic sensitivity of CMR. Interpretation of results can be confounded in the absence of pre-infection imaging, as subclinical myocarditis which occurred prior to the acute infection may result in incidental finding of myocardial fibrosis on late gadolinium imaging. A limitation of the use of LGE assessment of scar is that it is not possible to determine the age of an area of scar.

Outside of the US system, other research groups have used athletic screening networks to assess their populations. In Hungary, 147 athletes who had an acute illness ranging from moderate symptoms to asymptomatic underwent cardiac screening. The results identified CMR abnormalities in 4.7% (either late gadolinium enhancement or elevated native T1 or T2 values), but a diagnosis of myocarditis in only 1.4%. When compared with athletic controls, there were no differences in native T1 and T2 values[121].

In Spain, professional soccer players who had either asymptomatic or mild disease underwent cardiac screening. 24 players were included, and none had CMR findings other than standard athletic

adaptation to cardiac size and function. All were supervised during their return to sports training and none developed subsequent cardiac sequaelae[122].

The athletes' experience demonstrates the presence of cardiac involvement in a much smaller number of subjects than the hospitalised patient studies. This is likely a product of the fact that it is a younger population at less risk of severe disease, and with a better cardiovascular risk profile, in part a result of regular exercise training. However, many of the athlete studies demonstrate subclinical involvement which is seen on CMR but not seen on ECG or echo. It is possible therefore, that lack of symptoms or illness severity may not allow us to rule out inflammatory or fibrotic changes to the myocardium. Additionally, the age of these subjects ranges from 17-39, which is a younger group than the military. It is likely that the cardiac involvement in our population is somewhere between the athlete experience and the general patient results.

1.10 Rationale and Aims

Specifically, in my DPhil studies, I wanted to complete a full assessment of exercise in military personnel with post-COVID syndrome. I further wanted to understand the cardiovascular and respiratory contributions to any reduction in exercise capacity.

As the military population undertake a higher level of physical exercise than the general public, and have a lower level of pre-existing medical conditions, it was important to use a control group matched not only for age and gender, but also for occupation, so these individuals would be recruited from the same military population as the patient groups.

In order to explore longitudinal effects on exercise capacity, a second CPET assessment was performed 6 months after the baseline assessment. It was hypothesised that military personnel who have had SARS-CoV-2 will have a greater impairment in total exercise capacity (measured by peak $\dot{V}O_2$ and peak workload achieved) than those who have not had SARS-CoV-2. From the inception of the study, I considered it likely that those who were hospitalised during their acute infection would have a larger

reduction in their exercise capacity than those managed in the community. As other studies looking at older, hospitalised cohorts found deconditioning to be a prominent component of exercise limitation, I anticipated some improvement in exercise capacity over time.

During this thesis, I also sought to understand the role of deconditioning in the context of a period of illness as a possible explanation for any changes in exercise capacity, or residual cardiac or respiratory consequences of COVID-19.

1.10.1 Rationale and Aim for Chapter 3

Rationale - For organisations who require their workforce to undertake physical exercise, or work in austere environments, it is important to understand the effect of a disease as prevalent as SARS-CoV-2 upon exercise capacity. This applies not just to those individuals who were hospitalised, who in fact are mostly older and less likely to be engaged in physical work, but also to the majority of patients who were managed in the community. Once the effects of SARS-CoV-2 on the exercise capacity of the military population are understood, mitigations can be designed and implemented to protect the health of service personnel and to assure the success of future military operations.

Aim - To do this, I used CPET to investigate the effect of SARS-CoV-2 infection on exercise capacity in members of the UK Armed Forces. These data are presented in Chapter 3.

1.10.2 Rationale and Aim for Chapter 4

Rationale – Military personnel have reported cardiovascular symptoms, reduced exercise capacity and fatigue following COVID infection. To maximise exercise capacity, the cardiovascular and respiratory systems must work together to provide both a delivery system of oxygen (O_2) and a removal system of carbon dioxide (CO_2) to the working muscles. As such, the ability to perform physical exercise is directly related to 1) the cardiovascular capacity to supply O_2 to the muscles, 2) pulmonary ventilation and diffusion capacity to move O_2 / CO_2 between the lungs and the blood, 3) transport of O_2 and CO_2 in the blood, 4) capillary gas exchange, of O_2 / CO_2 between the capillary blood and the working

skeletal and cardiac muscle and, 5) skeletal and cardiac muscle mitochondrial electron transport chain (ETC) capacity to use the O_2 to facilitate ATP production. As such, documenting cardiac function and ATP metabolism are key components of exercise performance.

Aim - To do this, I used the combination of CMR imaging, ^{31}P magnetic resonance spectroscopy and rest and stress echocardiography to investigate the effect of SARS-CoV-2 infection on cardiac function and metabolism in members of the UK Armed Forces. These data are presented in Chapter 4.

1.10.3 Rationale and Aim for Chapter 5

Rationale – In addition to the role of respiratory gas exchange highlighted above, military personnel have previously reported increased respiratory symptoms during and after overseas deployments, and the deployed operational environment is associated with increased exposure to respiratory insults to which the civilian population are not exposed[123-125]. Furthermore, some military personnel operate in both hyper- and hypobaric environments, which can both cause hypoxia. Acceleration induced alterations in pulmonary blood flow, humidity changes from recycled cabin air and supplemental oxygen, and potential rapid pressure changes from decompression can also all challenge the respiratory system[126].

These added stressors and requirements for service personnel mean that it is imperative that the consequences of such a widespread infectious disease on the pulmonary mechanics in this population is known. If there is a severe or permanent reduction in function it could have serious consequences for the ability for defence forces to safely deploy personnel into austere environments or, possibly new screening measures might need to be implemented when recruiting personnel.

It is important then, to understand the respiratory effect of SARS-CoV-2 on the military population, so that the respiratory burden of post COVID-19 organ involvement is identified, and if needed, mitigations introduced to protect the health of service personnel and to assure the success of future operations in austere environments.

Aim To do this, I used lung function testing in combination with arterial blood gas sampling to investigate the effect of SARS-CoV-2 infection on respiratory function in members of the UK Armed Forces. These data are presented in Chapter 5.

Results chapters are written to enable comprehension when read alone, and therefore there is some repetition where required in the methods sections of each chapter.

Chapter 2- Methods

2.1 Ethical considerations

This study received favourable opinion from the Ministry of Defence Research Ethics Committee (MODREC) (1061/MODREC/20) and complied with the Declarations of Helsinki. Local approval was provided by the Defence Military Rehabilitation Centre (DMRC) Stanford Hall and by Oxford University Hospitals NHS Foundation Trust. All participants were given detailed written information prior to recruitment and provided written informed consent.

2.2 Study Design

This is an observational cohort study. The trial design included 113 participants across three groups covering the spectrum of COVID-19 infection. Group 1, patients with hospitalised COVID infection (n=35). Group 2, patients with community treated COVID-19 infection (n=52). Group 3, controls without prior COVID-19 infection (n=26). All group 3 patients were recruited from the military population and were confirmed as not having had previous infection with antinuclear antibody testing prior to inclusion in the study.

2.3 Study Data

Study data were collected and managed using REDCap electronic data capture tools hosted at the Royal Centre of Defence Medicine[127]. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: an interface for validated data entry; audit trails for tracking data manipulation and export procedures and automated export procedures for data downloads.

2.4 Study participants

2.4.1 Recruitment

Patients who were suitable for the study were identified from the referral list for the Defence COVID Recovery Service (DCRS), a clinic dedicated to investigating Armed Forces personnel with persistent symptoms post COVID-19 infection. All service personnel diagnosed with confirmed or probable COVID-19 were referred from primary care if they met criteria for severe acute COVID illness (pragmatically defined by 'requiring hospital admission'); if they had mild/moderate disease (defined as managed in the community) with prolonged symptoms (fatigue, exercise intolerance, shortness of breath) after resolution on acute illness (>12 weeks); or had features associated with elevated clinical risk (chest pain and ECG changes, elevated cardiac enzymes during acute illness and desaturation at rest or on exertion in primary care). Each individual's military electronic medical records were reviewed, and patients contacted by members of the clinical team in anticipation of their visit, and any who were eligible for participation were approached and given a participant information leaflet (PIL). Participants who had fully recovered from mild/moderate COVID-19 infection and participants without prior COVID-19 infection acting as healthy controls were also recruited from the military population. Volunteers expressing interest in participation were screened for eligibility and given a PIL. Those who still wished to take part were age and sex matched to the patient groups and enrolled onto the study. Control participants were matched as close as possible and recruited from local military health units.

2.4.2 Group Selection

On the first day of the visit, a consent interview was conducted either by me or another member of the clinic team, and written informed consent was obtained from those who were willing to participate. Group selection was undertaken at this time and confirmed following the clinical review by the reviewing consultant in the case of the clinical patients.

2.4.3 Inclusion criteria

All Participants

Serving members of the UK Armed Forces

Group 1- Hospitalised patients

- Previous COVID-19 infection requiring hospital admission
- Positive for COVID-19 antigen PCR test at the time of the acute illness, or clinically adjudicated COVID-19
- Negative for COVID-19 antigen PCR test at the time of recruitment to the study
- ≥4 weeks since resolution of fever/acute illness

Group 2- Community patients

- Previous COVID-19 infection with mild/moderate acute illness which was self-limiting and did not require admission to hospital
- Positive for COVID-19 antigen PCR test at the time of the acute illness, or clinically adjudicated COVID-19, or subsequent positive SARS-CoV2 antibody test
- Negative for COVID-19 antigen PCR test at the time of recruitment to the study
- ≥4 weeks since resolution of fever/acute illness

Group 3- Healthy volunteers

- No previous clinical suspicion of acute COVID-19 infection
- Negative for COVID-19 antigen PCR test and anti-nuclear antibody test at time of recruitment to study

2.4.4 Exclusion criteria

All Groups

- Unwilling or unable to give informed consent

- Active acute infection at the time of recruitment
- Pregnant at the time of recruitment
- Unable to meet the criteria of the MRI safety questionnaire
- Unable, or unwilling to complete any research investigations
- Past medical history of significant cardiac or pulmonary disease (mild asthma and controlled hypertension excluded)

Group 3 exclusion criteria

- Clinical suspicion of previous COVID-19 infection
- Contracting COVID-19 during the study period

2.5 Baseline assessment

2.5.1 Detailed medical history

As part of the screening process and following written informed consent, a detailed medical and drug history was taken, and where necessary, further information was obtained from the participant's military medical records.

2.5.2 Symptoms questionnaires

The following questionnaires were sent to participants as a web link via email for them to complete on the first day of their assessment. Answers were auto populated onto the REDCap database.

Physical Activity International Physical Activity Questionnaire- This is an internationally validated and standardised tool to enable quantification of physical assessment[128]. It assesses the amount and intensity of physical activity undertaken in the previous 7 days.

Mental Health Generalised Anxiety Disorder Scale (GAD-7)- This is a self-completion score of anxiety related symptoms that has been validated for use in clinical and research settings[129].

Patient Health Questionnaire 9 (PHQ-9)- This questionnaire is a self-administered test for symptoms of depression and has been validated for brief-intervention assessment of depressive disorders[130].

Posttraumatic Stress Disorder Checklist (PCL-5)- This is a self-report measure of post-traumatic stress disorder symptoms and severity[131].

Alcohol Use Disorders Identification Test (AUDIT)- This is a self-report tool for detecting at-risk alcohol consumption[132].

Quality of life European Quality of life 5 domain (EQ5D)- This self-completion measure asks participants to describe their health related quality of life across five domains (mobility, self-care, usual activities, pain/discomfort, anxiety/depression)[133].

Fatigue Fatigue assessment scale (FAS)- This 10 question tool scores participants on physical and mental symptoms of fatigue and has been validated against previous methods[134].

2.5.3 Height and weight, baseline cardiovascular measurements

Participant height and weight was measured using a combined scale and stadiometer, and recorded on the eCRF (Seca GmbH, Hamburg, Germany). Body Mass Index was calculated as weight (kg)/ height $(m)^2$. Resting heart rate, oxygen saturation and non-invasive blood pressure measurements were taken using an IPM 8 Mindray Patient Monitor (Mindray UK Ltd, Huntingdon, UK) according to standardised methods (5 minutes of seated rest prior to measurement, 3 measurements taken and lowest recorded). A standard 12-lead electrocardiogram was obtained.

2.5.4 Blood collection

Peripheral venous blood was sampled using a 22-gauge Vacutainer® Eclipse devices (Becton Dickinson, Plymouth, UK) from the participant's antecubital fossa. Samples were analysed in the pathology laboratory of the Nottingham University Hospitals NHS Trust, Queen's Medical Centre. Results were accessed within one week. Tests run included: full blood count, urea & electrolytes, serum creatinine,

liver function tests, thyroid function tests, creatine kinase, vitamin D levels, serum iron studies. Only those that were salient to the chapters in this thesis are included.

2.6 Cardiopulmonary exercise test

Cardiopulmonary exercise testing with capillary blood gas and capillary brain natriuretic protein measurement at rest and stress was conducted on a stationary upright cycle ergometer in the exercise laboratory at DMRC Stanford Hall (Figure 2.1, Lode Corival, Lode BV, Groningen, The Netherlands) and a Metalyzer3B (Cortex, Germany) calorimeter. $\dot{V}O_2$, workload, heart rate, ventilatory efficiency ($\dot{V}E/\dot{V}CO_2$), breathing reserve, oxygen uptake efficiency and oxygen pulse ($\dot{V}O_2$ peak/peak heart rate) were recorded throughout the test. Non-invasive blood pressure, oxygen saturations and relative perceived exertion (RPE; Borg 6-20 scale) were recorded every 2 minutes. The test commenced with two-minutes of quiet rest, followed by two minutes of unloaded pedalling, then a progressive ramp protocol (starting at 25W with a ramp of between 15-35 Watts/minute; the rate of increase in work was chosen based on age, sex, body mass and symptoms, to achieve a test lasting 10±2 mins) to volitional fatigue. Measurements of breath composition were performed by indirect calorimetry (Metalyzer® 3B Cortex Biophysik, Leipzig, Germany). Maximal testing was defined as a peak RER ≥1·10 and/or plateau in oxygen uptake with associated increasing workload. Predicted peak $\dot{V}O_2$, predicted peak workload and predicted O_2 pulse are based upon the Wasserman weight algorithm[135].

Capillary blood gas samples were taken from the left earlobe at rest and immediately post peak exercise using a Unistik 3 lancet (Owen Mumford Ltd, Oxford, UK). Initial drops were discarded, and subsequent blood was sampled in 125µL heparinised capillary tubes (safeCLINITUBES, Radiometer, Denmark). These were transferred to CG4+ and BNP iStat cartridge and measured immediately with an iStat analyser (Abbott, Chicago, USA).



Figure 2.1 Cardiopulmonary Exercise set up in the Defence COVID Recovery Service (Defence Medical Rehabilitation Centre Stanford Hall)

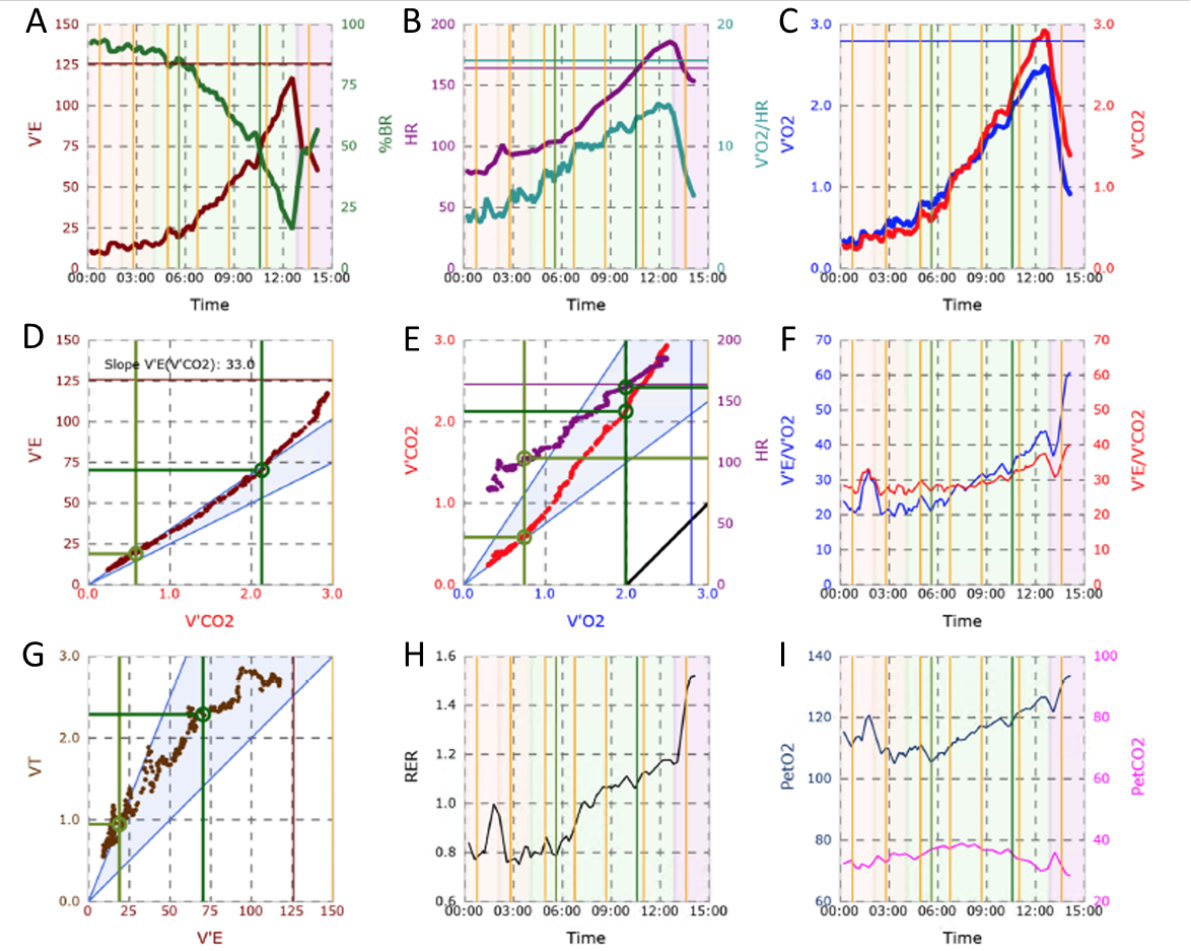


Figure 2.2 example panel CPET plot. Panel (A) Pulmonary Ventilation ($\dot{V}E$), expressed in litres per minute/time and % of predicted breathing reserve remaining/time. (B) Heart rate (HR)/time and $\dot{V}O_2$/HR against time. (C) Oxygen uptake ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) against time. (D) $\dot{V}E$ / against. (E) $\dot{V}CO_2$ against $\dot{V}O_2$. (F) $\dot{V}E$ /$\dot{V}O_2$ and $\dot{V}E$ /$\dot{V}CO_2$ plotted against time. (G) Tidal volume against minute ventilation, $\dot{V}E$. (H) Respiratory exchange ratio (RER). (I) End-tidal partial pressures of CO_2 ($petCO_2$) and O_2 ($petO_2$) plotted over time.

2.7 Pulmonary function testing

Pulmonary function testing was carried out using a Vyntus ONE Pulmonary Testing System (Vyaire Medical, USA) (Figure 2.3), using standard European Respiratory Society recommended protocols [136]. Spirometry, carbon monoxide diffusion using the single breath tracer gas method and static lung volumes using the nitrogen washout technique were performed in the sitting position[137,138]. Forced

vital capacity (FVC), forced expiratory volume in 1 second (FEV1), total lung capacity (TLC), diffusion capacity of the lung for carbon monoxide (DLCO), alveolar volume (VA) and carbon monoxide transfer coefficient (KCO) are measured using this test. In addition to raw values, a percent predicted value is calculated using a reference range provided by Vyaire.



Figure 2.3 The Vyntus One lung function system.

2.8 CMR scan protocols

2.8.1 ^{1}H Imaging for Cardiac Volumes and Function

All cardiac MRI scans for the assessment of myocardial mass, ventricular volumes, and ejection fraction were performed on a 3 Tesla MR system (Magnetom Trio, Siemens, Germany). Imaging was acquired using a steady state free precession (SSFP) sequence with an echo time (TE) of 1.5ms, a repetition time (TR) of 3.0ms, in plane resolution 1.5 x 1.5 mm^2, temporal resolution 47.84ms, flip

angle 60°, as previously described[139]. All imaging was performed supine, prospectively cardiac gated, and acquired during end-expiratory breath-hold. All analyses were performed using cmr42 © (Circle Cardiovascular Imaging Inc, Calgary, Canada) as previously described[140]. Endocardial and epicardial LV contours were drawn on the LV short axis and long axis images using a semi-automated process (Figure 2.4).

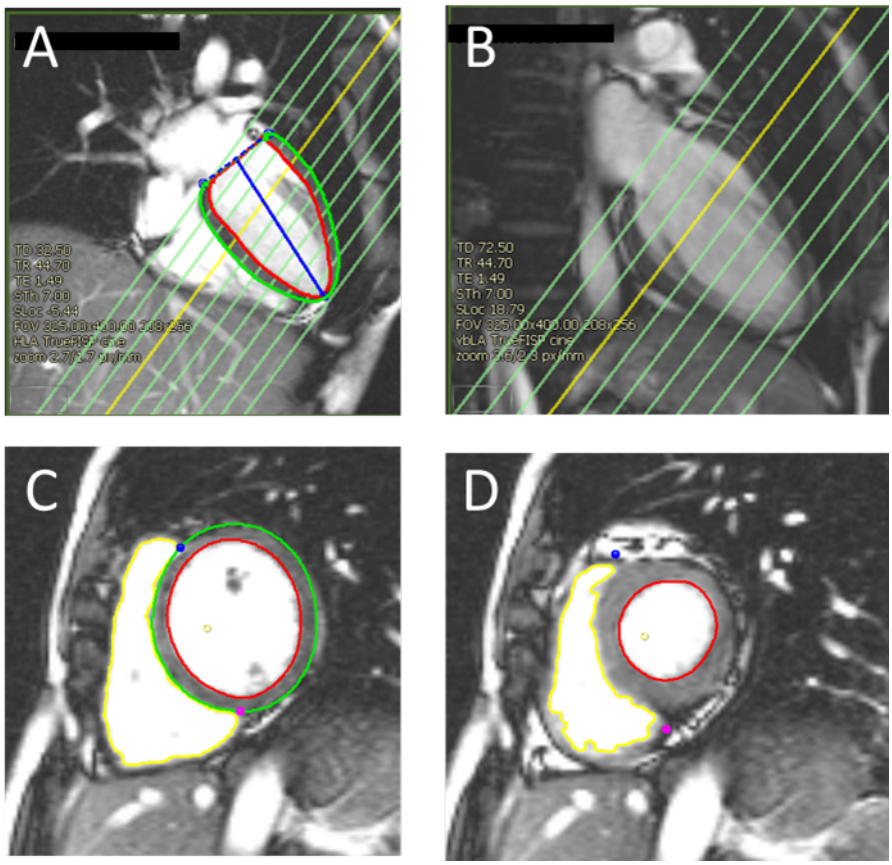


Figure 2.4: Example of long axis cines of horizontal long axis A) vertical long axis B), and manually contoured left and right ventricular short axis 1. 5T cine images at C) end-diastole and D) end-systole within Circle CVI 42.

2.8.2 Native T1 values

Native T1 maps were acquired for 5 LV slices using Shortened Modified Look Locker T1 Mapping sequences (ShMOLLI, Siemens) as described previously[141]. Endocardial and epicardial LV contours

were drawn using a semi-automated process and analysis was performed using cmr42 (Circle Cardiovascular Imaging Inc, Calgary, Canada, Figure 2.5).

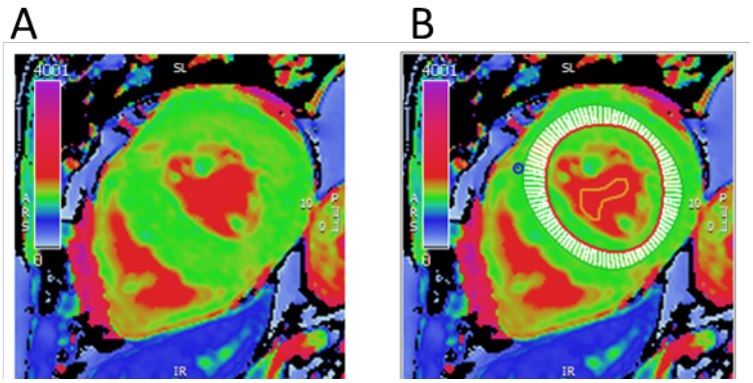


Figure 2.5*:* A) Acquired and (B) contoured Native mid-ventricular ShMOLLI T1 maps

2.8.3 Late Gadolinium Enhancement

Late gadolinium imaging was acquired by giving an intravenous bolus injection of 0.1mmol/kg of gadolinium-based contrast agent (Gadovist) through a peripheral cannula, followed by a T1-weighted, phase-sensitive inversion recovery sequence, using techniques previously described for extra cellular volume fraction calculation (ECV)[141,142].

2.8.4 ^{31}P Magnetic Resonance Spectroscopy

^{31}P MRS was performed at rest and during dobutamine stress on a 3 Tesla MRI scanner (Magnetom Trio, Siemens, Germany). Participants were laid prone over the center of a 3-element dual-tuned ^{1}H/^{31}P surface coil in the isocenter of the MR system.

Cardiac ^{31}P-MRS requires the signal of interest, (which is often the interventricular septum as this is usually the thickest part of the myocardium and suffers fewer motion discrepancy in comparison to other parts of the heart) to be localised in a voxel and separated from other potential signal contamination. The most common areas of potential signal contamination are the skeletal muscle, the liver and the ribs. To achieve this localisation, a non-gated 3D acquisition-weighted ultra-short echo

time (UTE) chemical shift imaging sequence was used with saturation bands placed over liver and skeletal muscle, as previously described (Figure 2.6)[143].

The raw data were subject to pre-processing correction before fitting spectral peaks using the advanced method of accurate, robust and efficient spectroscopic (AMARES) method, a semi-automated fitting protocol within a Matlab program created for our research department. Phosphocreatine, 2,3-diphosphoglycerate, phosphodiesters and α, β, γ - ATP peaks are fitted on the spectrum using established knowledge of relative peak frequencies. Correction for RF partial saturation effects was performed using the recorded excitation flip angle and known metabolite T_1 values at 3T (PCr 3.8s, PDE 1.11s, 2,3-DPG 1.39s, γ-ATP 2.4s, α-ATP 2.5s, β-ATP 2.7s). The ATP peak value was further corrected for contamination from the blood pool using DPG peak area[144]. PCr/ATP ratio was calculated using the average of the three ATP peaks. The quality of spectral fit was assessed using the coefficient of variation in the measured PCr/ATP ratio, based on Cramer-Rao lower bounds. Samples with a greater than 20% coefficient of variation were excluded.

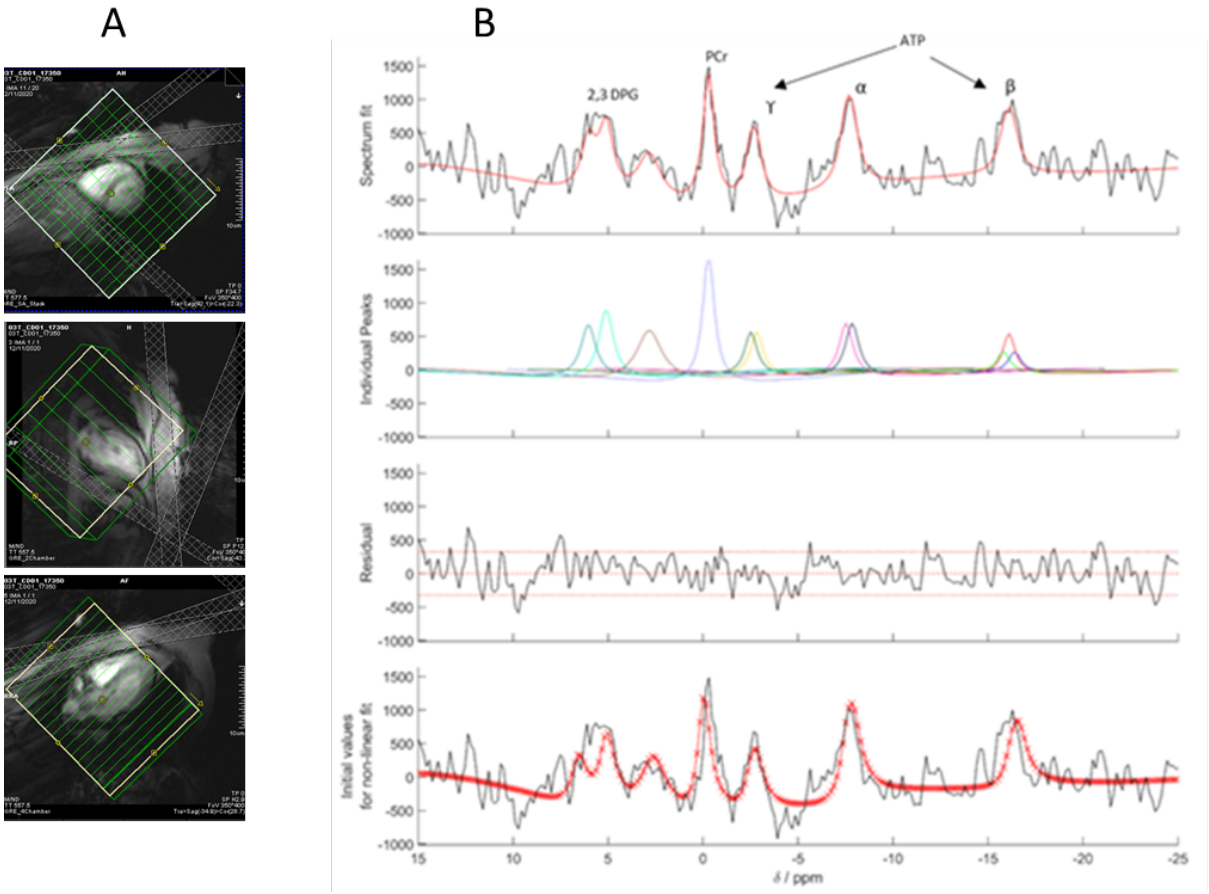


Figure 2.6 Example of ^{31}P acquisition and analysis (A) Grid placement and Saturation bands (B) Spectrum fit with labelled peaks.

2.8.5 Dobutamine stress

After ^{31}P MRS acquisition at rest, a peripheral dobutamine infusion was given using an intravenous cannula, as necessary to achieve the target heart rate of 65% maximum (maximum heart rate calculated as 220-age) as previously described[145]. ^{31}P-MRS measurements were repeated at this target to assess energetic response to stress.

2.9 Exercise echocardiography

Echocardiography was performed to assess diastolic function at rest and exercise stress. Images were acquired on a stationary upright cycle ergometer (Corival, Lode, The Netherlands) using a Philips CX50 cardiac ultrasound machine (Philips, The Netherlands). A standardised protocol was used which has been described previously[146]. Apical 2, 3, and 4-chamber views were taken for LV function estimation.

Pulsed-wave Doppler assessment of the inflow of the mitral valve was used to calculate the E/A ratio. Tissue Doppler velocities were measured at the medial and lateral mitral valve annulus to determine E/e' ratios[146]. Once resting measurements were completed, participants were asked to pedal on the cycle ergometer until they reached 75% of their peak heart rate from the cardiopulmonary exercise test. Measurements were then repeated at target heart rate to determine the change in diastolic function in response to exercise. If the heart rate dropped below the target, participants were asked to continue cycling until their heart rate was once again in range and a full dataset was acquired.

2.10. Arterial blood gas sampling

A resting sample of arterial blood was obtained from the radial artery following local anaesthetic infiltration. This sample was sent for point of care gas analysis (ABL 90 Flex, Radiometer, England)

2.11 Statistical analyses

Statistical analysis was performed using commercial software (IBM SPSS 27, Chicago, USA, and GraphPad Prism 9, San Diego, USA).

As this study investigated a novel disease, pilot data were not available to perform a power calculation, and thus group size was based on feasibility of recruitment. Agreed recruitment targets were as follows: Group 1: n=25-35 Group 2: n=20-60 Group 3: n=10-30

All data are presented as mean ± standard deviation unless otherwise stated. Comparison of healthy control groups vs post COVID-19 infection groups were conducted using Mann Whitney (non-parametric) or unpaired T-test (parametric). All groups were then compared using ANOVA for multiple comparisons with post hoc Bonferroni correction. In cases where the hypothesis suggested that increased severity of disease would cause a greater change from the control result, a Jonckheere Terpstra test for ordered medians was used (the order was defined as control>community infection>hospital care).

A p value of <0. 05 was determined to be significant.

Chapter 3-The Effect of SARS-CoV-2 on Exercise Capacity

3.1 Introduction

The UK Armed Forces (Royal Navy, British Army and Royal Air Force) require the majority of their personnel to undertake physical exercise, and many all operate in challenging environmental conditions. For operational success and safety, it is important to understand the effect of a prevalent disease such as SARS-CoV-2 upon exercise capacity. This supports the imperative to understand the effect of SARS-CoV-2 on the exercise capacity of military population, where required mitigations can be put in place to protect the health of service personnel and to support operational capability.

3.2 Prevalence of Exercise Related Symptoms in the M-COVID Study

As discussed in Chapter 1, fatigue and reduced exercise capacity are commonly reported symptoms post SARS-CoV-2 infection outside the military. As a first aim of Chapter 3 I analysed the standardised questionnaires to assess subjective breathlessness (modified BORG, 0-10 breathlessness scale); fatigue (fatigue assessment scale, FAS); anxiety (GAD-7); depression (PHQ-9) and post-traumatic stress (PCL-5), alcohol consumption (alcohol audit) to elucidate the prevalence of exercise related symptoms in the M-COVID cohort. This is shown in Table 3.1 with exercise intolerance highlighted in grey.

Cardiorespiratory symptoms were extremely common. Shortness of breath at any level of exertion was far more common in post COVID groups than controls (there were no occurrences of shortness of breath in the control group). Prevalence of 'any shortness of breath' appeared to be higher in those hospitalised versus those with community managed infections (92% community, vs 97% hospitalized).

CPET was used to quantify exercise capacity in members of the UK Armed Forces enrolled into M-COVID and to determine whether these symptoms translated to objectively reduced exercise capacity.

Table 3.1. Symptoms and Frequency within the three M-COVID groups. Column data are absolute value (percentage)

	Control n=25	**Community** n=52	**Hospitalised** n=35
Nonspecific			
Loss of smell	0 (0)	7 (13)	3 (9)
Fatigue	1 (3)	23 (44)	18 (51)
Fever	0 (0)	2 (4)	1 (3)
Sore throat	0 (0)	2 (4)	2 (6)
Runny nose	1 (3)	1 (2)	1 (3)
Muscle aches	0 (0)	8 (15)	8 (23)
Joint pain	0 (0)	5 (10)	8 (23)
Headache	0 (0)	8 (15)	4 (11)
Cardiorespiratory			
Persistent cough	0 (0)	3 (6)	2 (6)
Hoarse voice	0 (0)	0 (0)	1 (3)
Chest pain	0 (0)	12 (23)	6 (17)
Palpitations	1 (3)	11 (21)	5 (14)
Any Shortness of Breath	0 (0)	48 (92)	34 (97)
Shortness of breath at rest	0 (0)	8 (15)	5 (14)
Shortness of breath on mild activity	0 (0)	19 (37)	9 (26)
Shortness of breath on moderate activity	0 (0)	21 (40)	20 (57)
Neurological			
Dizziness	0 (0)	6 (12)	5 (14)
Generalised weakness	0 (0)	6 (12)	8 (23)
Localised numbness/hypersensitivity	0 (0)	1 (2)	3 (9)
Localised pain	0 (0)	4 (8)	5 (14)
Fainting	0 (0)	1 (2)	0 (0)
Gastrointestinal			
Difficulty swallowing	0 (0)	2 (4)	1 (3)
Abdominal Pain	0 (0)	4 (8)	1 (3)
Weight loss	0 (0)	2 (4)	0 (0)
Diarrhoea	0 (0)	2 (4)	0 (0)
Sleep			
Difficulty getting to sleep	1 (3)	16 (31)	2 (6)
Difficulty staying asleep	2 (7)	11 (21)	9 (26)
Nightmares	0 (0)	4 (8)	2 (6)
Mental Health			
Low mood	0 (0)	7 (13)	9 (26)
Anxiety	1 (3)	7 (13)	9 (26)
Poor memory	2 (7)	16 (31)	8 (23)
Poor concentration	1 (3)	16 (31)	14 (40)
Poor attention	1 (3)	11 (21)	4 (11)
Confusion	0 (0)	3 (6)	3 (9)

3.3 Methods

Detailed methods for the study can be found in Chapter 2. The study received favourable opinion from the Ministry of Defence Research Ethics Committee (1061/MODREC/20) and complied with the Declaration of Helsinki.

In brief, 113 volunteer participants from the UK Armed Forces (87 post SARS-CoV-2 infection, 26 healthy SARS-CoV-2 negative controls) were recruited from either the Defence COVID Recovery Service, or from local military units. Inclusion criteria for the post SARS-CoV-2 group were previous SARS-CoV-2 infection, with either a positive COVID-19 antigen PCR test at the time of their acute illness, or clinically adjudicated COVID-19; at least 4 weeks post their acute infection; negative COVID-19 antigen PCR test at the time of enrolment to the study. The control group inclusion criteria were no previous suspicion of acute COVID-19 infection and negative COVID-19 anti-nuclear antibody test at the time of enrolment to the study. Exclusion criteria were a past medical history of significant cardiac or pulmonary disease, active infection at the time of recruitment, pregnancy, and contraindication to MRI scanning, as detailed in Chapter 2.4. Additionally, the control group were excluded if they contracted COVID-19 during the study period.

Participants underwent the following investigations at their baseline visit, and again at 6 months (± 1 month) following the baseline measurements.

3.3.1 Anthropometric and symptom assessment

Height and weight were measured using a stadiometer. Resting heart rate, and non-invasive blood pressure was measured. Peripheral venous blood was sampled to measure a full blood count (specifically Hb) and creatinine. Symptom questionnaires were completed to assess physical activity undertaken in the past seven days (IPAQ), quality of life (EQ5D) and fatigue (FAS) [128,134].

3.3.2 Cardiopulmonary exercise test.

Exercise capacity was assessed using a cardiopulmonary exercise test on a cycle ergometer. The protocol included 2 minutes rest; 2 minutes unloaded pedalling followed by an individualised progressive ramp protocol to achieve a maximal test following 8-12 minutes of loaded pedalling. A maximal test was defined by a respiratory exchange ratio ≥1.1 and/or plateau in oxygen uptake over 30s of increasing workload. Peak oxygen uptake and workload were calculated using the Wasserman weight algorithm[135].

3.3.3 Rest and exercise capillary blood gas

Earlobe capillary blood samples were taken immediately prior to and post cardiopulmonary exercise testing, two minutes after application of topical Capsaicin. Blood gas analysis was undertaken using CG4+ iStat cartridges (Abbott, Chicago, USA). During the same puncture, drops of blood were drawn to measure serum BNP using an iStat BNP cartridge (Abbott, Chicago, USA).

3.3.4 Statistics

Statistical analysis was performed using commercial software (IBM SPSS 27, Chicago, USA, and GraphPad Prism 9, San Diego, USA). Data are presented as mean ± standard deviation. Normality was confirmed using Kolmogorov-Smirnov testing. Chi squared testing was used to compare nominal data. Continuous data were compared using an independent t-test for baseline data and a paired t-test when comparing baseline with follow up data. When comparing more than two groups, one-way ANOVA with post hoc Bonferroni correction was used. A p value of <0.05 was considered significant.

3.4 Results

A total of 113 volunteers were recruited into the study. 87 had had COVID-19 infection of whom 52 had been managed in the community and 35 required hospital care. 26 were COVID negative controls.

88% of the healthy volunteers were male, 84% of the community COVID-19 volunteers were male, and 88% of the hospitalised volunteers were male. Of those who completed their baseline visit, 88 were also available to attend a follow up visit at 6 months. (See Figure 3.1)

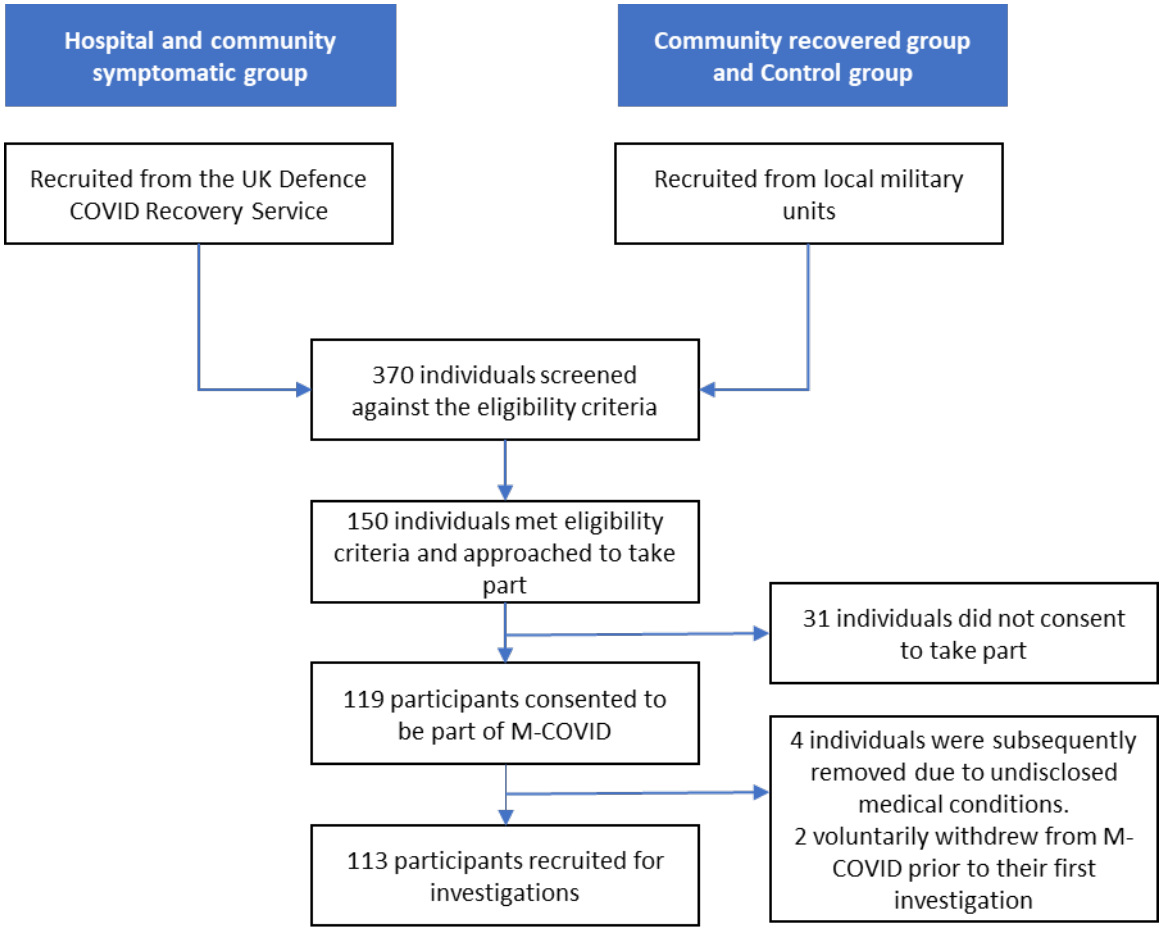


Figure 3.1 Consort diagram describing the recruitment process for M-COVID

3.4.1 Baseline Characteristics

For this analysis data are presented first as a comparison of control versus all participants with post-COVID syndrome (Table 3.2) and then with separate analysis of the COVID severity sub-groups (community vs. hospitalised groups) (Table 3.3).

There was no difference in the ages of all patients and controls (39.02 ±9.59 vs 38.27 ±8.22years p=0.7). Those who were hospitalised were older than those who were managed in the community (43years (±8) vs. 36 years (±9), p<0.001). The time from period of acute illness to baseline

assessment was a mean average of 159 ±7 days (approx. 22 weeks). Groups were well matched for sex between controls and those who had been infected (controls 88% male, post SARS-CoV-2 group 86% male, p=0.5) and between severity groups (community 84% male, hospitalised 88% male, p=0.8).

There was a significant between-group difference in body mass index. Patients had adverse body composition compared with controls (BMI 29.30 ±4.12 vs. 25.41 ±2.58 kg/m^2, p<0.0001), Hospitalised patients had higher BMI than those managed in the community (BMI 31.15 ±4.08 vs. 28.03 ±3.674 kg/m^2, p<0.01). There was no difference in systolic blood pressure between the two groups (although within the patient group, systolic blood pressure was higher in those who had been hospitalised compared with those treated in the community (126 ±10vs. 119±11 mmHg, p<0.01). There were no between group differences in diastolic blood pressure. Resting heart rate was higher in the patient group versus controls (81.64 ±13.05 vs. 72.96 ±8.28 bpm, p=0.001) with no difference between hospitalised and community groups (82.06±11.53 vs. 81.37 ± 14.07 bpm, p>0.9). There were no differences in baseline haemoglobin, creatinine or BNP (all p=>0.2).

Table 3.2. Baseline characteristics of study participants BMI- Body mass index, BSA- body surface area, SBP- systolic blood pressure, DBP- diastolic blood pressure, MAP- mean arterial pressure, HR- heart rate, Hb- haemoglobin, BNP- B-type natriuretic peptide. Counts expressed as number (%), continuous variables expressed as mean (±SD). *denotes Chi Squared Test

	Controls (n = 26)	**Post COVID (n =87)**	**p-value (unpaired t test)**
Age (years)	38.27 (±8.22)	39.02 (±9.59)	0.72
BMI (kg/m^2)	25.41 (±2.58)	29.30 (±4.12)	***<0.0001***
BSA (m^2)	2.0 (±0.14)	2.2(±0.22)	***<0.0001***
Male (%)	23 (88%)	76 (86%)	0.5*
SBP (mmHg)	121 (±11)	122 (±11)	0.70
DBP (mmHg)	79 (±6)	83 (±8)	***0.01***
MAP (mmHg)	92 (±6)	95 (±8)	0.06
HR (bpm)	72.96 (±8.28)	81.64 (±13.05)	***0.001***
Hb (g/L)	150 (±12)	151 (±10)	0.84
Creatinine (µmol/L)	87 (±18)	83 (±15)	0.30
BNP (ng/L)	21 (±14)	18 (±12)	0.26

Table 3.3. Baseline characteristics across severity of disease. BMI- Body mass index, BSA- body surface area, SBP- systolic blood pressure, DBP- diastolic blood pressure, MAP- mean arterial pressure, HR- heart rate, Hb- haemoglobin, BNP- B-type natriuretic peptide. Counts expressed as number (%), continuous variables expressed as mean (±SD). * denotes Chi Squared Test.

	Controls (n = 26)	**Community (n = 52)**	**Hospital (n =35)**	**p-value (one-way ANOVA w Bonferroni)**		
				Control v Community	**Control v Hospital**	**Community v Hospital**
Age (years)	38.27 (±8.22)	36.21 (±9.36)	43.19 (±8.44)	>0.9	0.10	***0.001***
BMI (kg/m^2)	25.41 (±2.58)	28.03 (±3.67)	31.15 (±4.08)	***0.009***	***<0.0001***	***0.0004***
BSA (m^2)	2.0 (±0.14)	2.1 (±0.24)	2.2 (±0.19)	***0.004***	***0.0003***	0.69
Male (%)	23 (88%)	44 (84%)	32 (88%)	0.8*	0.8*	0.8*
SBP (mmHg)	121 (±11)	119 (±11)	126 (±10)	>0.9	0.21	***0.009***
DBP (mmHg)	79 (±6)	82 (±8)	85 (±7)	0.27	***0 005***	0.14
MAP (mmHg)	92 (±6)	94 (±8)	98 (±7)	>0.9	***0.006***	***0.01***
HR (/min)	72.96 (±8.28)	81.37 (±14.07)	82.06 (±11.53)	***0.01***	***0.01***	>0.9
Hb (g/L)	150 (±12)	150 (±11)	151 (±9)	>0.9	>0.9	>0.9
Creatinine (µmol/L)	87 (±18)	83 (±13)	83 (±17)	>0.9	>0.9	>0.9
BNP	21 (±14)	16 (±7)	20 (±15)	0.39	>0.9	0.6

3.4.2 The effect of SARS-CoV-2 on workload achieved in military personnel

For this analysis, data are presented first as a comparison of control versus all participants with post-COVID syndrome (in Table 3.4) and then by severity of acute illness (determined by whether they were managed in the community or were hospitalised (Table 3.5, Figure 3.2).

All participants achieved a maximal test (defined by a respiratory exchange ratio at peak of greater than 1.1). Peak workload achieved was 18% higher in the controls, compared with the post SARS-CoV-2 volunteers (304 W (±65) vs. 257 W (±58), p<0.001). Of the post SARS-CoV-2 group, peak workload achieved was 18% lower in the post hospitalised group when compared with the community managed group (233 W (±33) vs. 274 W (±65), p<0.01). Despite these changes, when normalised to the standardised reference population, all groups achieved what would be considered a 'normal' workload during the test (Controls 127% predicted (±32), community group 106% predicted (±22), hospitalised group 98% predicted (±16), summary ANOVA p=0.0001).

There were also differences in the workload achieved at VT1, which approximates to the anaerobic threshold. Patients achieved 82 W (±28) at VT1, in comparison with 109 W (±34) for controls (p<0.0001). This represents a 33% reduction in the workload at the anaerobic threshold (AT), which is a marker associated with strong endurance exercise performance[147,148]. This value was 29% lower in hospitalised patients in comparison with community cases (70W (±15) vs. 90W (±32), p<0.01.

Table 3.4. Respiratory exchange ratio and workload achieved. RER- Respiratory exchange ratio, VT1-ventilatory threshold 1. Continuous variables expressed as mean (±SD)

	Controls (n = 26)	**Post COVID (n =87)**	**p-value (unpaired t test)**
RER at peak	1.22 (±0.07)	1.20 (±0.06)	***0.02***
Peak workload achieved (W)	304 (±65)	257 (±58)	***0.0006***
Peak workload achieved (% pred)	127 (±32)	103 (±20)	***<0.0001***
Workload achieved at VT1 (W)	109 (±34)	82 (±28)	***<0.0001***

Table 3.5 Workload achieved by clinical severity. RER- Respiratory exchange ratio, VT1- ventilatory threshold 1, or Anaerobic Threshold. Continuous variables expressed as mean (±SD)

	Controls (n=26)	**Community (n=52)**	**Hospital (n=35)**	**p-value (one-way ANOVA w Bonferroni)**		
				Control v Community	**Control v Hospital**	**Community v Hospital**
RER at peak	1.22(±0.06	1.19 (±0.07)	1.18 (±0.05)	0.11	0.12	>0.9
Peak workload achieved (W)	304 (±65)	274 (±65)	233 (±33)	0.08	**<0.0001**	**0.005**
Peak workload achieved (% pred)	127 (±32)	106 (±22)	98 (±16)	**0.0005**	**<0.0001**	0.35
Workload achieved at VT1 (W)	109 (±34)	90 (±32)	70 (±15)	**0.02**	**<0.0001**	**0.004**

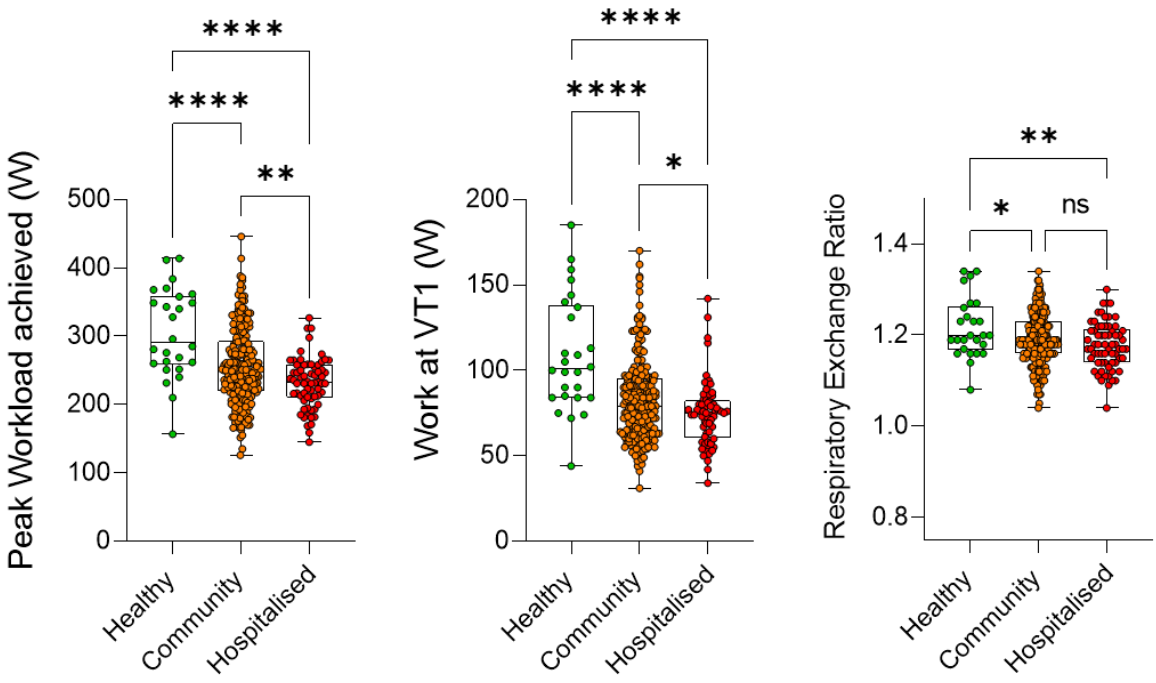


Figure 3.2 Respiratory Exchange Ratio, workload at VT1, and Peak Workload for the study groups

3.4.3 The effect of SARS-CoV-2 on oxygen consumption in military personnel

As for previous parameters, data are presented first as a comparison of control versus all participants with post-COVID syndrome (in Table 3.6) and then with separate analysis of the post COVID group divided into community managed and hospitalised groups, to determine the effect of illness severity (Table 3.7).

Volunteers with prior SARS-CoV-2 had a peak $\dot{V}O_2$ 26% lower than controls (35ml/kg/min (± 8) vs. 46 ml/kg/min (± 9), $p<0.0001$. As expected, hospitalised patients, had a peak $\dot{V}O_2$ 23% lower than those managed in the community (31 ml/kg/min (±5) vs. 38ml/kg/min (± 9), $p<0.001$). When normalised to the standard reference range, all groups achieved a peak $\dot{V}O_2$ greater than 100% predicted, albeit with lower values as the severity of the disease increased (controls 133% predicted (± 25) vs. community cases 115% predicted (± 20) vs. hospitalised cases 109% predicted (±16), ANOVA $p<0.0001$). All these mean values would be regarded as 'normal' in a clinical setting.

Oxygen consumption at VT1, or the anaerobic threshold, expressed as a percentage of the peak predicted $\dot{V}O_2$, is the standard way of describing the anaerobic threshold during a CPET study. Values which are ≥40% of the median peak predicted value for age, sex and body mass are regarded as normal[149-151]. Higher proportions of the predicted peak $\dot{V}O_2$ (50-60%) are seen with aerobic training and those less than 40% suggest a possible circulatory limitation or deconditioning. Post SARS-CoV-2, the $\dot{V}O_2$ at VT1 was 46% of peak (±10), whereas the value was 55% of peak (±15) in controls (p<0.001). There was no difference in the value between those hospitalised and those managed in the community (44% of peak (±7) vs. 47% of peak (±11), p=0.45). Once again, despite the reduction in total values, all remained within the acceptable range for a 'normal' clinical study.

Table 3.6. Oxygen consumption during exercise $\dot{V}O_2$- rate of oxygen consumption, VT1- ventilatory threshold 1, HR- heart rate. Continuous variables expressed as mean (±SD)

	Controls (n = 26)	**Post COVID (n =87)**	**p-value (unpaired t test)**
Peak $\dot{V}O_2$ (ml/kg/min)	46 (±9)	35 (±8)	**<0.0001**
Peak $\dot{V}O_2$ (% predicted)	133 (±25)	113 (±18)	**<0.0001**
$\dot{V}O_2$ @ VT1 (% of peak predicted)	55 (±15)	46 (±10)	**0.0002**
$\dot{V}O_2$/HR at peak (% predicted)	126 (±22)	105 (±20)	**<0.0001**

The $\dot{V}O_2$/HR at peak exercise was lower in post SARS-CoV-2 volunteers in comparison with healthy volunteers (105% pred. (±20) vs. 126% pred. (±22), p<0.0001). Additionally, there was a difference between each group according to severity of disease (community cases: 110% pred. (±20) vs. hospitalised cases: 99% pred. (±20), p<0.05). (See Table 3.7)

Table 3.7 Comparison of oxygen consumption during exercise by severity $\dot{V}O_2$- rate of oxygen consumption, VT1- ventilatory threshold 1, HR- heart rate. Continuous variables expressed as mean (±SD)

				p-value (one-way ANOVA w Bonferroni)		
	Controls (n = 26)	**Community (n = 52)**	**Hospital (n =35)**	**Control v Community**	**Control v Hospital**	**Community v Hospital**
Peak $\dot{V}O_2$ (ml/kg/min)	46 (±9)	38 (±9)	31 (±5)	***<0.0001***	***<0.0001***	***0.0002***
Peak $\dot{V}O_2$ (% predicted)	132 (±25)	115 (±20)	109 (±16)	***0.001***	***<0.0001***	0.47
$\dot{V}O_2$ @ VT1 (% of peak predicted)	55 (±15)	47 (±11)	44 (±7)	***0.007***	***0.0002***	0.45
$\dot{V}O_2$/HR at peak (% predicted)	126 (±22)	110 (±20)	99 (±20)	***0.003***	***<0.0001***	***0.038***

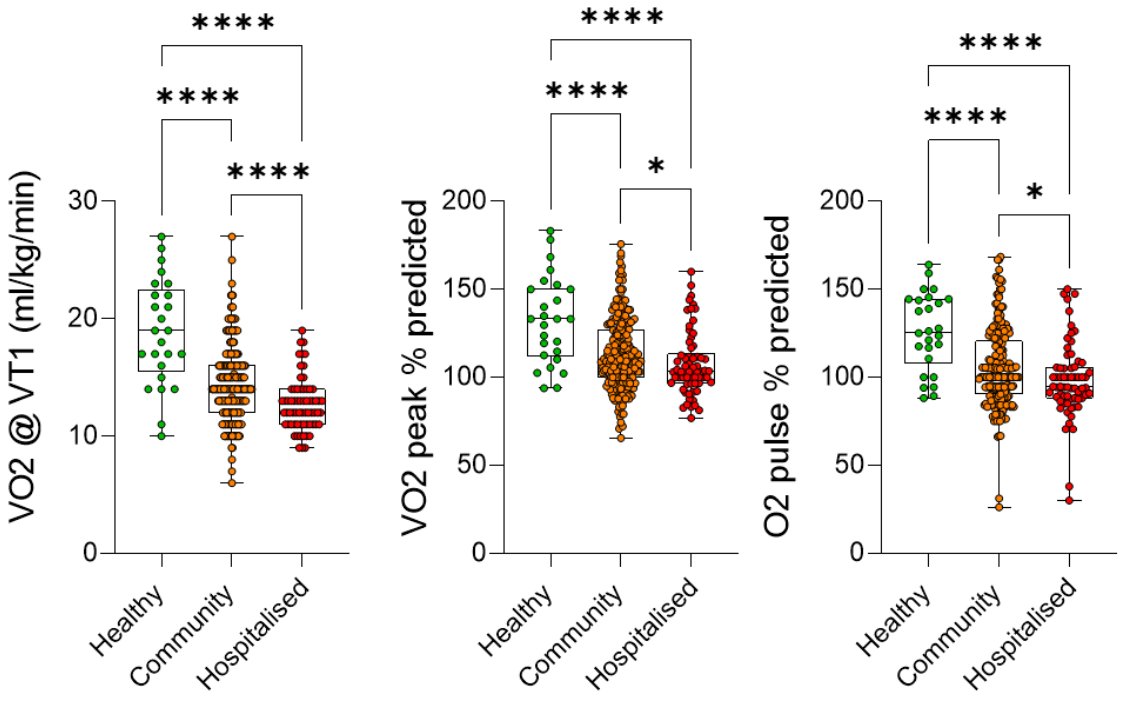


Figure 3.3 Selected CPET parameters for the study groups

3.4.4 The effect of SARS-CoV-2 on ventilatory efficiency and oxygen uptake efficiency achieved in military personnel

As for previous, parameters, data are presented first as a comparison of control versus all participants with post-COVID syndrome (in Table 3.8) and then with the COVID group separated by severity into community and hospitalised subjects (Table 3.9) No difference was seen in $\dot{V}E/\dot{V}CO_2$ slope between post SARS-CoV-2 volunteers and controls (27.6 (±5.7) vs. 25.5 (±2.6), p=0.07). However, those with the most severe disease did have a significantly elevated slope, indicating reduced ventilatory efficiency in comparison with the control population (29.2 (±5.2) vs 25.5 (±2.6), p=0.02).

The oxygen uptake efficiency slope was lower in post SARS-CoV-2 volunteers compared with controls (3.3 (±0.8) vs. 3.8 (±0.9), p=0.02), predominantly driven by the reduction in the hospitalised group (3.1 (±0.6)).

Table 3.8. Ventilatory efficiency, oxygen uptake efficiency, A-a gradient and BNP values $\dot{V}E/\dot{V}CO_2$-ventilatory efficiency, OUES- oxygen uptake efficiency slope, A-a – alveolar-arterial, BNP- b-type natriuretic peptide. continuous variables expressed as mean (±SD)

	Controls (n = 26)	**Post COVID (n =87)**	**p-value (unpaired t test)**
$\dot{V}E/\dot{V}CO_2$ slope	25.5 (±2.6)	27.6 (±5.7)	0.07
OUES	3.8 (±0.9)	3.3 (±0.8)	***0.02***
A-a gradient at rest (mmHg)	1.6 (±1.4)	2.7 (±1.9)	***0.01***
A-a gradient peak exercise (mmHg)	1.2 (±1.3)	2.8 (±1.8)	***<0.0001***
Δ A-a gradient with exercise (mmHg)	-0.4 (±1.4)	0.02 (±2.1)	0.48
BNP at rest (ng/L)	21 (±14)	18 (±12)	0.26
BNP at peak exercise (ng/L)	29.1 (±26)	28.6 (±29)	0.94
Δ BNP with exercise (ng/L)	10.5 (±20)	9.7 (±20)	0.87

There was an increase in the alveolar-arterial gradient (estimated by cap blood gases) in post SARS-CoV-2 volunteers when compared with controls both at rest (2.7 (±1.9) vs. 1.6 (±1.4), p=0.01) and with exercise (2.8 (±1.8) vs. 1.2 (±1.3), p<0.0001). When the COVID group is separated by severity of disease and compared using ANOVA, the hospitalised group demonstrate a higher A-a gradient at rest, and with exercise stress than the community group (community rest gradient 2.20 (±2.2) vs. hospitalised 3.29 (±1.5), p=0.02; community stress gradient 2.30 (±1.8) vs. hospitalised 3.48 (±1.6), p<0.01) but no difference is seen in the change from rest to exercise within each group (see Table 3.13).

There was no between-group difference in BNP, at rest or peak stress.

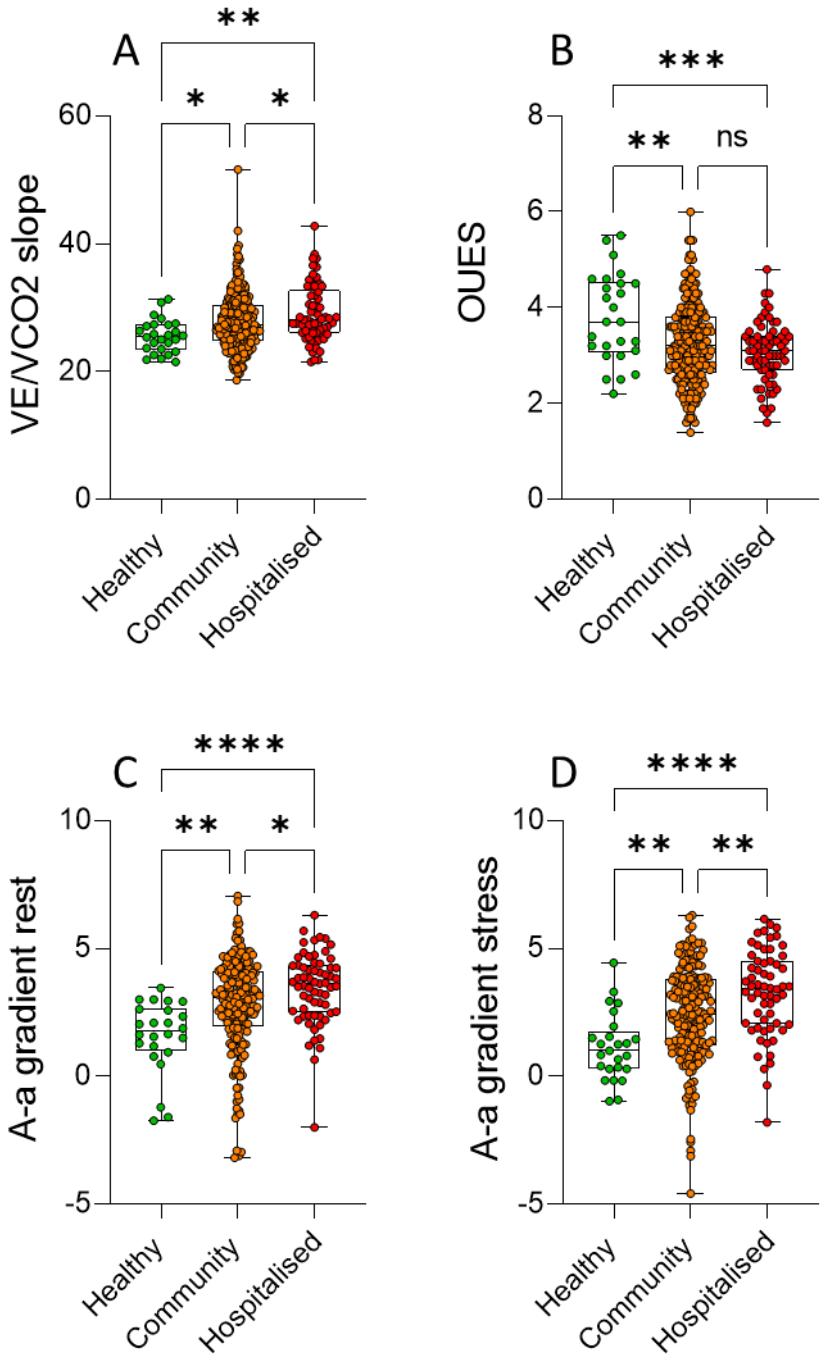


Figure 3.4 CPET parameters for the study groups A) $\dot{V}E/\dot{V}CO_2$ slope, B) OUES, C) A-a gradient at rest and D) A-a gradient at stress

Table 3.9. Ventilatory efficiency, oxygen uptake efficiency, A-a gradient and BNP values at rest and peak exercise by severity $\dot{V}E/\dot{V}CO_2$- ventilatory efficiency, OUES- oxygen uptake efficiency slope, A-a – alveolar-arterial, BNP- b-type natriuretic peptide. Continuous variables expressed as mean (±SD).

	Controls (n = 26)	Community (n = 52)	Hospital (n =35)	p-value (one-way ANOVA w Bonferroni) Control v Community	Control v Hospital	Community v Hospital
$\dot{V}E/\dot{V}CO_2$ slope	25.5 (±2.6)	26.6 (±5.8)	29.2 (±5.2)	>0.9	***0.02***	0.06
OUES	3.8 (±0.9)	3.5 (±0.9)	3.1 (±0.6)	0.41	***0.005***	0.11
A-a gradient at rest (mmHg)	1.6 (±1.4)	2.2 (±2.2)	3.3 (±1.5)	0.48	***0.001***	***0.02***
A-a gradient peak exercise (mmHg)	1.2 (±1.3)	2.3 (±1.8)	3.5 (±1.6)	***0.01***	***<0.0001***	***0.006***
Δ A-a gradient with exercise (mmHg)	-0.4 (±1.5)	-0.1 (±2.3)	0.1 (±1.7)	>0.9	>0.9	>0.9
BNP at rest (ng/L)	21 (±14)	16 (±7)	20 (±15)	0.39	>0.9	0.6
BNP at peak exercise (ng/L)	29 (±25)	28 (±23)	30(±37)	>0.9	>0.9	>0.9
Δ BNP with exercise (ng/L)	11 (±20)	11 (±17)	10 (±24)	>0.9	>0.9	>0.9

Table 3.10 Exercise Variables in relation to Symptoms Acronyms as per Table 3.14, * No SOB vs SOB at Rest, ' No SOB vs SOB Mild, # Not SOB vs SOB at Rest

	No SOB		**SOB Moderate Activity**		**SOB Mild Activity**		**SOB At Rest**		**ANOVA**
	Mean (SD)		**Mean (SD)**		**Mean (SD)**		**Mean (SD)**		**p**
Peak Workload achieved (W)	282.0	(±64.9) *	253.5	(±51.5)	243.9	(±41.5)	231.7	(±59.9)	**0.01**
Heart rate - Peak (bpm)	175.7	(±9.7)	172.8	(±16.1)	176.5	(±19.1)	168.9	(±16.3)	0.31
Systolic Blood Pressure- PEAK (mmHg)	166.9	(±20.3)	176.9	(±14.9)	169.3	(±15.0)	167.1	(±15.7)	0.37
Diastolic Blood Pressure- PEAK (mmHg)	70.0	(±22.1)	73.5	(±30.4)	80.9	(±15.3)	83.3	(±13.0)	0.12
$\dot{V}O_2$ @ PEAK (ml/kg/min)	40.4	(±10.2)*'#	32.6	(±5.9)	32.4	(±3.4)	30.9	(±8.8)	**<0.001**
% Predicted $\dot{V}O_2$ Peak (%)	122	(±23)*	113	(±15)	108	(±4)	102	(±13)	0.052
O2 pulse % predicted (%)	114.2	(±21.1)	105.5	(±18.1)	100.9	(±21.8)	95.4	(±26.5)	**0.01**
$\dot{V}E/\dot{V}CO_2$ Peak	32.7	(±11.3)	33.5	(±3.8)	34.4	(±5.7)	35.4	(±4.5)	0.78
OUES	3.9	(±2.5)	3.1	(±0.6)	3.1	(±0.7)	3.2	(±1.2)	0.37
Lactate Peak (mmol/L)	13.7	(±2.3)	12.4	(±2.3)	12.5	(±2.1)	12.1	(±2.9)	**0.04**
Arterial pH at Peak	7.26	(±0.05) *'#	7.30	(±0.06)	7.31	(±0.03)	7.31	(±0.04)	**<0.001**
pCO2 Peak (kPa)	4.6	(±0.7)	4.2	(±0.9)	4.4	(±0.7)	4.2	(±0.9)	0.25
pO2 Peak (kPa)	13.3	(±1.9)	13.2	(±3.5)	12.5	(±1.6)	12.6	(±2.4)	0.51
A-a gradient at Peak (kPa)	2.1	(±1.7)	2.5	(±2.9)	3.0	(±1.4)	3.2	(±1.8)	0.15
RPE Peak	17.0	(±2.0)	18.3	(±2.1)	17.7	(±1.6)	17.1	(±1.8)	0.13
BORG Breathlessness (0-10) Peak	6.5	(±2.4)	7.3	(±2.9)	6.7	(±2.0)	7.4	(±1.8)	0.45
FEV 1 (L)	3.9	(±0.6)	3.8	(±0.7)	3.9	(±0.7)	3.6	(±0.8)	0.47
Ventilation Peak (L/min)	137.8	(±27.0)	126.8	(±27.0)	135.7	(±29.5)	119.8	(±45.0)	0.19
SpO2 Peak (%)	97.2	(±1.7)	96.9	(±3.1)	97.4	(±1.2)	95.9	(±3.8)	0.25
Respiratory Exchange Ratio Peak	1.2	(±0.1)	1.2	(±0.1)	1.2	(±0.0)	1.2	(±0.1)	**0.04**
stress HR (bpm)	124.4	(±16.8)	127.5	(±20.7)	134.9	(±12.3)	125.5	(±10.0)	0.19
Visual LV EF stress (%)	70.5	(±6.2)	70.5	(±6.8)	71.3	(±8.3)	70.5	(±6.0)	0.98
Av E/e' STRESS	8.0	(±2.3)	7.7	(±2.5)	7.7	(±2.1)	7.7	(±1.9)	0.91

3.4.5 Relationship between Anthropometrics and Exercise Capacity

In order to understand the relationship between exercise performance and baseline anthropometric data of the study group a correlation matrix was performed (Figure 3.5). In line with the literature, this showed that Peak $\dot{V}O_2$, and Peak work were both negatively correlated with DBP, BMI and age, and positively correlated with biological sex (when coded 1 male, 0 female for this analysis), all $p<0.01$)

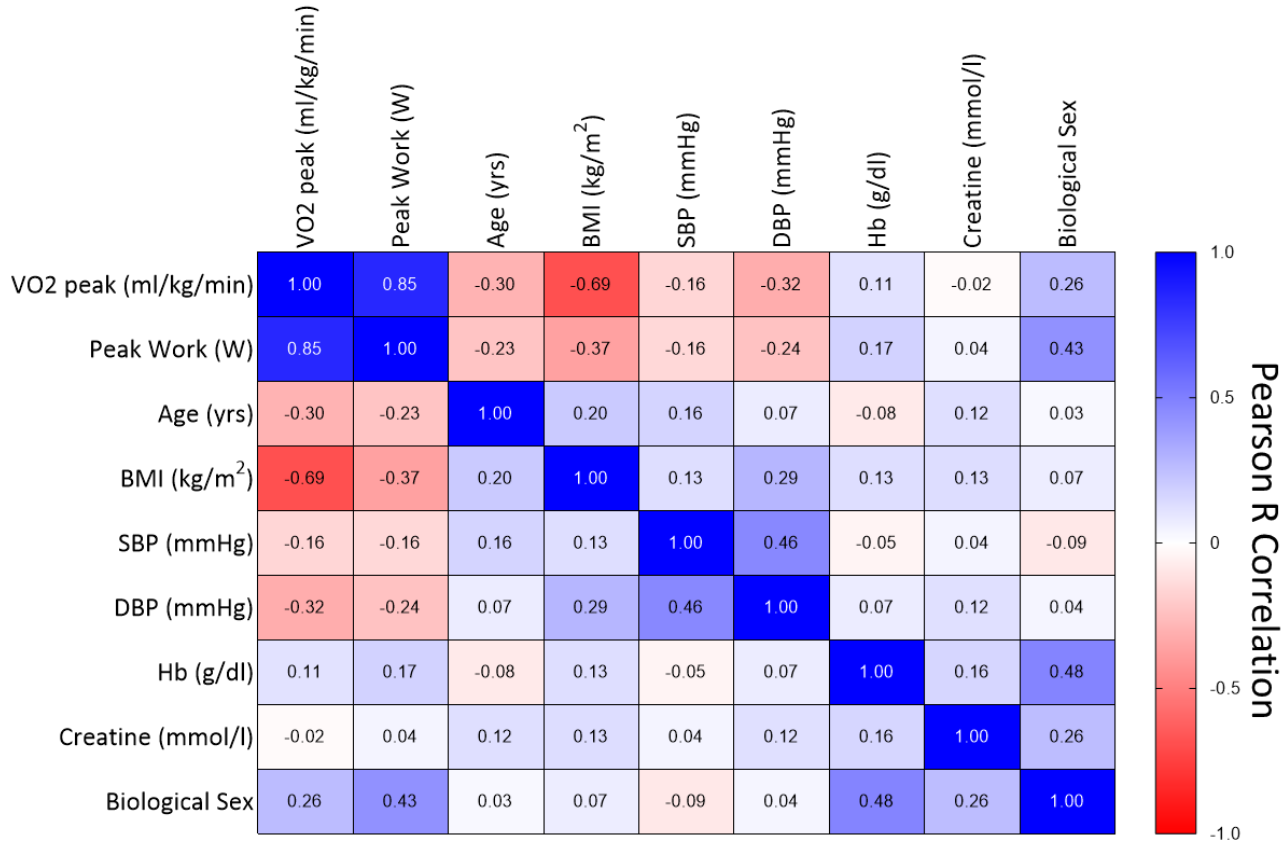


Figure 3.5 Correlation Matrix of baseline anthropometric data and Exercise Capacity

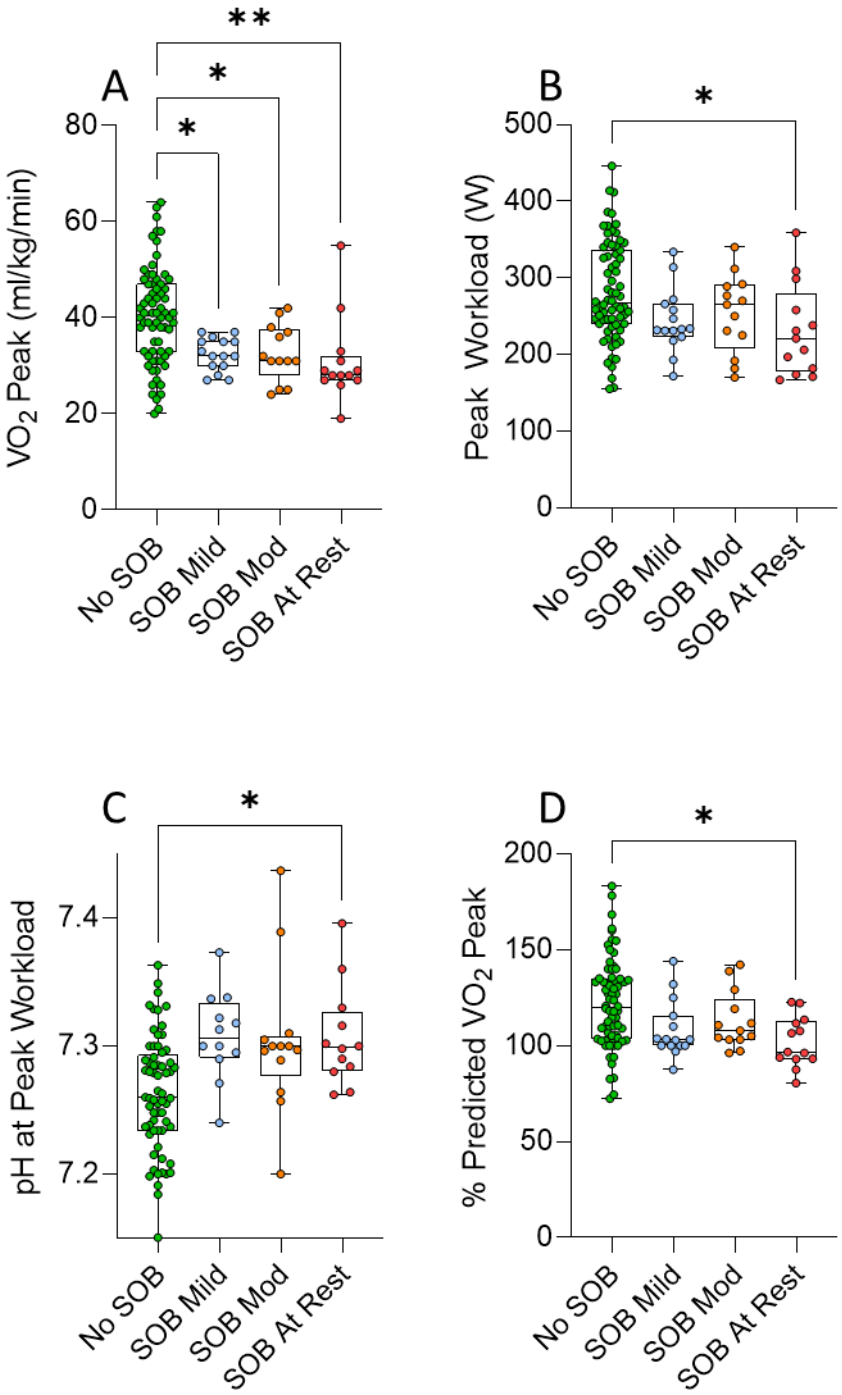


Figure 3.6 $\dot{V}O_2$ Peak, Peak Workload and pH at Peak separated by symptoms

The more severe symptom of shortness of breath (SOB) at rest was associated with lower $\dot{V}O_2$ Max, lower Peak work (W), and higher pH during CPET. In addition, there was a progressive reduction in median $\dot{V}O_2$ peak when moving from no SOB > SOB on mild exertion > SOB on moderate exertion > SOB at rest (Jonckheere Terpstra test $p<0.001$). Overall, this suggests that the perception of

symptoms is related to reduced exercise capacity, though it is not possible to determine whether the subjective sensations (symptoms) are a cause or effect. It should also be noted that all groups reach an average VO_2 peak greater than 100%, which would be regarded clinically as robustly normal.

3.5 Changes in exercise capacity at 6-month follow up after SARS-CoV-2 infection in military personnel

The difference between baseline and 6-month follow-up values are shown in Table 3.11. When the difference between baseline and 6-month follow-up values was compared in those who have had SARS-CoV-2 versus controls, there were no significant differences between the groups.

Table 3.11. Comparison of difference in exercise capacity from baseline to 6-month follow up visit RER- Respiratory exchange ratio, VT1- ventilatory threshold 1, $\dot{V}O_2$- rate of oxygen consumption, AT- anaerobic threshold, HR- heart rate, $\dot{V}E/\dot{V}CO_2$- ventilatory efficiency, OUES- oxygen uptake efficiency slope, A-a – alveolar-arterial. Continuous variables expressed as mean (±SD)

	Change from baseline to 6-month follow up		**Independent t test**
	Controls (n = 16)	**Post COVID (n 69)**	**p-value**
RER at peak	0.01 (±0.7)	0.03 (±0.1)	0.5
Peak workload achieved (W)	-0.68 (±18.9)	4.61 (±14.6)	0.3
Peak workload achieved (% pred)	-7 (±49)	9.2 (±39)	0.2
Workload achieved at VT1 (W)	3.75 (±33)	7.93 (±28)	0.7
Peak $\dot{V}O_2$ (ml/kg/min)	-1.4 (±7)	0.39 (±6)	0.4
Peak $\dot{V}O_2$ (% predicted)	-1.6 (±20)	3.4 (±18)	0.4
$\dot{V}O_2$ @ AT (% of peak predicted)	1.9 (±14)	2.6 (±13)	0.8
$\dot{V}O_2$/HR at peak (% predicted)	-2.9 (±28)	1.1 (±22)	0.6
$\dot{V}E/\dot{V}CO_2$ slope	0.53 (±2.8)	0.008 (±4.57)	0.56
OUES	-0.3 (±0.8)	0.06 (±0.9)	0.09
A-a gradient at rest	0.86 (±2.6)	0.31 (±2.6)	0.46
A-a gradient peak exercise	0.47 (±2.9)	-0.1 (±2.4)	0.49

When the paired values at baseline and 6-month follow up are compared, no difference was found in the control group. However, in the post COVID group, there were differences in the RER achieved at peak (baseline 1.18 (±0.06) vs. follow up 1.2 (±0.06) , $p<0.01$), peak workload achieved expressed as a percentage predicted (baseline 102% predicted (±20) vs. follow up 106% predicted (±20), $p=0.01$) and in the workload achieved at the anaerobic threshold (baseline 82W (±28) vs. follow up 89W (±26) , $p=0.03$).

Overall, this suggests that after 6 months there was an improvement in exercise capacity in those with post COVID syndrome.

Table 3.12. Comparison of exercise capacity at baseline and at 6-month follow up 4. RER- Respiratory exchange ratio, VT1- ventilatory threshold 1, $\dot{V}O_2$- rate of oxygen consumption, AT- anaerobic threshold, HR- heart rate, $\dot{V}E/\dot{V}CO_2$- ventilatory efficiency, OUES- oxygen uptake efficiency slope, A-a – alveolar-arterial. Continuous variables expressed as mean (SD)

	Controls at baseline (n = 25)	**Controls at 6 months (n = 16)**	**p-value (paired t-test baseline v follow up)**	**COVID at baseline (n=87)**	**COVID at 6 months (n = 69)**	**p-value (paired t-test baseline vs. follow up)**
RER at peak	1.22 (±0.07)	1.22 (±0.06)	0.6	1.20 (±0.06)	1.2 (±0.06)	***0.003***
Peak workload achieved (W)	304 (±65)	284 (±52)	0.5	257 (±58)	254 (±53)	0.1
Peak workload achieved (% pred)	127 (±32)	125 (±37)	0.8	103 (±20)	106 (±20)	***0.01***
Workload achieved at VT1 (W)	109 (±34)	103 (±32)	0.6	82 (±28)	89 (±26)	***0.03***
Peak $\dot{V}O_2$ (ml/kg/min)	46 (±9)	43 (±9)	0.5	35 (±8)	35 (±8)	0.6
Peak $\dot{V}O_2$ (% predicted)	133 (±25)	131 (±28)	0.7	113 (±18)	115 (±19)	0.1
$\dot{V}O_2$ @ AT (% of peak predicted)	55 (±15)	54 (±17)	0.5	46 (±10)	48 (±11)	0.1
$\dot{V}O_2$/HR at peak (% predicted)	126 (±22)	123 (±24)	0.7	105 (±20)	105 (±19)	0.7
$\dot{V}E/\dot{V}CO_2$ slope	25.5 (±2.6)	26.3 (±2.8)	0.5	27.6 (±5.7)	28 (±3.8)	0.7
OUES	3.8 (±0.9)	3.3 (±0.7)	0.1	3.3 (±0.8)	3.3 (±0.9)	0.6
A-a gradient at rest	1.6 (±1.4)	2.7 (±2.5)	0.4	2.7 (±1.9)	3.0 (±1.9)	0.3
A-a gradient peak exercise	1.2 (±1.3)	2.1 (±2.3)	0.6	2.8 (±1.8)	2.5 (±1.9)	0.2

Table 3.13 Comparison of exercise capacity from baseline to 6-month follow up by severity of disease. RER- Respiratory exchange ratio, VT1- ventilatory threshold 1, $\dot{V}O_2$- rate of oxygen consumption, AT- anaerobic threshold, HR- heart rate, $\dot{V}E/\dot{V}CO_2$- ventilatory efficiency, OUES- oxygen uptake efficiency slope, A-a – alveolar-arterial. Continuous variables expressed as mean (±SD

	Controls at baseline (n = 26)	**Controls at 6 months (n = 16)**	**p-value (paired t-test baseline vs. follow up)**	**Community baseline (n=52)**	**Community 6 months (n = 40)**	**p-value (paired t-test baseline vs. follow up)**	**Hospitalised baseline (n=35)**	**Hospitalised 6 months (n =29)**	**p-value (paired t-test baseline vs. follow up)**
RER at peak	1.22 (±0.06)	1.22 (±0.06)	0.6	1.19 (±0.07)	1.21 (±0.07)	***<0.001***	1.18 (±0.05)	1.20 (±0.07)	0.17
Peak workload (W)	304 (±65)	284 (±52)	0.5	274 (±65)	273 (±60)	0.2	233 (±33)	241 (±44)	0.1
Peak workload (% pred)	127 (±32)	125 (±37)	0.8	106 (±22)	108 (±17)	***0.01***	98 (±16)	101 (±22)	0.21
Workload achieved at VT1 (W)	109 (±34)	103 (±32)	0.6	90 (±32)	95 (±27)	0.3	70 (±15)	82 (±24)	***0.01***
Peak $\dot{V}O_2$ (ml/kg/min)	46 (±9)	43 (±9)	0.5	38 (±9)	37 (±7)	0.8	31 (±5)	31 (±7)	0.6
Peak $\dot{V}O_2$ (% predicted)	132 (±25)	131 (±28)	0.7	115 (±20)	118 (±18)	0.1	109 (±16)	110 (±20)	0.5
$\dot{V}O_2$ @ AT (% of peak predicted)	55 (±15)	54 (±17)	0.5	47 (±11)	48 (±11)	0.7	44 (±7)	49 (±13)	0.09
$\dot{V}O_2$/HR at peak (% predicted)	126 (±22)	123 (±24)	0.7	110 (±20)	110 (±17)	0.4	99 (±20)	99 (±19)	0.9
$\dot{V}E/\dot{V}CO_2$ slope	25.5 (±2.6)	26.3 (±2.8)	0.5	26.6 (±5.8)	27.6 (±3.6)	0.5	29.2 (±5.2)	28.4 (±4.0)	0.2
OUES	3.8 (±0.9)	3.3 (±0.7)	0.1	3.5 (±0.9)	3.4 (±0.9)	0.7	3.1 (±0.6)	3.1 (±0.7)	0.7
A-a gradient at rest	1.56 (±1.4)	2.7 (±2.5)	0.4	2.20 (±2.2)	2.8 (±2.1)	0.3	3.29 (±1.5)	3.4 (±1.6)	0.7
A-a gradient peak exercise	1.15 (±1.3)	2.1 (±2.3)	0.6	2.30 (±1.8)	2.1 (±1.9)	0.3	3.48 (±1.6)	3.0 (±1.8)	0.5

3.5.1 Changes in exercise capacity at 6-months follow up by severity of disease

Changes in exercise capacity at 6-months follow up by severity of disease are shown in Table 3.13 and 3.14. When the participants with severe disease (who were hospitalised) were compared with those with mild/moderate disease (who were managed in the community) and with controls, there was no significant difference in the differences between baseline and 6-month follow up (by one-way ANOVA).

Table 3.14. Change between baseline and 6-month exercise variables by severity. RER- Respiratory exchange ratio, VT1- ventilatory threshold 1, $\dot{V}O_2$- rate of oxygen consumption, AT- anaerobic threshold, HR- heart rate, $\dot{V}E/\dot{V}CO_2$- ventilatory efficiency, OUES- oxygen uptake efficiency slope, A-a – alveolar-arterial. Continuous variables expressed as mean (±SD)

	Difference baseline & 6-month			**p-value**
	Controls (n = 16)	**Community (n = 40)**	**Hospital (n =29)**	**(one-way ANOVA)**
RER at peak	0.1 (±0.8)	0.03 (±0.6)	0.02 (±0.8)	0.7
Peak workload achieved (W)	-7 (±50)	8.2 (±42)	10.6 (±35)	0.36
Peak workload achieved (% pred)	-0.68 (±19)	4.92 (±12)	4.2 (±18)	0.47
Workload achieved at VT1 (W)	3.8 (±33)	4.9 (±32)	12.0 (±24)	0.6
Peak $\dot{V}O_2$ (ml/kg/min)	-1.4 (±7.5)	0.28 (±6.7)	0.6 (±6.1)	0.6
Peak $\dot{V}O_2$ (% predicted)	-1.6 (±20)	4.1 (±17.2)	2.4 (±18.7)	0.5
$\dot{V}O_2$ @ AT (% of peak predicted)	1.9 (±14)	0.8 (±11)	5.0 (±29)	0.4
$\dot{V}O_2$/HR at peak (% predicted)	-2.9 (±7.1)	2.3 (±17.6)	-0.5 (±26 5)	0.7
$\dot{V}E/\dot{V}CO_2$ slope	0.5 (±2.8)	0.3 (±4.9)	-0.7 (±3.9)	0.6
OUES	-0.3 (±0.8)	0.13 (±0.9)	-0.7 (±0.9)	0.2
A-a gradient at rest	0.86 (±2.6)	0.44 (±2.9)	0.13 (±2.1)	0.6
A-a gradient peak exercise	0.47 (±2.9)	0.002 (±2. 3)	-0.21 (±2.7)	0.38

When the paired values at baseline and 6-month follow up are compared, the RER achieved at peak was higher in the community group (baseline 1.19 (± 0.07) vs. follow up 1.21 (±0.07), $p<0.001$), whereas there was no difference in the hospital group. Peak workload achieved expressed as a percentage predicted was also higher in the community group (baseline 105% (±22) vs. follow up 109% (±17), p=0. 01). Workload achieved at the anaerobic threshold (related to the endurance exercise capacity) improved in the hospitalised group (baseline 70W (±15) vs. follow up 82W (±24), p=0.01).

3.6 Discussion

In this study, the groups investigated were well matched for age, gender and employment. However, when the post SARS-CoV-2 group were divided into subgroups by severity, the hospitalised group were older than those managed in the community. This is commensurate with the increased severity of disease with age, and has been previously demonstrated across global populations[152]. The post SARS-CoV-2 group were overweight in comparison to the COVID negative controls, with a trend towards a higher BMI in the hospitalised group than the community group. Again, this is expected. The association of BMI with severe disease for both the acute illness as well for the risk of developing prolonged symptoms has been reported widely[153,154].

3.6.1 Workload, exercise capacity and oxygen consumption

Individuals post SARS-CoV-2 did not reach the same workload at peak exercise in comparison to COVID negative controls. Additionally, with more severe the acute infection (as determined in this study by acute illness which required hospitalisation), a lower peak is workload achieved, both at peak exercise and at the anaerobic threshold. Oxygen consumption at peak exercise and at the anaerobic threshold is similarly reduced at greater disease severity. This suggests not only that the total exercise capacity is reduced, but also that the anaerobic threshold is reached earlier in those with prior infection, and furthermore that those who had a more severe infection requiring hospitalisation, have a greater impairment than those with mild or moderate acute disease.

In studies of civilian populations, a reduction of $\dot{V}O_2$ is also seen, both at peak exercise and at the anaerobic threshold [88,89,108-111] However, in contrast to this study, they focused exclusively on hospitalised groups and a direct comparison with this cohort is not possible. There is also variability in the methodology between the studies. Some measured exercise capacity at hospital discharge, whereas others looked 3 months later, in comparison to this study, which reviewed exercise capacity at a minimum of 12 weeks after acute infection but in reality, was approximately 22 weeks post symptom onset (159±7 days). Despite this, the findings suggest that the same pathophysiological

effects are being seen in the military group as civilian groups, albeit over a different range of absolute values.

It is striking that although the same trends in exercise capacity are seen in this study (reduced workload, peak $\dot{V}O_2$ and $\dot{V}O_2$ at the anaerobic threshold), the values are quite different. Multiple studies have reported a peak $\dot{V}O_2$ of <80% predicted in individuals post SARS-CoV-2, whereas in this study, we have shown that although there is a trend, even the most severely affected group were able to achieve a mean peak $\dot{V}O_2$ of 109% predicted. This peak value is significantly lower than in the community group and the COVID negative controls but would be considered a normal result if performed in a clinical setting. The reference data for this calculation are automatically applied in the analysis software and are based on a civilian reference population from North America measured in the 1970s[155]. Any participants measured against this group who have higher baseline fitness will generate above average precent of predicted values. Given that there is a significant decline in exercise capacity in comparison to the control population, it is reasonable to assert that SARS-CoV-2 infection leads to a reduction in exercise capacity in the military population, and that this is more pronounced in severe disease. What is not certain, is whether the control population values are a true representation of the 'pre-infection' exercise capacity of the patient population. As we have already seen, there are more overweight and obese individuals in the post SARS-CoV-2 group, and they may represent a less athletic subset of the military population.

3.6.2 Possible mechanisms for difference in exercise capacity

Differences in other measures derived from the cardiopulmonary exercise test may suggest the mechanism of the differences seen in the post SARS-CoV-2 group. Clinical analysis of exercise limitation is in broad categories: circulatory limitations, ventilatory limitations, mixed limitations, impaired peripheral oxygen utilisation and deconditioning[156]. According to standard clinical criteria, these categories only apply to 'abnormal' tests: those with a peak $\dot{V}O_2$ of <85% predicted. On this basis, the military post-COVID group are not 'abnormal'.

3.6.3 Circulatory limitation

Given the concern at the start of the COVID-19 pandemic that there would be a large burden of cardiac morbidity associated with infection, particularly myocarditis, we should consider whether there is evidence of cardiac limitation in the exercise data. BNP samples before and at peak exercise demonstrate no significant change. However, BNP plays no part in routine clinical interpretation of the cardiopulmonary exercise test. The accepted measurement to suggest a circulatory limitation is an early anaerobic threshold, with $\dot{V}O_2$ at VT1 measurements of less than 40% of the peak predicted $\dot{V}O_2$[156]. In our study, none of the groups are at or below this value, although there is a definite trend towards a lower $\dot{V}O_2$ at VT1 with increasing severity of disease.

An alternative indicator that there might be a circulatory limitation is a reduction in peak $\dot{V}O_2$/HR, or an early plateau and then fall in this value. When there is no evidence of peripheral oxygen extraction (as is the case in this study), the $\dot{V}O_2$/HR can be viewed as a proxy marker for stroke volume, and a peak $\dot{V}O_2$/HR of <80% predicted suggests a possible limitation in the augmentation of stroke volume to match the exercise requirement[135]. As with the peak $\dot{V}O_2$ measurements, none of the studied groups had a $\dot{V}O_2$/HR of less than the 80% predicted cut off value, although there was a trend towards a lower value in the more severe cohort of the patient group. It is not clear whether such a sub-clinical trend can be considered 'limiting' in this overall above-average group who otherwise are able to achieve a 'normal' test. Of note, other studies which have used CPET, echocardiography and central mixed venous O_2 measurements have concluded that stroke volume does not change and that any reductions in $\dot{V}O_2$/HR are more likely secondary to reduced peripheral oxygen consumption[111].

3.6.4 Ventilatory limitation

SARS-CoV-2 appears to be primarily a respiratory disease, and as such, a ventilatory cause for exercise limitation would not be unexpected. Histopathology reports of fatal cases have shown diffuse alveolar damage, thrombosis and microvascular injury[157]. If this pathophysiological process is also present to

a lesser extent in survivors, it would be reasonable to postulate an impairment in pulmonary gas exchange and/or ventilation-perfusion matching.

In CPET analysis, any pathology or behaviour which impairs ventilatory efficiency will increase the minute ventilation required to excrete a given volume of CO_2. This can be quantified by examining the $\dot{V}E/\dot{V}CO_2$ at a given point (e.g., at rest or at the VT1) or by establishing the slope ($\dot{V}E/\dot{V}CO_2$) from one minute after loaded pedalling until the second ventilatory threshold: the respiratory compensation point (VT2 or RCP). The $\dot{V}E/\dot{V}CO_2$ value (and slope) rises with worsening ventilatory efficiency. In individuals with increased dead space ventilation or ventilation perfusion mismatch values are elevated[158]. $\dot{V}E/\dot{V}CO_2$ also rises with certain types of dysfunctional breathing, including ventilation in excess of the body's requirements. $\dot{V}E/\dot{V}CO_2$ slope values below 30 are considered normal. Values ≥30 are adverse prognostic markers, associated with an increased morbidity and mortality in patients with chronic heart failure[159].

In all groups studied, the $\dot{V}E/\dot{V}CO_2$ slope was less than 30, and although there was a trend towards higher values with increased severity of disease, this only demonstrated a significant difference when comparing the hospitalised group with the controls. It is possible that in the community group the sample size was too small to demonstrate a statistically significant difference from the controls, at a smaller effect size. As with exercise capacity, we have found that despite some individuals having life threatening severe disease during their acute presentation, the values in this population only reach sub-clinical levels of impairment.

Oxygen transfer across the alveolar/capillary interface can be quantified using the alveolar-arterial oxygen gradient (A-a gradient). This measure is known to increase slightly in normal individuals at peak exercise, as diffusion capacity becomes a limiting factor to increasing workloads, in particular owing to the increased transpulmonary flow and reduced time of blood in the pulmonary capillary at the diffusion interface[160]. However, much larger increases in this gradient at exercise have been seen

in patients with early fibrotic parenchymal changes in pneumoconiosis, and in individuals with early pulmonary hypertension as a result of chronic thromboembolic disease[161,162].

In the post SARS-CoV-2 patients, both the rest and exercise A-a gradients are significantly elevated in comparison to the control group. A severity trend is also present, with more elevated values in the severe disease population in comparison with the non-hospitalised, mild/moderate disease group. This finding is highly suggestive of a ventilatory cause for the differences in exercise capacity across the groups. Of note, when the A-a gradient values from rest to exercise are compared in each group, there was no statistically significant increase.

3.6.5 6-Month Follow Up Data

When comparing the 6-month follow up exercise data with the baseline tests, it is interesting to note that, although there were some improvements in exercise capacity (the peak workload and the anaerobic threshold have increased in the post COVID group), there were no significant differences in the total exercise capacity when measured by peak $\dot{V}O_2$. This suggests that for every unit of $\dot{V}O_2$ utilised at the 6 month follow up in the post COVID group, there is an increase in workload achieved. Thus, although the total oxygen consumption remains unchanged, there is an increase in the exercise *efficiency* and the functional exercise capacity following 6 months of recovery. That improvements are seen in both the peak workload achieved and in the workload at the anaerobic threshold suggests both increased efficiency at peak exercise and in endurance exercise capacity. No similar changes were seen in the control group when the baseline and 6 month follow up data were compared. It is not clear from this study whether a recovery in the oxygen consumption as measured by peak $\dot{V}O_2$ is also seen beyond the 6 month point to match COVID negative controls, or whether those changes are irreversible.

3.6.6 Strengths and limitations

This study included a comprehensive exercise dataset in a population with a spectrum of severity of acute illness. The group was employment and age matched. Additionally, it included a contemporaneously recruited age, sex and occupational role matched control population. Such a well-controlled and comprehensive population had not been reported in the literature previously. As participants had undergone their acute care at local healthcare facilities, all clinical data were not available to researchers, so those who required hospital care were considered 'severe' cases, and those who were managed in the community were considered 'mild-moderate' cases. However, other markers which might otherwise stratify severity of clinical cases, such as number of days spent in ICU, number of days with endotracheal intubation and ventilation, peak WCC/CRP levels, lowest arterial O2 levels etc, were not included.

As there has been no CPET study of the normal healthy population in the UK Armed Forces, there are no pre-pandemic baseline exercise data against which to compare the post-Covid participants. A number of participants were lost to follow up. This was in part due to control participants subsequently contracting SARS-CoV-2 and being excluded from the study. In other cases, changing work obligations after the periods of national lockdown prevented attendance within the defined time window.

We were also unable to control for clinical treatment in the post SARS-CoV-2 group. Some, but not all, were offered rehabilitation therapy, and this occurred at slightly different time points post acute illness.

3.7 Conclusion

We found that SARS-CoV-2 infection is associated with a persistent reduction in total exercise capacity, and a reduced anaerobic threshold in military personnel when measured by oxygen consumption. However, by 6 months, there is an improvement in efficiency of exercise and in workload achieved at

peak exercise and at the anaerobic threshold. The effect size of reduction in exercise capacity is associated with the severity of illness. Importantly, all values would fall within the sub-clinical range for a normal civilian population. There is some evidence that there could be either a ventilatory or circulatory component to the exercise limitation, but as the values are all within the normal range for the reference population, more work needs to be done to establish whether this is the case. The next chapter will examine whether the differences in exercise capacity are associated with abnormalities in cardiac function at the macro or metabolic levels.

Chapter 4- The Effect of SARS-CoV-2 on Cardiac Structure and Function

4.1 Introduction

In normal human physiology, exercise capacity is limited by cardiac output and a typical limiting symptom is leg fatigue, which is the result of inadequate skeletal muscle oxygen supply and subsequent anaerobic lactate accumulation. At peak exercise, the cardiac output can increase as much as 10-fold, and the skeletal muscle up to 30-fold compared with rest. The capacity to engage in physical activities therefore hinges on several pivotal factors, including cardiac function.

4.2 Determinants of Exercise Capacity

During exercise, the cardiovascular system adapts to provide working skeletal (and cardiac) muscles with a consistent supply of O_2 that is required ultimately to supply enough ATP to sustain muscle contraction. Cardiac output increases to pump sufficient blood across the interface of the pulmonary capillaries and alveoli to exchange the necessary O_2 and CO_2. The pulmonary mechanisms of ventilation and diffusion, which are responsible for the exchange of O_2 and CO_2 between the lungs and blood, ensure sufficient oxygen-rich blood is available to be pumped to the muscles and deoxygenated blood is returned to be re-oxygenated and CO_2 excreted. Haemoglobin concentration is crucial in this process, binding with O_2 to be transported to muscles and CO_2 for excretion at the alveolar interface. Capillary gas exchange at the capillary/muscle interface is another critical component of exercise capacity. Here, the exchange of O_2 and CO_2 occurs between capillary blood and myocytes. A healthy, dense capillary bed is required for this exchange. Lastly, at the cellular level, the mitochondrial electron transport chain (ETC) requires O_2 delivery to complex V to act as the electron acceptor. The ETC results in the pumping of protons from the interior of the mitochondrion into the 'interspace'

between the inner and outer membranes. It is the return of these protons, down their chemiosmotic gradient, which leads to the resynthesis of ATP.

As such, maximal exercise capacity requires the integration of many mechanisms, working together to supply, transport, and utilize oxygen efficiently across different physiological systems. Having documented reduced exercise capacity in patients with post COVID syndrome and symptoms of fatigue and breathlessness. SARS-CoV-2 infection is associated with a persistent reduction in total exercise capacity, and a reduced anaerobic threshold in military personnel. The effect size of reduction in exercise capacity is associated with the severity of illness. Therefore, documenting cardiac function and ATP metabolism are key components of exercise performance.

In order to do this, in this chapter I used the combination of CMR imaging, ^{31}P magnetic resonance spectroscopy and rest and stress echocardiography to investigate the effect of SARS-CoV-2 infection on cardiac function and metabolism in members of the UK Armed Forces.

4.3 Methods

Detailed methods for the study can be found in Chapter 2. The study received favourable opinion from the Ministry of Defence Research Ethics Committee (MODREC- ref: 1061/MODREC/20) and complied with the Declarations of Helsinki. Volunteer participants were the same as those who took part in Chapter 3 and were recruited from the Defence COVID Recovery Service, or from local military health units.

Inclusion criteria for the post SARS-CoV-2 group were: previous SARS-CoV-2 infection, with either a positive COVID-19 antigen PCR test at the time of their acute illness, or clinically adjudicated COVID-19, at least 4 weeks post their acute infection, and negative COVID-19 antigen PCR test at the time of enrolment to the study. The Control group inclusion criteria were: no previous suspicion of acute COVID-19 infection and negative COVID-19 anti-nuclear antibody test at the time of enrolment to the study. Exclusion criteria were a past medical history of significant cardiac or pulmonary disease, active

infection at the time of recruitment, pregnancy, and contraindication to MRI scanning, as detailed in Chapter 2. Additionally, the control group were excluded if they contracted COVID-19 during the study period.

Participants underwent the following at their baseline visit:

4.3.1 Cardiac Magnetic Resonance Imaging

Left and right ventricular structure, volumes and systolic function assessment, pre and post contrast T1 parametric mapping and myocardial energetics, were performed on a dedicated 3T system (Siemens, Erlangen, Germany). See Chapter 2 for protocol details.

4.3.2 Myocardial energetics

^{31}P MR Spectroscopy was used to determine septal phosphocreatine/ATP ratios using a ^{31}P surface coil (Pulse Teq, Chobham, UK). Measurements were taken at rest and again at stress (aiming for 65% predicted maximal heart rate using peripheral dobutamine infusion (see Chapter 2)). Spectra were analyzed using a semi-automated fitting of data from within OXSA toolbox, a MATLAB implementation of the AMARES fitting routine.

4.3.3 Exercise echocardiography

Echocardiography was performed using a Phillips CX50 (Phillips, Netherlands) using a standardised protocol. Pulsed wave Doppler was used to assess mitral valve inflow and calculate E/A. Tissue Doppler was used to measure velocities at the medial and lateral mitral annulus to measure e' and derive an average E/e' value for each patient. M-mode imaging was used to measure tricuspid annular plane systolic excursion (TAPSE). Measurements were taken from the apical view at rest whilst participants were sat on an upright cycle ergometer. After acquisition of the rest measurements, participants exercised to achieve a target heart rate of 75% of the maximum achieved during their CPET. Once at target heart rate, measurements were repeated immediately post exercise.

4.3.4 Statistics

Statistical analysis was performed using commercial software (IBM SPSS 27, Chicago, USA, and GraphPad Prism 9, San Diego, USA). Data is presented as mean ± standard deviation. Normality was confirmed using Kolmogorov-Smirnov testing. Chi squared was used to compare nominal data. Continuous data was compared using an independent t-test for baseline data and a paired t-test when comparing baseline with follow up data. When comparing more than two groups, one-way ANOVA with post hoc Bonferroni correction was used. A p value of <0.05 was considered significant. The effects of disease severity were also tested using Jonkheere-Terpstra test for ordered medians (order defined as control>community infection> hospitalisation for infection). A p-value of 0.05 (2-tailed) was taken to indicate statistical significance. To assess the major determinants of exercise capacity, a stepwise multiple linear regression model was performed. This multivariate model consisted of peak $\dot{V}O_2$ max (% predicted) as dependent variable and of independent variables that had a significant relation with peak $\dot{V}O_2$ max (% predicted) in the simple linear regression analysis. To limit collinearity variables with a variance inflation factor of > 4 were removed from the model.

4.4 Results

113 volunteers were assessed for this study (see Table 3.2 for baseline anthropometrics). 87 volunteers were post SARS-CoV-2 infection (52 managed during their acute illness in the community, and 35 in hospital) and 26 were COVID negative healthy controls. Groups were matched for age (Sars-CoV-2 group 39.02 ± 9.59 vs. controls 38.27 ±8.22years, p=0.72) and sex (86% male vs. 88% male, p=0.5), and all were members of the UK Armed Forces. Groups are not matched for BMI, and the post SARS-CoV-2 group have a higher BMI than the healthy controls (29.30±4.12 vs. 25.41 ±2.58 kg/m^2, p<0.0001).

4.4.1 The impact of SARS-CoV-2 on left ventricular size and function

No differences were seen between healthy controls and all post SARS-CoV-2 volunteers in left ventricular volumes, mass or systolic function. Between the two groups, there were also no differences observed between the average E/e' measurements at rest or exercise stress, although the size of change from rest to stress was larger in those post SARS-CoV-2 infection (1.48± 2.7 vs. 0.37 ± 2.9, p=0.03).

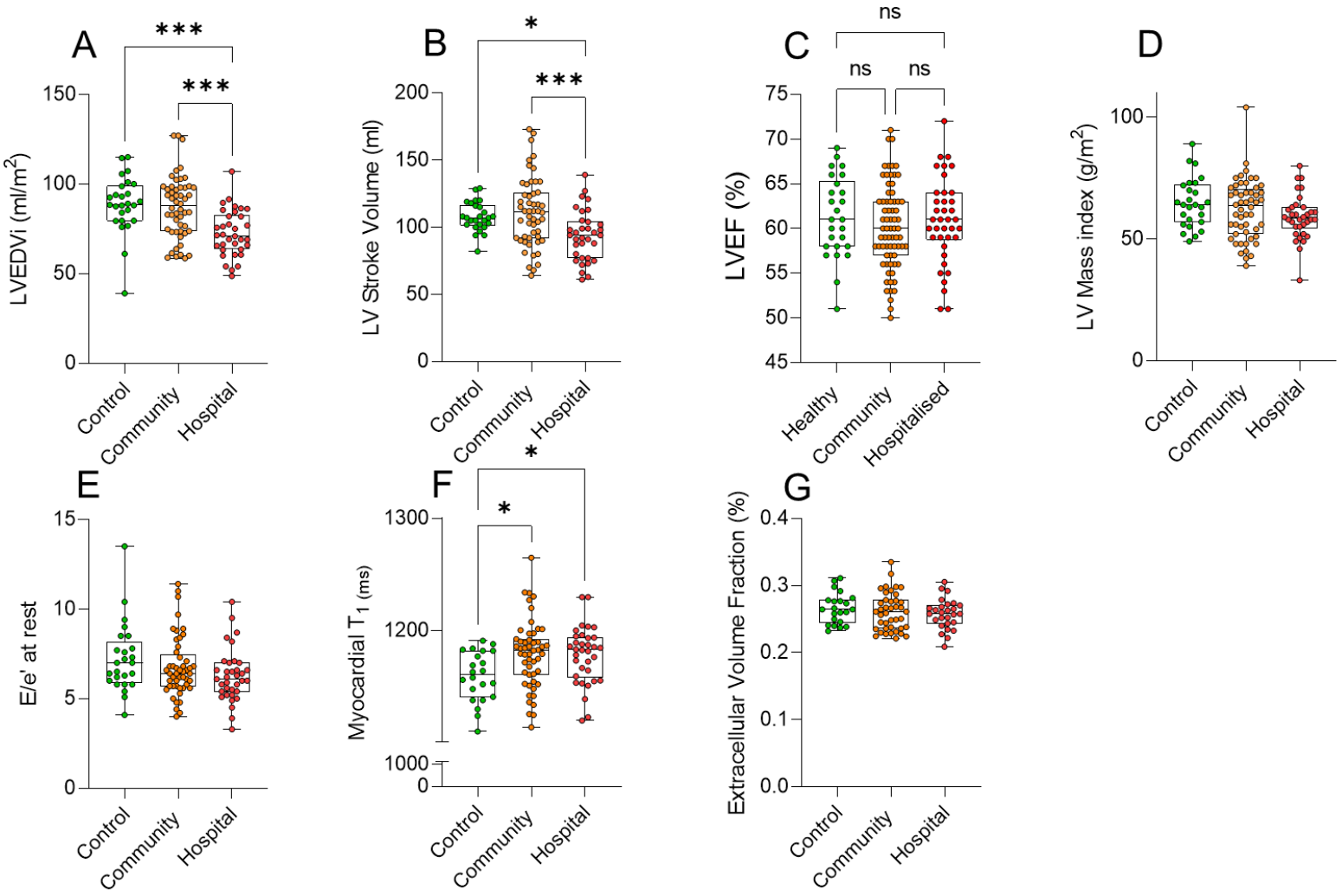


Figure 4.1 Left ventricular size and function by severity. A) LVEDVi- left ventricular end diastolic volume index, B) LVSV- stroke volume, C) LVEF- left ventricular ejection fraction, D) LV Mass Index, E) E/e' at rest, F) Native myocardial T1 and F) Extracellular Volume.

Table 4.1 Left ventricular size and function. LVEDV- Left ventricular end diastolic volume, LVEDVi- left ventricular end diastolic volume index, LV- left ventricle, SV- stroke volume, LVEF- left ventricular ejection fraction, E/e'- ratio of early diastolic mitral inflow velocity to early diastolic mitral annulus velocity. Continuous variables expressed as mean (±SD)

	Controls (n = 26)	**Post COVID (n =87)**	**p-value (unpaired t test)**
LVEDV (ml)	180 (±29.5)	173 (±41)	0.41
LVEDVi (ml/m^2)	89 (±16)	82 (±17)	0.06
LV SV (ml)	108 (±11)	104 (±25)	0.50
LVEF (%)	61 (±5)	60 (±5)	0.47
LV mass (g)	125 (±20)	129 (±27)	0.46
LV mass index (g/m^2)	65 (±9)	61 (±11)	0.11
E/e' rest	7.2 (±1.9)	6.6 (±1.6)	0.08
E/e' stress	7.6 (±1.9)	8.0 (±2.3)	0.41
Change in E/e'	0.37 (±2.9)	1.48 (±2.7)	**0.03**

When the post SARS-Cov-2 group is separated by severity, the hospitalised group demonstrate differences in left ventricular volumes in comparison with both the control group and the community illness group. Left ventricular end diastolic volume in the hospitalised group was smaller than in the community group (156ml±30 vs. 184 ml±43, p=0.002) and in the COVID negative control group (181ml±30, p=0 04), with those trends still present after indexing to body surface area. Left ventricular stroke volume was also lowest in the hospitalised group in comparison with both groups (94ml ±19 vs. controls 108ml ±11, p=0. 03, and vs. community 111ml ±26, p=0.001).

The differences seen in the change in diastolic function as measured by average E/e' were not seen when comparing the smaller numbers in the severity groups, as was seen in the post SARS-CoV-2 group as a whole.

Table 4.2. Left ventricular size and function by severity. LVEDV- Left ventricular end diastolic volume, LVEDVi- left ventricular end diastolic volume index, LV- left ventricle, SV- stroke volume, LVEF- left ventricular ejection fraction, E/e'- ratio of early diastolic mitral inflow velocity to early diastolic mitral annulus velocity. Variables expressed as mean (SD)

	Controls (n = 26)	**Community (n = 52)**	**Hospital (n =35)**	**p-value (one-way ANOVA w Bonferroni)**		
				Control v Community	**Control v Hospital**	**Community v Hospital**
LVEDV (ml)	181 (±30)	184 (±43)	156 (±30)	>0.9	**0.04**	**0.002**
LVEDVi (ml/m^2)	89 (±16)	87 (±17)	73 (±13)	>0.9	**0.0005**	**0.0002**
LV SV (ml)	108 (±11)	111 (±26)	94 (±19)	>0.9	**0.03**	**0.001**
LVEF (%)	61 (±5)	61 (±5)	61 (±5)	>0.9	>0.9	>0.9
LV mass (g)	125 (±20)	130 (±29)	129 (±22)	>0.9	>0.9	>0.9
LV mass index (g/m^2)	65 (±9)	62 (±13)	59 (±9)	0.79	0.19	0.94
E/e' rest	7.2 (±1.9)	6.8 (±1.8)	6.3 (±1.5)	0.77	0.11	0.66
E/e' stress	7.6 (±1.9)	8.1 (±2.3)	7.8 (±2.3)	>0.9	>0.9	>0.9
Change in E/e'	0.37 (±2.9)	1.63 (±2.6)	1.26 (±2.8)	0.19	0.65	>0.9

4.4.2 The impact of SARS-CoV-2 on right ventricular size and function

In the post SARS-CoV-2 participants, the indexed right ventricular end diastolic volume is smaller than in the healthy controls (84ml/m^2 ±20 vs. 99ml/m^2 ±13, p=0.0004), but the difference is not seen in non-indexed volumes. There was no difference between groups in right ventricular systolic function or stroke volume. Changes in right ventricular longitudinal function with exercise were measured using the difference in tricuspid annular plane systolic excursion (TAPSE) at rest and exercise stress. There was a large difference in the change in RV longitudinal function in the control population in comparison to the post SARS-CoV-2 participants (2.8cm ±6.3 vs. 0.72cm ±1.9, p=0.01).

Table 4.3. Right ventricular size and function RVEDV- right ventricular end diastolic volume, RVEDVi- right ventricular end diastolic volume index, RV- right ventricle, SV- stroke volume, RVEF- right ventricular ejection fraction, TAPSE- tricuspid annular plane systolic excursion. Continuous variables expressed as mean (SD)

	Controls (n = 26)	**Post COVID (n =87)**	**p-value (unpaired t test)**
RVEDV (ml)	194 (±30)	179 (±46)	0.11
RVEDVi (ml/m^2)	99 (±13)	84 (±20)	***0.0004***
RV SV (ml)	109 (±11)	103 (±25)	0.23
RVEF (%)	57 (±5)	58 (±5)	0.23
Change in TAPSE	2.8 (±6.3)	0.72 (±1.9)	***0.01***

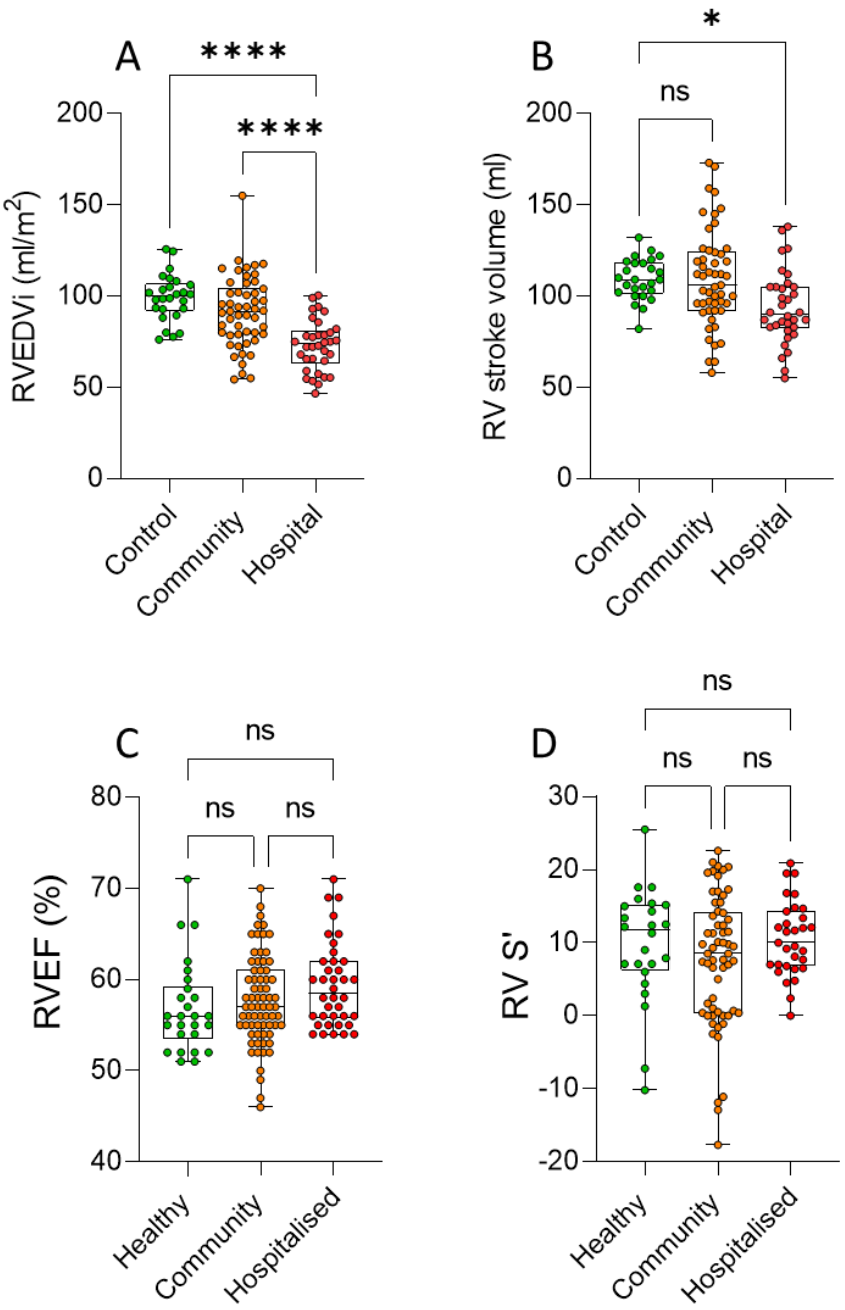


Figure 4.2 Right ventricular size and function by severity. A) RVEDVi- right ventricular end diastolic volume index, B) RVSV- stroke volume, C) RVEF- left ventricular ejection fraction, D) RV S by echocardiography

When the post-COVID group is divided by disease severity, further differences can be seen. Both indexed and non-indexed right ventricular end-diastolic volumes demonstrate a trend of decreasing

size with COVID-19 disease severity (RVEDVi controls 99±13 ml/m^2 vs community 91±19 ml/m^2, vs hospitalised 73±14 ml/m^2, all comparisons $p<0.0001$). The difference between the control and community managed populations was not significantly different. Although no difference was seen in RV stroke volume between all post SARS-CoV-2 participants, there were changes seen between those who were treated in hospital and those managed in the community (93ml ±20 vs 110ml ±26, $p=0.003$), and the COVID negative controls (109ml ±11, $p=0.02$).

Table 4.4 Right ventricular size and function by severity RVEDV- right ventricular end diastolic volume, RVEDVi- right ventricular end diastolic volume index, RV- right ventricle, SV- stroke volume, RVEF- right ventricular ejection fraction, TAPSE- tricuspid annular plane systolic excursion. Continuous variables expressed as mean (SD)

				p-value (one-way ANOVA w Bonferroni)		
	Controls (n = 26)	**Community (n = 52)**	**Hospital (n =35)**	**Control v Community**	**Control v Hospital**	**Community v Hospital**
RVEDV (ml)	194 (±30)	193 (±47)	158 (±34)	>0.9	***0.002***	***0.0005***
RVEDVi (ml/m^2)	99 (±13)	91 (±19)	73 (±14)	0.16	***<0.0001***	***<0.0001***
RV SV (ml)	109 (±11)	110 (±26)	93 (±20)	>0.9	***0.02***	***0.003***
RVEF (%)	57 (±5)	57 (±5)	60 (±4)	>0.9	0.11	0.11
Change in TAPSE	2.8 (±6.3)	0.3 (±0.8)	1.3 (±2.9)	***0.01***	0.36	0.64

4.4.3 Myocardial inflammation and fibrosis post SARS-CoV-2

Global native T1 values are elevated post SARS-CoV-2 in comparison with healthy controls (1177ms ±31 vs. 1159ms ±22, P=0.01), with no difference between the community and hospitalised groups (1179ms ±30 vs. 1178ms ±25, p>0.9). However, all group values are within the normal range for the 3T MR system used (1114-1222).

There was no significant difference in the extra cellular volume fraction between any groups.

Table 4.5. Measures of tissue characterisation Continuous variables expressed as mean (SD)

	Controls (n = 26)	**Post COVID (n =87)**	**p-value (unpaired t test)**
Global native T1 (ms)	1159 (±22)	1177 (±31)	**0.01**
Extracellular volume fraction (%)	0.27 (±0.02)	0.26 (±0.03)	0.29

Table 4.6. Tissue characterisation by severity; continuous variables expressed as mean (SD)

	Controls (n = 26)	**Community (n = 52)**	**Hospital (n =35)**	**p-value (one-way ANOVA w Bonferroni) Control v Community**	**Control v Hospital**	**Community v Hospital**
Global native T1 (ms)	1159 (±22)	1179 (±30)	1178 (±25)	***0.01***	***0.03***	>0.9
Extracellular volume fraction (%)	0.27 (±0.02)	0.26 (±0.02)	0.27(±0.05)	>0.9	>0.9	>0.9

4.4.4 Myocardial energetics at rest and catecholamine stress

No differences were seen in the PCr/ATP ratio at rest or with dobutamine stress between the healthy controls and the post SARS-CoV-2 group, either as a whole or when analysed by severity.

Table 4.7. Myocardial energetics at rest and with dobutamine stress PCr- Phosphocreatine, ATP- adenosine triphosphate. Continuous variables expressed as mean (SD)

	Controls (n = 26)	**Post COVID (n =87)**	**p-value (unpaired t test)**
PCr/ATP rest	2.23 (0.3)	2.33 (±0.3)	0.17
PCr/ATP stress	2.15 (±0.3)	2.06 (±0.4)	0.33
Δ PCr/ATP	-0.18 (±0.8)	-0.25 (±0.7)	0.69

Additionally, there was no difference in the size of change between rest and stress of the PCr/ATP ratio.

Table 4.8. Myocardial energetics at rest and with dobutamine stress by severity PCr- Phosphocreatine, ATP- adenosine triphosphate. Continuous variables expressed as mean (SD)

	Controls (n = 26)	**Community (n = 52)**	**Hospital (n =35)**	**p-value (one-way ANOVA w Bonferroni)**		
				Control v Community	**Control v Hospital**	**Community v Hospital**
PCr/ATP rest	2.2 (±0.3)	2.2 (±0.3)	2.3 (±0.3)	0.24	>0.9	0.59
PCr/ATP stress	2.2 (±0.3)	2.0 (±0.4)	2.1 (±0.4)	0.77	>0.9	>0.9
Δ PCr/ATP	-0.2(±0.8)	-0.2 (±0.7)	-0.4 (±0.7)	>0.9	>0.9	0.81

4.5 Correlation of Left Ventricular Parameters with Exercise Performance Metrics

In order to understand the relationship between LV parameters and exercise performance a correlation matrix was performed (Figure 4.3 and Table 4.9). This showed that Peak $\dot{V}O_2$, OUES and $\dot{V}E/\dot{V}CO_2$ were positively correlated with LVEDVi, LVESV, LVSV and LV mass index (all $p < 0.01$) and with the exception of OUES, negatively with LVEF (all $p<0.01$). While stress energetics were related to stress diastolic function, neither were correlated with peak $\dot{V}O_2$ indices.

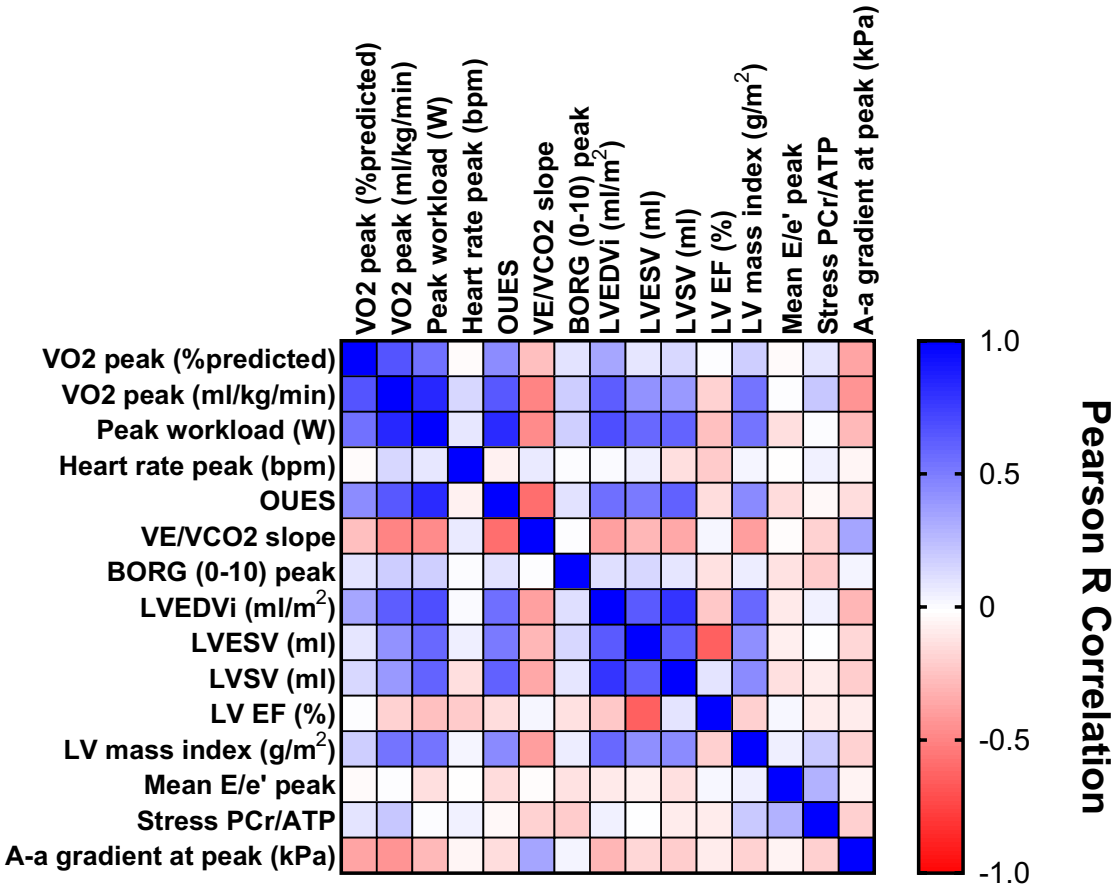


Figure 4.3 - A Visual Correlation Matrix of CPET and LV parameters

Table 4.9 - A Pearson R Correlation Matrix of CPET and Left Ventricular parameters with significance p<0.001, **p<0.05**

Correlations	VO2 peak (%)	VO2 peak	Peak work (W)	Peak HR	OUES	VE/VCO2	BORG peak	LVEDVi	LVESV	LVSV	LV EF	LV mass index	Mean E/e' peak	Stress PCr/ATP	A-a peak
VO2 peak (%)	1.000														
VO2 peak	0.670	1.000													
Peak work (W)	0.556	0.846	1.000												
Peak HR (bpm)	-0.017	0.156	0.093	1.000											
OUES	0.448	0.653	0.833	-0.057	1.000										
VE/VCO2 slope	-0.255	-0.484	-0.457	0.080	-0.572	1.000									
BORG (0-10) peak	0.109	**0.193**	0.184	0.009	0.111	0.008	1.000								
LVEDVi (ml/m^2)	0.349	0.635	0.692	0.020	0.561	-0.376	0.124	1.000							
LVESV (ml)	0.096	0.426	0.592	0.061	0.521	-0.286	0.156	0.643	1.000						
LVSV (ml)	0.149	0.398	0.609	-0.128	0.616	-0.343	0.096	0.794	0.629	1.000					
LV EF (%)	0.006	-0.180	-0.251	-0.204	-0.137	0.032	-0.118	-0.217	-0.624	0.105	1.000				
LV mass index	**0.192**	0.544	0.542	0.036	0.456	-0.384	0.070	0.589	0.435	0.451	-0.184	1.000			
Mean E/e' peak	-0.020	0.005	-0.128	-0.007	-0.140	-0.016	-0.116	-0.086	-0.065	-0.122	0.029	0.062	1.000		
Stress PCr/ATP	0.104	**0.218**	0.009	0.054	-0.031	-0.180	-0.203	0.052	0.002	-0.078	-0.075	**0.211**	0.299	1.000	
A-a peak (kPa)	-0.358	-0.421	-0.276	-0.042	-0.137	0.349	0.041	-0.288	**-0.159**	-0.199	-0.075	-0.177	-0.050	-0.186	1.000

4.5.1 Correlation of Right Ventricular Parameters with Exercise Performance Metrics

A correlation matrix between the RV parameters and exercise performance metrics was performed to assess the relationship between the two (Figure 4.4 and Table 4.10). In this analysis, peak $\dot{V}O_2$, and peak workload are positively correlated with RVEDVi, RVESV and RVSV, but there is no correlation involving $\dot{V}E/\dot{V}CO_2$ or A-a gradient and the RV parameters.

Figure 4.4 - A Visual Correlation Matrix of CPET and RV parameters

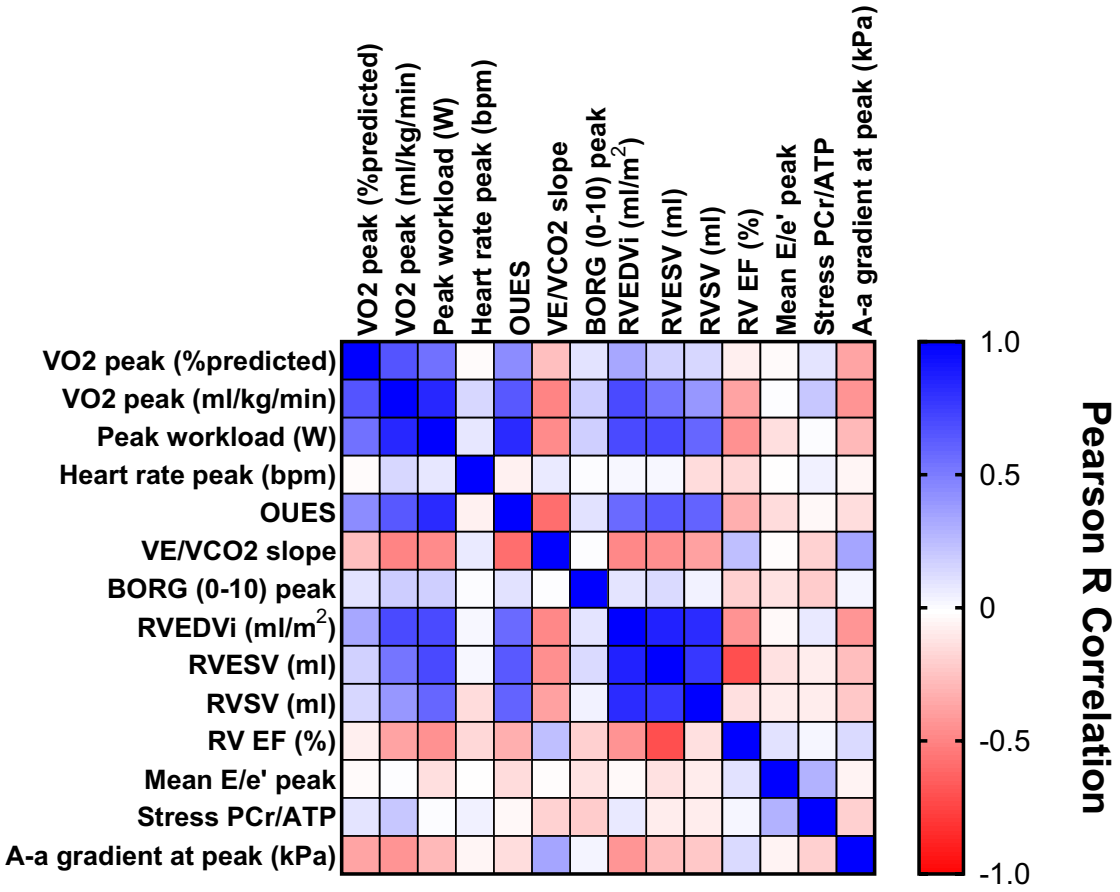


Table 4.10 - A Pearson R Correlation Matrix of CPET and Right Ventricular parameters with significance p<0.001, **p<0.05**

Correlations	VO2 peak (%)	VO2 peak	Peak work (W)	Peak HR	OUES	VE/VCO2	BORG peak	RVEDVi	RVESV	RVSV	RV EF	Mean E/e' peak	Stress PCr/ATP	A-a peak
VO2 peak (%)	1.000													
VO2 peak	0.670	1.000												
Peak work (W)	0.556	0.846	1.000											
Peak HR (bpm)	-0.017	0.156	0.093	1.000										
OUES	0.448	0.653	0.833	-0.057	1.000									
VE/VCO2 slope	-0.255	-0.484	-0.457	0.080	-0.572	1.000								
BORG (0-10) peak	0.109	**0.193**	0.184	0.009	0.111	0.008	1.000							
RVEDVi (ml/m^2)	0.345	0.706	0.706	0.029	0.582	-0.467	0.103	1.000						
RVESV (ml)	0.180	0.538	0.713	0.029	0.649	-0.441	0.143	0.870	1.000					
RVSV (ml)	0.156	0.405	0.600	-0.140	0.612	-0.371	0.048	0.831	0.783	1.000				
RV EF (%)	-0.064	-0.364	-0.435	-0.154	-0.316	**0.248**	-0.186	-0.426	-0.691	-0.122	1.000			
Mean E/e' peak	-0.020	0.005	-0.128	-0.007	-0.140	-0.016	-0.116	-0.025	-0.121	-0.075	0.113	1.000		
Stress PCr/ATP	0.104	**0.218**	0.009	0.054	-0.031	-0.180	-0.203	0.086	-0.073	-0.074	0.034	0.299	1.000	
A-a peak (kPa)	-0.358	-0.421	-0.276	-0.042	-0.137	0.349	0.041	-0.417	-0.257	**-0.218**	**0.143**	-0.050	-0.186	1.000

4.5.2 Cardiac predictors of Exercise performance in Post COVID syndrome

To assess the major determinants of exercise capacity, a stepwise multiple linear regression model was performed. This multivariate model consisted of peak $\dot{V}O_2$ as the dependent variable and of independent variables that had a significant relation with peak $\dot{V}O_2$ in the simple linear regression analysis above. To limit collinearity variables with a variance inflation factor of > 4 were removed from the model. Multivariate analysis revealed LVEDVi ($\beta = 0.4$, $p < 0.001$) along with A-a gradient at stress ($\beta = -1.1$ $p = 0.023$), and $\dot{V}E/\dot{V}CO_2$ slope ($\beta = -0.3$, $p = 0.04$), as the main independent predictors of peak $\dot{V}O_2$ predicted (overall $R^2 = 0.55$, $p<0.001$).

4.6 Discussion

Overall, this chapter demonstrates that the changes in cardiac size and function seen in some of the general hospitalised patient populations which have been reported are not seen in the UK Armed Forces population. When comparing all post COVID-19 subjects with controls, there were no changes between the left ventricular volumes or systolic function. When analysed by subgroup, there are reductions in left ventricular end diastolic volumes and left ventricular systolic volumes, with the severe illness, hospitalised groups having smaller volumes than the community or the control cohorts. There are no changes in LV mass or systolic function as measured by LV ejection fraction. In the US military study, a reduction in LV mass was seen, and in other studies which look at patients with illness severe enough for hospitalisation, the main finding was an increase in LVEDV, and a reduction in systolic function.

Looking at the changes in the hospital group, it is interesting to note that despite the difference, the values remain in the normal range. The mean peak $\dot{V}O_2$ was over 100% of the predicted, whereas in previous studies it is reported to lie between 74-89% [108,163]. Considering that this group were in hospital for their treatment it is reasonable to assume that they were in bed or sedentary for the majority of the day. We already know that endurance sport and frequent training leads to volume loading and causes chamber dilatation, and it may be that in this case the reduction in LV size

represents a reverse remodelling of a previously trained ventricle [164-166]. Alternatively, as obesity is an independent risk factor for severe COVID-19 illness, it is possible that these differences do not represent a change following infection, but rather, are a difference relating to increased baseline exercise training in the control and mild disease groups, in comparison to the group who were hospitalised.

Sub-clinical cardiovascular changes have been reported in several studies including both community treated and hospitalised patients post-COVID-19. The most commonly reported changes are elevated native T_1 and subclinical reductions in LV ejection fraction and strain [167]. In this study, prior COVID-19 infection was associated with reduced LV and RV cavity size, reduced stroke volumes, and elevated T1 time, suggesting a mild degree of residual inflammation.

This is in keeping with previous studies in the military population where a trend to reduced LV cavity size was also seen[91]. It is reported that 3 weeks following COVID 19 infection in athletes, when compared to athletes without prior COVID 19, that there is reduced LVEDV and elevated T2 time (suggesting oedema) [168]. In addition, as LVEDV, RVEDV and LVSV were all correlated with peak $\dot{V}O_2$ in this study, it is likely that reduced chamber volumes are implicated in the reduced exercise performance seen in the post COVID group. Furthermore, the observed stepwise trend to lower LV mass index when comparing control to community to hospitalised infection would support this.

There were more marked changes in the right ventricular volumes. When all COVID-19 subjects were compared with controls, the indexed right ventricular end diastolic volume (RVEDVi) was smaller. When analysed by subgroup, the difference which reached significance was between the hospitalised, severe illness group, and the mild infection and control groups.

In addition to smaller diastolic volumes, the right ventricular stroke volume was also lower. One interpretation of this finding could be that COVID-19 infection reduced the size of the RV due to an underlying mechanism of the disease. However, reduced right sided volumes are not seen in the general population studies, where, as with the left ventricle, volumes generally increased. As COVID-

19 is primarily a respiratory illness and causes widespread inflammatory changes in the most serious cases, the subsequent fibrotic changes result in a prolonged increase in the pulmonary vascular pressures. This pressure change leads to a pressure loaded right ventricle which dilates over time [169,170]. Though present in the general population studies, this is not seen in the majority of the athlete studies, nor in the US military study [91].

The changes are also not explained by the difference in body size between the groups. In cases of obesity cardiomyopathy, it is more common to find increased right ventricular size and diastolic dysfunction, whereas we did not see this in either of the post COVID-19 sub-groups, who both had a higher average BMI than the control population[171]. Given that the difference is predominantly seen in the severe disease group who required hospital care, it may not be the pathophysiology of the viral illness, so much as that the severity of the infection required these subjects to take a period of bedrest. As with the left ventricle, right ventricular remodelling is seen in athlete populations and can be reversed by a period of detraining (or bedrest)[172].

Although it is unlikely that all the subjects in the hospitalised group undertake elite level amounts of exercise on a regular basis, the detraining mechanism would explain the findings, and also support the findings in Chapter 3 which suggest deconditioning as a mechanism for the difference in exercise capacity. This would particularly be the case if the control group demonstrated evidence of a training effect. As with the left ventricle, despite the differences seen, all values remain within the normal clinical range, regardless of severity of infection.

Whilst there were no differences seen in the baseline diastolic function between the groups, the change in E/e' measurement with exercise was higher in the control group in comparison to the post COVID-19 group. A dynamic increase in E/e' with exercise has been used as a technique to identify patients with heart failure and a preserved ejection fraction [173]. Impairment in diastolic function can occur prior to impairment in systolic function, or as an isolated finding. It is related to the relaxation of the ventricular myocardium during diastole and can be associated with fibrotic changes in the

tissue. Such fibrotic changes can be seen as maladaptive mechanisms in response to an insult, including from inflammation[174]. A change in E/e' with exercise was seen in another COVID-19 study, which found a change in diastolic measurements with exercise from the normal range to the abnormal range in patients who were 6 months post COVID-19 [175]. Although the trend seen in changes to exercise diastolic function agrees with the wider literature, our data do not reach the threshold values for abnormal diastolic function.

This study has also revealed a measurable difference in native global T1 values, when the control group is compared with all post COVID subjects, and when each severity group is compared with the controls separately. However, despite the changes, the values obtained still fall within the normal values reported for our centre [176]. This echoes findings from other studies at our centre who have looked at post-COVID hospital populations and the experiences of other centres who have used native T1 mapping [86,88]. Native T1 mapping is a validated clinical tool used to identify diffuse tissue fibrosis and inflammation not evident from other techniques, such as late gadolinium enhancement. An increase in the native T1 value which still falls within the normal range is suggestive of sub-clinical inflammation of the myocardial tissues in the post-COVID subjects, which falls short of the threshold for a diagnosis of fibrosis. In contrast with the non-military studies, where non-ischaemic fibrosis from myocarditis and perimyocarditis was a headline finding, the quantitative measure of myocardial fibrosis we have investigated, the extracellular volume, demonstrates no change, so any differences which may be present at group level are too subtle to demonstrate using this technique.

Previous studies which have demonstrated a difference with catecholamine stress on groups with heart failure of other aetiologies have been relatively homogeneous in comparison to this population. As COVID-19 is a novel virus and myocardial energetics have not been tested in this population before, it is possible that this is an underpowered sample, which might explain why no change was detected. Alternatively, as the markers of fibrosis (native T1 and ECV) have changed either minimally or not at all, it is possible that in this population the cardiac changes are too small to confer a meaningful change

to cardiac metabolism, and thus no differences were seen. Given that the native T1 changes and the exercise diastolic changes are all still within the normal range, it is likely that this is the case.

4.7 Limitations

At the time this study was conceived, early published experiences of clinical consequences of COVID-19 suggested that myocarditis would be a significant pathology of concern in the health outcomes for patients[86]. Internationally recognised guidelines recommend tissue characterisation of myocarditis using a CMR protocol which includes oedema sensitive T2 weighted imaging[177]. However, owing to resource constraints, namely the time available to complete all the study investigations, we were unable to add T2 weighted images to the protocol.

Although the control group is well matched in terms of age, gender and employment group, they are not matched for body size. As overall athleticism and bodyweight are potential confounders for the changes we see in this chapter, it would have been ideal to have pre-study cardiac imaging available to compare the changes against the patient population group.

4.8 Conclusion

In this study we have found that previous COVID-19 is associated with some structural changes in cardiac volumes and diastolic function. There are also signals to suggest that there remains a degree of sub-clinical myocardial inflammation as seen in the elevated T1 values. However, this does not translate into substantial scar formation and differences in the extracellular volume, and it also doesn't affect the myocardial energetics, either at rest or with dobutamine stress. The most likely explanation for these changes is deconditioning, given that the control group have upper limit of normal chamber volumes, and the trend of reducing ventricular volumes across the severity of disease would not be explained by pressure overload of either the right or the left sides, and it is not explained by the response of the cardiovascular system to obesity. It is, however, recognised in studies of detraining athletes. Given that we have already seen that there is a difference in the total exercise

capacity and workload achieved following COVID-19, these changes are most likely a consequence of this, rather than a limiting factor causing that reduced exercise capacity.

In the next chapter I will look at the other possible system which could contribute to the changes seen in exercise capacity. As COVID-19 is primarily a respiratory illness with multisystem effects, it may be that an underlying cause for the differences is seen in the lungs.

Chapter 5- The Effect of SARS-CoV-2 on Pulmonary Function

5.1 Introduction

It is clear from Chapter 4, that not only is exercise capacity reduced, but also that symptoms of shortness of breath in military personnel with post COVID syndrome are commonplace. As occupational deployment can increase exposure to respiratory insults including both hyper- and hypobaric environments,[123-125] there is an increased risk of hypoxia. Moderate and high intensity exertion in a respirator, acceleration induced alterations in pulmonary blood flow, humidity changes from recycled cabin air, rapid pressure changes from decompression and exposure to a range of chemical and environmental lung irritants are all additional potential hazards for the respiratory system which are not seen in the general population[126].

As such, it is imperative that the consequences of COVID 19 are documented and understood in this population. A permanent impairment of lung function has potential serious consequences for safe deployment of Defence personnel in austere environments.

In this chapter, I have used lung function testing, in combination with arterial blood gas sampling to explore the effect of SARS-CoV-2 infection on respiratory function in members of the UK Armed Forces. In addition, I examine the relationship with exercise capacity.

5.2 Methods

Detailed methods for the study can be found in Chapter 2. The study received favourable opinion from the Ministry of Defence Research Ethics Committee (MODREC- ref: 1061/MODREC/20) and complied with the Declarations of Helsinki. Volunteer participants were the same as those who took part in previous chapters and were recruited from the Defence COVID Recovery Service, or from local military units.

Inclusion criteria for the post SARS-CoV-2 group were: previous SARS-CoV-2 infection, with either a positive COVID-19 antigen PCR test at the time of their acute illness, or clinically adjudicated COVID-19, at least 4 weeks post their acute infection, and negative COVID-19 antigen PCR test at the time of enrolment to the study. The Control group inclusion criteria were: no previous suspicion of acute COVID-19 infection and negative COVID-19 anti-nuclear antibody test at the time of enrolment to the study. Exclusion criteria were a past medical history of significant cardiac or pulmonary disease, active infection at the time of recruitment, pregnancy and contraindication to MRI scanning, as detailed in Chapter 2. Additionally, the control group were excluded if they contracted COVID-19 during the study period.

All pulmonary function testing was performed using a Vyntus ONE Pulmonary Function System (Vyaire Medical Inc, Illinois, USA). Participants underwent the following investigations at their baseline visit and at 6 months post baseline:

Forced spirometry flow-volume loops were measured according to ATS/ERS 2005 spirometry guidelines[178]. Diffusion capacity of carbon monoxide and alveolar volume were performed using the Single Breath Method on the Sentry Suite 'Diffusion SB Realtime' program (Vyaire Medical Inc, Illinois, USA) using a methane/tracer gas mix. The software program meets the quality criteria described in the ATS/ERS 2017 guidelines[179]. Static lung volumes were measured using the nitrogen washout method [180] Measurements were collected as raw data and also expressed as a percentage of the predicted value using reference equations in the Sentry Suite program which account for sex, age and height.

5.2.1 Statistics

Statistical analysis was performed using commercial software (IBM SPSS 27, Chicago, USA, and GraphPad Prism 9, San Diego, USA). Data are presented as mean ± standard deviation. Normality was confirmed using Kolmogorov-Smirnov testing. A Chi squared test was used to compare nominal data. Continuous data were compared using an independent t-test for baseline data and a paired t-test

when comparing baseline with follow up data. When comparing more than two groups, one-way ANOVA with post hoc Bonferroni correction was used. A p value of <0.05 was considered significant. The effects of disease severity were also tested using Jonkheere-Terpstra test for ordered medians (order defined as control>community infection> hospitalisation for infection). A p-value of 0.05 (2-tailed) was taken to indicate statistical significance. To assess the major determinants of exercise capacity, a stepwise multiple linear regression model was performed. This multivariate model consisted of peak $\dot{V}O_2$ (% predicted) as dependent variable and of independent variables that had a significant relation with peak $\dot{V}O_2$ (% predicted) in the simple linear regression analysis. To limit collinearity variables with a variance inflation factor of > 4 were removed from the model.

5.3 Results

108 volunteers underwent initial pulmonary function testing. 83 volunteers were post SARS-CoV-2 infection (50 managed during their acute illness in the community, and 33 in hospital (severe disease)) and 25 were COVID negative healthy controls. Groups were matched for age (SARS-CoV-2 group 38 years ± 8 vs. controls 39 years ±10, p=0.7) and sex (86% male vs. 88% male, p=0.5), and all were members of the UK Armed Forces. 81 volunteers underwent repeat testing after 6 months (16 COVID negative healthy controls, 37 subjects who had COVID managed in the community and 28 who were managed in hospital). The primary reasons for dropout were non-attendance of post-COVID participants and, in the case of the COVID negative healthy controls, contracting SARS-CoV-2 and becoming ineligible. A small number were unable to undergo repeat pulmonary function testing due to equipment failure.

5.3.1 The impact of SARS-CoV-2 on pulmonary function at baseline measurement

There was no difference measured in the Forced Vital Capacity (FVC) between post SARS-CoV-2 patients and healthy controls when raw numbers at baseline measurement were compared. However, when corrected for predicted values, the FVC in the post SARS-CoV-2 group was lower than the control group (100.8% predicted ±12.9 vs. 108.6% predicted ±12.2, p=0.008). The Forced

Expiratory Volume in 1 second (FEV1) was lower in subjects post COVID, when compared to healthy controls. This was the case for both the raw values (3.9L ±0.7 vs. 4.21L ±0.6, p=0.04) and for corrected values (95% predicted ±12.3 vs. 102.5% predicted ±14, p=0.01). There was no between-group difference in FEV1/FVC ratio.

Total Lung Capacity (TLC) was reduced in the post SARS-CoV-2 group in comparison to the healthy control group, both uncorrected (5.97L ±1.3 vs. 6.59L ±1, p=0.03) and when corrected (85.6% predicted ±13.8 vs. 94.5% predicted ±14.4, p=0.006). Diffusion capacity of the lungs for carbon monoxide (DLCO) was reduced in the post SARS-CoV-2 group in comparison to the healthy control group. This was the case for both the raw values (8.91min/kPa ±2.3 vs. 10.04min/kPa ±1.37, p=0.02) and for corrected values (86.7% predicted ±17.3 vs. 97.4% predicted ±8.8, p=0.004).

Alveolar volume (VA) was also reduced in the post SARS-CoV-2 group in comparison to healthy controls, in both the measured (5.70L ±1.3 vs. 6.45L ± 0.8, p=0.006) and corrected values (86.5% predicted ±15.4 vs. 98.2% predicted ±10.9, p=0.0006). No difference was found in the CO transfer coefficient (KCO) between groups.

Table 5.1. Pulmonary function testing at baseline visit. Continuous variables expressed as mean (SD)

	Controls (n =25)	**Post COVID (n =83)**	**p-value**
FVC (L)	5.52 (±0.7)	5.09 (±1.0)	0.05
FVC (% pred)	108.6 (±12.2)	100.8 (±12.9)	**0.008**
FEV1 (L)	4.21 (±0.6)	3.9 (±0.7)	**0.04**
FEV1 (% pred)	102.5 (±14.0)	95 (±12.3)	**0.01**
FEV1/FVC	76.3 (±5.3)	76.8 (±5.9)	0.7
TLC (L)	6.59 (±1.0)	5.97 (±1.3)	**0.03**
TLC (% pred)	94.5 (±14.4)	85.6 (±13.8)	**0.006**
DLCO (min/kPa)	10.04 (±1.37)	8.91 (±2.3)	**0.02**
DLCO (% pred)	97.4 (±8.8)	86.7 (±17.3)	**0.004**
VA (L)	6.45 (±0.8)	5.70 (±1.3)	**0.006**
VA (% pred)	98.2 (±10.9)	86.5 (±15.4)	**0.0006**
KCO (min/kPa/L)	1.56 (±0.1)	1.57 (±0.2)	0.7
KCO (% pred)	99.1 (±8.5)	100.5 (±14.9)	0.6

FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second, TLC- Total Lung Capacity, DLCO- Diffusing capacity of the lungs for carbon monoxide, VA- alveolar volume, KCO-Carbon monoxide transfer coefficient. %pred- percent predicted.

When the post SARS-Cov-2 group is separated by severity, the hospitalised group generally demonstrate larger reductions in pulmonary function measures than subjects who were managed in the community. Significant differences in function measurements were seen from the community to the hospital groups for FVC (5.33L ±1.0 vs. 4.72 ±0.9, p=0.01), TLC (6.27L ±1.4 vs. 5.5L ±1.02, p=0.02), DLCO (9.49min/kPa ±2.4 vs. 8.02min/kPa ±1.7, p=0.004) and VA (6.05L ±1.2 vs. 5.17L ±1.3, p=0.003). No differences between these groups were seen in FEV1 or KCO. Between the control population and the community managed subjects, many variables were not significantly different. Only FEV1 when expressed as a percentage of the predicted value was significantly reduced in the community group

vs. the control group (94.3% predicted ± 10.9 vs. 102.5% predicted ± 14.0, p= 0.03). It is important to note that both of these values lie comfortably inside the normal clinical range.

KCO values remained unchanged across all severity groups.

5.3.2 Correlation of Lung Function Tests with Exercise Performance Metrics

To understand the relationship between lung function tests and exercise performance a correlation matrix was performed (Figure 5.1 and Table 5.2). There was a strong correlation between Peak workload and TLC, FEV1, FVC, VA and DLCO. Peak $\dot{V}O_2$ (% predicted) was not correlated with resting lung function parameters. There was a weak inverse correlation between peak workload and KCO. A relationship was identified between OUES and TLC, FEV1, VA and DLCO as might be anticipated.

Figure 5.1 A heat map Correlation matrix of Lung Function Tests against CPET parameters

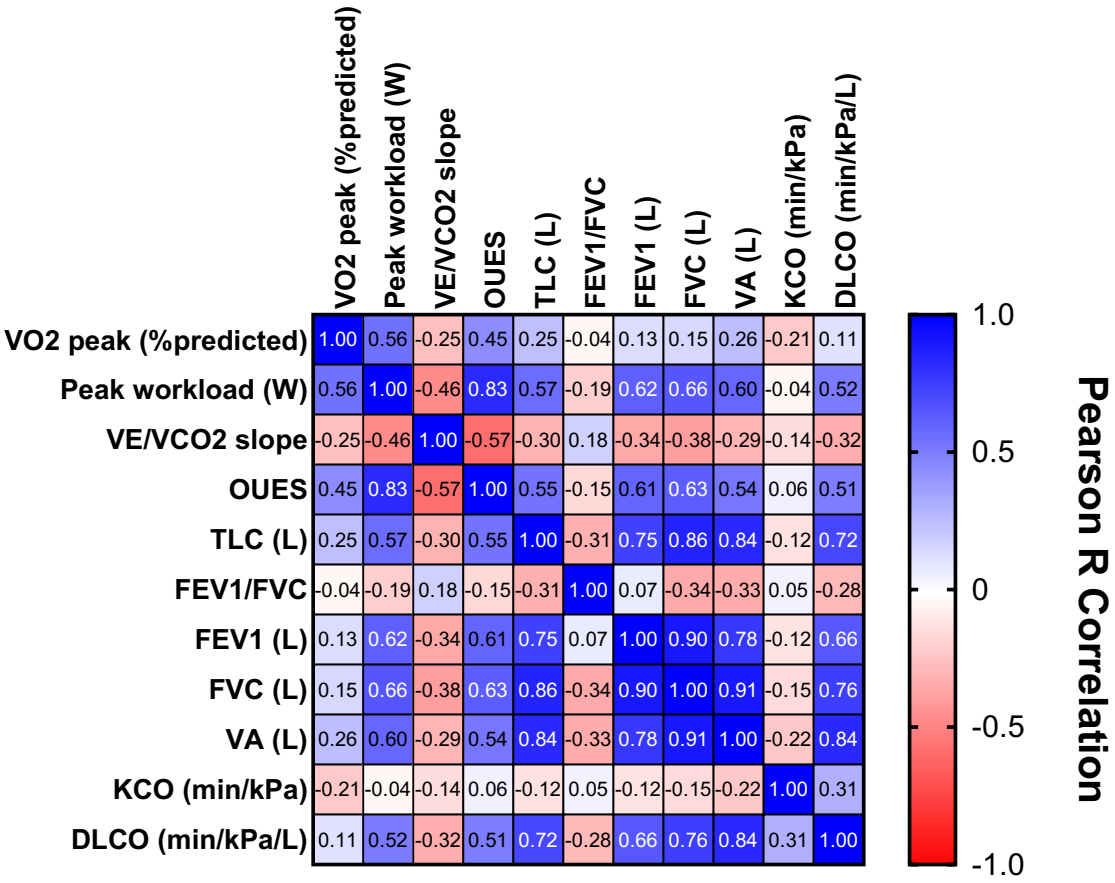


Table 5.2 A Pearson R Correlation Matrix of CPET and Lung Function Test parameters with significance p<0.001, **p<0.05**

Correlations	VO2 peak (%)	VO2 peak	Peak work (W)	VE/VCO2	OUES	TLC (L)	FEV1/FVC	FEV1 (L)	FVC (L)	VA (L)	KCO	DLCO
VO2 peak (%)	1.000											
VO2 peak	0.670	1.000										
Peak work (W)	0.556	0.846	1.000									
VE/VCO2 slope	**-0.255**	-0.484	-0.457	1.000								
OUES	0.448	0.653	0.833	**-0.572**	1.000							
TLC (L)	**0.251**	0.432	0.574	-0.304	0.548	1.000						
FEV1/FVC	-0.035	-0.143	**-0.193**	0.182	-0.154	**-0.309**	1.000					
FEV1 (L)	0.135	0.517	0.624	-0.344	0.606	0.748	0.075	1.000				
FVC (L)	0.154	0.520	0.660	-0.382	0.634	0.862	-0.335	0.902	1.000			
VA (L)	0.259	0.505	0.595	**-0.292**	0.536	0.841	**-0.329**	0.783	0.906	1.000		
KCO (min/kPa/L)	**-0.214**	-0.123	-0.037	-0.137	0.057	-0.121	0.055	-0.117	-0.148	**-0.219**	1.000	
DLCO (min/kPa)	0.114	0.389	0.517	**-0.322**	0.507	0.719	**-0.275**	0.658	0.756	0.841	**0.308**	1.000

5.3.3 Predictors of reduced exercise capacity

To assess the predictors of reduced exercise capacity a stepwise multiple linear regression analysis of all exercise and pulmonary data was performed across all the participants. This analysis draws data from the previous two chapters. This multivariate model consisted of peak $\dot{V}O_2$ (% predicted) as the dependent variable and of independent variables that had the most significant relationships with peak $\dot{V}O_2$ (% predicted) in the previous simple linear regression analysis (BMI, TLC, Va, LVEDVi, LVSV, A-a gradient at stress, Oxygen Uptake Efficiency Slope (OUES) and $\dot{V}O_2$/W slope). To limit collinearity variables with a variance inflation factor of >4 were again removed from the model. Multivariate analysis revealed LV stroke volume (β = -0.51 p = 0.001), LVEDVi (β = 0.50, p = 0.012), A-a gradient at stress (β = -3.53 p = 0.003), OUES (β = 11.2, p = 0.012) and $\dot{V}O_2$/W slope (β = 4.9, p = 0.03), as the main independent predictors of peak $\dot{V}O_2$ % predicted (overall R^2 = 0.43, p<0.001).

5.3.4 Correlation of Lung Function Tests and Right ventricular size and function

A correlation matrix was performed to explore the relationship between right ventricular size and function and lung function test parameters. (Figure 5.2 and Table 5.3)

Strong correlation is seen between the RV volumes, and FVC and FEV1 measurements. Additionally, RVSV positively correlates with TLC, VA and DLCO. Of note, there is no positive correlation seen with FEV1/FVC, KCO3nd RV EF in relationship to any other parameter in the analysis.

Figure 5.2 A heat map Correlation matrix of Lung Function Tests against Right Ventricle parameters

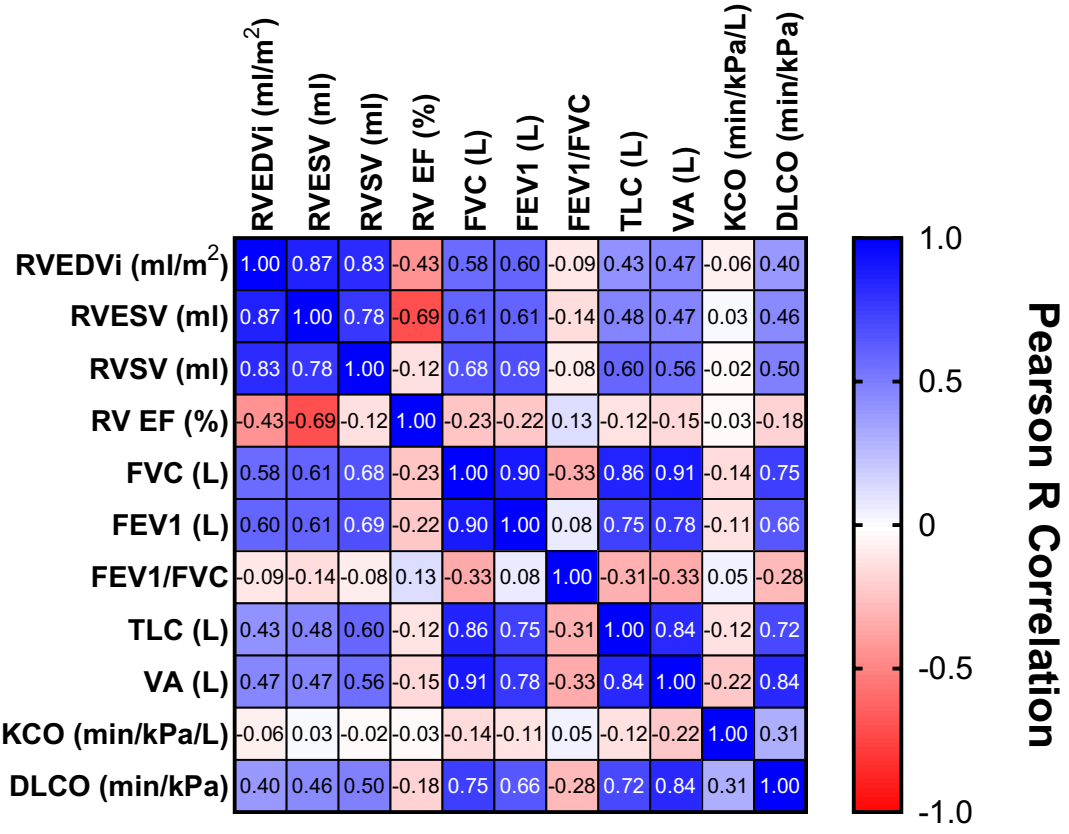


Table 5.3. A Pearson R Correlation Matrix of RV and Lung Function Test parameters with significance p<0.001, **p<0.05**

Correlations	RVEDVi	RVESV	RVSV	RV EF	FVC	FEV1	FEV1/FVC	TLC	VA	KCO	DLCO
RVEDVi (ml/mm^2)	1.000										
RVESV (ml)	0.870	1.000									
RVSV (ml)	0.831	0.783	1.000								
RVEF (%)	-0.426	-0.691	-0.122	1.000							
FVC (L)	0.577	0.608	0.678	**-0.234**	1.000						
FEV1 (L)	0.605	0.609	0.687	**-0.217**	0.901	1.000					
FEV1/FVC	-0.095	-0.135	-0.075	0.133	-0.335	0.077	1.000				
TLC (L)	0.431	0.479	0.596	-0.117	0.861	0.746	**-0.308**	1.000			
VA (L)	0.474	0.475	0.562	-0.151	0.906	0.782	**-0.330**	0.841	1.000		
KCO (min/kPa/L)	-0.060	0.025	-0.024	-0.027	-0.144	-0.113	0.052	-0.118	-0.217	1.000	
DLCO (min/kPa)	0.403	0.457	0.497	**-0.175**	0.755	0.656	**-0.276**	0.717	0.839	**0.314**	1.000

Table 5.4. Pulmonary function by severity at baseline visit

				p-value (one-way ANOVA w Bonferroni)		
	Controls (n = 25)	**Community (n = 50)**	**Hospital (n =33)**	**Control v Community**	**Control v Hospital**	**Community v Hospital**
FVC (L)	5.52 (0.7)	5.33 (1.0)	4.72 (0.9)	>0.9	**0.005**	**0.01**
FVC (% pred)	108.6 (12.2)	102.1 (11.9)	98.7 (14.1)	0.1	**0.01**	0.7
FEV1 (L)	4.21 (0.6)	3.9 (0.7)	3.7 (0.7)	0.6	**0.02**	0.2
FEV1 (% pred)	102.5 (14.0)	94.3 (10.9)	96.4 (14.2)	**0.03**	0.2	>0.9
FEV1/FVC (%pred/%pred)	76.3 (5.3)	75.6 (6.5)	78.7 (3.9)	>0.9	0.3	**0.04**
TLC (L)	6.59 (1.0)	6.27 (1.4)	5.5 (1.02)	0.8	**0.003**	**0.02**
TLC (% pred)	94.5 (14.4)	88.7 (13.5)	80.9 (13.0)	0.2	**0.0008**	**0.04**
DLCO (min/kPa)	10.04 (1.37)	9.49 (2.4)	8.02 (1.7)	0.8	**0.0008**	**0.004**
DLCO (% pred)	97.4 (8.8)	90.1 (17.8)	81.7 (15.6)	0.2	**0.0006**	0.05
VA (L)	6.45 (0.8)	6.05 (1.2)	5.17 (1.3)	0.4	**0.0001**	**0.003**
VA (% pred)	98.2 (10.9)	90.5 (12.9)	80.2 (17.0)	0.08	**<0.0001**	**0.004**
KCO (min/kPa/L)	1.56 (0.1)	1.56 (0.2)	1.59 (0.3)	>0.9	>0.9	>0.9
KCO (% pred)	99.1 (8.5)	98.9 (12.5)	103.0 (17.9)	>0.9	0.8	0.5

FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second, TLC- Total Lung Capacity, DLCO- Diffusing capacity of the lungs for carbon monoxide, VA- alveolar volume, KCO-Carbon monoxide transfer coefficient. %pred- percent predicted. Continuous variables expressed as mean (SD)

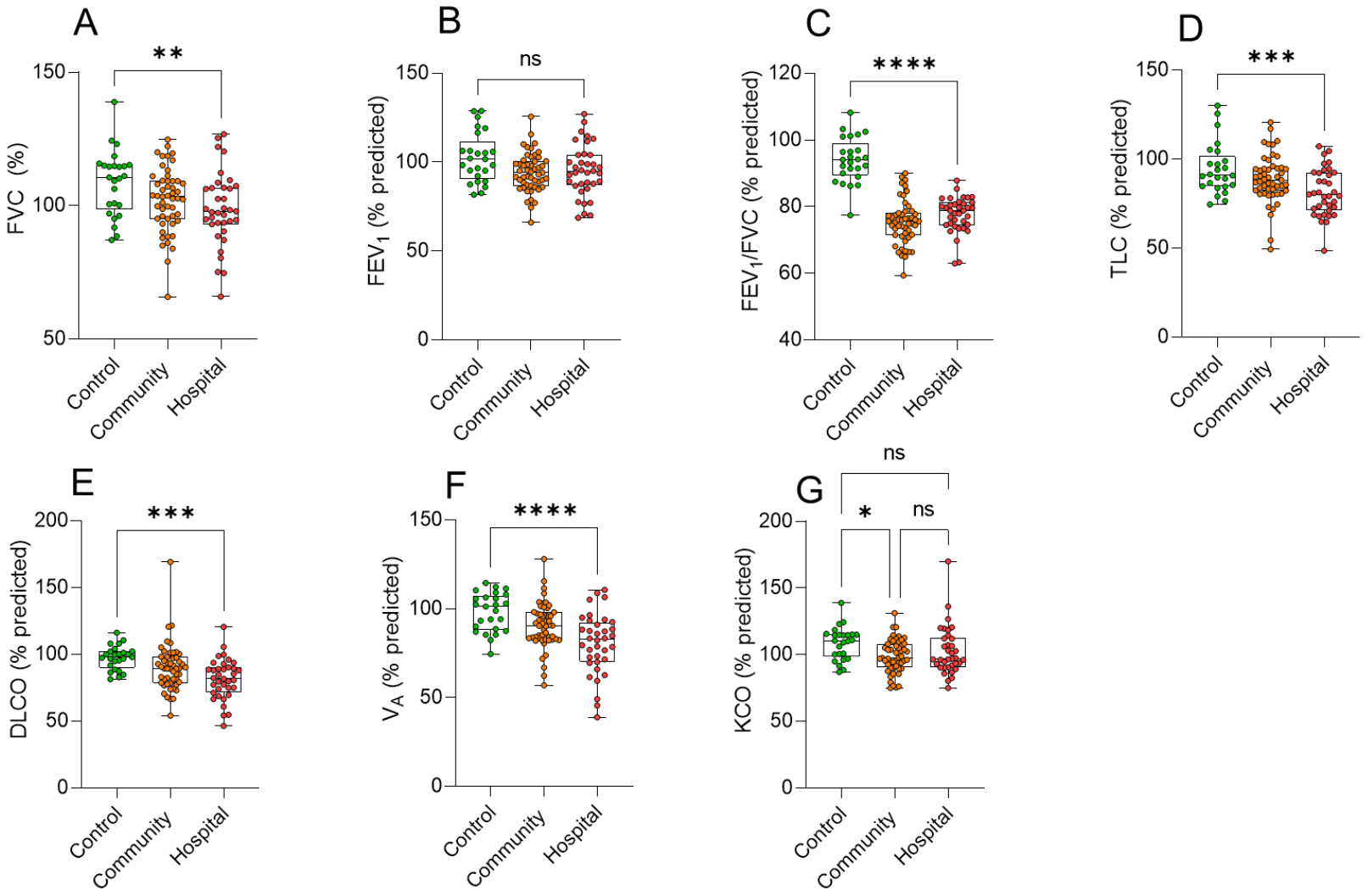


Figure 5.3 Lung Function Tests segregated by group presented as % of predicted. FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second, TLC- Total Lung Capacity, DLCO- Diffusing capacity of the lungs for carbon monoxide, VA- alveolar volume, KCO-Carbon monoxide transfer coefficient.

Table 5.5- A Pearson R Correlation Matrix of CPET and Lung function tests with significance **p<0.001**, p<0.01

Correlations	$\dot{V}O_2$ %	W %	$\dot{V}E/\dot{V}CO_2$	OUES	TLC	FEV1	FVC	FEV1/FVC	Va	KCO	DLCO
$\dot{V}O_2$(% Predicted)	1.00										
Peak Workload W (% predicted)	0.05	1.00									
$\dot{V}E/\dot{V}CO_2$	-0.09	-0.20	1.00								
OUES	0.09	**0.25**	**-0.50**	1.00							
TLC (L)	0.01	0.06	**-0.28**	**0.37**	1.00						
FEV1	0.01	-0.02	**-0.29**	**-0.43**	**0.83**	1.00					
FVC (L)	0.03	0.00	**-0.33**	**0.45**	**0.91**	**0.94**	1.00				
FEV1/FVc	-0.08	-0.05	0.16	-0.13	**-0.35**	0.10	**-0.34**	1.00			
Va (L)	0.07	0.08	**-0.27**	**0.39**	**0.90**	**0.85**	**0.93**	**-0.38**	1.00		
KCO	-0.06	-0.21	-0.12	0.04	**0.30**	**-0.33**	**-0.31**	0.05	0.23	1.00	
DLCO	0.10	-0.04	**-0.30**	**0.38**	**0.79**	**0.75**	**0.82**	**-0.32**	**0.88**	**0.53**	1.00

5.4 Pulmonary function metrics at 6-month follow-up

At the 6-month follow up point, there are fewer differences between the healthy controls and the post SARS-CoV-2 group. FVC is lower in the post SARS-CoV-2 group than in the healthy controls when expressed as corrected values (101.6% predicted ±12.1 vs. 111.7% predicted ±9.3, p=0.003), although there was no difference without correction for age, sex and body surface area.

FEV1 was reduced in the post SARS-CoV-2 group in comparison to the control group when corrected for age, sex and body surface area (95.5% predicted ±12.0 vs. 105.6% predicted ±12.6, p=0.005), but was not significantly different when the raw values were compared.

The post SARS-CoV-2 group had a reduced DLCO in comparison to the healthy control group, which was significant when expressed as a percentage of the predicted value (87.1% predicted ±12.8 vs. 97.3% predicted ±11.7, p=0.005), but not if uncorrected. The same pattern of difference was seen when comparing the VA measurements (post SARS-Co-V group 87.7% predicted ±12.0 vs. 98.2 ±11.6, p=0.002) FEV1/FVC, TLC and KCO demonstrated no change between groups at the 6-month follow-up point.

Table 5.6. Pulmonary Function Testing at 6-month follow-up

	Controls (n = 16)	**Covid (n = 65)**	**p-value**
FVC (L)	5.28 (±0.8)	5.10 (±1.0)	0.6
FVC (% pred)	111.7 (±9.3)	101.6 (±12.1)	**0.003**
FEV1 (L)	4.02 (±0.6)	3.86 (±0.7)	0.4
FEV1 (% pred)	105.6 (±12.6)	95.5 (±12.0)	**0.005**
FEV1/FVC (%pred/%pred)	76.7 (±6.9)	76.0 (±6.3)	0.7
TLC (L)	6.11 (±1.3)	6.04 (±1.3)	0.8
TLC (% pred)	91.4 (±13.5)	80.4 (±22.3)	0.06
DLCO (min/kPa)	9.39 (±1.9)	8. 88 (±1.8)	0.3
DLCO (% pred)	97.3 (±11.7)	87.1 (±12.8)	**0.005**
VA (L)	6.20 (±1.7)	5.81 (±1.2)	0.3
VA (% pred)	98.2 (±11.6)	87.7 (±12.0)	**0.002**
KCO (min/kPa/L)	1.52 (±0.2)	1.54 (±0.2)	0.7
KCO (% pred)	98.7 (±8.7)	99.4 (±14.1)	0.7

FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second, TLC- Total Lung Capacity, DLCO- Diffusing capacity of the lungs for carbon monoxide, VA- alveolar volume, KCO-Carbon monoxide transfer coefficient. %pred- percent predicted. Continuous variables expressed as mean (SD)

5.4.1 Change in pulmonary function from baseline to 6-month follow up by severity.

When the pulmonary function values for each group at baseline were compared to the same measurements at 6-month follow up, no changes were seen in any group for FVC, TLC or VA. Additionally, there were no significant changes across any variable in the healthy control group between baseline and the 6-month follow-up.

In the hospital group, the measurement of DLCO improved from baseline to 6-months (8.02min/kPa ± 1.7 vs. 8.5min/kPa ± 1.4, p=0.01).

In the group managed in the community, FEV1 measurements improved from baseline to 6 months (3.9L ± 0.7 vs. 3.93L ± 0.7, p=0.016). The community group also demonstrated a small, significant reduction in the KCO values from baseline to 6 months (1.56 min/kPa/L ± 0.2 vs. 1.49 min/kPa/L ± 0.2, p=0.04).

No other differences measured between the baseline and 6-month points achieved statistical significance.

Table 5.7 Paired comparison of pulmonary function measurements by severity at baseline and at 6-month follow up. All data shown with P value representing paired T-Test for available paired datasets.

	Controls			**Community**			**Hospitalised**		
	Baseline (n = 25)	**6 months (n = 16)**	**p-value**	**Baseline (n=50)**	**6 Months (n = 37)**	**p-value**	**Baseline (n=33)**	**6 Months (n =28)**	**p-value**
FVC (L)	5.52 (±0.7)	5.28 (±0.8)	0.46	5.33 (±1.0)	5.6 (±1.1)	0.25	4.72 (±0.9)	4.8 (±0.9)	0.49
FVC (% pred)	108.6 (±12.2)	111.7 (±9.3)	0.27	102.1 (±11.9)	102.7 (±11.9)	0.89	98.7 (±14.1)	100.1 (±12.3)	0.44
FEV1 (L)	4.21 (±0.6)	4.02 (±0.6)	0.22	3.9 (±0.7)	3.93 (±0.7)	**0.016**	3.7 (±0.7)	3.76 (±0.7)	0.49
FEV1 (% pred)	102.5 (±14.0)	105.6 (±12.6)	0.27	94.3 (±10.9)	93.6 (±11.2)	0.19	96.4 (±14.2)	97.9 (±12.9)	0.46
FEV1/FVC (%pred/%pred)	76.3 (±5.3)	76.7 (±6.9)	0.81	75.6 (±6.5)	73.9 (±7.1)	0.06	78.7 (±3.9)	78.8 (±3.8)	0.94
TLC (L)	6.59 (±1.0)	6.11 (±1.3)	0.27	6.27 (±1.4)	6.39 (±1.3)	0.78	5.5 (±1.02)	5.59 (±1.0)	0.21
TLC (% pred)	94.5 (±14.4)	91.4 (±13.5)	0.47	88.7 (±13.5)	84.4 (±21.7)	0.08	80.9 (±13.0)	75.1 (±22.5)	0.34
DLCO (min/kPa)	10.04 (±1.37)	9.39 (±1.9)	0.06	9.49 (±2.4)	9.2 (±2.1)	0.08	8.02 (±1.7)	8.5 (±1.4)	**0.01**
DLCO (% pred)	97.4 (±8.8)	97.3 (±11.7)	0.29	90.1 (±17.8)	87.3 (±12.2)	0.1	81.7 (±15.6)	86.9 (±13.8)	**0.02**
VA (L)	6.45 (±0.8)	6.20 (±1.7)	0.27	6.05 (±1.2)	6.17 (±1.3)	0.94	5.17 (±1.3)	5.36 (±1.0)	0.15
VA (% pred)	98.2 (±10.9)	98.2 (±11.6)	0.68	90.5 (±12.9)	91.2 (±11.5)	0.96	80.2 (±17.0)	83.1 (±11.3)	0.12
KCO (min/kPa/L)	1.56 (±0.1)	1.52 (±0.2)	0.29	1.56 (±0.2)	1.49 (±0.2)	**0.04**	1.59 (±0.3)	1.61 (±0.3)	0.53
KCO (% pred)	99.1 (±8.5)	98.7 (±8.7)	0.62	98.9 (±12.5)	95.35 (±9.9)	**0.04**	103.0 (±17.9)	104.8 (±17.0)	0.59

FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second, TLC- Total Lung Capacity, DLCO- Diffusing capacity of the lungs for carbon monoxide, VA- alveolar volume, KCO-Carbon monoxide transfer coefficient. %pred- percent predicted. Continuous variables expressed as mean (±SD)

5.4.2 Comparison of changes from baseline to 6-month follow up between severity groups

The magnitude of change over time between the severity groups was non-significant across measures of spirometry or lung capacity. There was a significant difference in the change in DLCO values from baseline to 6-month follow up when expressed as measured values (controls -0.67min/kPa/L ±1.3, vs. community -0.39min/kPa/L ±1.3, vs. hospital 0.7min/kPa/L ±1.5, p=0.002) and when corrected for age, sex and body surface area (controls -1.84% predicted ±6.8, vs. community -3.53% predicted ±12.5, vs. hospital 7.20% predicted ±16.1, p=0.006).

Table 5.7 Comparison of the change between baseline and 6-month follow up of different severity groups. p value is between groups difference from one-way ANOVA

	Δ baseline and 6-month			**p-value**
	Controls (n = 16)	**Community (n = 36)**	**Hospital (n = 28)**	
FVC (L)	-0.12 (0.6)	-0.04 (0.2)	0.10 (0.7)	0.4
FVC (% pred)	2.7 (9.3)	-0.1 (4.5)	1.5 (9.7)	0.5
FEV1 (L)	-0.11 (0.4)	-0.07 (0.2)	0.08 (0.6)	0.2
FEV1 (%pred)	2.5 (8.5)	-1.1 (4.9)	1.4 (9.4)	0.2
FEV1/FVC (%pred/%pred)	0.31 (5.2)	-0.84 (2.6)	-0.06 (3.9)	0.5
TLC (L)	-0.33 (1.2)	-0.04 (0.8)	0.18 (0.7)	0.2
TLC (%pred)	-2.6 (14)	-5.4 (18)	-4.6 (25)	0.9
DLCO (min/kPa)	-0.67 (1.3)	-0.39 (1.3)	0.7 (1.5)	**0.002**
DLCO (%pred)	-1.84 (6.8)	-3.53 (12.5)	7.20 (16.1)	**0.006**
VA (L)	-0.25 (0.9)	-0.01 (0.5)	0.29 (1.0)	0.08
VA (%pred)	-1.0 (9.8)	0.1 (6.9)	4.4 (14.2)	0.2
KCO (min/kPa/L)	-0.04 (0.1)	-3.67 (10.1)	0.04 (0.3)	0.2
KCO (%pred)	-0.04 (0.1)	-0.05 (0.2)	0.04 (0.2)	0.3

FVC- Forced Vital Capacity, FEV1- Forced Expiratory Volume in 1 second, TLC- Total Lung Capacity, DLCO- Diffusing capacity of the lungs for carbon monoxide, VA- alveolar volume, KCO-Carbon monoxide transfer coefficient. %pred- percent predicted. Continuous variables expressed as mean (SD)

5.5 Discussion

Previous chapters have described the reduction in exercise capacity in the UK Armed Forces population who developed SARS-CoV-2. Values for peak $\dot{V}O_2$ remained above the threshold for a 'normal' test in the general population, but they were significantly reduced in comparison to healthy controls. Additional metrics were suggestive of deconditioning as the primary reason for the change, although differences in the A-a gradient at rest and at peak exercise directed us to consider whether a pulmonary component may be driving the difference in exercise capacity. This chapter has endeavoured to explore this concept further.

As the same participant population was used throughout this work, the groups were well matched for age, gender and employment. When the post SARS-CoV-2 group is compared by severity, the post-hospital group were older than those who were managed in the community, which reflects the fact that age is an independent risk factor for severe disease. Participants post SARS-CoV-2 had a higher BMI when compared to those in the healthy control group, who were not infected. Again, this reflects the association between infection and obesity, which is now well acknowledged.

5.5.1 Spirometry and lung volumes

Post SARS-CoV-2 infection there is a persistent reduction in forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and total lung capacity (TLC) in comparison to controls. This echoes the findings of many of the general population studies, which highlighted the same abnormalities in their respective populations, although they typically did not have a control population to compare against[65]. In the civilian population studies, the cut-off for abnormality was the number of subjects with recorded values of <80% of the predicted value, which correlates with the usual thresholds in clinical interpretation of the tests. In our population, none of the recorded group means were below 80% predicted, although a small number of individuals in the cohort were. So, although trends towards both obstructive/restrictive respiratory physiology are seen, the population values in this military cohort do not meet the definition of either obstructive or restrictive lung disease. This

difference is likely to reflect a higher degree of severe illness in published civilian studies due to recruiting practices (recruitment from hospital discharge, with a higher proportion of intensive care admissions, longer ICU length of stay and longer duration of invasive ventilation as a consequence). Alternatively, better premorbid physical status in the military population may explain these differences, or possibly a combination of these factors. It might be expected then, for those subjects to have a larger degree of impairment than those who had only mild disease.

As this study recruited from across the spectrum of disease, from those with asymptomatic illness to those who received intensive hospital care, the magnitude of the difference could be expected to be less. When the data was analysed by severity of illness, those who received hospital treatment for SARS-CoV-2 had greater reductions in all metrics than those with community disease. The community managed 'mild disease' cohort did not differ with the control population in for lung function parameters, with the exception of the FEV1 measurement. When the hospital group are analysed separately, the corrected means for key lung parameters do tend towards the ≤80% predicted threshold.

In the military population, severe SARS-CoV-2 is associated with more abnormalities of spirometry and lung function results post recovery, in comparison with mild or asymptomatic infection. These findings are consistent with the literature. The military population who suffered severe disease by our pragmatic case definition (hospitalisation) behaves like the patients in published civilian studies of hospitalised COVID patients. The military group who suffered mild/moderate disease in the community are better reflected in published studies of athletes)[115].

5.5.2 Gas transfer

As noted in published literature for both previous SARS-CoV-1 infection and for SARS-CoV-2 infection in the general population, a reduction is seen in diffusion of carbon monoxide across the alveolar capillary membrane (DLCO), when compared to healthy controls[179]. When assessed by severity, the severe, hospitalised patient group had lower DLCO values than those who had mild, asymptomatic or

sub-clinical disease (community group). As the majority of general population studies are recruiting from the hospitalised patient population, and the athlete studies are recruiting from the generally well, mild illness, younger individual group, this would suggest that there are no differences in trends when looking at the military population specifically. The mixture of community recruited participants and hospitalised patients most likely reflects the spectrum of disease seen in wider society, although as the military population have lower comorbidities and are younger, the total values of the DLCO levels are higher than those seen in the post hospital civilian cohorts.

The main measurable components of DLCO are the alveolar volume (VA) and the carbon monoxide transfer coefficient (KCO). This study noted a reduction in VA in both all post COVID and in the severe disease group in comparison to healthy controls, whereas there was no change seen in the KCO values. These differences, together with the spirometry values are used in clinical practice to determine the pathological cause of the impairment in DLCO when absolute numbers are below the acceptable normal limit. When KCO is preserved, a likely cause of the reduced transfer capacity is a result of intrapulmonary damage, rather than that of extrapulmonary aspects that can affect these findings (e.g., pre-capillary pulmonary hypertension)[181]. My findings are consistent with loss of effective alveolar volume rather than widespread damage to the alveolar-capillary interface. It is possible that the mechanism for these deficits is related to the pro-inflammatory processes that occur in the alveolar-capillary interface during the acute infection, particularly as evidence of alveolar hyperplasia, macrophage deposition and endothelial dysfunction have been noted in biopsies from individuals who did not go on to require supported ventilation[182]. However, given that the DLCO in this cohort does remain within clinical norms, the concept of widespread disruption of the respiratory exchange surface as the mechanism for the difference is less plausible, and relative preservation of a reduced total alveolar volume is more likely.

5.5.3 Change over time.

When the 6-month follow up data are compared, a significant difference between those post SARS-CoV-2 and the healthy controls remains. The difference in the TLC is no longer apparent, but FVC, FEV1 and DLCO and VA relative deficits persist. When the severity of the illness is considered, a significant, but clinically trivial improvement is seen in the FEV1 in community cases. There is a more substantial, and clinically relevant increase in the DLCO seen in the hospitalised cases. Very long follow up studies of persistent deficits post SARS-CoV-2 are in progress but not yet published, however, following the SARS-CoV1 epidemic resolution of lung function was not seen until 2 years post acute infection, with complete recovery not achieved in only those with the most severe post infection fibrotic changes[183]. In studies which were conducted on hospitalised patients, albeit generally younger and more active than the SARS-CoV-2 population, deficits in lung function were seen to start to improve after the 6-month point, and some persisted beyond the 12-month point[58,59]. It remains to be seen whether the differences will fully resolve over time.

5.5.4 Other Causes of Reduced Exercise Capacity Not Investigated

5.5.5 Deconditioning as a Contributor to Reduced Exercise Capacity

Exercise capacity is known to deteriorate quickly, and significantly following bed rest. $\dot{V}O_2$ max reduces, steeply by around 15% after 2 weeks and plateaus at around a 30% reduction after 90 days[184]. It has also been shown that this decrease in $\dot{V}O_2$ max accompanying bedrest results from both central and peripheral factors. Central cardiovascular deconditioning with reduced cardiac cavity size and stroke volume has been observed with as little as 2 weeks of bedrest. Further changes are seen at 6 weeks[185]. In addition, impairment of peripheral gas exchange has also been shown to result from bedrest. Decreased muscle oxidative capacity, mitochondrial density, and oxidative enzyme activity have all been demonstrated, paralleling a decrease in skeletal muscle mass[186].

In keeping with this, previous studies reporting CPET in post COVID syndrome have highlighted deconditioning as a significant cause of exercise intolerance, with an early anaerobic threshold, earlier termination of effort, lower peak work and peak oxygen pulse and a reduced $\dot{V}O_2$/WR slope[187]. As many of these changes were also identified in this study it is likely that deconditioning is playing a large role in many of the changes seen in this study.

When combined, the cardiovascular changes seen in this relatively active, young, military population, generally mimic those seen following bedrest and deconditioning, with reduced $\dot{V}O_2$ max, reduced peak workload, reduced cardiac chamber size, reduced cardiac stroke volume and reduced O_2 pulse all seen following both COVID 19 and bedrest. As such, we believe that it is likely that physical inactivity and bedrest, which are more significant in those with hospitalised illness, will account for many of the changes seen here. Importantly, these mechanisms are not COVID 19 specific, and would occur after prolonged prostrating illness of any sort. The lack of difference in global systolic and diastolic function and in cardiac energetics also support this conclusion.

5.5.6 Peripheral Changes

Involvement of the musculoskeletal system in post COVID syndrome is well reported with reduced peripheral causes of peak $\dot{V}O_2$ also shown in invasive CPET studies[188]. Weakness and myalgia are commonplace. Mechanisms underlying muscle dysfunction as a direct result of COVID-19 include inflammation, mitochondrial dysfunction, mitochondrial volume loss and dysregulation of the renin-angiotensin system[189]. In this study, whilst no specific measurement of muscle physiology was undertaken, it is likely that inflammatory or disuse myopathy is contributing to the reduced exercise capacity seen following COVID.

5.6 Limitations

This study was planned and conceived when information on the effects of COVID-19 were scarce. Indeed, this was the reason for the study. As a result, it was not possible to recruit to ‘size’ the groups

on the basis of a power calculation., group sizes were based on feasibility rather than the number needed to demonstrate significance. Furthermore, the COVID negative healthy control participant group was smaller by the 6 month follow up visit in part due to subsequent waves of SARS-CoV-2 spreading through the population, and those individuals being excluded. It is therefore possible that certain differences may have been missed (Type 2 error), that would have been evident with a larger sample size.

Pulmonary function testing is not part of routine health screening in the UK Armed Forces, so there are no pre-infection results with which to compare changes following the pandemic. This has been partially mitigated with a matched control group, but pre-existing 'normal' data would have been helpful. Finally, some of the post SARS-CoV-2 participants had attended an exercise rehabilitation program, and some had not. It is not known whether this impacted their pulmonary function test results, although their utility post ARDS has been proven and they are recommended in international guidelines. The selection of those individuals for those resources was outside of the control of the study organisers[190].

5.7 Conclusion

In this study, I have explored the differences in pulmonary function of members of the UK Armed Forces after SARS-CoV-2 and their colleagues who were not infected. We have demonstrated reductions in spirometry measurements, lung capacity and gas transfer across the alveolar-capillary membrane after COVID-19 infection, which is more marked in severe disease requiring hospitalisation. When followed up 6 months after the initial test, deficits in spirometry and gas transfer persist. A plausible mechanism for this is a loss of effective lung parenchyma demonstrated by the reduced alveolar volume, without a generalised deterioration in the alveolar-capillary gas exchange function of the remaining lung. Such a process would explain a reduced DLCO that is proportionate to the demonstrated loss in lung volume, without a significant change in KCO. The fact that the Armed Forces population have a lower incidence of medical co-morbidities than the general population are more

active and have a higher baseline level of cardiorespiratory fitness, is likely to account for these differences lying largely in the sub-clinical range. However, they could contribute to the differences in exercise capacity noted in Chapter 3.

Future work in this area could re-measure the population after a further period of 'convalescence' to identify whether the pulmonary changes will normalise. This work also prompts a requirement to establish 'normal' data for the military population, to better assess the impact of future pandemics, environmental illnesses and even attacks (such as chemical, biological and radio/nuclear injury) upon the Armed Forces population. Such a reference dataset would help to inform occupational medicine clinicians on the safety of deploying personnel to austere environments after recovery from pulmonary insults.

Chapter 6- Summary and Future Work

6.1 Chapter 3 Summary

The novel coronavirus SARS-CoV-2 which emerged in 2019 has spread globally. Although the mortality rate for this disease is low, prior to the development of an effective vaccine, morbidity was unknown in a working age population. There have been reports of persistent symptoms which affect day to day functioning in some. It is not clear how SARS-CoV-2 affects working age populations, individuals who were managed outside of the hospital setting, and what the implications may be for individuals who undertake strenuous exercise in their occupational role, or who work in high hazard occupations.

If exercise capacity is affected, what is the extent of the deficit and what are the possible underlying mechanisms. To determine if SARS-CoV-2 infection affects the exercise capacity in military personnel, 113 members of the UK Armed Forces (87 post SARS-CoV-2, 26 COVID negative controls) were recruited to undergo exercise testing. Cardiopulmonary exercise testing with earlobe capillary blood sampling was performed at baseline and at 6 month follow up. To summarise the results, when compared to controls, post COVID participants achieved a lower peak workload, anaerobic threshold and peak $\dot{V}O_2$ (all $p<0.01$). Those with severe illness (requiring hospitalisation) achieved the lowest values. $\dot{V}O_2$/HR at peak exercise was lower after SARS-CoV-2 than in controls (105% pred. (±20) vs. 126% pred. (±22), $p<0.0001$) and the A-a gradient at rest (2.7 (±1.9) vs. 1.6 (±1.4), $p=0.01$) and with exercise (2.8 (±1.8) vs. 1.2 (±1.3), $p<0.0001$) was higher. Hospitalised participants demonstrated elevated $\dot{V}E/\dot{V}CO_2$ in comparison with controls (29.2 (±5.2) vs 25.5 (±2.6), $p=0.02$). Despite these differences, the values for peak $\dot{V}O_2$ remained within the limits of normal for a sedentary person (>85% predicted).

At 6 months follow up, the peak workload and the workload at the anaerobic threshold had increased in the post COVID participants, but other values did not change. This suggests that with time, the exercise capacity does improve via increased exercise efficiency at peak exercise and with endurance

exercise. To summarise Chapter 3, COVID-19 infection is associated with a reduction in exercise capacity and anaerobic threshold in military personnel. These differences are observed in moderate and severe disease, although they are greater if the acute illness was severe. There are still differences present at re-testing after 6 months, although some recovery in exercise capacity is noted in the post infection group by way of increased exercise efficiency. Differences in $\dot{V}O_2$/HR and diastolic function suggest a cardiac limitation or deconditioning as the underlying mechanism, alternatively, a relatively elevated A-a gradient could suggest a respiratory cause.

6.2 Summary Chapter 4

Post SARS-CoV-2, a reduction in exercise capacity remains in military personnel in comparison with COVID negative controls. Studies have suggested that myocarditis or other cardiac involvement may be a contributing factor. If persistent myocardial inflammation is the cause of reduced exercise capacity, it would have significant implications for members of the Armed Forces to deploy to austere environments and undertake strenuous physical exercise. To determine if observed differences in exercise capacity post SARS-CoV-2 infection could be caused by impairment in cardiac function or persistent, post-viral myocardial inflammation, 113 members of the UK Armed Forces (87 post SARS-CoV-2, 26 COVID negative controls) were recruited to undergo cardiac investigations, including cardiac MRI, ^{31}P MRS at rest and catecholamine stress, and rest and exercise stress diastolic assessment by echocardiography. To summarise the results, no changes were seen in left ventricular volumes between post COVID and controls. By severity, those with the most severe disease (requiring hospital admission) had smaller left ventricular volumes than those who were managed in the community or controls ($p<0.05$). Post COVID participants had smaller RV indexed end diastolic volumes than controls ($84ml/m^2$ ±20 vs. $99ml/m^2$ ±13, $p<0.01$). Change in TAPSE and average E/e' with exercise was also blunted in the post COVID group ($p<0.05$). An increase in global native T1 was seen in the post COVID group (1177ms ±31 vs. 1159ms ±22, P=0.01) although the values remained in the normal range. No changes were observed in extracellular volume or myocardial energetics. To summarise Chapter 4,

whilst a small, subclinical difference in native T1 values is seen between groups, there is no evidence to support the hypothesis of persistent cardiac injury in this population post COVID-19 infection. Changes that are seen, particularly in chamber size and in augmentation of longitudinal function with exercise, suggest that a detraining mechanism could be responsible, and further support deconditioning as a possible cause of the observed differences in exercise capacity.

6.3 Summary Chapter 5

SARS-CoV-2 is a multisystem disease with a primary respiratory component. It is known to cause significant morbidity in those with both severe and non-severe acute illness. We have demonstrated that in participants drawn from the UK Armed Forces there is a reduction in exercise capacity after SARS-CoV-2 which has not fully normalised by 6 months. Possible components responsible for this are deconditioning or a ventilatory limitation. To determine whether there is a difference in pulmonary function between UK military personnel who have had SARS-CoV-2 and a matched control population, 108 military personnel were recruited, 83 with prior SARS-CoV-2 infection (38±8 years, 86% male; 50 mild (community), 33 severe (hospitalised) and 25 healthy volunteers (mean age 39±10 years, 88% male). They underwent pulmonary function testing (spirometry, static lung volumes and diffusion capacity of carbon monoxide) at their baseline visit and 6 months later at follow up. In brief, prior infection with SARS-CoV-2 was associated with lower FVC (100.8% predicted± 12.9 vs. 108.6% percent predicted ± 12.2, p=0.008), FEV1 (95 % predicted ± 12.3 vs. 102.5% predicted ± 14, p=0.01), TLC (85.6% ±13.8 vs. 94.5% ±14.4, p=0.006) and DLCO (86.7% predicted ±17.3 vs. 97.4% predicted ±8.8, p=0.004) compared to an uninfected control group. Severe illness requiring hospitalisation resulted in the largest deficits. After the 6 month follow up, the post SARS-CoV-2 group pulmonary function was generally improving but was still reduced in comparison to those with no history of SARS-CoV-2 infection (FVC 101.6% predicted ± 12.1 vs. 111.7% predicted ± 9.3, p=0.003; FEV1 95.5% predicted ± 12.0 vs. 105.6% predicted ± 12.6, p=0.005; DLCO 87.1% predicted ± 12.8 vs. 97.3% predicted ± 11.7, p=0.005). To summarise Chapter 5, post SARS-CoV-2 infection there is a reduction

in lung capacity, lung volumes and gas transfer properties in military personnel. This is more pronounced in individuals who were hospitalised with severe infection. There is some improvement after 6 months, but a persistent deficit in gas transfer remains. This deficit could explain the difference in exercise capacity noted between those with and without history of SARS-CoV-2 infection, and the differences seen in the A-a gradient at rest and with exercise. This does seem more likely on the basis that the more affected post-COVID groups also had the least efficient pulmonary ventilation: demonstrated by a higher VE/VCO2 slope. A possible unifying mechanism could be post inflammatory damage to the alveolar-capillary membrane, although at the KCO values were unchanged, it is more likely that the reduced total gas exchange is a result of smaller alveolar volume with preserved gas transfer across the alveolar membrane.

6.4 Several future directions can be anticipated.

We need a comprehensive long COVID research agenda that builds on the existing knowledge acquired since the pandemic. This has focused on, but is not limited to brain, cardiovascular system, skeletal muscle and pulmonary function. There is clear scope to delve further into the psychological, biological and physiological mechanisms underlying long COVID. This would include studying the virus's impact on the immune system, inflammatory processes, metabolic profiling, endothelial function, mitochondrial fragmentation, antiviral and metabolic phenotypes, hypoperfusion/cerebral blood flow, overlaps with connective tissue disorders, metabolomics and proteomics, and the effects of vaccination. Whilst this is beyond the scope of this DPhil, this body of work does provide the basis for further investigation. With a better understanding of post COVID physiology, research can now focus on developing effective treatments targeted towards cardiac, respiratory and skeletal muscle physiology, as well as improved management of mood and thinking, and in tandem create rehabilitation protocols that are tailored to individual needs.

Publications

Some of the ideas, data and figures in this body of work have appeared previously in the following publications:

Exercise capacity following SARS-CoV-2 infection is related to changes in cardiovascular and lung function in military personnel.

Chamley RR, Holland JL, Collins J, Pierce K, Watson WD, Green PG, O'Brien D, O'Sullivan O, Barker-Davies R, Ladlow P, Neubauer S, Bennett A, Nicol ED, Holdsworth DA, Rider OJ. (2024) Int J Cardiol. Jan 15;395:131594.

Cardiopulmonary, Functional, Cognitive and Mental Health Outcomes Post-COVID-19, Across the Range of Severity of Acute Illness, in a Physically Active, Working-Age Population.

O'Sullivan O, Holdsworth DA, Ladlow P, Barker-Davies RM, ***Chamley R***, Houston A, May S, Dewson D, Mills D, Pierce K, Mitchell J, Xie C, Sellon E, Naylor J, Mulae J, Cranley M, Talbot NP, Rider OJ, Nicol ED, Bennett AN. 2023. Sports Med Open. Feb 2;9(1):7.

Cardiopulmonary exercise testing excludes significant disease in patients recovering from COVID-19.

Holdsworth DA, Barker-Davies RM, ***Chamley RR***, O'Sullivan O, Ladlow P, May S, Houston AD, Mulae J, Xie C, Cranley M, Sellon E, Naylor J, Halle M, Parati G, Davos C, Rider OJ, Bennett AB, Nicol ED. 2022 BMJ Mil Health. Nov 28. Online ahead of print.

Defence Medical Rehabilitation Centre (DMRC) COVID-19 Recovery Service.

O'Sullivan O, Barker-Davies R, ***Chamley R***, Sellon E, Jenkins D, Burley R, Holden L, Nicol AM, Phillip R, Bennett AN, Nicol E, Holdsworth DA. 2023 BMJ Mil Health. Jun;169(3):271-276.

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