Electromagnetic Wearable Sensors: A Solution to Non-Invasive Real-Time Monitoring of Biological Markers during Exercise

By

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Dedicated to my Parents

Lisa and Gareth

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In Loving Memory of

Martin Greene

(1938 – 2019)

PUBLISHED WORK

Contained within this thesis are findings that have been published throughout the duration of this research project.

1. Greene, J., Louis, J., Korostynska, O. and Mason, A., 2017. State-of-the-Art Methods for Skeletal Muscle Glycogen Analysis in Athletes—The Need for Novel Non-Invasive Techniques. *Biosensors*, *7*(1), p.11. DOI 10.3390/bios7010011
2. Mason, A., Korostynska, O., Louis, J., Cordova-Lopez, L.E., Abdullah, B., Greene, J., Connell, R., and Hopkins, J., 2017. Non-Invasive In-situ Measurement of Blood Lactate using Microwave Sensors. IEEE Explore, DOI 10.1109/TBME.2017.2715071
3. Jacob Greene, Julien Louis, Olga Korostynska, and Alex Mason., 2017. Non-invasive in-situ measurement of blood lactate using microwave sensors during progressive incremental exercise. LJMU Faculty of Engineering and Technology, Research Week Proceedings. ISSN 2398-6611 p.44
4. J. Greene, O. Korostynska, J. Louis, A. Mason, "In-Vitro Quantification of Glycogen Using a Novel Non-Invasive Electromagnetic Sensor", International Conference on Sensing Technology (ICST2017), DOI: 10.1109/ICSensT.2017.8304477, 2017.
5. J. Greene, J. Louis, O. Korostynska, A. Mason., 2018. Non-Invasive Monitoring of Glycogen in Real-Time Using an Electromagnetic Sensor. LJMU Faculty of Engineering and Technology, 2018 Research Week Proceedings. ISSN 2398-6611
6. J. Greene, B. Abdullah, J. Cullen, O. Korostynska, J. Louis and A. Mason, "Non-Invasive Monitoring of Glycogen in Real-Time Using an Electromagnetic Sensor", in Modern Sensing Technologies, S. C. Mukhopadhyay, O. Postolache and K. P. Jayasundera Eds. Springer International Publishing, pp. 1-15, DOI: 10.1007/978-3-319-99540-3_1, 2019.

TABLE OF CONTENTS

ABSTRACT

Wearable sensing technology enables greater insights into the performance and health status of athletes during training and competition, which are currently unattainable through traditional laboratory-based techniques. The process of collecting accurate data from complex metabolic parameters usually requires the use of specialised equipment and methods that are often expensive and invasive. This research proposes the novel use of a purpose-built electromagnetic (EM) sensor to non-invasively detect biological markers in humans during exercise. Three parameters were selected for investigation: sweat sodium, blood lactate, and skeletal muscle glycogen. Each of these parameters were selected based on their significance to athletic performance monitoring, as well as their current methods of analysis being impractical for real-time monitoring during exercise.

Four human studies and two in-vitro sample-based studies were conducted, accumulating in 140 sweat samples, 523 blood lactate samples, and 21 glycogen samples, collected from a combined total of 71 participants, 56 males, and 15 females. The research presented within this thesis demonstrated that a hairpin EM sensor operating at microwave frequencies could detect and measure changes in sodium concentration within human sweat samples at 1.6 GHz (R^2 = 0.862). Further sensor development is required for on-subject monitoring of sweat sodium during exercise (R^2 = 0.149), findings suggest this was a result of the microwave sensor's design, rather than sensing capabilities. Additionally, the sensor was shown to measure blood lactate concentration in untrained participants at 3.4-3.6 GHz (R^2 = 0.78), and within endurance-trained

participants at 3.2-3.8 GHz (R^2 = 0.757). Furthermore, results showed that the sensor could detect changes in glycogen sample concentration at 2.11 GHz (R^2 = 0.87) and monitor skeletal muscle glycogen in humans when concentrations were grouped into exercise specific ranges at 2.0-2.25 GHz (R^2 = 0.91).

This research presents an accurate, cost-effective, and efficient method of detecting biological markers non-invasively and continuously during exercise. With future research and development, a single microwave sensor could ultimately lead to improvements in human performance monitoring, enabling individualised and real-time fuelling strategies during training and competition. Further assessment of this technology is needed within a real-world setting to understand if this remains a feasible solution outside of a controlled environment.

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LIST OF ACRONYMS

ATP	Adenosine Triphosphate
aw	Water Activity
BEST	Built Environment and Sustainable Technologies
BF	Biceps femoris muscle
C	Carbon
CHO	Carbohydrate
CF	Cystic Fibrosis
DEXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
EM	Electromagnetic
EU	European Union
FDA	Food and Drug Administration
GPS	Global Positioning System
H	Hydrogen
HFSS	High Frequency Structural Simulation
La-	Lactate
LT	Lactate Threshold
LJMU	Liverpool John Moores University
MLSS	Maximal Lactate Steady State
MMP	Maximum Minute Power
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy

MUT	Material Under Test
Na^+	Sodium
NIR	Near-Infrared
NMR	Nuclear magnetic resonance
NN	Neural Network
OECT	Organic Electrochemical Transistor
P	Phosphorus
PAS	Periodic Acid-Schiff
PCR	Principle Component Regression
pH	Power of Hydrogen
POC	Point of Care
PMI	Pairwise Mutual Information
PPO	Peak Power Output
RPE	Rating of Perceived Exertion
RH	Relative Humidity
RMSE	Root Mean Square Error
SHLT	Short-Open-Load-Through Calibration
SMA	SubMiniature version A
STHA	Short Term Heat Acclimation
VNA	Vector Network Analyser
VO_2 max	Maximum Oxygen Consumption
W	WATT

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 - INTRODUCTION

Over recent decades, technology has evolved and revolutionised the professional sporting environment, with ever more acceptance that meticulous attention to detail and greater insight can provide a competitive advantage, ultimately making the difference between winning and losing. Advances in technology have permitted athletes, sports teams, and physicians to monitor player movements (Loader et al., 2012), workloads (Mooney et al., 2011), and biometric indicators (Foster et al., 2010) enabling optimisation of athletic performance and also reducing the risk of injury. The introduction of wearable sensor technology has proved a useful tool for improving sporting performance. Wearable sensors are currently used to track a multitude of performance-related parameters such as physiological, performance, biomechanical and environmental data. The ability to analyse and interpret such data during training and competition can provide sports scientists, practitioners, and athletes more significant insights previously unattainable. This data can lead to improvements in training, competition, recovery, and safety.

As well as physical and biomechanical performance, being able to measure and accurately monitor physiological biomarkers is of first importance in sport science. Indeed, physiological markers such as blood lactate, intramuscular glycogen stores, blood glucose, blood oxygenation or the mineral concentration are often measured in sport science protocols in an attempt to assess the effects of the exercise itself on the organism or to evaluate the efficacy of a specific training or nutritional intervention. Collecting accurate data requires adapted equipment and methods often expensive and

invasive, such as muscle biopsies to estimate glycogen concentration or venepuncture to estimate blood minerals and lactate. This collaborative research program fits into this context of the optimisation of equipment and methods to measure accurately physiological markers classically studied in sport science and directly used in the field. This project might find applications not only in sport science but also in the medical domain, thanks to the potential development of innovative non-invasive techniques to measure physiological parameters also classically monitored in medicine.

1. 1 **Problem statement**

Modern medicine relies on the ability to observe and monitor human anatomy to understand human physiology. In a sporting context, being able to track essential performance-related parameters is critical to understanding athletes' exercise output. The ability to monitor athletes and patients over prolonged period of times and within challenging environments is only feasible with the development of wearable sensors. Technology in sport is used to monitor an athlete's performance, identifying exactly where improvements are needed, and which weaknesses need to be addressed. To date, few technologies allow the analysis of real-time physiological factors. Sensors that determine physiological responses during competition and training allow many benefits for sports practitioners, namely, nutritionists, injury rehab professionals, strength, and conditioning coaches, etc. This ability undoubtedly will lead to a more individualised approach for the athletes, which is vital at the elite end of the performance spectrum. Currently, the challenge faced today regarding wearable sensor technologies is that they only have the capabilities to monitor rudimentary physiological parameters such as heart rate, muscle activity, temperature, and oxygenation levels. Monitoring complex

performance-related parameters requires more advanced and often invasive techniques such as muscle biopsies and blood sampling which can only provide a snapshot in time rather than monitoring the athlete in real-time.

1. 2 **Overview of the physiological parameters selected for investigation**

Three distinct physiological parameters have been selected, chosen to undergo human trials using a novel non-invasive EM sensor. These parameters have been selected based on their significance to elite sporting performance, as well as the current methods of analysis being impractical for real-time monitoring during exercise. Little is known about how an EM sensor would react to monitoring physiological parameters during exercise and how well it could penetrate through human tissue. Therefore each of the three selected parameters are present within the different layers of human tissue, as shown in Figure 1.1.

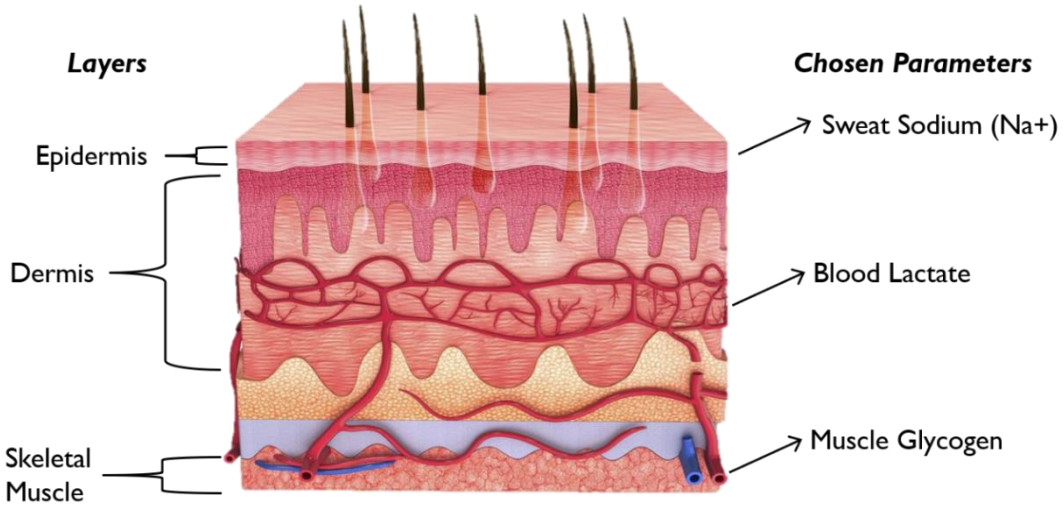

Figure 1.1 - Illustration highlighting where the parameters of focus are located within the anatomy of the skin and the tissues beneath

The first stage was to determine if the sensor's EM field would interact with the surface of the skin and the epidermis (top layer). Secondly, investigating the sensors capability at detecting a parameter situated within the dermis; this segment of tissue includes blood capillaries. Finally, assessment the functionality of the sensors to detect through the skin and into the skeletal muscle.

1. 2. 1 **Parameter 1: Sweat sodium**

Water (H_20) is the most important abundant inorganic (i.e. lacking carbon) compound in the body making it play an essential role and making up roughly 60-70% total body mass. Going only a matter of days without water can lead to severe long-term health complications and in serious cases, even death. However, athletes involved in weight-making sports such as boxing or MMA (Mixed Martial Arts), where they participate in dehydration techniques to "cut weight" leading up to the weigh-in and endurance athletes who are exposed to extreme environmental conditions, are at much higher risks associated with dehydration and electrolyte imbalances caused by excess sweating and in cases this has even led to death (Crighton, Close and Morton, 2016) (Rosner, 2015).

To prevent our bodies from overheating during exercise, water is used to regulate body temperature via increased sweating. When sweat is evaporated, heat is taken away from the surface of the skin, however, dehydration will occur if this fluid is not replaced. The onset of dehydration, as little as 2% body mass loss, has a significant detrimental impact on exercise performance (Armstrong et al., 1985). Currently, there is no real-time method of analysing hydration status during exercise. Current techniques which aim to monitor hydration levels are either as rudimentary as taking body weight measurements, and

checking the colour of urine, to complex and invasive techniques such as urine osmolality and blood tests.

However, monitoring sweat loss during exercise and specifically the compounds contained in sweat can give us a way to determine hydration levels during exercise. Sweat contains approx. 99% water with the other 1% comprised of electrolytes and urea (Shirreffs and Maughan, 1997), sweat analysis as a means of obtaining biochemical data has continued to gain interest in recent years. The monitoring of sweat electrolyte concentrations can yield much information on the physical and chemical state of the human body (Buono, Ball and Kolkhorst, 2007). Sodium (Na^+) is the primary ion in sweat, playing a pivotal role in maintaining water balance and fluid retention, and is essential for the generation of action potentials for muscle contraction (Baker, 2017). Monitoring sodium loss in sweat therefore can be used as marker of hydration status. Currently devices and techniques to monitor Na^+ loss can only provide a snapshot in time due to their single-use approach and provide no real indication of hydration status.

1. 2. 2 **Parameter 2: Blood lactate**

In sports physiology, the metabolite lactate within the blood is one of the most frequent and useful parameters to measure during both clinical exercise trials and real-world performance monitoring. Lactic acid is produced in our muscles during high-intensity exercise (Karlsson and Saltin, 1970) and is synonymous with the pain caused when muscles are pushed to their limits. Blood lactate concentrations are collected to monitor an athletes training intensity and output prescribed by the practitioner or coach.

The terms lactic acid and lactate are often used interchangeably. When lactic acid is produced, it is quickly ionised by releasing a hydrogen ion. It's traditionally believed

that the remaining lactate was a waste product, but we now know the remaining lactate compound can provide an additional source of energy (Leverve, Mustafa and Péronnet, 1998). The lactate threshold can be used as a good predictor of performance, exercise intensity and is a good indication of training adaptation. Currently, there are very few non-invasive or continuous monitoring systems available for this parameter. Being able to monitor lactate levels continuously during exercise will help to modify training and improve exercise performance and aerobic capacity.

1. 2. 3 **Parameter 3: Skeletal muscle glycogen**

Carbohydrates are the major fuel and energy supply for muscles, more so during prolonged or intense bouts of exercise. Carbohydrate (CHO) is predominately stored as glycogen in both the muscle and the liver, with a small amount of glucose circulating in the blood stream. Glycogen is the storage form of glucose, which is a form of CHO that can be mobilised as an energy source for exercise. Glycogen depletion is a major factor in fatigue during exercise. CHO availability has long been recognised as a key contributor to improved exercise performance since the early research by Christensen and Hansen in the late 1930's (Leverve, Mustafa and Péronnet, 1998) and by Bergstrom *et al* in the late 1960's and onwards (Ahlborg et al., 1967; Bergström et al., 1967).

The ability to monitor glycogen during exercise would allow for optimal fuelling and re-fuelling strategies during competition as well as playing a vital role in both preparation, and recovery, maximising overall physical performance. However, despite the need to monitor carbohydrate availability (especially in competitive athletes) due to its role in exercise capacity, the current gold standard method to detect skeletal muscle glycogen (the storage form of carbohydrates) is to use invasive muscle biopsies in a controlled

laboratory environment. This approach limits real-world application and ultimately does not allow for the monitoring of carbohydrate availability during exercise. This invasive technique has allowed insight into the role of glycogen in cellular adaptation and exercise performance, as well as an understanding of the sites of storage of this important metabolic fuel. However, the nature of this procedure limits the practicality of being able to monitor an athlete's glycogen stores outside of a controlled setting. A non-invasive technique that can accurately measure glycogen continuously will improve the accuracy of research protocols and allow for further insight into the physiological adaptations to exercise.

1. 3 **Novelty**

The use of EM sensors operating at microwave frequencies for the detection of physiological parameters is an emerging field offering a vast range of applications.

- To date, there is no work detailing how microwave sensors operate during exercise, nor is there any research examining if the parameters presented within this thesis can be detected or monitored in humans.
- This project is investigating the feasibility of an microwave sensor to detect and monitor blood lactate, skeletal muscle glycogen and sodium loss in sweat in human subjects during an exercise protocol tailored for each parameter.
- This innovative approach would enable greater insights and understanding into human exercise physiology and athletic performance monitoring, currently unattainable with current technology.

1. 4 **Aim and objectives**

This project aims to investigate the feasibility of a non-invasive microwave sensor to detect and monitor the physiological responses of three chosen parameters, sweat sodium, blood lactate, and skeletal muscle glycogen in humans during exercise. The objectives set out to achieve this aim are detailed below:

- To identify and investigate three biological parameters which would benefit from non-invasive continuous measurement, which will enable greater insights into human physiology during exercise
- Design and develop appropriate research methodology to enable measurement of sweat, blood and skeletal muscle during exercise in human participants to assess an EM sensor comparing against the current gold standard or best practice devices. Each experimental study should also aim to ensure parameters can provide a full range of naturally occurring concentrations to ensure validity of the sensor
- Investigate if, and to what level of accuracy, a microwave sensor can detect each parameter via a combination of in-vitro laboratory analysis and in-vivo analysis during a controlled exercise protocol
- Determine if the microwave sensor is a feasible option for use in sports performance monitoring

1. 5 **Thesis overview**

In order to obtain the objectives of this research, the thesis is structured into chapters. The initial chapter has discussed the importance and benefits of monitoring

physiological parameters during exercise and specifically that of sweat sodium, blood lactate and skeletal muscle glycogen concentration.

Chapter 2 reviews all the literature that consists of five sections namely, an overview of current sensor technology in sport and healthcare, theoretical backgrounds, and current commercially available methods of monitoring for sweat sodium, blood lactate and muscle glycogen. The literature will highlight the need for the development of non-invasive real-time sensors using electromagnetic wave sensors. This section will look at the theory behind electromagnetic waves and microwave sensing systems as a proposed method of detecting physiological parameters during exercise, detailing different sensor materials and design.

Chapter 3 seeks to explore the theory and use cases of the electromagnetic spectrum. How the spectrum was first discovered and subsequently allowed for the development of a variety of technologies each providing us with capabilities previously unimaginable at the time. The chapter will then discuss the use of an electromagnetic sensor operating at microwave frequencies and how this can be used to monitor biological tissues in real-time offering non-destructive measurement of these materials. The chapter will conclude by exploring microwave sensor design and fabrication requirements from existing research.

Chapter 4 will detail the general underpinning of the research methodology used throughout the research project, including participant information, sample preparation, experimental approaches, and data analysis. Additionally, this chapter will provide detailed accounts of the electromagnetic sensing system used and how the set-up

differed during in-vitro and in-vivo experiments and how anatomical positioning was a factor in monitoring each parameter.

Chapter 5 will be the first of three technical chapters. Each technical chapter will focus specifically on one of the three physiological parameters. Chapter 5 will detail the experimental approaches taken to enable sufficient quantities of sweat were produced in human participants during exercise and will also detail how a full spectrum of concentrations throughout the trials. To fully assess the EM sensor's capability to monitor sodium concentration in sweat, measurements during exercise was conducted, as well as additional laboratory-based analysis using the same sensor and sweat samples.

Chapter 6 will explore the second parameter chosen for investigation. To monitor the full spectrum of blood lactate concentrations in healthy adults, two experimental studies were conducted using the same exercise protocol and methodology. Study 1 assesses the blood lactate levels of untrained healthy participants, whereas study 2 assessed endurance trained participants. This chapter will investigate whether an EM sensor can detect the changes in blood lactate concentrations during a bout of progressive incremental exercise.

Chapter 7 examines the third and final parameter of the thesis, skeletal muscle glycogen. Initially this chapter explores the use of an EM sensor to detect glycogen within a laboratory environment analysing in-vivo solutions. Secondly, human trials are conducted using the gold standard technique to assess muscle glycogen which is an invasive muscle biopsy to provide data to compare the EM sensor.

Chapter 8 will bring together all the main findings from each experimental chapter and discuss the commonalities and differences between each parameter analysed and their significance. A general discussion is then given which focuses on the critical evaluation of the non-invasive electromagnetic sensor's capabilities in detecting each parameter. Additionally, specific attention is given to the use of the EM sensor to detect each parameter during exercise in professional sport. The discussion will seek to identify the future implications of EM technology as a feasible option for non-invasive measurement of key parameters associated to physical performance monitoring, as well as examine any limitations and methods to improve the current sensor set-up, in line with the data generated from each experimental chapter. The thesis concludes the overall body of work, outlining the impact of the thesis. It will then close with potential directions for future research.

CHAPTER 2 - LITERATURE REVIEW

In this chapter, the literature review will begin by focusing on the current wearable technology that are commonly used to monitor physical activity. Then, each of the three chosen parameters, sodium released in sweat, blood lactate, and skeletal muscle glycogen will be critically reviewed. For each parameter, the relationship to exercise performance will be explored, the current invasive and non-invasive methods used to monitor these parameters in humans will be provided. Finally, a summary of the key findings within the literature will be provided, along with discussion of the need for a novel non-invasive continuous wearable sensing solution to detect all three parameters.

2. 1 **The application of wearable sensing technologies to monitor physical activity and health status**

The need for non-invasive, real-time monitoring of biomarkers is prevalent in sport and healthcare settings. Over the past few decades, the interdisciplinary collaboration between technology and healthcare monitoring has led to some huge leaps in the field of advanced sportswear systems. This technology is designed to aid in athletic performance and allow the athlete to reach their desired goals and full potential by additional means, this technology is then filtered down and can be readily used by amateurs and novice populations. Wearable sensors are a relatively new addition to the professional sport due to the advances in wireless technology in recent years. These sensors can act as useful tools for monitoring and improving sports performance. They can provide physiological, performance and other insightful data before, during and

after sporting activity. These sensors can be designed alongside medical practitioners and used by coaches to optimise training and recovery and ensure athlete safety in more severe conditions. Significant progress has been made in the design and usability of wearable sensors across team and individual sports and provided substantial arguments in how they can assist athletes in improving many aspects of performance management.

In recent years, advances in technology have allowed athletes, sports teams, and physicians to monitor performance workload indicators (Aroganam, Manivannan and Harrison, 2019), rudimentary biological markers (Lightman, 2016; Seshadri et al., 2017; Camomilla et al., 2018) and examine athletes' movements in meticulous detail (Rein and Memmert, 2016; Jackson et al., 2018). The most common examples of available wearables which are used in sport today is the use of ECG (electrocardiogram) systems to monitor heart rate combined with GPS (Global Positioning System), accelerometers and gyroscopes to monitor physical activity. These systems have now become everyday routine across all sports and have now filtered down into the general population where basic understanding of cardiovascular function is widespread and is recognised as an important parameter to monitor during all types of activity. Application of wearable technology in sport comes in many different forms. The most popular body worn devices are on the wrist and chest, this is a trend that is forecast to grow past 2020, as well as sensors which are embedded into smart textiles (Kamišalić et al., 2018). An advantage of these sensors is that they enable users to monitor their performances without hindering any movement (Aroganam, Manivannan and Harrison, 2019).

Wearable technology in sports is not just about training performance. Monitoring health is also a key factor in the function of wearables. This type of technology enables health

monitoring outside of the constraints of a hospital or laboratory. There are wearable sensors which now meet clinical standards which are built into readily available smart devices (Koltowski et al., 2019). This level of accuracy is playing a key role in improving the future of healthcare. Collecting this data can play a critical part in self-diagnosis, monitoring current exercise activity and preventing more serious conditions from taking place. Currently, there are several techniques being used to monitor healthcare, medical and physical activity parameters. However, the majority of wearable devices on the market do not meet medical standards and aim to monitor physical indicators such as step count, heart rate, blood pressure, and blood oxygen and even glucose. The major limitations faced by these devices are in relation to accuracy levels, calibration, and reliability.

With the increased availability of wearable sensors, the quality of the data collected has vastly improved, often devices include their own data software packages and applications to analyse live streamed data. Professional sports utilise data analytics to assess the performance of their teams and athletes. Systematic, objective, and reliable performance monitoring can reinforce the link between research and coaching practice (Camomilla et al., 2018). Being able to track critical information about an athlete during exercise has the potential to provide further insights into key aspects of human performance. The future of wearable technology is to develop new predictive models, (e.g. objective measures to identify risk of injury, physical fatigue, enhancing or accelerating performance in line with the data output), these benefits would be widespread throughout sports and exercise science and would span out of just sport into areas where humans are placed in extreme physical and environmental conditions such

as during exploration and in military scenarios. The challenge becomes to enable more intelligent, real-time, accurate data, allowing coaches and athletes to act accordingly (Hynes, O'Grady and O'Hare, 2013; Lightman, 2016).

These revolutionary methods allow for observations and analysis of performance and health status indicators that were previously impossible to monitor outside of a controlled laboratory environment. However, all these technologies have common limitations, as to date, there has not been a practical method to track complex physiological parameters non-invasively in real time.

Despite recent advances in wearable technology, many state-of-the-art health and exercise monitoring systems rarely consist of more than a single sensing modality. However, the future of health and athletic monitoring requires multiple physiological parameters to be monitored simultaneously and using as few individual devices as possible. With recent studies researching the effects of different levels of glycogen (Bradley et al., 2016a), lactate (Kirsch et al., 2019), and sodium (Hoffman and White, 2020) levels during training and actual competition (Kiely et al., 2019), the need for real time non-invasive measuring equipment would be ever more valuable. This would help solve many of the issues faced by scientists and coaches when using invasive equipment outside of the laboratory environment.

2. 2 **Parameter 1: Relevance of sweat sodium for sport and exercise and the current measurement methods**

During exercise, sweat is secreted through the skin, serving in the regulation of body temperature through evaporation. Physical exertion in hot and humid conditions can

cause humans to lose vast amounts of water and consequently electrolytes contained within sweat (Meyer et al., 1992). The result of this can have damaging effects on health and athletic performance, water loss equating to only 2% of body weight can reduce physical work capacity (Murray, 2007). More severe levels of dehydration (i.e.>4-5%) have been linked to increased risk of heat-stoke (Howe and Boden, 2007), impaired glycogen use (Febbraio, 2000; Jentjens, Wagenmakers and Jeukendrup, 2002), nervous system fatigue due to increased core temperature (Nybo et al., 2001), cardiovascular strain (Cheuvront, Carter and Sawka, 2003) and reductions in the metabolic acid buffer system (Noakes, 1992). These side effects of dehydration highlight the critical importance of monitoring markers of hydration, not only from a performance point of view but also a health perspective.

It is commonly recognised that drinks containing electrolytes will promote improved performance better than water alone, especially for athletes who display considerable losses of fluid and electrolytes from prolonged or excessive sweating (Sawka et al., 2007). The monitoring of sweat electrolyte concentrations, therefore, can yield much information on the physical and chemical state of the human body. Sodium (Na^+) is the primary ion in sweat, playing a pivotal role in maintaining water balance and fluid retention (Sawka et al., 2007), and is essential for the generation of action potentials for muscle contraction (Weber and Murray, 1973). Current evidence suggests that maintaining pre-exercise levels of serum osmolality and serum Na^+ concentrations is a determinant of the beneficial effects of fluid ingestion on cardiovascular function and thermoregulation (Wendt, Van Loon and Lichtenbelt, 2007). However, competing in hot humid conditions such as ultra-endurance events can lead to severely low levels of Na^+,

brought on by competing with excessive drinking rates and increased levels of Na^+ loss. If Na^+ loss is not monitored, this combination can lead to a condition known as exercise-induced hyponatraemia (Hew-Butler et al., 2005). However, if athletes are exposed to heat frequently, the onset of physiological adaptations is referred to as heat acclimation. Heat acclimation can cause a linear decrease in sweat sodium ion concentration; this response can be rapid and has even been reported to occur following only two days of heat exposure (Buono et al., 2018). This evidence also suggests that heat acclimation can rapidly improve the rate of sodium-ion reabsorption (Buono et al., 2018). Therefore, it is recommended that fluid and Na^+ should be ingested at rates that closely match an athlete's rate of total sweat and Na^+ Loss.

The recommended guidelines for sodium replacement during exercise lasting more than 1 hr are to consume 20-30 $mmol.L^{-1}$ (Von Duvillard et al., 2004). However, as Na^+ loss is highly dependent on the individual and exercise conditions, being able to monitor Na^+ loss real-time will provide a more useful approach. However, current gold standard methods to detect markers of hydration status rely on complex procedures, analysing body fluids such as sweat, blood, urine, and saliva, only providing a snapshot in time (Armstrong, 2007). Real-time on-patient monitoring of Na^+ also would provide a clinical interest. Sweat electrolyte is currently used in the diagnosis of Cystic Fibrosis (CF), indicated by abnormally high Na^+ levels in sweat (Schazmann et al., 2010). Although currently CF diagnosis is determined in a technically complex manner, not providing real-time feedback (Schazmann et al., 2010). Additionally, in the treatment of CF, during the rehabilitative physical exercise stages, high-frequency real-time sweat electrolyte data could provide advantages to clinicians providing greater insight. Sweat monitoring

is dependent on three necessary steps, irrespective of the approach. Firstly, sweat stimulation, followed by sweat collection and storage and finally the analytical method (Schazmann et al., 2010).

2. 2. 1 How environmental stressors affect sweat sodium loss during exercise

Exercising in hot and humid conditions poses a significant thermoregulatory challenge on the body, affecting the control of the internal temperature, this onset is due to increased rates of metabolic heat gain and heat production from the environmental conditions. Exercising in the heat can, therefore, increase the risk of heat-related illnesses, especially in individuals who are unaccustomed to such environments (Casadio et al., 2017; Périard et al., 2017). Athletes, who are aiming to compete at optimal levels in sporting events held over summer months where high temperatures are expected, will face many challenges, and there is an increased chance of developing heat-related injury, especially for those unfamiliar to exercising in such environments (Casadio et al., 2017). A strategy employed to reduce the adverse effects of competing in hot environments is to implement a heat acclimation protocol. Heat acclimation is a process achieved through what is known as controlled hyperthermia. This process involves controlling the body's internal temperature over a period of time to achieve the necessary physiological adaptations to allow for prolonged exercise in hot conditions. Athletes can benefit from effective heat acclimation protocols due to the numerous physiological adaptations, e.g., a reduction in resting and exercising core temperature and heart rate, plasma volume expansion, earlier onset of sweating and higher sweat output. Combined, these adaptations contribute to improvements in cardiovascular

stability and sweating capacity. Overall, the athletes are better equipped to deal with heat stress due to improvements in thermoregulation and sporting performance can be improved during competition in the heat (Gagge, Stolwijk and Hardy, 1967; Corbett et al., 2018).

The most common form of heat acclimation is to use an active strategy, the three main techniques within the literature are; firstly, thermal clamping, referred to as "controlled hyperthermia technique" where a chosen core temperature is maintained; secondly, exercising at a constant predetermined workload in a hot and/or humid environment , known as the "constant work rate technique" and finally, where the individual chooses their working rate during the exercise trial in a hot and/or humid conditions, referred to as the "self-regulated technique" (Taylor and Cotter, 2006; Périard, Racinais and Sawka, 2015; Heathcote et al., 2018). Heat acclimation protocols are usually programmed for a specific duration. The most regular are short-term (< 7 Days), medium-term (8-14 days) and long-term (> 14 days) (Garrett, Rehrer and Patterson, 2011). Short term heat acclimation protocols provide a convenient solution when athletes have busy training programs ahead of an event or competition (Garrett et al., 2012; Zurawlew et al., 2016), yet medium-term protocols offer more significant physiological adaptations resulting in improved performances within hot conditions (Guy et al., 2015). However, the greatest adaptations occur during long-term heat acclimation programmes (Tyler et al., 2016). Although there are multiple methods and time durations to achieve heat acclimation to best suit the individual needs of the athlete, current recommendations within the literature state to achieve the physiological and performance benefits, athletes should train on consecutive days, across 1-2 weeks (Casadio et al., 2017).

Extreme hot conditions, especially dry heat can present a severe challenge to homeostasis. However, humans are extraordinarily well equipped to withstand and adapt to extreme physiological stresses commonly found in nature within desert environments (Kenney, DeGroot and Holowatz, 2004). The primary means of heat dissipation during exercise in hot conditions is through evaporation through sweating, and this is coupled with an elevation in skin blood flow. Maintaining functional sweat levels, therefore, plays a vital role in thermoregulation in such extreme environments (Kenney, DeGroot and Holowatz, 2004; Lim, Byrne and Lee, 2008). However, in the absence of fluid and electrolyte replacement, progressive dehydration and cardiovascular strain will follow. The time a person can tolerate in dry heat, therefore, depends on a sustainable sweating rate and the capacity to withstand the resulting dehydration (Kenney, DeGroot and Holowatz, 2004). During heat acclimation, however, the sweating response is enhanced and therefore aids in the ability to avoid thermal overload and maintain thermal balance overload (Henane and Valatx, 1973). Furthermore, sodium concentration within the sweat is significantly reduced following heat acclimation. It is well known within the literature that heat acclimation results in significant reductions in sweat sodium concentration (Allan and Wilson, 1971; Kirby and Convertino, 1986; Buono, Ball and Kolkhorst, 2007). There have been reports of sodium concentration being reduced as much as 50% following a 10-day active heat acclimation protocol in 40°C (Kirby and Convertino, 1986). More recently, it was shown that sodium concentration in sweat decreased during short term heat acclimation showing a linear response, on as little as only two days of active heat exposure (Buono et al., 2018). Such results suggest that heat acclimation can rapidly improve the reabsorption of sodium ion.

2. 2. 2 **The current techniques to monitor sweat sodium loss in humans**

Sweat monitoring is commonly used amongst scientists and practitioners to determine water and electrolyte losses in athletes during exercise. The data provided by monitoring sweat can be used to provide an individualised model to ensure sufficient fluid and electrolyte replacement strategies, leading to optimal athletic performance. Monitoring sweat electrolyte concentration and sweating rates in humans can be very individualised, variability in sweat can be caused by differences in collection methods (whole body or localised), the timing/duration of sweat collection, skin cleaning procedure, sweat sample handling/storage, and analytical method (Kolbinger and Lames, 2017). Measuring the sodium concentration in sweat plays an essential role in preventing fluid and electrolyte imbalances, as this allows the athlete to quantify the loss and replace with the appropriate amount of sodium. Monitoring sweat sodium concentration determined from anatomical sites can be used to predict whole-body sweat with regression equations. Sodium concentration in sweat can be highly individual and can vary significantly from person to person, and typical ranges are from approximately 10 to 90 mmol/L.

The primary sweat glands responsible for thermoregulatory sweating (Wilke et al., 2007) are the eccrine sweat glands. These sweat glands respond to thermal stimuli, such as increases in body core temperature (Nadel, 1979) and increases in skin temperature (Wingo et al., 2010) and are located across most of the body's surface. The three main methods to induce sweat to allow for sample collection are pharmacological, passive thermal (heat) stress, and exercise (Baker, 2017). Pharmacological methods involve the

stimulation of the muscarinic receptors on the sweat glands and induce sweat secretion by using a small electrical current (iontophoresis) to propel charged cholinergic agonists (usually pilocarpine) (Gibson and Cooke, 1959). Pilocarpine iontophoresis is the current gold standard method for sweat testing for the diagnosis of cystic fibrosis (Mishra, Greaves and Massie, 2005). However, there are significant differences in the local sweating rates between pharmacological and thermal and/or exercise-induced sweating. Local sweating rate has been shown to be consistently higher in thermal stress and during exercise when compared to pilocarpine iontophoresis (Vimieiro-Gomes et al., 2005; Taylor and Machado-Moreira, 2013). Comparison between the composition of pharmacological and thermal sweat has shown mixed results within the literature, although it is recommended when monitoring athletes, sweat should be collected from exercise and within relevant thermal stress environments (Baker, 2017).

Monitoring sweat sodium loss during exercise continuously in real-time is currently unavailable on the market. Current best practice recommendations for measuring electrolyte loss, including sodium in athletes, is to use an absorbent patch technique to measure sweat. Sweat is usually tested for sodium using an ion chromatography technique which is often done within a controlled laboratory environment. However, athletes needing to monitor sodium loss do not readily have access to such facilities, and the delay in results hinders any real-world use apart from providing an estimate of sodium loss in such conditions. Instead of using complex ion chromatography to measure sodium concentration in sweat, portable devices such as the LAQUAtwin Na^+ meter (Horiba) shown in Figure 2.1 can provide quick and reliable measurements of collected sweat samples, using an ion-selective membrane. The devices are compact and

only required a small amount of sweat to conduct measurements; this requires analysis to be assessable outside of a laboratory setting making it more suitable for real-world application (Baker et al., 2014).

However, to detect sodium concentrations in human sweat, firstly the sample site must be cleaned using deionised water and dried. The use of sterile sweat collection patches is required, and these are usually placed upon the forearm. The sweat patches are then collected from the subject once appropriate levels of sweat have saturated the patch following strenuous exercise or in hot environments. Once the sweat has been extracted, a small amount is placed upon the sensor of the LAQUAtwin Na^+ which then triggers the measurements. The sensor can only measure one sample at a time, to measure another sweat sample, firstly the device needs to be cleaned with deionised water and dried. This easy-to-use interface is simple and allows for a convenient and accurate method to monitor sodium concentration in sweat samples (Baker et al., 2014). However, there remains a need for a continuous device which can monitor sodium in real-time during exercise to ensure appropriate fluid and electrolyte replacement.

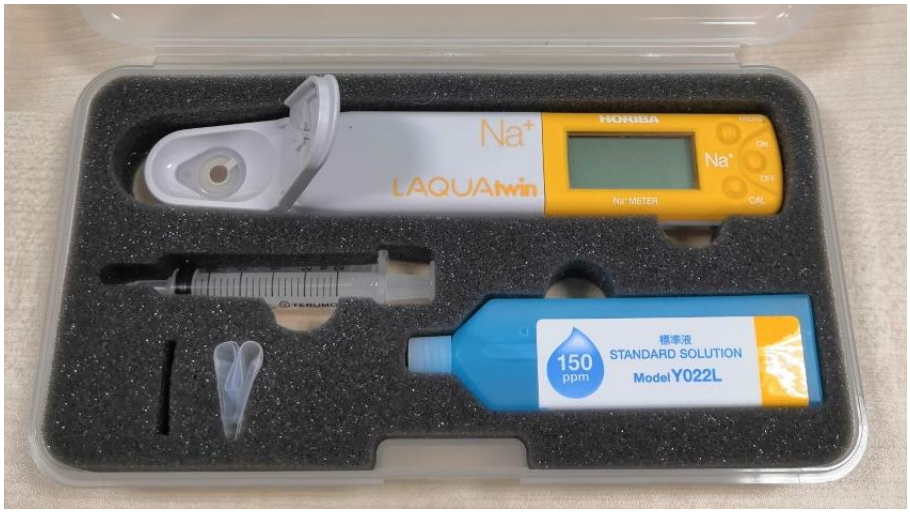

Figure 2.1 - Horiba LAQUAtwin Na+ meter with standard calibration solution

Currently, within the literature, sweat analysis is evolving from sample collection and laboratory analysis to development of fully integrated wearable platforms to monitor sweat sodium concentration. There have been various attempts at sweat analysis in the type of devices and sweat sampling techniques for wearable sensor application. Sensors have been embedded within clothing using textile-based sweat sensing techniques (Matzeu, Florea and Diamond, 2015; Parrilla et al., 2016), additionally sensors have been developed onto plastic and epidermal temporary-transfer tattoos to monitor sweat analytes (Bandodkar et al., 2014; Rose et al., 2014; Bandodkar, Jia and Wang, 2015; Kim et al., 2016). However, these devices are limited and can only provide a snapshot in time with a single measurement or lack on-site signal processing circuitry and sensor calibration mechanisms for accurate analysis. Developing a fully integrated wearable sensor can ensure accurate measurements in real-time and can offer continuous measurement with the appropriate sample regeneration (Gao et al., 2016b; McCaul et al., 2018). Commercially these devices are currently not available, and further research is needed to confirm the technology and its robustness especially during exercise and under different environmental conditions.

2. 2. 3 **Summary**

In summary, monitoring sweat can provide scientists, practitioners, and athletes with a tool to estimate sweating rate and monitor sodium loss. This data can help improve fluid and electrolyte replacement strategies that will aid in performance and improve the safety of an athlete, especially when exercising in hot and humid environments. However, further research is needed to assess how current all-in-one techniques can

manage outside of laboratory conditions, as no current commercially available sodium sensors can provide continuous monitoring, essential for practical application.

2. 3 **Parameter 2: Relevance of blood lactate for sport and exercise and the current measurement methods**

Blood lactate concentration is one of the most commonly measured parameters during clinical exercise testing and during athletic performance monitoring (Goodwin et al., 2007). Lactate is vital in two fundamental metabolic processes, glycolysis and oxidative phosphorylation, which serve as the primary source of energy production within the human body (Bakker, Nijsten and Jansen, 2013a). Glycolysis is the process of converting glucose into the intermediate molecule pyruvate. Oxidative phosphorylation completes the process, in conjunction with oxygen, to form carbon dioxide; and this results in the production of adenosine triphosphate (ATP) which provides energy to the cells for normal function (Goodwin et al., 2007). Under resting or non-active conditions, lactate levels in the bloodstream are maintained at a relatively low steady-state. According to Andropoulos (Andropoulos, 2012), whole blood lactate should be in the range 0.2 – 1.7 mmol/L in a healthy patient, with some variation, noted based on age. However, when stress is introduced to the body (e.g. via exercise or acute illness), the energy requirements of the body can alter significantly.

In response to progressive, incremental exercise, blood lactate concentration increases gradually at first and then more rapidly as the exercise intensity increases. The lactate threshold is a term used to describe the point at which lactate levels increase exponentially as shown in Figure 2.2 and is a better predictor of performance than VO_2

max which is maximum rate of oxygen consumption measured during incremental exercise and is a better indicator of exercise intensity than heart rate. Lactate threshold can therefore be held as the best single predictor of overall endurance performance (Sjödin and Jacobs, 1981; Tanaka et al., 1983; Allen et al., 1985; Yoshida et al., 1987; Coyle et al., 1988). In response to maximal physical exertion, lasting in the range of 30 – 120 seconds, peak blood lactate concentrations could be between 15-25 mM, usually observed 3-8 minutes post-exercise. In a healthy person post-exercise, the lactate level will steadily drop back to normal levels, with oxidative phosphorylation being able to clear the excess lactate.

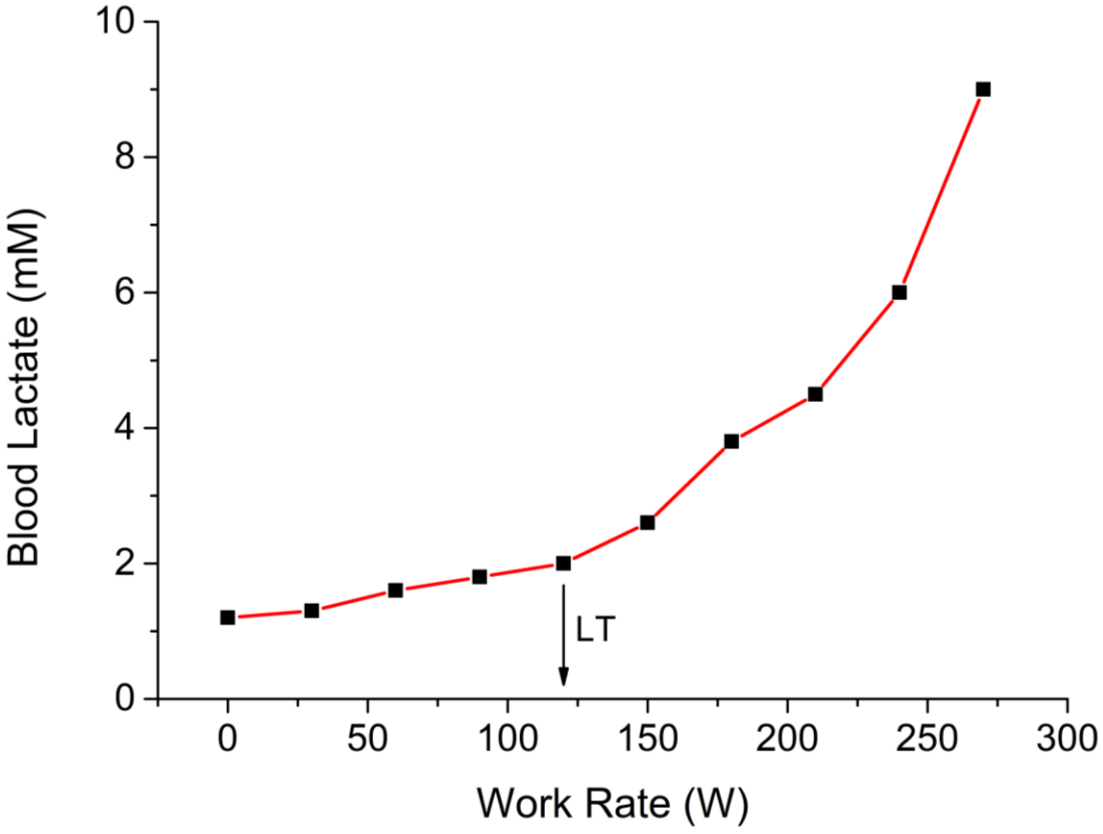

Figure 2.2 - A typical blood lactate response during progressive, incremental exercise. LT indicates the "lactate threshold". Adapted from Smith et al 1997 (SMITH et al., 1997)

When the stress placed on the body is due to illness, the tendency for the body to accumulate lactate is prolonged, perhaps resulting in lactic acidosis. It is therefore commonplace in contemporary medicine for lactate to be used as a means by which to evaluate the severity of acute illness, diagnose disease states, predict mortality, and assess response to resuscitation (Watson and Heard, 2010). Furthermore, in sport, lactate is one of the most often measured parameters when performance testing athletes and prescribing exercise intensities (Goodwin et al., 2007).

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Current off-the-shelf Point of Care (POC) technologies necessitate a blood sample. While steps have been taken to speed up the process of measurement and analysis, the requirement of extracting blood is still considered a major inconvenience. In a hospital environment, this carries significant infection control risks, and the frequency of sampling is rarely sufficient for clinicians to understand whether intervention is necessary. Even if patient blood is sampled and measured 4-6 times per day, as may be the case in intensive care environments, this does not readily enable one to understand if the lactate level is rising (i.e. worsening condition) or falling (i.e. recovery). For athletes, the issue of blood volume is less challenging since they are typically adult and

in a good state of health. However, blood sampling is still cumbersome in sport since athletes typically must reduce exercise intensity (or stop altogether) to provide a measurement which prohibits continuous high-resolution monitoring during exercise.

2. 3. 1 **Current invasive methods used to monitor blood lactate concentration**

In a clinical environment blood gas analysis has become an integral part of patient monitoring, particularly in the case of acute illness (i.e. in emergency wards or intensive care units), with clinical staff relying upon inclusion of blood gas analysers (BGAs) to assist in diagnostic workups and development of treatment plans (Arias-Oliveras, 2016). A BGA, such as that shown in Figure 2.3, can directly measure pH, partial pressure of oxygen (PO_2) and carbon dioxide (PCO_2), a variety of electrolytes, and various metabolites including glucose, lactate, blood urea nitrogen, and creatinine (Gonzalez and Waddell, 2016). Compared with laboratory analysis, a BGA offers rapid measurement time (approx. 1 minute, excluding sampling and transit times) and a wealth of information upon which assessment of patient condition can be made. It is no surprise, therefore that the BGA has become the gold standard against which many clinicians compare emerging point of care technologies.

Measurement with a BGA is not without its drawbacks, the process of extracting blood from a patient is an invasive procedure, with potential complications which include artery occlusion, digital embolisation leading to digital ischaemia, sepsis, local infection, pseudoaneurysm, haematoma, bleeding, and skin necrosis (Brzezinski, Luisetti and London, 2009). As a result of this infection control risk, resource availability, and also consideration of patient capacity to provide blood, BGA does not give a high-resolution

assessment of patient condition over time, which many clinicians argue would provide information relevant to understanding the necessity and form of intervention

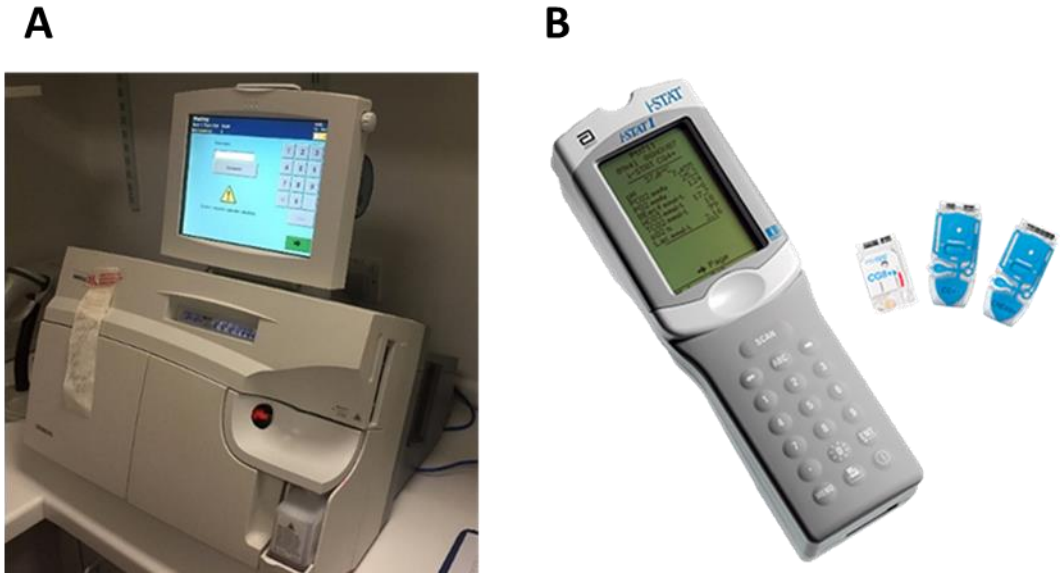

Figure 2.3 - A) An example of a Siemens® BGA, with the capacity to measure and predict 24 different parameters based on an input of approx. 0.1 ml of blood, and B) a handheld Abott i-STAT BGA offering a higher level of portability while retaining a broad range of measurement capability, needing 65 µL blood volume

Furthermore, the drive toward more point of care monitoring equipment located at the patient bedside has clinicians looking toward smaller and more portable devices. Some attempts to produce portable BGAs, such as the Abott i-STAT device illustrated in Figure 2.3B, have been commercialised and studies show they give levels of accuracy for lactate comparable with larger desktop systems (Ismail et al., 2015). However, the required blood volume (65 µL), sampling time (approx. 65 seconds) and skilled handling procedure preclude its use at the bedside. BGAs offers a broad range of measurements, but several devices have been released to the market which offer single metabolite measurement. These are typically based on an electrochemical principle, using an electrochemically sensitised strip which, when exposed to blood, changes its electrical

properties. When inserted into a device designed to interface with these strips, users can obtain a lactate reading within 15-60 second's time. While these devices still require blood to be extracted from a subject, the volume requirement is significantly lower than a BGA – for example, the Lactate Pro V2 LT–1730 system (Figure 2.4), requires only five μL of blood.

Figure 2.4 - Lactate Pro V2 LT–1730 in use to detect blood lactate levels via a finger prick.

An in-depth study was carried out by Bonaventura *et al* (Bonaventura et al., 2015) considering the reliability of such hand-held electrochemical devices, which concluded that although all devices tested exhibited varying characteristics (error, accuracy), all could be used for longitudinal studies and have particular relevance in prescribing exercise regimes. A smaller study by (Singh et al., 2016) also demonstrates that such electrochemical sensors give acceptable results in clinical settings, and some are approved for medical use. However, there is little evidence to show significant uptake in this context. This is perhaps due to uncertainty regarding the unknown sources of error with point of care devices (e.g. temperature, operator training, equipment condition, etc.) when compared with clinical laboratory facilities (Boldt et al., 2001), and

the remaining infection control risk due to extraction of blood, albeit in smaller volumes. In addition, some caution against the use of a fingertip test for lactate due to inferior accuracy. (Gaieski et al., 2013) note that this may not be an issue in all patients but compare the case of those undergoing intensive care with those presenting at emergency departments. In the former case, patients will be given significant volumes of intravenous fluid which, coupled with continued capillary leak and decreased intravascular osmotic pressure, can lead to diffuse tissue oedema (Koomans and Boer, 1997). In the latter case, however, patients are often hypovolemic, potentially decreasing the amount of extravascular fluid that enters a fingertip blood sample.

Devices such as the Lactate Pro and i-STAT represent the current state-of-the-art in terms portable point of care systems for determining absolute blood lactate, and work continues in this field to improve cost, reliability, and accuracy. A comprehensive review of electrochemical sensor techniques to realise lactate measurement has been produced by (Rathee et al., 2015) and other research continues to improve this field through new fabrication techniques and methods to move toward wearables, with researchers utilising sweat rather than blood for lactate measurements (Gao et al., 2016c; Tur-García et al., 2017). The desire for devices to be wearable is well known across a range of blood metabolites, to remove the need for blood extraction altogether and revolutionise healthcare practices.

2. 3. 2 **Non-invasive methods of detecting blood lactate**

Conventional methods for lactate detection rely on invasive or partially invasive sampling methods such as finger pricks to assess lactate measurements. These methods are currently not suited to POC or personal health monitoring devices looking to

incorporate an all-in-one solution, which can monitor lactate levels during exercise. The alternative to non-invasive monitoring in sport currently on the market is the BSX Insight lactate prediction system shown in Figure 2.5.

Figure 2.5 - BSX Insight athlete lactate prediction system. A) with the sensor removed from its wearable sleeve. B) Worn by a cyclist during exercise

This wearable device has been shown to predict the lactate threshold, the point at which the concentration of blood lactate begins to exponentially increase during exercise (Borges and Driller, 2016). This system uses near-infrared (NIR) sensors inside a compression material sleeve which monitors muscle oxygenation in the gastrocnemius muscle, using a patented algorithm, and can detect inflexion points in the muscle oxygenation curve at increased workloads. Other optical-based techniques for monitoring lactate are evident in the literature (McCormack et al., 1997; Li et al., 2002; Arnold et al., 2003; Lafrance, Lands and Burns, 2004; Wang et al., 2016); however, little

of that work appears to have made a significant presence on the POC market. These types of devices combine a chemical approach (i.e. a colour change) which then infers a lactate concentration. However, these suffer from the same drawback as current electrochemical methods, namely the limited reusability of the sensitive elements of the devices themselves.

Currently, research into wearable sensing systems is attempting to incorporate complementary sensors to monitor a more robust fitness-monitoring platform rather than just physical and electro-physical sensors alone. Research has been completed into the development of a wearable chemical-electrophysiological hybrid biosensing system in the bid to monitor lactate during exercise (Imani et al., 2016). Using a single epidermal patch, comprised of three-electrode amperometric lactate biosensors and two electrocardiogram electrodes. The combination of these sensors is tailored to monitor physical exertion. The ability to monitor multiple physiological parameters using a single wearable patch allows for greater understanding of a person's health status and is also more convenient for the user.

Analysing epidermal sweat is showing promising results in detecting lactate. A non-invasive tattoo based lactate sensor using a flexible substrate has been developed (Jia et al., 2013). However, tattoo-based electrochemical sensors show significant calibration issues and face questions regarding how they cope under exercise conditions due to stability and their durability (Windmiller et al., 2012). Another device which uses sweat to monitor lactate levels is the organic electrochemical transistor (OECT) sensor with an ionogel solid-state electrolyte on a flexible biosensor. The sensor supports a wearable design in a bandage-type form, well suited to exercise during health monitoring

(Khodagholy et al., 2012). Furthermore, innovative developments have emerged in the non-invasive POC sensors, with advancements in flexible and lightweight materials essential for wearable devices (Labroo and Cui, 2013). An example is the development of contact lenses to monitor biological information, eliminating the need for any sample collection. The contact lens-based lactate sensor is minimally invasive and designed to monitor lactate levels in tear fluid (Thomas, Lähdesmäki and Parviz, 2012). Another innovative approach is the development of an amperometic electrochemical lactate biosensor that can be fitted with nose-bridge pads which attach to eyeglasses. This platform uses wireless connection and screen printed electrochemical sensors to detect lactate in sweat in the nose region, and it has been reported to continually sense lactate for a dependent period within a couple of hours (Sempionatto et al., 2017). The challenges faced by POC devices and areas that need to be addressed are the long-term stability, selectivity, sensitivity, power consumption and biocompatibility. With POC devices efforts need to be made in ensuring reliability during rigorous use in real-world environments. Factors such as resiliency and cost-effectiveness need to be considered if steps are to be taken away from traditional blood sampling techniques.

2. 3. 3 **Summary**

Blood lactate is one of the most measured parameters in sports performance monitoring due to its role in determining both the exercise intensity of a session but also the overall fitness levels of an athlete. Currently however, the gold standard and the method which is readily used in sports is using a device which can detect lactate levels from a small drop of blood. This invasive technique is not the ideal solution and the need to develop a reliable continuous device which an athlete can wear whilst exercising can be seen in

the literature. Many of the technologies which are emerging cannot provide a reliable measurement, so blood sample analysis will remain the preferred method of choice, until a reliable device comes to market.

2. 4 **Parameter 3: Relevance of skeletal muscle glycogen to sport and exercise and the current measurement methods**

The capability to monitor skeletal muscle glycogen, conveniently, non-invasive and in real-time remains elusive. This literature review aims to critically review the current gold standard and current state-of-the art techniques to monitor skeletal muscle glycogen.

2. 4. 1 **The role of skeletal muscle glycogen**

Muscle glycogen provides the main source of energy during anaerobic exercise. Furthermore, total glycogen stores within the body also contribute significantly to energy metabolism in endurance-type events lasting longer in duration. Therefore, endurance based events lasting up to three require strategic preparation of carbohydrate (CHO) based fuels (muscle and liver glycogen, blood glucose and blood muscle and liver lactate) to sustain the high demands for energy production (Burke, van Loon and Hawley, 2016; Leckey et al., 2016; Torrens et al., 2016). Glycogen and the enzymes responsible for glycogen synthesis (glycogenesis) are contained within the cytoplasm of liver and muscle cells. Excess glucose under normal circumstances following the ingestion of carbohydrate, enters the pathways of energy metabolism where it's either stored as glycogen, or converted to fat. Glycogenesis is the formation of glycogen from glucose. The demand for glucose and adenosine triphosphate (ATP) depends on the rate

that glycogen is synthesized. If both glucose and ATP are present in substantial amounts, then the surplus of insulin stimulates glycogenesis for storage in the liver and muscle cells. Glycogen is the principal d-glucose storage polymer in humans. Most human cells have glycogen, but only liver and skeletal muscle cells are able to store significant quantities of this molecule (Baba, 1993). Glycogen is a polysaccharide, compose of hundreds of glucose molecules (monosaccharides) joined end to end, with prevalent branches. Osmotic pressure is dependent on the number, not the size, of dissolved substances.

A single glycogen molecule may contain 5,000 glucose units compared to that of 5,000 individual glucose molecules. This explains why glycogen is a convenient way to store glucose inside cells without affecting cell osmotic pressure (Tiidus, Tupling and Houston, 2012). Glycogen contains a number of hydroxyl **(**OH) groups, which allows for the interaction with water in the cell, this means that in terms of weight, glycogen is a substantial fuel as shown in Figure 2.6 (Ahlborg et al., 1967). Rates of post-exercise glycogen synthesis are integral to an athlete's regime, this allows the athlete to ensure sufficient energy stores for the following day, a fundamental component in events which take course over many days. Without consumption of CHO post-exercise, glycogen synthesis occurs at rates of 1-2 mmol/kg wet weight (w.w) of muscle/h through gluconeogenesis (Maehlum and Hermansen, 1978). However, when large amounts of CHO are consumed post-exercise, glycogen synthesis improves greatly, rates of resynthesizing increase to within the range of 5-10 mmol/kg w.w./h and then continue throughout the recovery stage (Burke, van Loon and Hawley, 2016). Maximising muscle glycogen synthesis in between important exercise sessions and events therefore is

dependent on matching fuel stores closely with the demands of the intended exercise intensity and duration.

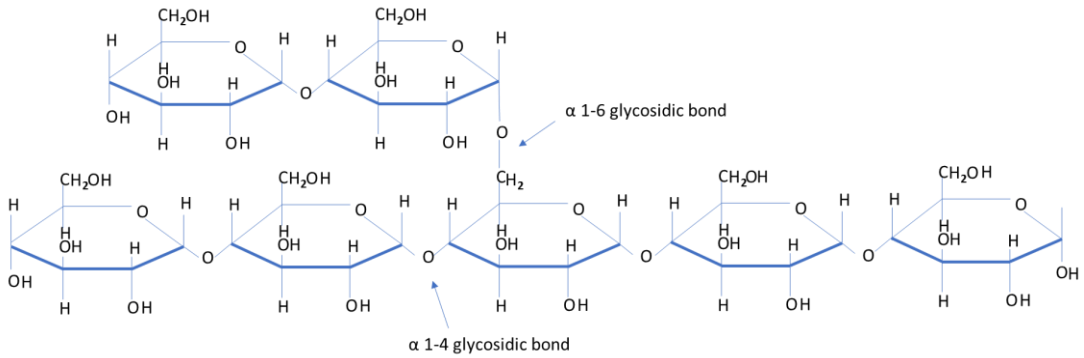

Figure 2.6 - A section of a glycogen molecule illustrating individual glucosyl units. It shows the two different types of glycosidic bonds used to make up glycogen

Carbohydrates are an important source of fuel and energy for intense and prolonged bouts of exercise. Glycogen is one of the main energy sources for ATP production to facilitate muscle contraction during a wide range of exercises, from brief high-intensity exercises to endurance exercises as shown in Figure 2.7 (Bergström et al., 1967). It has been reported that muscle glycogen content is associated with muscle performance and its depletion by high-intensity exercise leads to a decline in performance, also known as muscle fatigue (Costill and Hargreaves, 1992). Glycogen stores in human muscle and liver are determined and will vary dependent on the individual's activity status and how much CHO they consume (Burke, van Loon and Hawley, 2016). Normal levels of muscle glycogen stores for a well-trained athlete can usually fuel sporting activity for up to 60-90 minutes (Yeo et al., 2008a). Muscle glycogen levels have a profound impact on an athlete's sporting performance, thus measurement is vital. Although fatigue is a complex process involving many variables, there is a large amount of evidence to suggest the main cause of fatigue during endurance exercise is reduced muscle glycogen and blood

glucose availability, which reduces the availability of substrate required to maintain the high CHO oxidation rates necessary to sustain high power outputs (Maclaren and Morton, 2012). During exercise, CHO availability to the working muscle and central nervous system could become compromised due to the athlete exceeding endogenous stores of CHO when fuel cost is more than expected during either training or competition, reducing performance (Hawley and Morton, 2014).

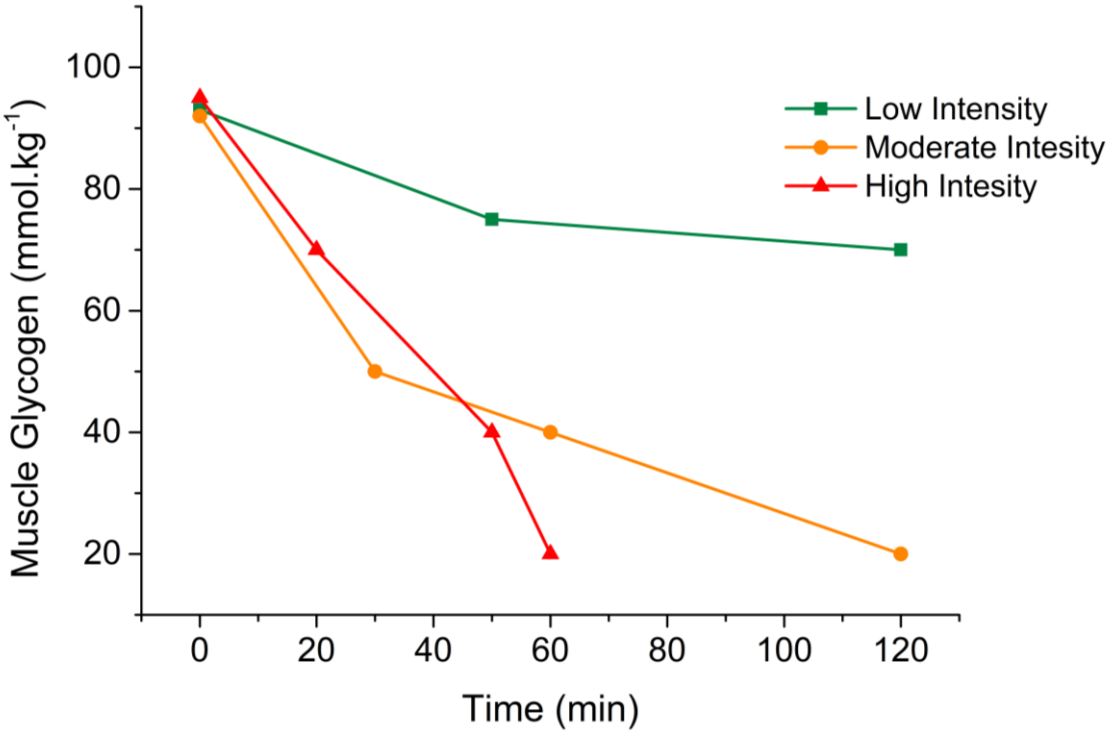

Figure 2.7 - Muscle glycogen use during exercise at different intensities (adapted from Gollnick et al., 1974)

The promotion of high CHO availability for prolonged exercise is widely established (Bartlett, Hawley and Morton, 2015b) to ensure there is enough muscle substrate to match the demands of the intensities and volume of endurance training and competition shown in Figure 2.8. To do this one of the most regular requests by a nutritionist or coach to an athlete is to undergo CHO loading to super-compensate muscle and liver glycogen

stores in the days before a major endurance competition. As well as ensuring a diet high in CHO, during competition the athlete will also be advised to ingest drinks, bars and gels with a high CHO content (Bartlett, Hawley and Morton, 2015b).

Muscle glycogen is widely recognised as the primary fuel source for sustaining contractile activity in human skeletal muscle (Bergström et al., 1967) Thus, the ability of skeletal muscle to perform repeated contractions (exercise) is seriously compromised when muscle glycogen reserves reach low levels (Bergström et al., 1967; Hermansen, Hultman and Saltin, 1967a; Balsom et al., 1999a) demonstrating a clear association between muscle glycogen and fatigue resistance during both prolonged and high-intensity exercise, this is illustrated in Figure 2.8.

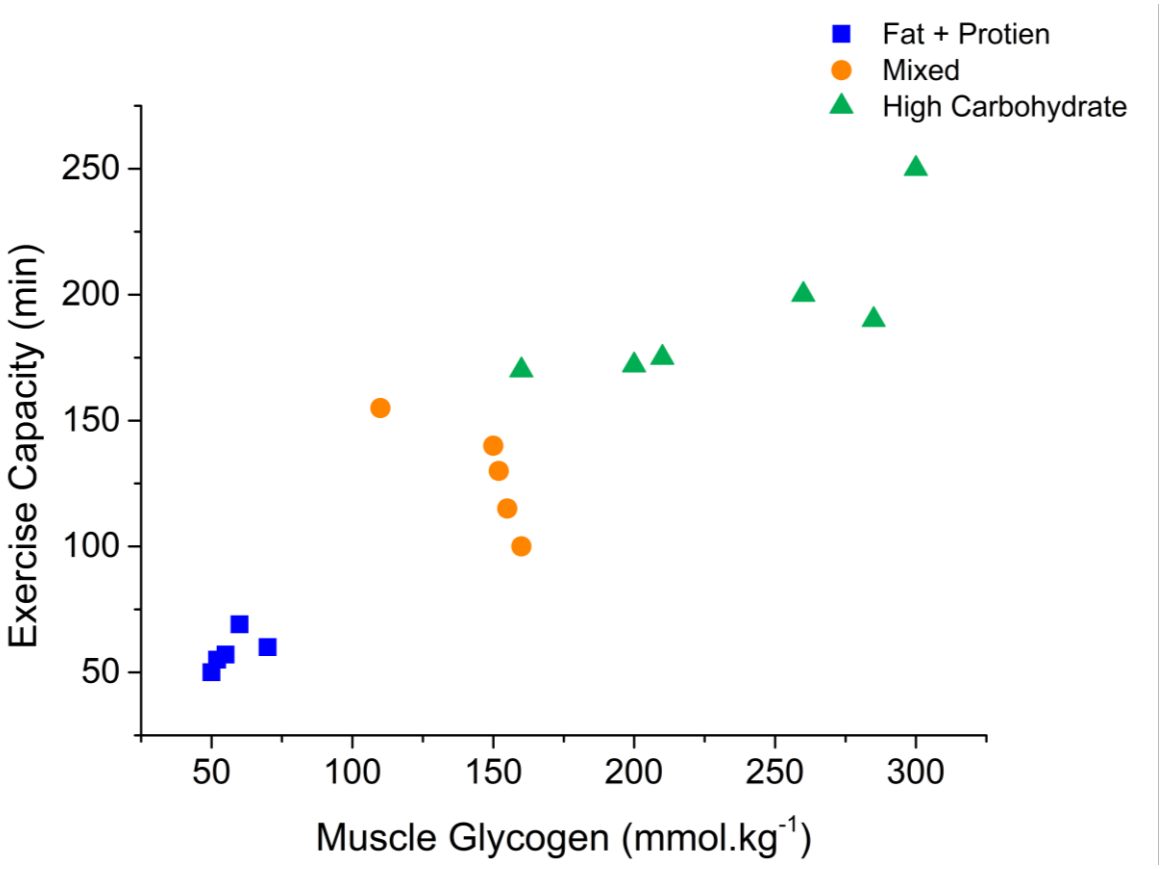

Figure 2.8 - Relationship between muscle glycogen content, exercise capacity and diet (adapted from Bergstrom et al. 1967)

To this end, it is widely recommended that exercise should be commenced with high CHO availability in order to optimise performance and delay fatigue (Burke et al., 2011). On the other hand recent research has also demonstrated that deliberately reducing the CHO availability around training sessions (i.e. by using fasted training, sleeping low, recovering low, training twice a day) is also shown to up-regulate the physiological adaptation to training (Burke et al., 2011). Consequently the concept of CHO periodisation has been introduced to help athletes both enhance the adaptive response to training (through low CHO availability around certain training sessions) and enhance exercise capacity in competition (through high CHO availability during all competitions) (Louis et al., 2016; Marquet et al., 2016).

2. 4. 2 **The need for non-invasive real-time detection of muscle glycogen during exercise**

Given the high training loads of elite athletes, traditional nutritional guidelines have typically advised a high CHO diet in addition to exogenous CHO provision during exercise and within the immediate recovery period following exercise (Burke et al., 2011). However, research gathered over the last decade has established that systematically commencing exercise with low muscle glycogen and limiting CHO intake during exercise, supplements a number of markers of mitochondrial biogenesis (Bartlett, Hawley and Morton, 2015b). Current recommendations involve the periodization of carbohydrates, alternating periods of low of high CHO availability according to the training load (Philp, Hargreaves and Baar, 2012). As such, most recent guidelines for CHO intake for training and competition (Burke et al., 2011) recognise that there is a need for a flexible and individual approach to the intake of CHO, dependent on such

factors as training status, type of training and the time to competition. Current guidelines are seen to promote a sliding scale of CHO intake with the goal of matching the predicted energy expenditure of the athlete's training and recovery (Burke et al., 2010). This research therefore shows the need to be able to monitor an athlete's glycogen stores to suit the specific needs of the athlete during the cycles of training and competition.

Untrained individuals consuming a mixed diet normally have a skeletal muscle glycogen content of ~80–90 mmol kg^{-1}, however for athletes involved in regular endurance training; this amount is higher at around 125 mmol kg^{-1} (Hawley et al., 1997). As 1 g of glycogen is usually stored with 2-3 g of water, a negative of glycogen loading is that the athlete's body mass will likely increase by around 1-2% after a period of several days CHO 'loading' (Bartlett, Hawley and Morton, 2015b). CHO loading is the process which endurance athletes undertake prior to competition to super-compensate glycogen stores to reduce the effects of muscle glycogen depletion on fatigue and exercise capacity (Bergström et al., 1967).

A practical example of CHO loading is reported by Bussau et al (Bussau et al., 2002a) who reported that elevated muscle glycogen stores may be achieved in as little as 24–36 h of rest and high CHO intake (8–12 g kg day^{-1}), which is a strategy for athletes who are participating in weekly cycles of competition. Being able to monitor glycogen stores during real time will allow development of strategic training programs which cater for specific needs of athletes. Recent studies which used a variety of strategies to reduce CHO stores manipulating CHO availability in both endogenously and/ or exogenously during short term training interventions have reported strong up-regulation of training

adaptation including increased whole body fat oxidation and increased activities of oxidative enzymes, when they were compared with exercising with normalised glycogen stores and high CHO availability (Yeo et al., 2008a; Morton et al., 2009a; Bartlett et al., 2013) as well as increasing whole-body and intramuscular lipid oxidation (Yeo et al., 2008a; Hulston et al., 2010a).

The practical application of training with lowered CHO availability (typically called "train low") is still in its early states as there are known limitations and risk factors associated with training consistently with low CHO stores. Training repeatedly with low CHO stores is reported to lead to an inability to maintain the preferred training intensity (Yeo et al., 2008a; Hulston et al., 2010a) this could furthermore lead to a substandard training impulse (i.e. volume x intensity). CHO restriction during training which is of high-intensity or long in duration can also have negative effects on an athlete's health, making them more susceptible to illness and infection, this is due to the role CHO have in offsetting exercise-induced immunosuppression (Gleeson, Nieman and Pedersen, 2004).

Another factor to consider is the increase of muscle protein breakdown, especially with conditions of low muscle glycogen (Howarth et al., 2010). The advantages and limitations of altering CHO stores throughout training has widely become one of the most debated topics for athletes, coaches, nutritionists and scientists. The importance of being able to record and measure CHO stores therefore is essential to provide real time non-invasive data, providing practical methods for real world situations.

2. 4. 3 **Methods of measuring muscle glycogen in athletes**

2. 4. 3. 1 **The elusive gold standard**

Currently the typical method to measure muscle glycogen requires an invasive muscle biopsy. Involving the use of needles, muscle biopsies have been the standard method to measure muscle glycogen. This procedure is common among sport science but does have its draw backs due to its invasive nature. The percutaneous biopsy technique is known to obtain skeletal muscle tissue specimens from human subjects. Duchenne (1806-1875) is recognised for the construction of the first needle with a trocar to obtain skeletal muscle from living subjects using this biopsy method (Shanely et al., 2014b). Bergström in the 1960's developed a needle similar to that previously used by Duchenne (Bergstrom, 1962; Bergström, 1975). The modified Bergström technique which is still being used today was developed in the 1980's by Evans *et al* (Evans, Phinney and Young, 1981) as shown in Figure 2.9.

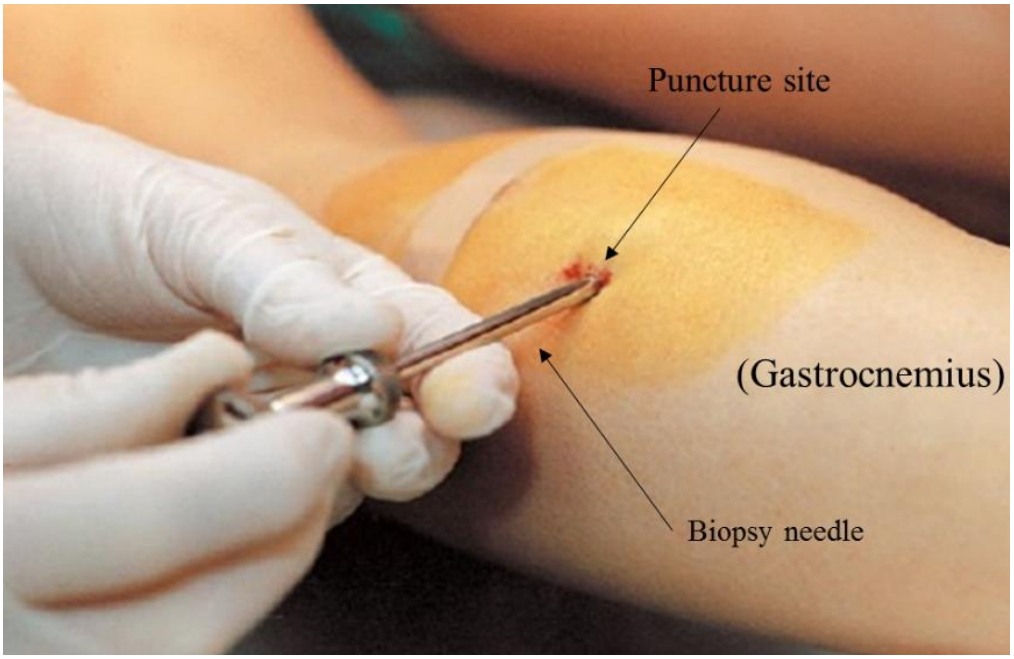

Figure 2.9 - Illustration of an invasive muscle biopsy being performed on the gastrocnemius muscle

This technique uses the addition of suction (700 TORR) to the inner bore of the biopsy needle after the needle has been inserted into the subject's muscle. The suction is designed to pull the surrounding muscle tissue into the needle, consequently ensuring the taking of a larger piece (X = 78.5 mg) (Evans, Phinney and Young, 1981). The advantages of this technique eliminate the need for recurring biopsies because of inadequate muscle sample size and improve the validity of subsequent analysis procedures (Hennessey et al., 1997), thus making it a recognised method in clinical and biomedical research environments.

The modified Bergström is invasive but ensures that it causes as little damage as possible, making the procedure relatively safe. The technique elevates the quality of the sample collected during the testing, whilst doing so under minimal time contraints for what is needed. Multiple biopsies can be taken from one subject during that specific session and the procedure can be completed quickly when the correct preparation is in place (Shanely et al., 2014b); this allows for pre-, mid-, and post-exercise biopsies to be taken. Another advantage of biopsies is it allows the measurement of many different outcome variables, not only the analysis of muscle glycogen stores, being useful when other parameters are investigated. Other outcome measures include for example, fibre typing, muscle damage, different fuel substrate stores, mitochondrial biogenesis and respiration, enzyme activity, shifts in metabolites and protein synthesis (Shanely et al., 2014b).

A current example of a muscle biopsy needle that is used in a sporting context is Monopty 12G, disposable core biopsy instrument (BARD, Brighton, UK), this needle has been used to provide field data in professional rugby players to measure muscle

glycogen utilisation pre- and post-game (Bradley et al., 2016b) shown in Figure 2.10. Once the biopsy has been taken it is then essential to immediately snap freeze in liquid nitrogen and stored at −80 °C for later analysis, this therefore shows the delay in time and resources that is required to gain a true muscle glycogen reading and why a non-invasive sensor could provide a practical and time saving method to the professional world of elite performance.

Figure 2.10 - Illustrates the Monopty 12G, disposable core biopsy instrument (BARD, Brighton, UK) inserted into an athlete's vastus lateralis

Although the use of biopsies is relatively safe, practicality in a sports setting to regularly measure skeletal muscle for the analysis of CHO stores is limited due to the invasive nature of the testing. Indeed, biopsies generally must take place within a biomedical research setting in order to limit the risk of infection. After the athlete has undergone a biopsy, it usually takes up to 5-7 days for soreness and swelling to fully dissipate. Although it is very rare, infection can accrue post procedure due to several factors. Tarnoplsky *et al* research reports taking 13,914 biopsies in both adults and children, with a total of 22 complications throughout (Tarnopolsky et al., 2011). Complications were as

follows, local skin infections (8 cases), arterial bleed (2 cases), ecchymosis/haematoma (2 cases), pain persisting for more than 3 days (5 cases) and a small area local numbness distal to the biopsy (5 cases) (Tarnopolsky et al., 2011).

Most subjects experience local soreness and stiffness in the leg for two or three days after the biopsy similar to a deep bruise, which is a key factor in why performing a biopsy before competition can be difficult to achieve due to the distractions it can cause for the athlete. There is a very low risk of internal bleeding at the biopsy site, which can result in more prolonged pain and stiffness in the leg. On occasions, a small lump of scar tissue may form under the site of the incision, but this normally disappears within 2-3 months, or within a few weeks if massaged. A small visible scar often remains from the biopsy incision. There is the possibility of a small area of numbness (approx. 25mm) around the biopsy site. This usually resolves over 5 – 6 months. There is a very low risk (estimated at less than 1/5000) of damage to small nerve branches within the muscle. This would result in partial weakness of the muscle and would likely have no impact on day-to-day activities. Nerve injuries like this usually resolve in 8 – 12 months, but there is a theoretical risk of mild leg weakness.

2. 4. 3. 2 **Histochemical methods**

Periodic Acid-Schiff (PAS) stain is based on the reaction of periodic acid with the diol functional groups in glucose and other sugars, oxidizing them to form aldehyde, which in turn reacts with the Schiff reagent to give a purple/magenta stain as shown in Figure 2.11B (Prats et al., 2013). PAS stain is therefore not specific to glycogen; it also stains glycoproteins and proteoglycans. In order to single out glycogen from the other PAS-

reactive cellular components cryosections can be pre-treated with the glycogenolytic enzyme diastase (Baba, 1993) shown in Figure 2.11A.

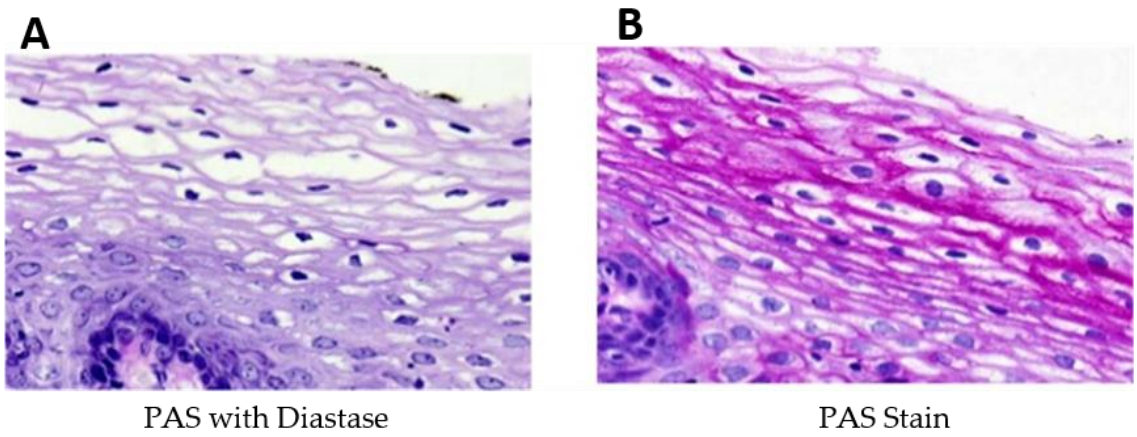

Figure 2.11 - The presence of glycogen is shown by the loss of staining after enzyme treatment when compared to the untreated segments. A) Glycogen sample pre-treated with Diastase. B) Glycogen sample after PAS stain

Once muscle biopsy samples have been removed, standard procedure requires the sample is immediately frozen in liquid nitrogen and stored at -80^{o}C. Collagen, blood, and other non-muscle fibre materials are then removed from the sample from under a microscope by a trained lab technician. The sample of muscle fibre (2-3 mg) is then weighed and 500 μl of 1 mol hydrochloric acid/L is added. After heating for 3 hours at 100^{o}C to hydrolyse the glycogen to glycosyl units and cooling down to room temperature, the solution is then neutralized by adding 267 mL tris/KOH. To conclude the procedure, 150 μl is then analysed for glucose using a calibrated specialised glycogen assay kit. After a muscle biopsy has been performed and the sample is prepared for use, glycogen content can be measured by biochemical techniques; however, such techniques are most often performed on muscle homogenates, and can therefore not discriminate between intramyocellular and extramyocellular glucose stores, and do not allow for

muscle fibre typing. In order to measure only intramyocellular energy stores and to differentiate between the different fibre types, histochemical methods have been extensively used (Prats et al., 2013).

The down side of this technique is that many published studies don't use this process and therefore glycogen content can be overestimated (Prats et al., 2013). A study by Fairchild & Fournier (Fairchild and Fournier, 2004) revealed that thawing and air drying muscle cryosections result in glycogen degradation. However, it is common practice in laboratories, where histochemical measurements of glycogen in tissue cryosections are done by PAS staining, to still thaw and dry tissue cryosections after cutting and before fixation (Prats et al., 2013).

New research which enables laboratories to optimise skeletal muscle preservation and increase stain specificity is to use the monoclonal anti-glycogen IgM antibody (Nakamura-Tsuruta et al., 2012). For optimal preservation of glycogen stores, muscle cryosections should not be air-dried. Any cycle of freezing/thawing should be avoided due to the resulting effect in loss of glycogen particles (Fairchild and Fournier, 2004). To increase the specificity of the glycogen staining, the use of a monoclonal antibody is recommended (Prats et al., 2013).

2. 4. 3. 3 **Magnetic resonance spectroscopy**

The need for extremely accurate methods to detect small changes in glycogen levels began when it was discovered that in diabetic subjects, responses to physiologic hyperinsulinemia caused changes in glycogen concentrations which were too small to be detected by the current biopsy techniques (Shulman et al., 1990). It was recognised that this was first done by obtaining the ^{13}C nuclear magnetic resonance (NMR) spectra

of human muscle glycogen in vivo from the 1.1 percent carbon nuclei that naturally occurs as this isotope (Jue et al., 1989a). Furthermore, NMR measurements of glycogen concentrations can be made more accurate by infusing ^{13}C-enriched glucose (Jue et al., 1989b). ^{13}C NMR spectroscopy was validated by Taylor *et al* (Taylor et al., 1992), the study compared the NMR to muscle biopsies and direct biochemical assay for glycogen concentrations. The results reported that in vivo, ^{13}C NMR measurement of human muscle glycogen can be considered just as accurate as biopsy results as well as delivering a higher precision measurement than a biopsy with a direct biochemical assessment.

Magnetic resonance spectroscopy (MRS) also offers a non-invasive method of detecting skeletal muscle glycogen accurately. MRS measures the chemical content of MR-visible nuclei, which include the metabolic elements of hydrogen (^{1}H), carbon (^{13}C), and phosphorus (^{31}P) (Befroy and Shulman, 2011). Whereas magnetic resonance imaging (MRI) establishes the spatial distribution of water (and lipid) protons within the site of interest (Befroy and Shulman, 2011). MRS is performed using the same machine as a conventional MRI scanner, using a powerful magnet, radio waves, and a computer to create detailed images. Spectroscopy is a series of tests that are added to the MRI scan across specific regions of the body for chemical metabolism. There are no known health risks associated with the magnetic field or the radio waves used in either MRI or MRS and all contrast agents used are all deemed safe and are FDA-approved. During the procedure, a radiology technologist performs the test in the MRI suite in a hospital's radiology department or an outpatient imaging centre. MRS is used to non-invasively measure tissue glycogen by using either ^{13}C natural abundance levels or ^{13}C atoms

incorporated into glycogen by ^{13}C substrate received through ingestion or intravenous administration.

The other method is to use the water signal with chemical exchange saturation transfer imaging (glycoCEST) (Avison et al., 1988; Kogan, Hariharan and Reddy, 2013). Recent advances over the last two decades within the field of MRS technology now allows the ability to non-invasively detect changes, in a variety of different intramuscular fuel sources, such as muscle glycogen, non-invasively (Taylor et al., 1992; Price, 2000; Fuchs et al., 2016).

Although MRS allows for the non-invasive method to analyse muscle glycogen, has a fast time resolution making for fast results, can be repeated as many times as necessary, and provides very accurate date. The limitations of this technology lie with its practicality for use within a sporting context. Gaining access to this expensive and specialised piece of equipment is limited MRI machines are also not portable and cannot distinguish between muscle fibre types, further reasons why alternative methods are more common to assess skeletal muscle glycogen within a sporting context.

2. 4. 3. 4 **Musculoskeletal high-frequency ultrasound**

Ultrasound has functioned as a valuable imaging modality in medicine for many decades, in more recent years it has gained increasing practical application and attention in the area of sports medicine. Ultrasound is currently established for evaluating the cardiovascular status among athletes, musculoskeletal pathology diagnosis and therapeutic interventions, and to visualise and monitor real-time movement of muscles and tendons (Yim and Corrado, 2012; Sikdar, Wei and Cortes, 2014) as shown in Figure 2.12. Musculoskeletal ultrasound has established more promise as a point of care device

to use within the field, rather than having to incorporate specialised laboratories and technicians. Musculoskeletal ultrasound is not only being utilised as a diagnostic tool but has a therapeutic use in treating a vast range of different musculoskeletal conditions affecting athletes (Yim and Corrado, 2012).

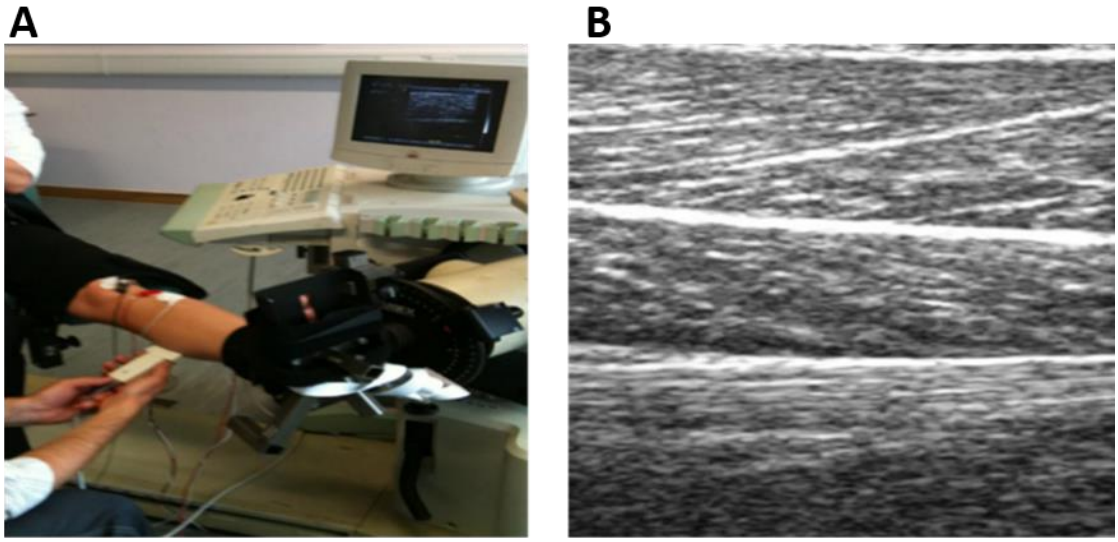

Figure 2.12- A) Application of ultrasound and equipment. B) Example of a greyscale image of the skeletal muscle fibres produced by the ultrasound scan when placed directly upon the skin

Furthermore, ultrasound velocity now allows for the possible detection of hydration status (Sarvazyan, Tatarinov and Sarvazyan, 2005). This technique was used in a recent study assessing changes in hydration status among National Collegiate Athletic Association Wrestlers, the protocol included the wrestlers undergoing acute bouts of dehydration followed by a 2-hour rehydration period. The results demonstrated the potential of ultrasound technology being deployed as a means of assessing the field-based hydration status of athletes (Utter et al., 2010). In comparison to the aforementioned methods, ultrasound technology prompts a practical solution in providing a non-invasive and relatively cheap alternative procedure to detect muscle glycogen. This technique determines muscle glycogen content within the muscles by

detecting the variations in the greyscale image, assessing the association between water content and glycogen values (Bone et al., 2016). Ultrasound technology is used frequently in medicine, and compared to the previous techniques mentioned within this review, has promising advantages such as portability, low cost, no harmful ionizing radiation, real-time, and also causes no discomfort or any long-term side effects.

Recently methods have been designed to try and overcome the invasive nature of biopsies. MuscleSound© have attempted to design software to enable the ability to use ultrasound to measure skeletal muscle glycogen. MuscleSound© methodology is based upon the measurement of the water content which is associated with glycogen in the muscle. When muscle glycogen is high, the ultrasound image is hypoechoic (dark), and when used for a muscle which has low stores of glycogen and has water loss, the image is hyperechoic (brighter) (Nieman et al., 2015). The idea behind the MuscleSound© software is to then quantify the observed changes in muscle glycogen levels using image processing and analysis through segmentation of the area that is of interest and measurement of the mean signal intensities (Nieman et al., 2015).

Research by Nieman et al.,2015 assessed the use of the ultrasound method using a high-resolution GE LOGIQ-e ultrasound machine (GE Healthcare, Milwaukee, WI) alongside MuscleSound© software for the ability to non-invasively measure exercise-induced changes in skeletal muscle glycogen content. Well-trained cyclists endured in a 75-km cycling time trial. Muscle biopsy samples and ultrasound measurements were acquired pre- and post-exercise. Ultrasound images were pre-processed to isolate the muscle area under analysis, with the mean pixel intensity averaged from the three scans and scaled (0 to 100 scale) to create the glycogen score. Pre- and post-exercise muscle biopsy

samples were acquired at the vastus lateralis location using the suction-modified percutaneous needle biopsy procedure and analyzed for glycogen content. MuscleSound® change scores attained from an average of three ultrasound scans at the vastus lateralis site correlated significantly with the change in vastus lateralis muscle glycogen content (Nieman et al., 2015). The data found in this specific study showed that MuscleSound® methodology was able to accurately and non-invasively estimate exercise-induced decreases in vastus lateralis skeletal muscle glycogen content.

However, further research is still needed to ensure this is a viable method to measure muscle glycogen in athletes under a number of variations. Recent examination of this technique by Bone et al (Bone et al., 2016) reported that ultrasound technology failed to measure indirect estimates of muscle glycogen concentrations. The study aimed to validate ultrasound technology for the measurement of muscle glycogen concentrations in well-trained individuals under different conditions which were previously tested by Neiman et al. These conditions included normal glycogen levels, depleted glycogen levels and loaded levels of glycogen. In addition, creatine loading was consumed by some subjects to provide a possible confounding effect on muscle water content (Bone et al., 2016). Again MuscleSound® software was used to interpret the ultrasound images and was compared to that of the suction-modified percutaneous needle biopsy procedure. The results from this study were unable to validate the use of ultrasound technology to estimate muscle glycogen or increases/decreases in these stores across a range of scenarios including exercise-depletion, normalised stores, carbohydrate loading and concomitant creatine loading (Bone et al., 2016).

2. 4. 1 **Summary**

With the array and emergence of new advances in medical and sporting technology over recent years, biopsies with the addition of histochemical assay remain the preferred method of measuring muscle glycogen regardless of their invasive nature. This will remain so until an alternative method is available which reaches a high standard of results and allows for portable, cost efficient, real-time and non-invasive assessments. MRS is leading the way with the non-invasive measurements of glycogen; when MRS and muscle biopsy samples are used in conjunction, the biopsy can be used to measure other metabolic variables such as enzymatic activities leaving MRS to detect glycogen levels, this is a rewarding combination for a coach and sports scientists who can then develop a strategy to optimise athletic performance. However, MRS is not a feasible option in the world of elite sport due to the high costs associated with the equipment, as well as the specialised facilities and practitioners needed for assessment to be completed. To date research into the measurement of muscle glycogen using MRS focuses mainly on the clinical side rather than athletic performance. Although data from musculoskeletal high-frequency ultrasound initially showed promising results, further research has shown the flaws and further development needs to be completed before this technique can be applied to the ever-changing circumstances of a professional athlete's regime.

2. 5 **Summary of literature**

It is clear from the literature that advancements in technology allow for greater insights into human athletic performance. Current wearable sensors are providing previously lab-based measurements to be attainable during real-world scenarios. In the world of

elite human performance, marginal gains in performance can be the difference between winning and losing. There is ever more focus on data collection as a training aid, performance monitor and an injury prevention tool. The next step in wearable technology is to enable data collection of physiological measurements which have been previously difficult to monitor such as sweat composition and parameters which require invasive measurements such as blood lactate and skeletal muscle glycogen. This literature review has detailed each parameter (sweat sodium, blood lactate, and muscle glycogen) and assessed and critiqued all current methods of detection.

Similarly, the need for non-invasive methods is present in the world of elite human performance and to provide accurate nutritional guidelines and improve performance strategies by assessing sodium loss, blood lactate levels and glycogen stores in real-time. This would help solve many of the issues faced by scientists and coaches using invasive and single measurement equipment.

CHAPTER 3 - ELECTROMAGNETIC SENSING THEORY

This chapter will detail the theoretical background of the electromagnetic spectrum, detailing its broad industrial and scientific use over the centuries. Section 3.2 will then explore the underpinnings of microwave sensing application and discuss how EM waves at specific frequencies can be utilised to identify and detect changes in different materials. Section 3.3 will investigate microwave sensor design and fabrication to ensure appropriate functionality and manipulation of the EM field.

3. 1 **Electromagnetic spectrum**

In 1800, Sir William Herschel discovered infrared light by exploring how much heat was contained by each of the colours in the visible spectrum. He found that the highest temperature was surprisingly just beyond the red coloured light. The following year, Johann Wilhelm Ritter investigated beyond the purple end of the spectrum, leading to the discovery of ultraviolet light. These findings led to James Clerk Maxwell to suggest the existence of electromagnetic waves and mathematically predict their properties before any observations had been made of this phenomenon. Maxwell established that light itself was an electromagnetic wave, along with many other forms of electromagnetic radiation which we are familiar with today, such as radio waves, X-rays and microwaves. All modern electrical and optoelectronic devices we use today owe their existence to Maxwell's work. In 1887, Heinrich Hertz demonstrated the existence of these waves by producing radio waves (Kraus, 1988). Initial assumptions on the

electromagnetic radiation were that it would propagate through a supporting medium, as sound can travel through air but not a vacuum. However, in 1887, Albert Michelson and Edward Morley's experiments showed that the speed of light is the same in all reference frames, referred to as the "most famous failed experiment", showing strong evidence against the existence of luminiferous ether. This principle eventually leads Albert Einstein to build his Special Theory of Relativity in 1905.

The electromagnetic spectrum covers a huge range with wavelengths reaching the size of an atom to almost the width of the universe as shown in Figure 3.1. The corresponding photon energies occupy another vast range, from the unmeasurable to the extremely dangerous. Ever since these fundamental steps in understanding the different wavelengths of the electromagnetic spectrum, scientists, engineers, and practitioners have used this radiation for a multitude of purposes, ranging from communication through to medical diagnosis.

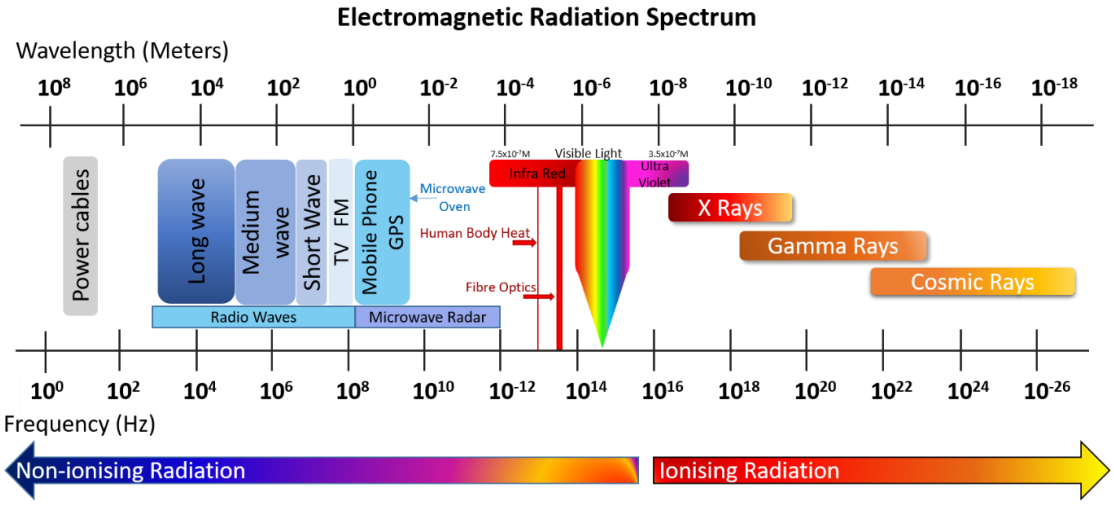

Figure 3.1 - The electromagnetic spectrum, illustrating frequency range applications (Adapted from Lawson 2005)

3. 2 **Application of electromagnetic sensors**

EM waves are energy that travels through a vacuum at the speed of light, which is approximately c=3x10^8 m/s (Staelin, Morgenthaler and Kong, 1994; White, 2016). Microwaves are EM waves that operate in the frequency range between 0.3GHz and 300GHz. EM waves can characteristically be defined by any of the following three physical properties: the frequency f, wavelength λ, or photon energy E. The frequency f (Hertz) of the wave is inversely proportional to the wavelength λ (meters) and is given by the relationship;

$$f = c / \lambda \qquad (1)$$

EM wave sensors operating at microwave frequencies are becoming increasingly used as alternative solutions to previously complex procedures in industries such as water pollution (Korostynska, Mason and Al-Shamma'a, 2014; Frau et al., 2018), food manufacturing (Korostynska et al., 2013; Bjarnadottir et al., 2015; Agranovich et al., 2016) and healthcare (Mason et al., 2013b; Mason et al., 2014; Choi et al., 2015; Mason et al., 2017). The diversity of EM sensor function is due to their range of altered designs, namely resonance-based, wideband and planar sensors, which can be developed for the task desired.

The theory behind this method is founded on the interactions between the changes in the transmitted (S_{21}) and reflected (S_{11}) microwave signals, providing a unique signal spectrum signatures at specific frequency intervals which can be linked to the composition of the material under test (MUT) (Kharkovsky and Zoughi, 2007; Ateeq et al., 2017) as shown in Figure 3.2.

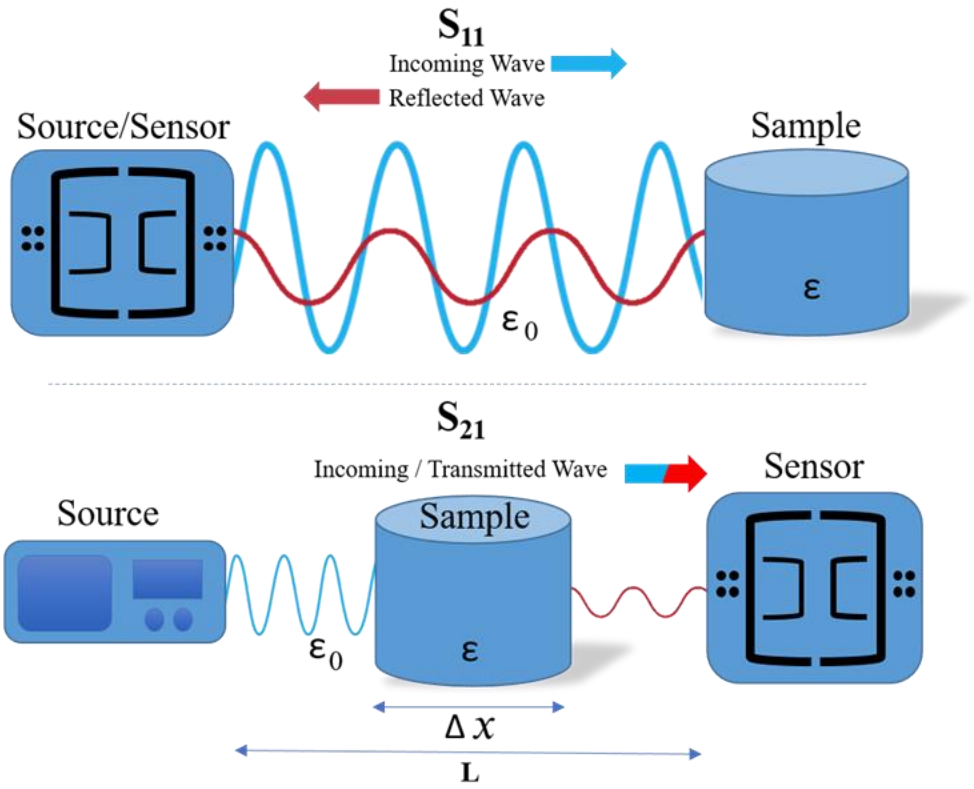

Figure 3.2 - Schematic showing the change in the reflected (S_{11}) and transmitted (S_{21}) microwave signal, interacting with a sample determining the composition and concentration

S-parameters are measured by quantifying the energy stored dielectric constant, which is when the EM wave pass through the material, some energy is stored, the rest is released slowly, this is measured and can be used to identify materials with varying dielectric values (Yilmaz, Foster and Hao, 2014). As well as the dielectric loss, which determines the reduction in the applied EM waves magnitude, causing the molecules to rotate causing friction which causes energy loss, reducing the magnitude of the wave (Oon et al., 2016).

As energy is coupled into the sensor, both the S_{11} and S_{21} signals vary depending upon properties of the analyte presented to the sensor, such as conductivity and permittivity (Choi et al., 2015). Conductivity is a measure of a material's ability to conduct an electric

current, whereas permittivity is the measurement the effect of a dielectric medium on the applied electric field (Kumar et al., 2001). This is determined by the ability of a material to polarise in response to the field, and reduce the total electric field inside the material. Therefore, permittivity (εr) as defined in (2) relates to a material's ability to transmit an electric field and is a complex value which varies with frequency, and accounts for both the energy stored by a material (ε) as well as any losses of energy (ε) which might occur (Mason et al., 2017).

$$\varepsilon r = \varepsilon' + j\varepsilon'' \quad \textbf{(2)}$$

The permittivity of a material is derived from several characteristics (e.g., temperature, chemical structure, molecular composition, etc.) and is a measurement of varying polarisation phenomena that occur over different frequency ranges when exposed to an alternating EM field (Blakey and Morales-Partera, 2016). This causes in dipolar polarisation in polar molecules, causing them to rotate over a time period proportional to their dipole moment and local conditions (e.g., viscosity) (Leanhardt et al., 2011), leading to a dielectric relaxation in the microwave region of the EM spectrum. A number of mathematical models have been developed by Cole and Cole (Cole and Cole, 1941) and Cole and Davidson (Davidson and Cole, 1951) to explain this relaxation phenomena. It is based upon these principles that EM wave sensors, operating at microwave frequencies, should be able to selectively detect biological parameters.

Most biological materials from the interest of microwave propagation can be considered as "lossy" dielectrics due to being commonly macroscopically or microscopically heterogeneous. Dielectric loss and thereby the energy transferred to the samples depends on the nature of the material and the frequency range of the microwave signal

(Roberts and Cook, 1952). Early research into the dielectric properties and behaviours of human tissue at microwave ranges was completed by T.S England and H.F. Cook in the early 1950s (England and Sharples, 1949; Cook, 1951). This research presented that the dielectric behaviour of skin, blood and muscle tissues in the microwave region is governed by the relaxation of "free" water and by ionic conductivity. The dispersion of these tissues can be approximately described using the Debye dispersion equations with a single relaxation time if additional dielectric loss due to ionic conductivity is allowed for (Cook, 1951; Cook, 1952). However, the dispersion of fatty tissues and bone is not so easily described in the microwave ranges due to significant differences in dielectric constant (Cook, 1951).

Microwave sensing technology is an emerging field for the detection of healthcare parameters (Hofmann et al., 2013). Microwave sensors using frequencies around 1GHz range can penetrate the tissue by a few centimetres, well suited to physiological monitoring, providing safe, fast and continuous monitoring of parameters beneath the surface of the skin (Choi et al., 2015). They are also non-ionizing with a low power output of approximately 1mW (0dBM) while maintaining good penetration depth (Mason et al., 2014), making the sensors safe for the use during prolonged monitoring during exercise or rest (Fok et al., 2015). The particular benefit of this technology is its non-invasive and continuous nature, which, in contrast with current techniques, holds the potential to monitor complex biological parameters in real-time. Recently there have been successful attempts to monitor blood glucose non-invasively using microwave sensing technology aiding self-monitoring for diabetic patients (Abedeen and Agarwal, 2018; Zhang et al., 2018). The prerequisite for non-invasive detection was due to the need to avoid the

complex, costly and painful nature of conventional (invasive) glucose monitoring. Glucose monitoring is of particular importance because of its involvement in the human metabolic process, giving promise to the future of non-invasive glycogen and blood lactate sensors. This shows that application of this technology could provide masses of benefits to a variety of different practitioners within the world of health care and sport and exercise performance who want to be able to quantify previously difficult performance markers both in the labs and in the field.

3. 3 **Microwave sensor design and fabrication considerations**

The standard microwave sensing system consists of three distinct parts, a sensor head, vector network analyser (VNA) and a graphical user interface (GUI). VNA's are an instrument commonly used for Radio Frequency (RF) applications, VNA's are regularly used in microwave and radio frequency (RF) devices, they allow for the measurements to be characterised in terms of network scattering parameters, or S parameters. This data can be then presented using magnitude, phase and complex data (real and imaginary). Advantages of VNA's are that they produce a high number of sweep points, allowing for greater sensitivity. The GUI is usually a computer or smartphone that is in full control of the VNA, it is responsible for configuration settings and initiation (e.g. S-Parameters, frequency, power settings, recording duration etc.), capturing the sensor data via the VNA, and can be used to analyse and display the data in real-time (visually and numerical predictions).

There are a wide variety of sensors which are classified based on their design, type of operation and range of frequency they employ. The application of these sensors is usually split into two major categories. Firstly, corresponding to the radiometric, topographic and radar sensing applications, involved in distance, movement and shape measurement (Ulaby et al., 2014). Secondly, sensors such as resonance cavities, microwave waveguides and transmission or reflection sensors, applications of which include measurement of material properties, such as liquids and gases which can flow through or be placed upon the sensors (Nyfors, 2000). Furthermore, microwave resonant sensors although they are traditionally used in communication systems, can offer the most suitable microwave components for developing a biosensor (Miranda et al., 2000). Recently there have been many microwave resonator sensors which have been designed to detect the electromagnetic wave interaction with water-based substances or biological materials. The application of a planar microwave filter was used to detect biological cells discrimination using impedance spectroscopy (Dalmay et al., 2010).

In healthcare, sensor ergonomics needs to be considered, if the sensor is to be placed upon the patient, a biocompatible material needs to be used and the shape and size of the sensor will also determine the sensor's real-life wearability and functionality. A patch type sensor is ideal for anatomical measurements due to its small dimensions and versatile designs; this class of sensors is called a resonant sensor. Resonant sensors respond sensitively and produce a high accuracy of measurements. Resonant sensors usually consist of three primary components, ground plane, substrate and a patch (Balanis, 2005) shown in Figure 3.3.

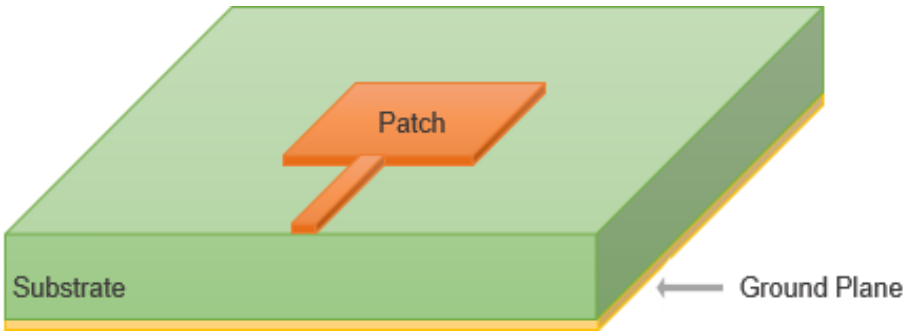

Figure 3.3 - Illustration of a standard resonance patch sensor. *not to scale

The design and geometries of the sensor play an essential role in the resonance frequency generated. Increases in electric field energy storage can be seen if the sensor is loaded with materials, leading to a decrease in the resonance frequency. Although water content can lead to a decrease in resonance frequency, subsequently, bandwidth is increased.

A half wavelength microstrip transmission line open-circuited at both ends and turned into a U shape is referred to as a hairpin resonator (Lee, Su and Haldar, 2012). Hairpin resonator devices were first introduced in the 1970s and were in demand due to the need for systems requiring lightweight and small size microwave bandpass filters (Cristal and Frankel, 1972). In the late 1980s, miniature hairpin resonator filters had application to receiver front-end microwave integrated circuits (MIC's) (Sagawa, Takahashi and Makimoto, 1989). Hairpin resonator devices can be designed in a variety of ways and be used for many microwave applications (Hong and Lancaster, 1998). These early designs used a multilayer structure of hairpins; however, modern designs use two adjacent hairpins resonating with capacitive coupling, meaning there is no physical connection between them (Joshi et al., 2016) as shown in Figure 3.4. A Hairpin shape also allows the size of the resonant sensor to be reduced, the specific resonance frequency is

determined via the length and characteristics of the impedance ratio (Makimoto and Yamashita, 2013).

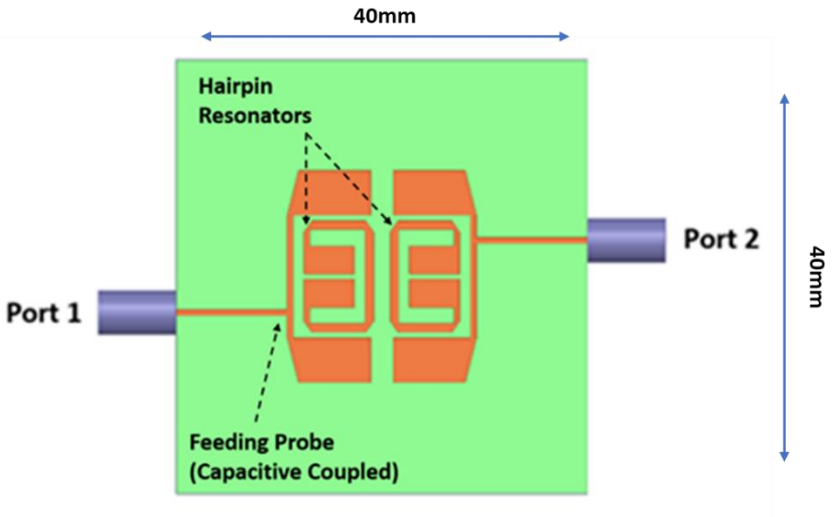

Figure 3.4- Schematic of sensor design and dimensions. High Frequency Structure Simulation Software (HFSS) model of a hairpin resonator sensor. dimensions are 40 x 40 x 1.6 mm (l x w x h)

Additionally, the materials the sensor is made up of play a crucial role in how much electric charge it can store. Choosing an appropriate substrate means considering many factors to meet the needs of the sensor, namely, the dielectric constant, temperature and frequency variation, homogeneity, thermal coefficient, temperature range, humidity and ageing, thickness etc.

FR-4 (flame retardant 4) substrate is a NEMA (The National Electrical Association) grade designation for a glass-reinforced epoxy laminate material. FR-4 is well accepted within industry as the standard material used for PCB manufacture as it functions well as an electrical insulator, is flame resistant, and has a good strength to weight ratio (Coonrod, 2011). FR-4 is used for a wide range of electrical and mechanical applications as it's known to retain high mechanical values and electrical insulating qualities in both dry

and humid conditions (Lando, Mitchell and Welsher, 1979; Vanlathem et al., 2006; Laskar et al., 2007).

3. 4 **Summary**

This chapter has introduced electromagnetic wave theory and microwave sensing systems as a novel solution, which could be potentially used to detect biological parameters in humans during exercise. This chapter highlighted the potential uses of this technology to detect parameters across a wide variety of applications in both industry and in scientific/medical uses. EM wave sensor technology is leading the way in providing non-destructive detection of different materials, however the use of microwave sensor technology in sport and human performance monitoring is still absent and the following research will aim to answer these unknown questions.

CHAPTER 4 - GENERAL RESEARCH METHODOLOGY

To investigate whether a microwave sensor could detect three distinct physiological parameters, a robust and thorough research methodology was required. This chapter will detail the general experimental protocols and procedures used throughout each of the following chapters, detailing the microwave sensor used and set-ups used for both in-vitro laboratory analysis and during human trials.

4. 1 **Experimental protocol**

To determine if a microwave sensor could detect sodium, lactate, and glycogen non-invasively in humans during exercise, three major studies were conducted. Each of the chosen parameters required a specific experimental design to ensure optimised data collection and analysis.

In-vitro measurements can provide useful information before moving on to human trials. Observations such as the frequency ranges and the sensitivity between concentration increases can be of benefit, when designing in-vivo trials. Therefore, it was critical to ensure that the microwave sensor could measure each parameter under in-vitro conditions prior to human trials. The use of an microwave sensor to detect both sodium and lactate in a water solution have already been researched (Kapilevich and Litvak, 2007; Mason et al., 2013a), and we conducted in-vitro analysis of glycogen as discussed later Chapter 7. However, as sweat is made up of multiple ions and waste

products, we also conducted in-vitro trials to measure sodium concentration in collected human sweat samples.

During human trials, we used a specifically chosen exercise protocol for each of the three parameters. This approach allows for optimal manipulation of the parameter. To increase sweating rates and assess changes in sodium loss during prolonged exposure to heat we conduced continuous steady state exercise in 30°C within an environmental chamber. To ensure a full range of blood lactate measurements participants completed a bout of progressive incremental exercise until voluntary exhaustion. To monitor glycogen stores, athletes consumed three different diets prior to the trials and completed a glycogen depletion interval protocol. Each protocol is explained in depth in the following chapters, the experimental studies are shown in Figure 4.1.

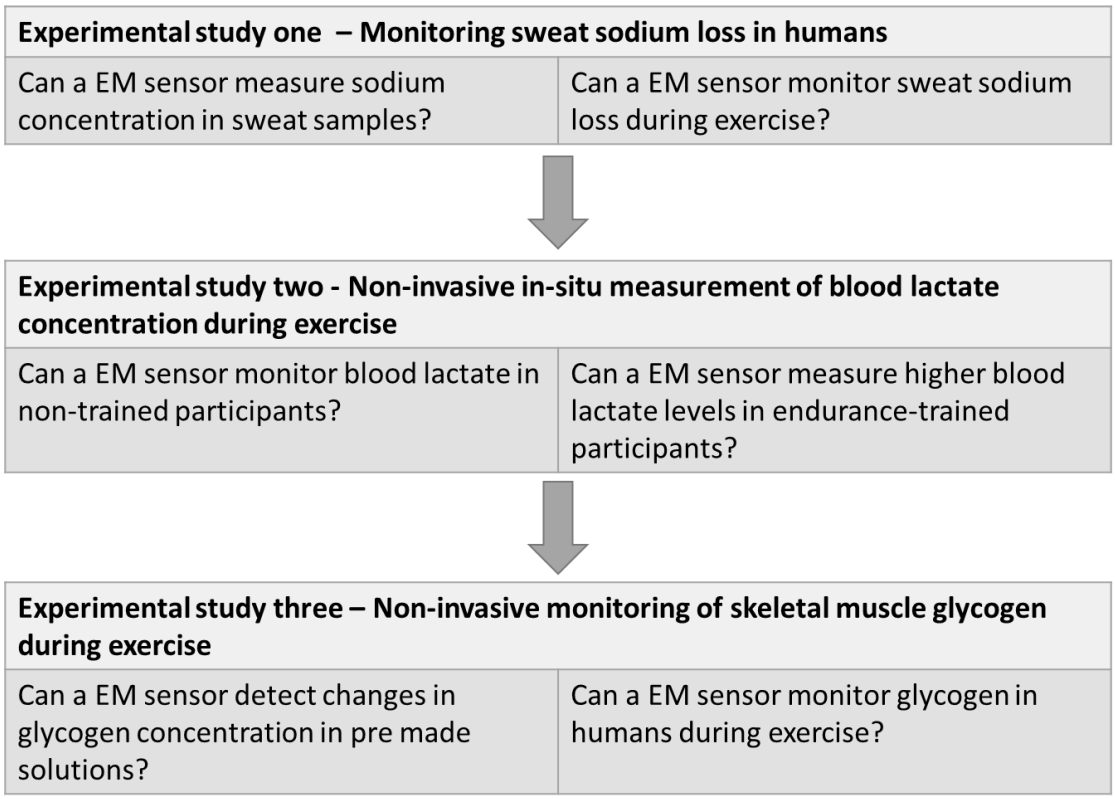

Figure 4.1 - An illustration of the experimental design throughout the thesis

These experiments accumulated in 140 sweat samples, 523 blood lactate samples and 14 muscle biopsy samples for analysis from a combined total of 61 participants, 56 males, and 15 females as shown in Table 4.1.

Table 4.1 - A summary detailing participant information and the total number of samples for each experimental study

Study	No. of Participants	No. Samples	Fitness Status	Gender	Age	Height	Weight
Sweat Sodium	10	140 sweat samples	Healthy non-trained	Male:4 Female:6	20-32	154cm – 182cm	46kg-82kg
Blood Lactate	44	523 blood samples	Endurance trained & Healthy non-trained	Male: 45 Female:9	18-45	158cm – 198cm	52kg-101kg
Skeletal Muscle Glycogen	7	14 (biopsy samples) 7 (In-vitro samples)	Endurance trained cyclists	Male:7	18-30	172cm-187cm	57kg – 80kg

4. 2 **Microwave sensor design and application**

A purpose-built "hairpin resonator" microwave sensor has been developed by the authors within the Faculty of Engineering and Technology at LJMU (Liverpool John Moores University) shown in Figure 4.2. The sensor is manufactured using a standard etching process and the substrate is an FR4 epoxy glass coated with a biocompatible membrane, preventing any copper leeching during use, designed to be placed directly over the skin. Being able to apply the sensor during exercise and collect real-time continuous data is a key advantage of using this technology and sets it apart from other techniques.

Measuring human participants required additional precaution and consideration regarding data quality as well as the participant remaining comfortable throughout the duration of the exercise trial. As mentioned in the previous chapter, ergonomic considerations were put into place when designing the sensor to ensure a fit to the forearm of the participant without requiring the sensor to be flexible to fit the natural curvature of the arm. A benefit of choosing and developing a hairpin sensor ensured that the EM field is concentrated close to the surface of the sensor, this is of particular benefit when placing the sensor onto the surface of the skin. The aim of this design was to allow full coverage of the different layers of the skin ranging from the surface through to the layers of the skeletal muscle. As well as enabling the sensor to non-invasively penetrate through the tissue, maintaining the EM field close to the surface has some advantages, namely that of reducing the interference and noise from outside sources.

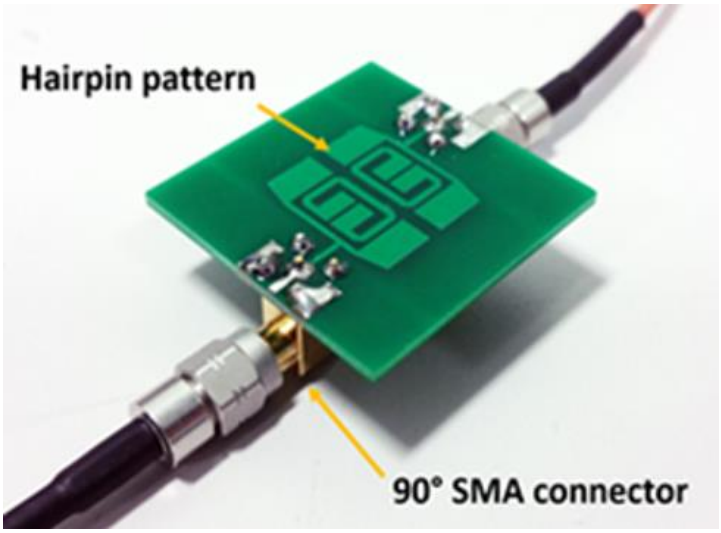

Figure 4.2- Working Ridged FR4 PCB microwave sensor attached to coaxial (SMA) cables.

The sensor has a discontinuous ground plane, isolating port 1 from port 2. This provides the sensor with shielding from external interference. The design of the hairpin sensor ensures the EM field is concentrated close to the sensor, allowing the microwaves to

penetrate through the skin and interact with the tissue beneath. The EM field can penetrate through the skin of a target and interact with the fluids beneath up to 10mm shown in Figure 4.3A. Maintaining a field close to the sensor surface has some advantages, namely that of reducing interference from objects other than the surface to which it is directly attached. The hairpin configuration of the device supports this notion well and has the primary reason for its use. Prior to the main experimental chapters, pre-trial S-parameter measurements for the sensor in air and water samples were measured to determine the repeatability of sensor measurements. Data collected show that the sensor resonates at approximately 2.3 GHz in air and shifts to 2.12 GHz when measuring deionized water as seen in Figure 4.3B.

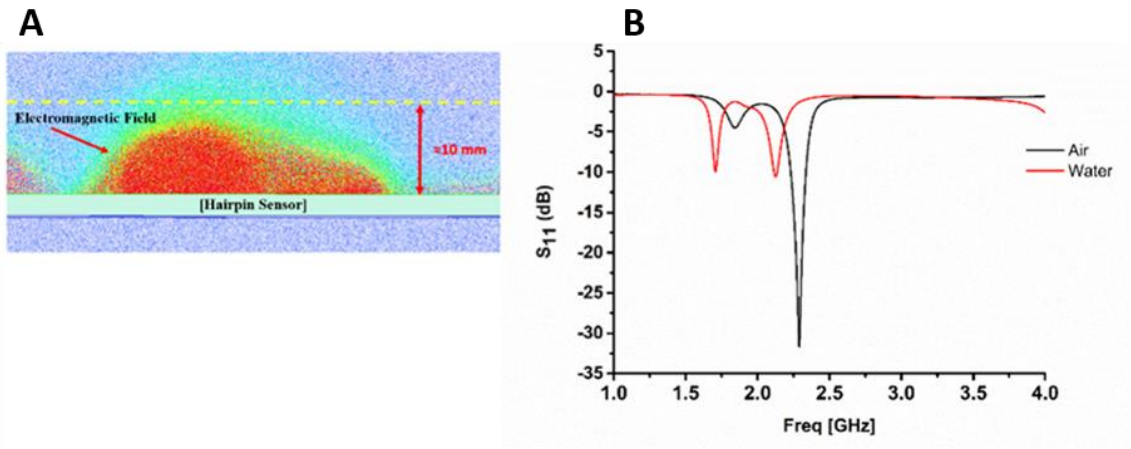

Figure 4.3 – A) A side view of the sensor using an Ansys HFSS model to illustrate the EM field of the hairpin resonator being most prominent up to approximately 10 mm from the sensor surface. B) Baseline S_{11} (dB) reflection coefficient signal distribution between 1Ghz and 4GHz frequency range using the microwave sensor in air and a deionised water sample.

The multi-parameter nature of microwave analysis allows for the captured microwaves to be presented in the form of scattering parameters. For data acquisition the sensor is connected either a portable 2 port Rohde & Schwarz ZVL13 VNA or a stationary 4 port

Rohde & Schwarz ZVL24 VNA shown in Figure 4.4, which emit non-irradiating electromagnetic waves in very low power emission. The R&S®ZVL VNA is often used in research studies due to its powerful measurements and increased analysis efficiency. However, to develop the sensor into a more wearable solution, a more portable and cost-effective microwave source can be attained, with the necessary VNA functionalities for the required frequencies. The VNA enables the S_{11} reflection coefficient and S_{21} transmitted coefficient through the sensors 2 port system. When operating an microwave sensor, systematic effects such as leakage, test post mismatch and frequency can corrupt the measurement (Huang, Liu and Chen, 2017). Within a controlled environment these effects can be eliminated when using a VNA, pre-analysis calibration was conducted via both in-vivo and post hoc in-vitro experiments using a short-open-load-through (SOLT) calibration.

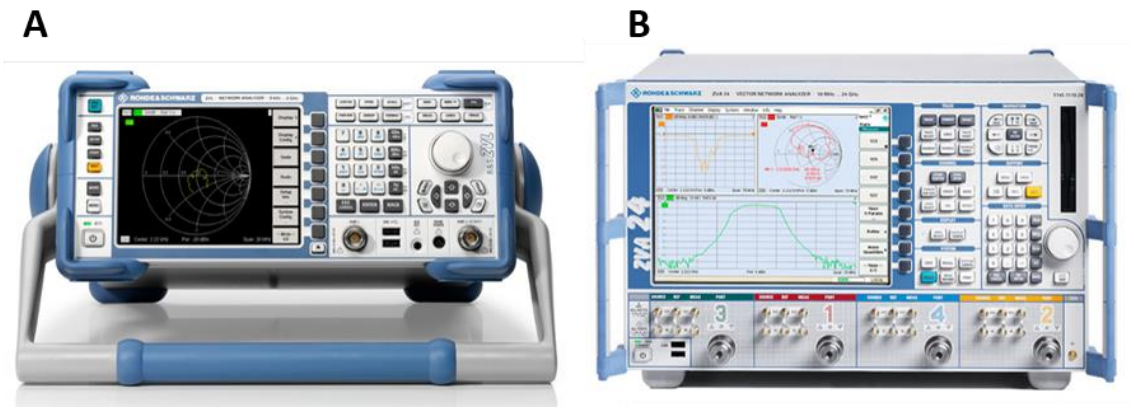

Figure 4.4 - A) Portable 2 port Rohde & Schwarz GmbH & Co KG ZVL13 VNA. B) Stationary 4 port Rohde & Schwarz GmbH & Co KG ZVL24 VNA

4. 2. 1 **Experimental set up for in-vitro microwave sensor analysis**

As in-vitro sample analysis took place within the Faculty of Engineering and Technology laboratories within LJMJ James Parsons Building. As this research was conducted within

a controlled laboratory environment, a stationary Rohde and Schwarz ZVA24 VNA was used for data acquisition via two coaxial cables shown in Figure 4.5,. A desktop PC was linked to the VNA enabling the microwave sensor to produce and capture a full frequency sweep between 10MHz to 4GHz producing 60,000 data points per sample.

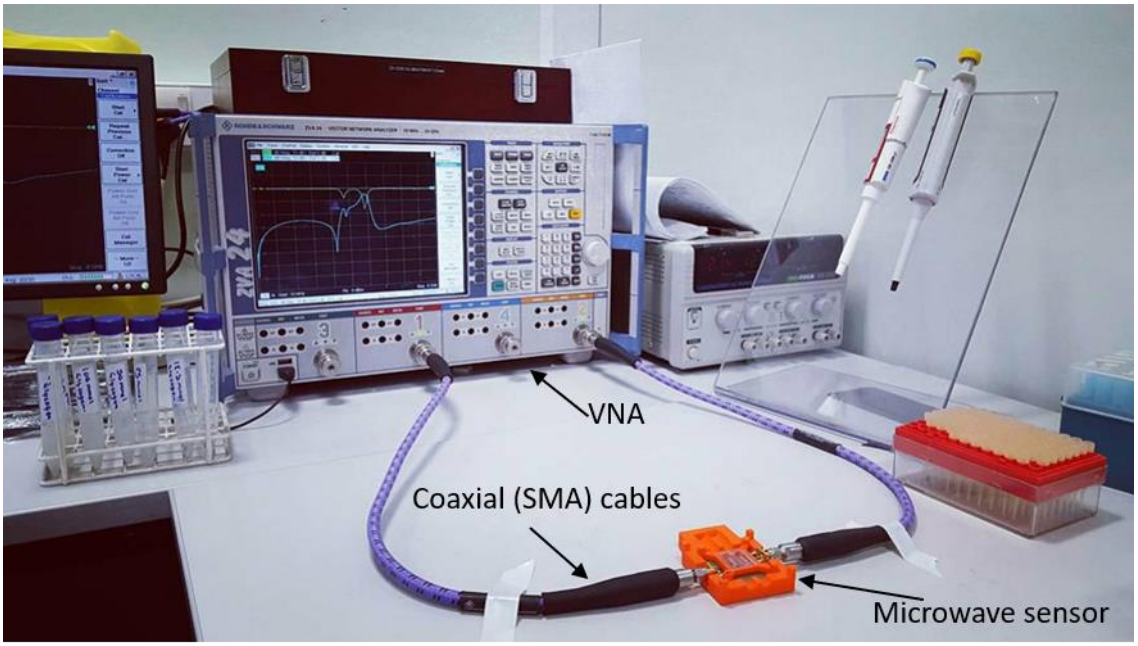

Figure 4.5 - Measurement Set-up showing Rohde & Schwarz ZVA24 and a microwave sensor connected via 2 coaxial cables

A custom-made plastic casing was engineered to give stability and even surface, ensuring no movement once testing had begun. It was critical to ensure the same volume of sample throughout this thesis for the in-vitro analysis as changes in water can influence the resonated near-field and can result in lower or higher resonant frequencies if water is reduced or increased (Dziedzic et al., 2014). To ensure consistent sample size, a plastic slot is placed directly over the sensor, this allows for the 200µl of solution to be inserted consistently during each new sample. The slot will then be cleaned with water and dried prior to e ach new sample.

4. 2. 2 **Experimental set-up for in-vivo microwave sensor measurement during exercise**

During human trials, each experimental protocol took place within LJMU Tom Reilly Sports and Exercise Laboratories. All protocols used either a cycle ergometer or a WATT Bike Pro depending on the specific requirements. To generate the chosen frequencies the microwave sensor was attached to a portable Rohde & Schwarz ZVL13 VNA during each exercise trial to enable a more convenient solution than the in-vitro trials. The sensing configuration remained the same and enabled the S_{11} reflection coefficient and S_{21} transmitted coefficient to be generated through the sensors 2 port system and was still able to produce the vast frequency sweep ranging from 10MHz to 4GHz with a reduced number of data points (4,000) per sweep as shown in Figure 4.6. A laptop was linked to the VNA to provide a bespoke LabVIEW software solution, enabling the microwave sensor to produce and capture a full frequency sweep every 30 seconds and recording continually throughout the entire exercise protocol.

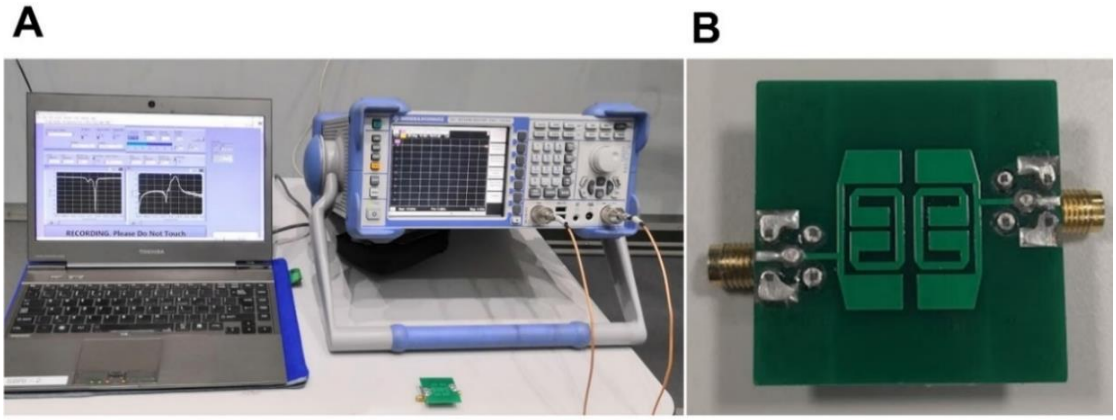

Figure 4.6 – A) Measurement Set-up showing Rohde & Schwarz ZVL13 VNA linked to appropriate data acquisition software with sensor placed for scale. B) Close up of Hairpin 2-port biocompatible microwave sensor

The VNA was set up in close proximity to the participant as shown in Figure 4.7A ensuring as limited movement of the cables as possible. The participant would be set up in the correct and comfortable position on the cycle ergometer used. The sensor was fixed to the participant using kinesiology tape, providing a flexible and stable attachment throughout the exercise protocol. Signal magnitude area cables were also taped to the participant, relayed to the VNA on a nearby platform shown in Figure 4.7B.

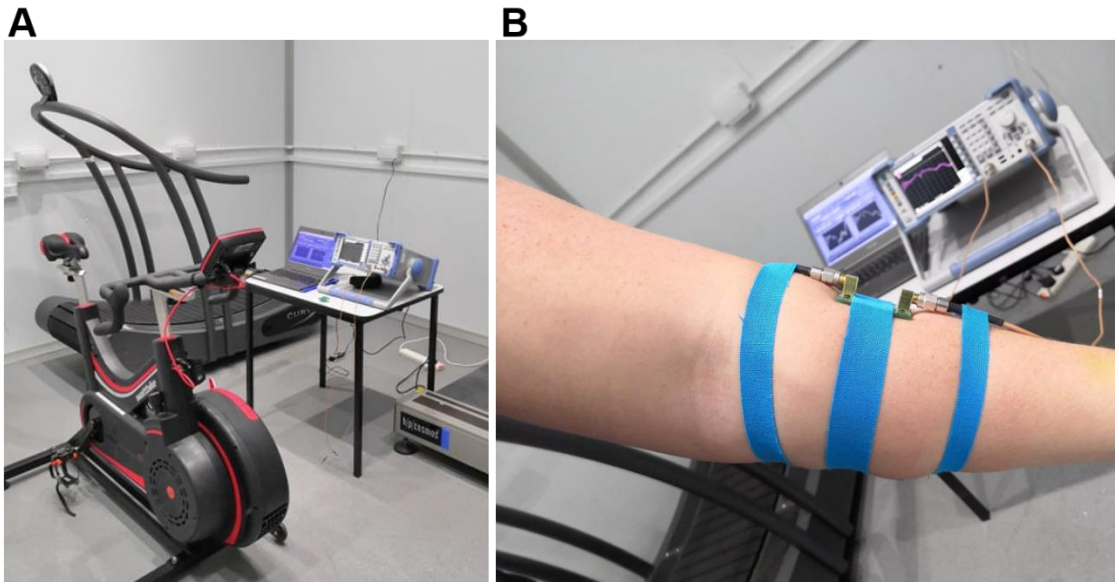

Figure 4.7 – A) experimental set-up in-vitro assessment of participants during exercise indicating positioning of VNA and cycle ergometer. B) Attachment of microwave sensor using kinesiology tape upon the forearm with SMA cables connected to VNA.

4. 3 **Summary**

This chapter has detailed the general underpinning of the research methodology used throughout the research project, including participant information, sample preparation, experimental approaches, and data analysis. This chapter has additionally described the specific microwave sensor design, materials and sensing capabilities used throughout for both in-vitro and in-vivo analysis, any individual differences between the studies throughout each of the experimental protocols will be discussed in further detail in the following chapters.

CHAPTER 5 - PARAMETER 1:

MEASUREMENT OF SWEAT SODIUM LOSS IN HUMANS DURING EXERCISE

In this chapter, the use of a microwave sensor for the detection of sweat sodium during exercise will be demonstrated. To achieve this, two measurement techniques will be used, section 5.2 will detail the methodology for both assessments. Results obtained from these experimental studies will be reported in section 5.3 and discussed throughout section 5.4.

5. 1 **Introduction**

Sodium plays a major role in body functions such as maintain fluid levels, absorption of nutrients, nerve in pluses to enable muscle contractions and maintaining normal cognitive function. Sodium loss through sweat varies largely amongst individuals and can depend on the environmental conditions and exercise intensity (Sawka et al., 2007). Severely low concentrations of sodium in the blood can lead to a condition known as hyponatremia. Hyponatremia has become more widespread during ultra-endurance events, where athletes compete at high intensity in hot, humid conditions with increased chance of excessive drinking behaviour (Reynolds Jr, Schumaker and Feighery, 1998). This combination can cause the onset of exercise-associated hyponatremia (EAH) if sodium loss is not regularly monitored (Hew et al., 2003).

Currently the alternative "gold standard" method to monitor sodium loss in athletes is to collect sweat samples which then require further analysis using an ion selective electrode device which can only providing a snapshot in time. Therefore, being able to monitor sodium losses real time will provide with a more individualised approach for sodium intake recommendations.

Microwave sensing technology presents a promising solution for the monitoring of Na^+ concentration in sweat during exercise. If applicable, microwave spectroscopy will aim to offer an alternative method for Na^+ analysis, offering both continuous and real-time data. Within this context, this study seeks to assess the microwave sensors interactions with sweat and determine if it can accurately monitor changes in Na^+ concentration. The aim of this chapter is to validate the feasibility of a new continuous electromagnetic sensor monitoring Na^+ concentration in sweat, during a 5-day heat acclimation protocol using an environmental chamber.

5. 2 **Methodology**

To determine if a microwave sensor can measure Na+ within human sweat, this chapter details two measurement approaches. Human sweat samples were collected during a bout of continuous exercise within a heat chamber and measured using a commercially available Na+ sensor detailed below. The first section of the methodology describes the methodology used to measure sweat Na+ concentration during exercise during in-vivo monitoring using the microwave sensor placed upon the surface of the skin. The second section of the research methodology for this chapter details the analysis of the collected sweat sample under in-vitro laboratory conditions using the same microwave sensor.

5. 2. 1 **Participants**

One hundred and forty sweat samples were collected from ten recreationally active participants, four male, six female (mean ± SD: age, 25 ± 5 years; body mass, 70.4 ± 14.6 kg; height, 170.4 ± 7.7 cm). Mean estimated VO_{2peak}, peak power output (PPO) and max minute power (MMP) for the participants were 42.3 ± 4.1 mL kg^{-1} min^{-1}; 364 ± 117 W and 249.7 ± 42.8 W, respectively. None of the subjects had any history of musculoskeletal or neurological disease, nor were they under any pharmacological treatments during the testing period. All participants provided written informed consent and all procedures conformed to the standards set by the Declaration of Helsinki (2008). This study was approved by the local Research Ethics Committee of Liverpool John Moores University.

5. 2. 2 **Experimental protocol (STHA)**

The exercise protocol for this study consisted of five consecutive days of cycling at 60% MMP for 45 mins in 30°C heat inside an environmental chamber as shown in Figure 5.1. As the study was assessing the effects of sodium loss rather than actively seeking to improve exercise output in elite athletes, these conditions we're ideal due to the practicality and effectiveness that a five day protocol can achieve (Garrett et al., 2012), without exposing the participants to unnecessary environmental conditions and durations. Each participant completed a 10-minute warm-up at a self-determined pace prior to each session. In the week prior to the main exercise protocol, participants were required to attend the laboratory to complete an initial fitness assessment. Following a familiarisation session the day before, each participant underwent a fitness screening and assessment. A "submaximal ramp test" and the "three-minute test" was performed to identify the current fitness levels of each participant such as PPO and MMP to ensure

each participant was exercising at the same relative intensity throughout the exercise protocol.

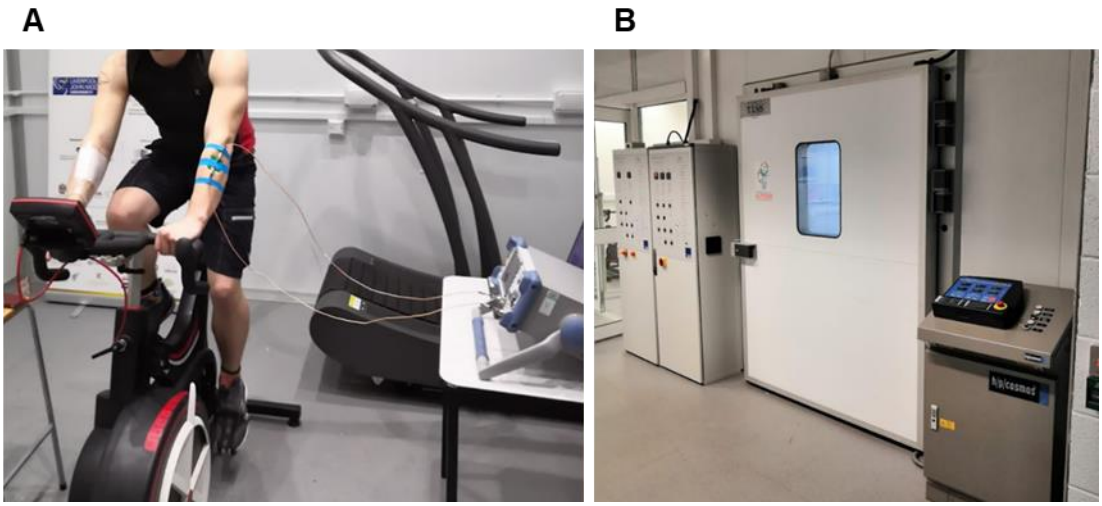

Figure 5.1 – A) Experimental set-up. Participant is exercising on a WATT bike whilst sensor is attached upon his right forearm, sweat collection patch is placed upon left forearm. B) Environmental Chamber Used at LJMU Sports and Exercise Faculties used to induce sweat and replicate exercising in hot environmental conditions

After a minimum of five days' rest, the second week required the participants to come to the environmental chambers to commence the short-term heat acclimation (STHA) protocol. All acclimation protocols were carried out in the winter and spring, minimising seasonal acclimation effects and all participants were unacclimitized prior to testing. Participants were recommended to arrive well hydrated by following hydration recommendations (min 1.5L ingested the day before + 0.5L before the exercise on the days of the experiment) and were weighed prior and post-testing along with the water consumption calculated. Participants' heartrate (BPM), Watt, rating of perceived exertion (RPE), temperature scale and the tympanic temperature (°C) was recorded every 5mins throughout the duration of the study.

5. 2. 3 **Sweat Na+ collection**

Sweat was collected using a standardised local absorbent patch technique to determine athletes' forearm sweat Na^+ (Baker et al., 2016). The forearm was used because it is one of the more accessible anatomical sites of athletes in sports attire. In addition, forearm sweat Na^+ has also been shown to be highly and significantly correlated with whole-body sweat Na^+ ($r = 0.96$) (Baker et al., 2016). During each of the five sessions inside the environmental chamber, sweat was collected three times (+15mins, +30mins, +45mins), these intervals were selected to ensure the time between collections enabled the absorbent patch to collect sufficient quantities of sweat for sample analysis. It has been demonstrated in previous studies that, when collecting sweat during exercise, the initial samples appear to contain higher mineral concentrations (Montain, Cheuvront and Lukaski, 2007; Ely et al., 2013). This is believed to be caused by samples mixing with the minerals that are trapped within the pores, and this is subsequently flushed out as the sweating is induced (Ely et al., 2011). Prior to each trial, participants forearms were meticulously cleaned using soap and then wiped down using medical grade alcohol wipes, rinsed with distilled water, and dried before application of the microwave sensor and wrap. Sweat was collected using an adapted method using a sterile, deionised patch wrapped in polyvinyl chloride (PVC). After each collection, the surface of the forearm was cleaned with deionised water and dried with an electrolyte-free medical wipe before reapplication. A LAQUA Twin B-772 Sodium Meter (Horiba, Kyoto-Japan) provided sodium reading for the study. Patches were changed every 15 mins as this provided us with sufficient absorbance. The patches were removed using sterile forceps and placed

into a syringe and drained into a plastic tube for later analysis. Sweat samples were analysed within a maximum of 24 hours and kept at 4 °C (refrigerated).

5. 2. 4 **Electromagnetic sensor**

5. 2. 4. 1 **In-vivo monitoring during exercise**

The sensor is attached to the forearm using kinesiology tape. The non-invasive sensor uses electromagnetic waves using a VNA ROHDE & SCHWARZ Zul- Network Analyser 9 KHz- 13.6 GHz. The VNA (vector network analyser), which emits non-irradiating electromagnetic waves in very low power emission, is connected via standard coaxial cables. Once in place, the device is set to record sodium continuously until the exercise protocol has ceased. For this experiment, only S_{11} was monitored due to this being the strongest correlating and more stable scattering parameter in previously reported studies (Mason et al., 2017; Greene et al., 2019). Pre-measurement calibration was conducted via both in-vivo and post hoc in-vitro experiments using a short-open-load-through (SOLT) calibration.

5. 2. 4. 2 **Post hoc in-vitro analysis**

In addition to real-time monitoring of sweat during exercise, the sweat samples which were taken for analysis were stored and then analysed using the sensor under laboratory conditions shown in Table 5.1.

Collecting sweat samples provided a unique opportunity to measure the sweat in both conditions for validation and repeatability assessment. The 2-port Hairpin microwave sensor used frequency sweeps between 10MHz and 4GHz, an ideal range to identify Na^+ electromagnetic signature. A Rohde and Schwarz ZVA24 VNA was used for data acquisition via two coaxial cables (Figure 5.2A).

Table 5.1 - Measurement Specifications

No. of Measurements	140 Samples x 5 Repetitions = 700
Range (Na^+)	300 – 4500ppm
Microwave Sensor	2 Ports (S_{11} only)
Volume of Samples	200ml
Temperature	20 °C
Frequency Sweep	10MHz – 4GHz
Channel Base Power	0 dBM

A plastic reservoir was placed directly over the surface, allowing for 200µl of sweat to be pipetted into for measurements shown in Figure 5.2B: this was repeated five times for each sample. The slot was cleaned out with deionised water and dried prior to each new sample.

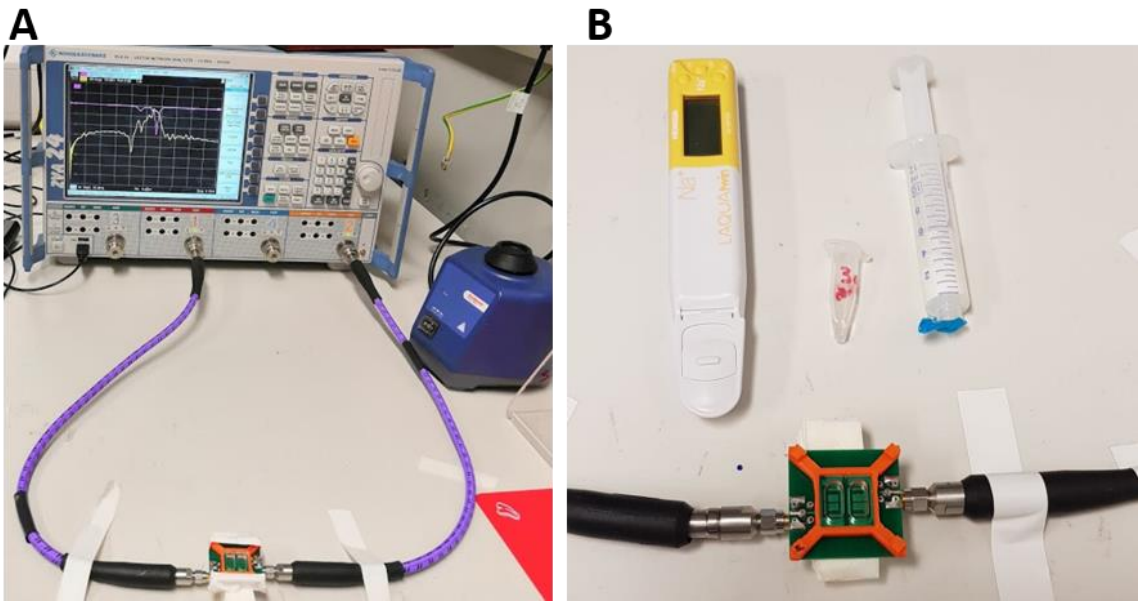

Figure 5.2 – A) Experimental set-up of microwave sensor under laboratory conditions to measure post hoc human sweat samples using a calibrated 2-port ZVA 24 VNA system. B) Close up of sensor set up, alongside the LAQUA Twin B-772 Sodium Meter (Horiba, Kyoto-Japan) and one individual sweat sample.

5. 2. 5 **Statistical analysis**

Statistical analysis was performed using Enterprise IBM SPSS 22 statistical analysing package to determine the significance of the data. A bivariate (Pearson) two tailed analysis was used to report the correlation between the microwave sensors S-parameter S_{11} (dB) and LAQUA Twin B-772 electrochemical sodium readings, the correlation was significant at the 0.01 level. Origin Pro 9 was used for the visual interpretation of the data. Peak analysis was run on each individual set of data, outliers which drop below 50 (dB) shall be removed from the data set to avoid corruption of the data.

5. 3 **Results**

The results of this chapter are divided into three sections. Firstly, the results will present the physiological data reported during the bout of continuous exercise. This section will report the actual Na^+ when measured with the LAQUA Twin B-772 and total water loss for each of the 5 days during the STHA protocol. Section 5.3.2 will then report the data from the microwave sensor when positioned directly on the skin and if it was able measure Na^+ concentration to obtain a correlation to the LAQUA Twin B-772. Section 5.3.3 reports the findings when measuring each of the collected sweat samples post exercise within a controlled laboratory environment using a microwave sensor to detect Na^+ and report any obtained correlations with the LAQUA Twin B-772.

5. 3. 1 **Sodium and water loss during a short-term heat acclimation trial**

The effects of a five-day heat acclimation protocol on sweat sodium loss and total body water loss can be observed in Figure 5.3. Within the box charts, the square indicates the mean, the horizontal line within the box shows the median, the box boundaries are the

25th and 75th percentile, whilst the whiskers indicate the min and max values. Figure 5.3A presents all forearm Na^+ concentrations collected for all participants throughout the duration of the five-day exercise protocol within the environmental chamber to stimulate passive thermal stress. This data shows that there was a linear reduction in Na^+ concentration from day one to day five with an R^2 of 0.56. Total body weight was measured in all subjects pre- and post- exercise session to determine their water loss in relation to their body weight shown in Figure 5.3B.

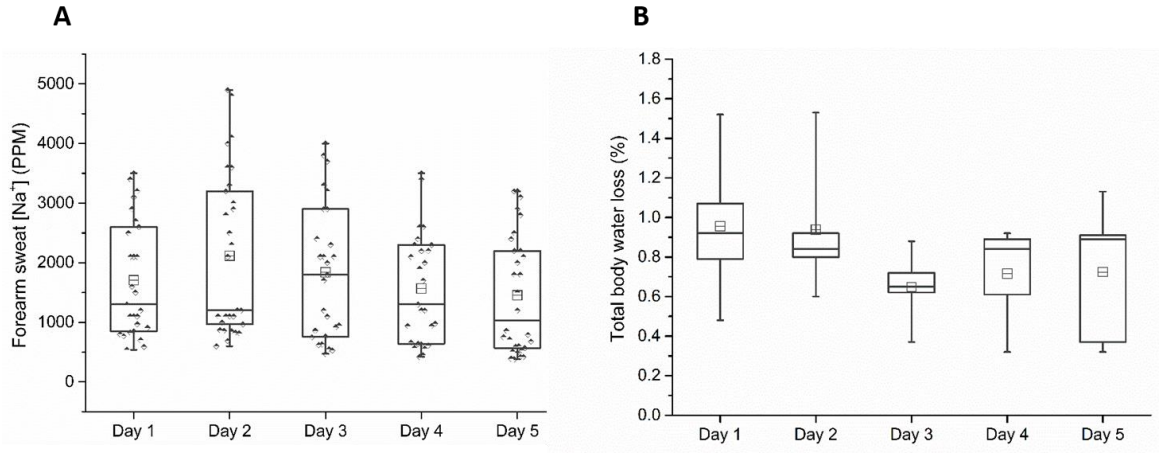

Figure 5.3 – A) Box plot for total forearm sweat sodium concentration (Na+) for all 10 subjects during the trial. B) Box plot for Total body water loss (percentage) for all 10 subjects during the trial.

5. 3. 2 **In-vivo measurement of sweat sodium concentration during exercise**

S_{11} measurements for the sensor when placed on the forearm during exercise were recorded for the duration of the exercise, after initial time stamping to match the sodium measurements by the LAUQAtwin Na^+ meter. The highest correlation between forearm sweat Na^+ and S_{11} measurement frequencies ranging from 1MHz to 4GHz when placed on the arm for all 140 samples was an $R^2 = 0.171$ at a frequency of 1.17 GHz. Further

assessment was done to assess S_{11} measurements between 1.5-1.7 GHz, which were successful during control analysis and the highest correlation was $R^2 = 0.149$ at 1.57 GHz. This shows there was no correlation shown between sweat Na^+ and S_{11} measurements when the sensor was placed upon the forearm.

5. 3. 3 **Post hoc analysis of sodium concentration in human sweat samples**

Each spectrum represents the sodium values detected for each sweat sample obtained during the trial for each participant. Peak observations show a distinct interaction of S_{11} amplitude at two specific points highlighted in Figure 5.4.

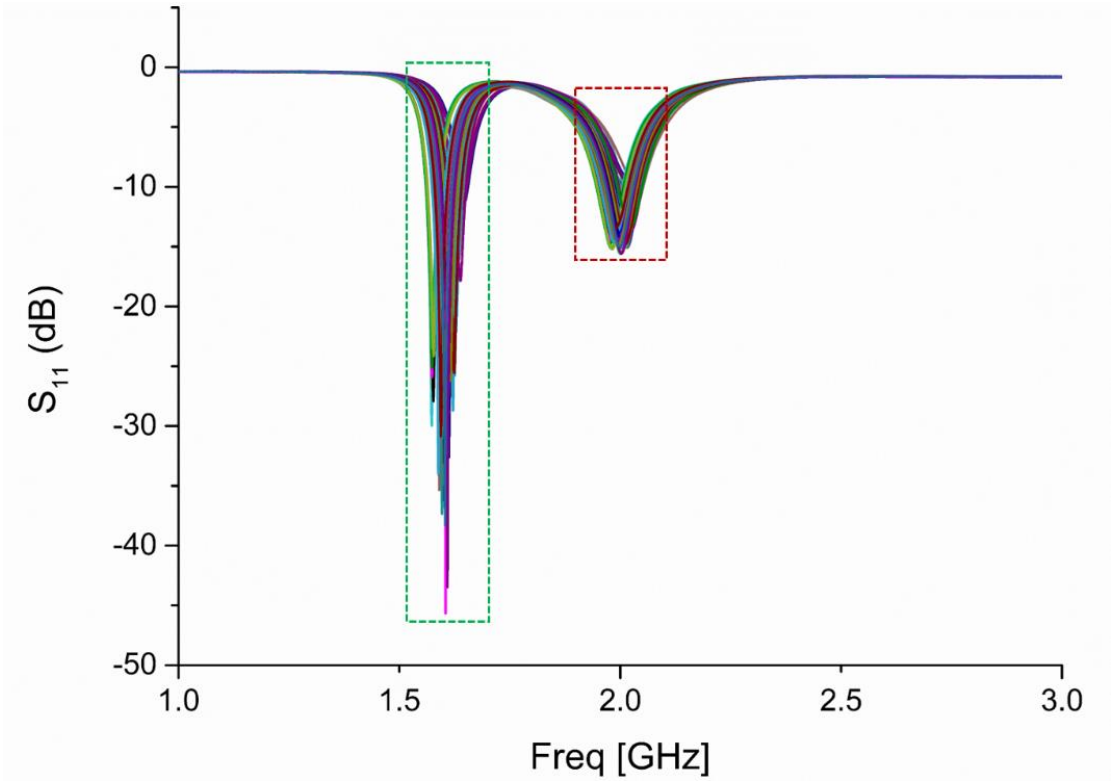

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Figure 5.4 - S_{11} (dB) reflection coefficient signal distribution between 1.0 GHz and 3.0 GHz frequency range using the microwave sensor monitoring sodium concentration (Na+) in 140 individual sweat samples

Results illustrate the microwave the full S_{11} scattering parameters for each of the 140 individual sweat samples sensors operating across a wide frequency range between 1-3 GHz. Moreover, the peaks on the right were produced at frequencies between 1.57- 1.63 GHz (indicated by the green dashes) and the peak on the left were produced within a frequency range between 1.98 – 2.02 GHz (indicated by the red dashes).

Determining if the S_{11} scattering parameter matched sodium's electromagnetic footprint in either frequency range required further investigation. Observations at ~1.6 GHz indicate a clear downward trend between S_{11} (dB) amplitude and the ranging concentrations of Na^+ in order of weakest through to highest concentration, shown in Figure 5.5.

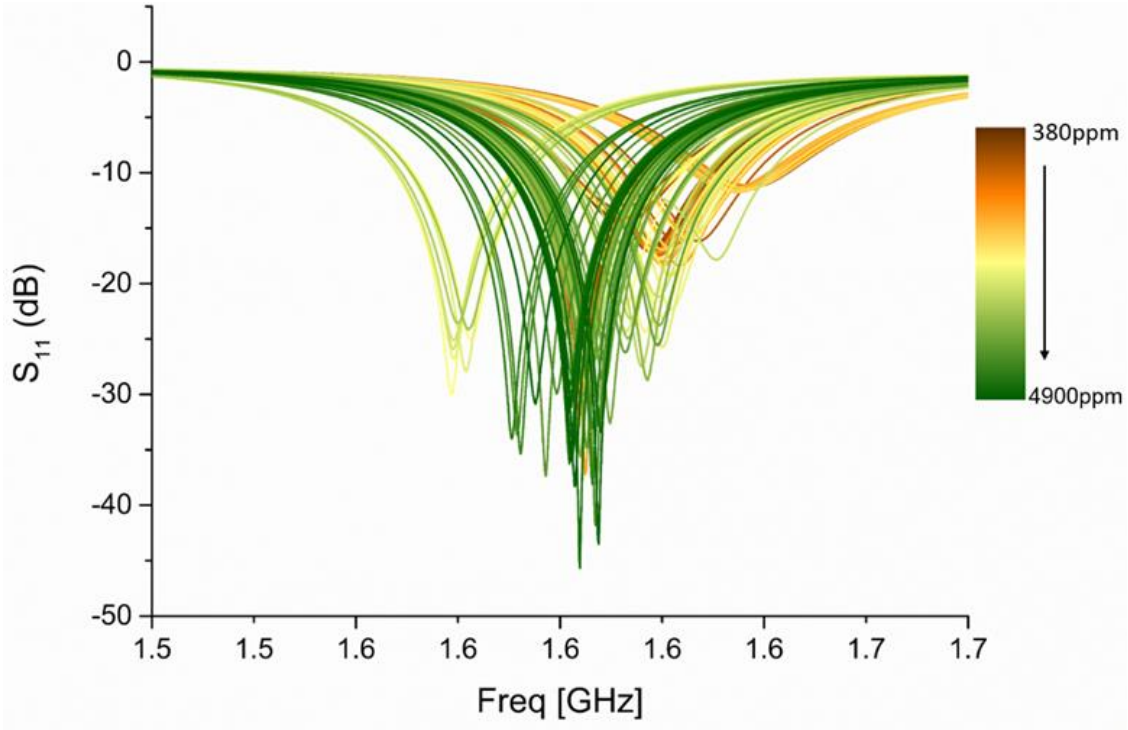

Figure 5.5 - Zoomed in view of S_{11} (dB) reflection coefficient signal distribution between 1.5 GHz and 1.7 GHz frequency range using a microwave sensor monitoring sodium concentration in all 140 sweat samples (Na+). Colour bar represents Na+ concentration from lowest through to highest

Further analysis illustrated that here was a strong linear relation between sweat Na^+ concentrations and S_{11} (dB) scattering parameter ($R^2 = 0.862$). The correlation is observed in a frequency range of 1.6 GHz, as observed in Figure 5.6.

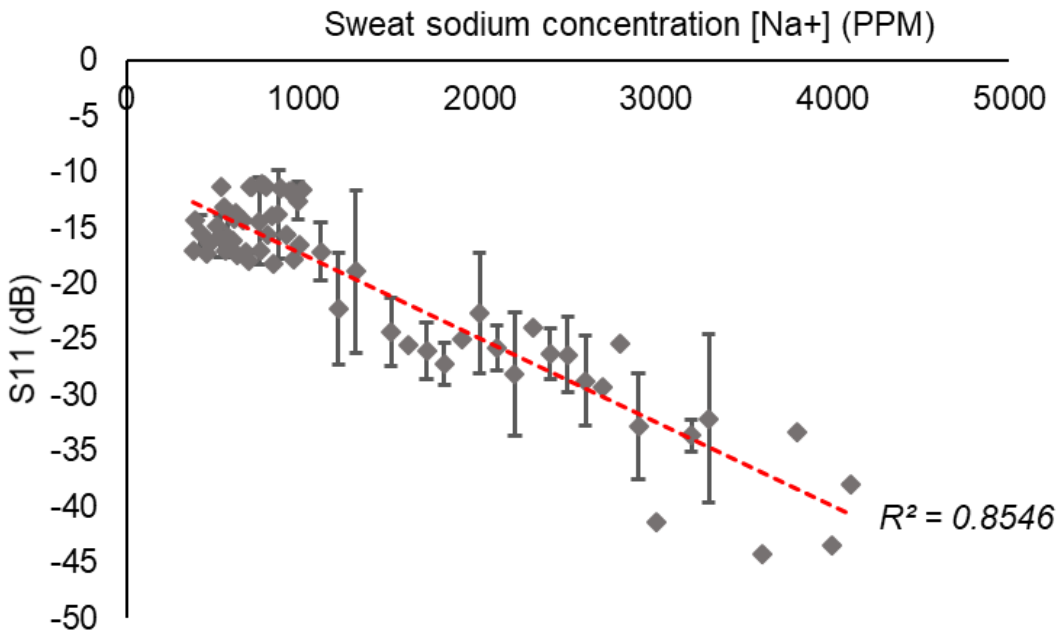

Figure 5.6 - The linear relationship between the generated microwave sensor S11 (dB) scattering parameter and sodium concentration within human sweat samples. Means ± SD indicate samples with the same sodium concentration.

5. 4 **Discussion**

This study was the first of its kind, determining the feasibility of a microwave resonator sensor to detect Na^+ concentrations in human sweat. This study is the initial step to enable development of an all-in-one electromagnetic sensor to monitor human sweat samples to obtain biochemical data via non-invasive sampling. The microwave sensor presents a solution to real-time, continuous monitoring of human sweat samples for the detection of Na+ through non-invasive techniques. Comparison with a commercial

grade sodium analyser allows for an ideal indication as to whether the microwave sensor can accurately provide consistent measurements.

The need for reliable continuous monitoring of Na^+ loss during exercise, rather than post exercise analysis, is reported within the literature, current methodological practices of collecting sweat samples from participants can cause significant unwanted variability in sweat-testing measurements (Baker, 2017). This is due to factors such as skin surface contamination and initial sweat (Ely et al., 2013), timings and duration of sweat collection (Morris et al., 2013), sample storage (Dziedzic et al., 2014) and the methods used to analyse the sweat (Baker, 2017). One of the major advantages in non-invasive real-time devices is the ability to monitor live patient information, as mentioned, current methods of sweat analysis can only provide a snapshot in time measuring one sample rather than continuously. Without continuous real-time monitoring, reliance on prediction models and detailed data collection are required, both being time-consuming and unavoidably reactive rather than preventative. Under laboratory conditions, the sensor performed as expected showing a clear strong correlation with Na^+.

5. 4. 1 **Monitoring sodium loss in human sweat during exercise**

Monitoring sweat during exercise physical activity in humans can enable insights into key biomarkers relevant to performance and health. Sodium is the key marker for electrolyte imbalance, monitoring sodium loss in hot and humid environments would allow for appropriate refueling strategies of electrolytes and fluids to improve performance and athlete safety (Bandodkar et al., 2014).

Results from this chapter reported that when assessing the microwave sensor to monitor Na^+ in real-time during the exercise protocol when it was attached to the forearm, results

generated showed a very low linear correlation, the highest correlation being $R^2 = 0.171$. The sensor used for this experiment has not been adapted to measure sweat levels exclusively and is intended to monitor a variety of parameters beneath the surface of the skin such as blood lactate shown in Chapter 5 and skeletal muscle glycogen shown in Chapter 6.

A key consideration before the trial was to limit sensor movement during the exercise trial, electromagnetic sensors are notorious for data being interrupted or producing noise interference if they are unstable and movement could allow the sensor to lift from the skin providing an inconsistent surface and factors such as airflow could affect readings. To counteract this, the sensor was positioned flush onto the forearm of the participant and no barrier was placed between the skin and the sensor. However, as the sensor was placed flush onto the skin with no added method to wick the sweat away and keep a consistent renewal of sweat, the sensor did not meet a high level of accuracy. As the sensor was shown to detect Na^+ concentration in the identical sweat samples, just not on the surface of the skin, it is likely a result of a design flaw in the sensor.

Recently, there has been a push for the development of wireless sensors to detect parameters in sweat including but not limited to sodium and other electrolytes. These sensors are now adding subtle to more sophisticated solutions to wicking or pumping sweat away from the skin and providing a neutral space for their sensors to analyse the solution. (Gao et al., 2016a; Dang et al., 2018; Lim et al., 2019).

5. 4. 2 **Post hoc analysis of sodium concentration in human sweat samples**

Although the data from this study appears to indicate that a microwave sensor is unable to attain measurement of Na^+ during exercise, it was imperative to identify the cause of this and determine if the microwave sensor was capable of detecting Na^+ when isolated within a controlled laboratory environment and if changes could be made to the existing sensor platform to improve sensing performance. The results from post hoc analysis, when sweat volume was consistent and placed directly over the sensor show the electromagnetic S_{11} scattering parameter displays two distinct spectra signal responses. Analysis of the S_{11} peaks identified the frequency of 1.6 GHz provides the strongest correlation with Na^+, 1.6GHz was located within the first range of spectra signal responses, and therefore it can be observed in **Error! Reference source not found.** that this has a more considerable variance between peaks, ideal for identifying Na+ concentration and providing higher accuracy.

The microwave sensor in this study provided reliable detection of Na^+ when measuring a consistent volume of 200ul of sweat, future research and sensor fabrication will be needed to discover the minimum required volume of sweat to obtain Na^+ and a pump-like system will be an ideal solution for the sensor to monitor the sweat in an isolated environment, allowing for an all-in-one wearable device. Under laboratory conditions, the sensor performed as expected showing a clear, strong correlation with Na^+. However, the initial results when the sensor was attached to the arm show inconsistencies. The design of this study was to determine whether a microwave sensor could detect sodium concentrations in sweat, this has been achieved under controlled laboratory conditions.

As results illustrate no significant correlation between the microwave sensor and Na^+ concentration in sweat when placed upon the forearm, but a high correlation when measuring under a laboratory setting using sweat from the same participants and the same volume of sweat for each measurement, we can draw reasonable conclusions that when measuring a consistent volume of sweat and when the sweat has opportunity to regenerate naturally then an microwave sensor can detect changes in Na^+ in sweat. Future research and sensor fabrication will be needed to discover the minimum required volume of sweat to obtain Na^+ and a pump-like system will be an ideal solution for the sensor to monitor the sweat in an isolated environment, allowing for an all-in-one wearable device. This trial has indicated the promise of a wearable sensor indicating that a microwave sensor can detect Na^+ in human sweat samples in real-time, an effort now needs to focus on development of the sensor to wick the sweat away from the skin to allow for consistent sweat flow.

5. 4. 3 **Summary**

In summary, this study presents the novel application of a microwave hairpin resonator sensor measuring changes in Na^+ concentration in human sweat samples under post hoc in-vitro conditions, when operating in S_{11} configuration at 1.6 GHz. Thus, with further research and development, it has the potential of becoming a novel approach as an alternative non-invasive method to monitor changes in sodium levels in-vivo. This technique shows a promising future and with the right adjustments made to the sensor, currently gives optimism to a practical non-invasive solution for the continuous monitoring of sodium. This would solve many of the issues faced by scientists and coaches using invasive equipment, restricted to laboratory environments and single use

measurements. Sweat measurement during exercise is a useful tool to aid in performance monitoring, providing a tool to estimate an athlete's sweat rate, and sweat Na^+ loss to enable appropriate replacement of fluid and electrolytes which can improve performance and ensure the athlete avoids hyponatraemia and the serious health risks associated with this condition.

CHAPTER 6 - PARAMETER 2: NON-INVASIVE MEASUREMENT OF BLOOD LACTATE DURING EXERCISE

This chapter will investigate a hairpin electromagnetic sensor operating at microwave frequencies to detect blood lactate in human subjects during exercise in real-time. The chapter will cover the methodology employed to ensure a full range of blood lactate measurements to allow for sensor readings observed at low lactate concentrations through to high levels of lactate produced by healthy human subjects. To ensure the full spectrum of lactate was measured, two sets of participants were used (endurance trained participants and untrained participants). As this chapter presents two separate studies which use the same exercise methodology/protocol and microwave sensor set-up, with two separate populations and methods of analysis, the sections are labelled "Blood lactate study 1" and "Blood lactate study 2" for clarity throughout.

6. 1 **Introduction**

There is a growing demand for non-invasive point of care devices to monitor metabolites in blood such as lactate. In hospital environments, having such tools would reduce the risk of infection, increase the frequency of measurements, and ensure timely intervention only when necessary. Monitoring blood lactate levels is a fundamental necessity for athletes seeking training adaptations and an overall improvement in performance. Tracking levels of lactate accumulation can allow for improvements of their lactate

threshold (Hall et al., 2016). The lactate threshold indicates the physical training level of an athlete, showing a finely tuned interplay of parameters that influence the balance between lactate production and lactate clearance (Goodwin et al., 2007).

The normal lactate response to progressive, incremental exercise is that lactate increases gradually at first and then more rapidly as the exercise becomes more intense, it is reported that normal resting values of blood lactate range from 0.5 to 1.5mmol/L (Phypers and Pierce, 2006). During the initial onset of exercise, lactate changes little, during easy to moderate intensity exercise (i.e. between 50% and 65% VO_2 max), lactate values remains low, most likely due to production of lactate being balanced out due to clearance by oxidation. However, at approximately 65% of VO_2 max, coinciding with a rise in adrenaline, muscle lactate release starts to increase steeply, during exercise above 65% VO_2 max values can be as high as 12mmol/L (Stanley et al., 1985). This sudden increase occurs when the glycolytic flux and lactate production exceeds that which can be cleared by mitochondria through oxidation, then the excess glycolytic flux is released as lactate. In the course of extensive physical exercise and extreme conditions this level may further increase upwards of 25mM which may also be observed 3-8 minutes post exercise (Glaister et al., 2007). The accumulated lactate is a reflection of the imbalance between the rate of lactate oxidation and lactate release, this suggests that humans are more limited by their oxidation capacities than their release capacities.

As detailed within the literature review, the main method to detect blood lactate concentration involves repeated application of a lancet device to prick the fingertip of the athlete to take a blood sample to frequently monitor blood lactate levels, this has brought on the need to create a solution to create non-invasive devices. The main

limitation of current invasive methods such as the Lactate Pro 2 is that the device uses single-point measurements. This process also requires many blood samples which can cause a lot of added stress for both the athlete and the patient alike. With this method, lactate is only seen as a snapshot in time rather than providing data on lactate fluctuations during critical points of analysis (Rassaei et al., 2014).

6. 1. 1 **Effects of training status**

Endurance training results in the onset of many physiological and metabolic adaptations which function to delay the onset of fatigue at a given exercise intensity. Adaptations to endurance training are usually noted with an increased maximal oxygen uptake, as well as an improved lactate threshold. Among well-trained distance runners, VO_2 max and running velocity at VO_2 max explains differences in performance better than velocity at lactate threshold or percentage of VO_2 max at the lactate threshold (Mclaughlin et al., 2010). Maximal 1 hour power, power at lactate threshold, and VO_2 at the lactate threshold are strong predictors of endurance cycling performance, but absolute VO_2 max is not a strong predictor of endurance cycling performance (Gregory, Johns and Walls, 2007). In elite runners, running velocity at maximal lactate steady state (MLSS) and VO_2 at MLSS are very trainable and account for nearly all improvement in running performance. VO_2 max, however, demonstrates limited improvement in response to training and correlates poorly with improvements in athletic performance (Tanaka et al., 1986). To summarise, a "trained" athlete will exhibit a higher lactate threshold and a lower blood lactate concentration than a "poor" or "untrained" athlete at any absolute intensity above resting allowing for longer duration during the implemented procedure

in study 2. This will allow us to test the sensor's capabilities at much higher concentrations of lactate than those observed in study 1 with the untrained subjects.

The aim of this chapter was to determine whether an electromagnetic sensor was capable of detecting changes of blood lactate in both untrained healthy adults and endurance-trained athletes during progressive incremental exercise.

6. 2 **Methodology**

To ensure the full spectrum of blood lactate values, measurement of two different population types was completed. Participants who did not regularly participate in physical activity (less than 3 times per week) and secondly endurance trained participants, who took part in endurance based physical activity (3 times or more a week). The purpose of this is due to the physiological adaptations that occur when you exercise regularly and your body's ability to managed blood lactate and the threshold and regulation of lactate increases. The combination of both trained and untrained participants therefore should allow us to measure consistently low levels of lactate and higher levels of lactate in the final stages of the exercise trial. It is worth noting, there was careful consideration in regard to the exercise trial used in this protocol. This method is a common technique to induce high levels of blood lactate and push the participant to their physical limits.

6. 2. 1 **Participants**

A total of 44 health participants (35 males, 9 females) aged between 18 and 40 years old resulted in 523 collected blood lactate samples over two trials.

6. 2. 1. 1 **Untrained participants**

The initial investigation was to collect blood lactate samples from subjects from the general population who did not participate regularly in endurance-based exercise. 34 participants (27 males, 7 females) aged from 20 to 40yrs. There was a total of 367 blood lactate measurements; on average there were 11 blood samples taken per participant. Naturally, this varied depending on the fitness level of each participant, and thus their ability to maintain a steady cadence despite the increasing work rate.

6. 2. 1. 2 **Endurance trained participants**

10 (8 males, 2 females) endurance trained participants (min 3 sessions of endurance training per week of 1h for at least 1 year), Aged from 18 to 40yrs, and free of any known health risks or disease. A total of 156 blood samples were collected and used as a direct comparison with the microwave sensor measurements.

6. 2. 2 **Experimental protocol**

The subjects are required to perform a bout of progressive incremental exercise commencing at 80 W staying between 60-80 RPM (revolutions per minute) for a duration of five minutes on an ergometer bike, at the end of this period measurements were recorded and exercise intensity increased by 20 W every minute whilst measurements were taken every 2 minutes. Once the participant reached maximal voluntary exhaustion, there was a recovery period of 10 minutes with measurements recorded every 2 minutes. During the study results for blood lactate concentration (mmol/L) were collected and analysed (LactatePro 2, Arkray) in the final 10 seconds of each stage, via finger prick capillary blood samples of only 0.3 μL, adhering to standard procedures. Blood samples were taken using a safety-lancet (Sarstedt, Germany).

To begin testing, firstly subjects were adjusted to fit the ego bike, two separate sensors were attached over the forearm directly over the flexor digitorum profundus, as well as the quadriceps, positioned over the rectus femoirs and the vastus lateralis to ensure maximal muscle activation during the protocol. Measurements were recorded pre-test (-5 min) to get baseline results blood lactate (mmol/l). Pre-test (0 min) results were noted. Once the test commenced the Rhode & Schwarz 9 KHz- 13.6 GHz VNA which is connected to the sensor is set to begin and was set to monitor lactate levels in alignment with the blood lactate sample collection points. Testing began at 80 W whist the subjected stayed between 60-80 RPM (revolutions per minute) for a duration of five minutes, at the end of this period measurements were noted and exercise intensity increased by 20 W every minute whist measurements were taken every 2 minutes,.i.e.. (100 W, 140 W, 180 W, 220 W). Exercise was ceased following maximal voluntary exhaustion or RPM) below 60, remaining on the bike for a further 10 minutes remaining stationary throughout whist results were recorded every 2 minutes during the recovery phase.

6. 2. 2. 1 **Sensor Placement**

The sensor was placed on the left arm and leg of each participant; the leg due to this being the source of lactic acid production during exercise, and the arm due to blood being drawn from the fingertip for lactate measurement. Specific placement on the leg was over the Rectus femoris muscle and on the wrist approx. one-third distance between the wrist and elbow joints, where there would be significant blood flow owing to the arteriovenous fistula.

The left side of each participant was chosen simply due to accessibility within the testing space itself. The sensors were fixed to the participant using 75 x 100 mm surgical dressings, modified by cutting to allow the right-angled SMA connectors to protrude. Cables were secured to the limbs of the participant using a surgical tape, primarily for mechanical strength. Prior to placement, the sensor and area under test was cleansed with an alcohol wipe. No shaving or other preparation of the skin was undertaken.

6. 2. 3 **Statistical analysis**

A number of techniques were considered for providing robust analysis and models to test the correlation between microwave wave sensor outputs and lactate level measured via Lactate Pro V2. Typical linear models, which have proven successful for in-vitro laboratory-based tests yield relatively low correlation across the complete data set.

6. 2. 3. 1 **Blood lactate study 1: Untrained participants**

Due to the large data set collected for this parameter within this population group, a Pairwise Mutual Information (PMI) was applied, combined with Neural Networks (NN) to test the correlation between the EM wave sensor outputs and the lactate measurements of the nontrained participants. A number of reduced datasets were produced using the PMI method, based on the top 10, 20, 50, 100, 250 and 500 frequencies of interest per measurement with the microwave sensor, where originally data was acquired at 4,000 discrete frequencies between 10 MHz and 4 GHz. This was replicated for data collected from both the arm and leg of each participant, as well as for each measurement mode, i.e., S_{11} and S_{21}.

The NN approach was applied in Mathworks MatLab software for each dataset. The data was split into a training set (225 values, 65%), validation set (75 values, 22%) and

test set (45 values, 13%). Splitting of the data was performed at random and 10-fold cross validation was performed, such that 300 lactate values were used for training the NN, and the remainder were used to test the resultant models. It is noted, as in Figure 6.1, that the volume of data available for lactate levels exceeding 15mmol/L is limited and so this part of the dataset was excluded from this machine learning exercise. Thus, the total number of lactate measurements available was reduced from 367 to 345.

6. 2. 3. 2 **Blood lactate study 2: Endurance trained**

Enterprise IBM SPSS 22 Statistical analysing package was used to determine the significance of the data. A bivariate (Pearson) two tailed analysis was used to report the correlation between the sensors S-parameters and the blood lactate sample, the correlation was significant at the 0.01 level. Origin Pro 9 was used for the visual interpretation of the data.

6. 3 **Results**

6. 3. 1 **Response to progressive incremental exercise in untrained participants**

Overall, as shown in Figure 6.1, the majority of data collected is in the lactate range of 0-5 mmol/L, with an approximately even split then between the 6-10 and 11-15 mmol/L groups. This is reasonable given participants would spend 5 minutes resting at the beginning and end of the resting regime, and a further 5 minutes warming up with little exertion (for most) experienced in this period. Few participants were able to raise their lactate level above 15 mmol/L, and so the data availability > 15 mmol/L for the purposes

of creating relevant models linking microwave sensor output with actual lactate level is limited.

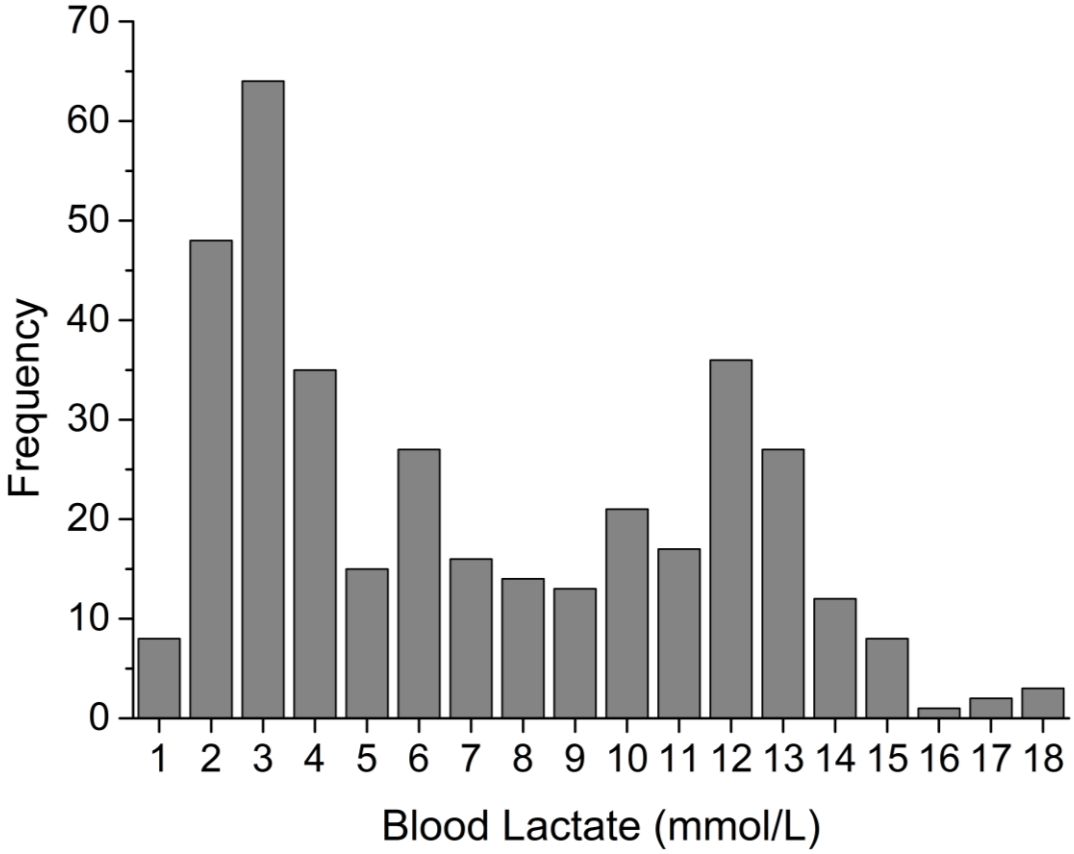

Figure 6.1 - Bar chart showing the spread of blood lactate data collected from participants across all lactate intervals in absolute values

When observing the average blood lactate measurements for the untrained population it was observed that overall, participants could reach a maximum of 300 W on average peaking at 13 mmol/L of blood lactate as shown in Figure 6.2. It was also observed that a rapid increase in blood lactate levels at around the 90-WATT stage, this inflexion point is referred to as the lactate threshold and is the point at which lactate will begin to accumulate more quickly than the body can remove it. The lactate threshold can then be then used as a determinant of fitness.

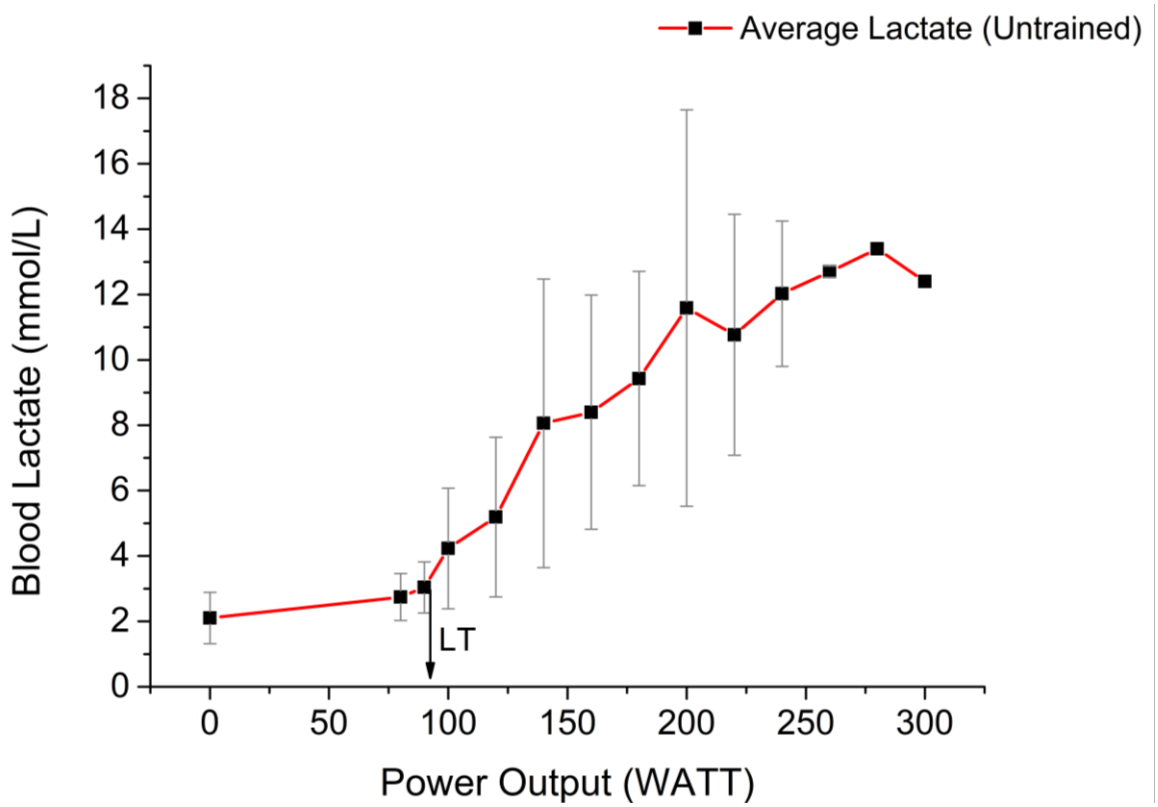

Figure 6.2 - Illustrates (mean ± SD) blood lactate levels for all untrained subjects during progressive incremental exercise until maximal voluntary exhaustion. Lactate threshold (LT)

6. 3. 2 **Response to progressive incremental exercise in endurance trained participants**

Overall, the majority of lactate values were in the range of 0-5 mmol/L, with an approximately even split then between the 6-10 and 11-15 mmol/L ranges. This is reasonable given participants would spend 5 minutes resting at the beginning and end of the testing regime, and a further 5 minutes warming up with little exertion (for most) experienced in this period. Few participants were able to raise their lactate level above 15 mmol/L, and so the data availability > 15 mmol/L for the purposes of creating relevant models linking microwave sensor output with actual lactate level is limited. A key observation made so far is that endurance trained subjects are able to sustain higher rates of lactate accumulation in the regions of 7-18mmol/L as seen in Figure 6.3. The

producing of substantially higher lactate values in the latter part of the exercise regime gives us a much wider spectrum of lactate values to compare with the adjacent sensor data.

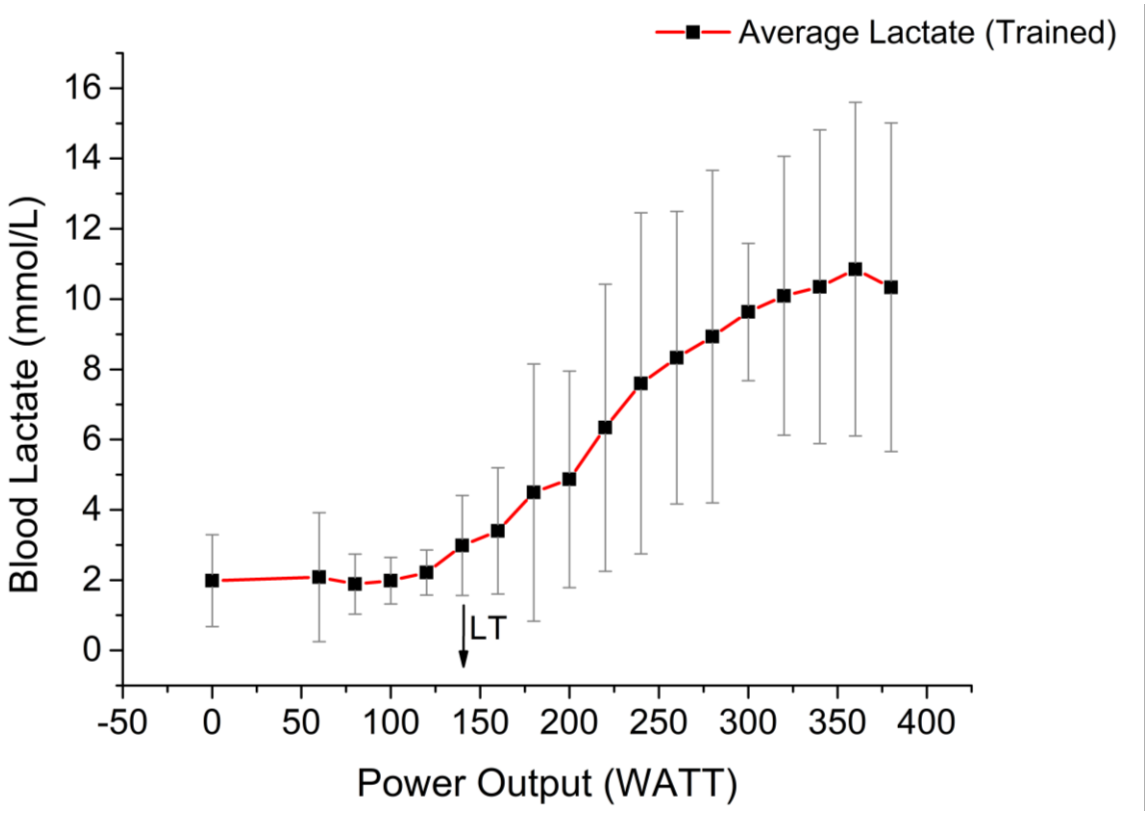

Figure 6.3 - Illustrates (mean ± SD) blood lactate levels for all endurance trained subjects during progressive incremental exercise until maximal voluntary exhaustion. Markers indicate blood lactate collection

6. 3. 3 **Comparison of blood lactate response and exercise capacity between untrained and trained participants**

Data collection occurred across two separate studies involving untrained and trained participants. However, the methodology used for both studies was identical allowing for a direct comparison between the two groups to evaluate if a full spectrum of naturally occurring blood lactate concentrations was obtained in healthy adults. Both sets of participants followed the same warm up routine and stopped the exercise protocol when

the participant deemed that they could not go any further or until the RPM dropped below 60. The average blood lactate response during the exercise trial is illustrated in Figure 6.4.

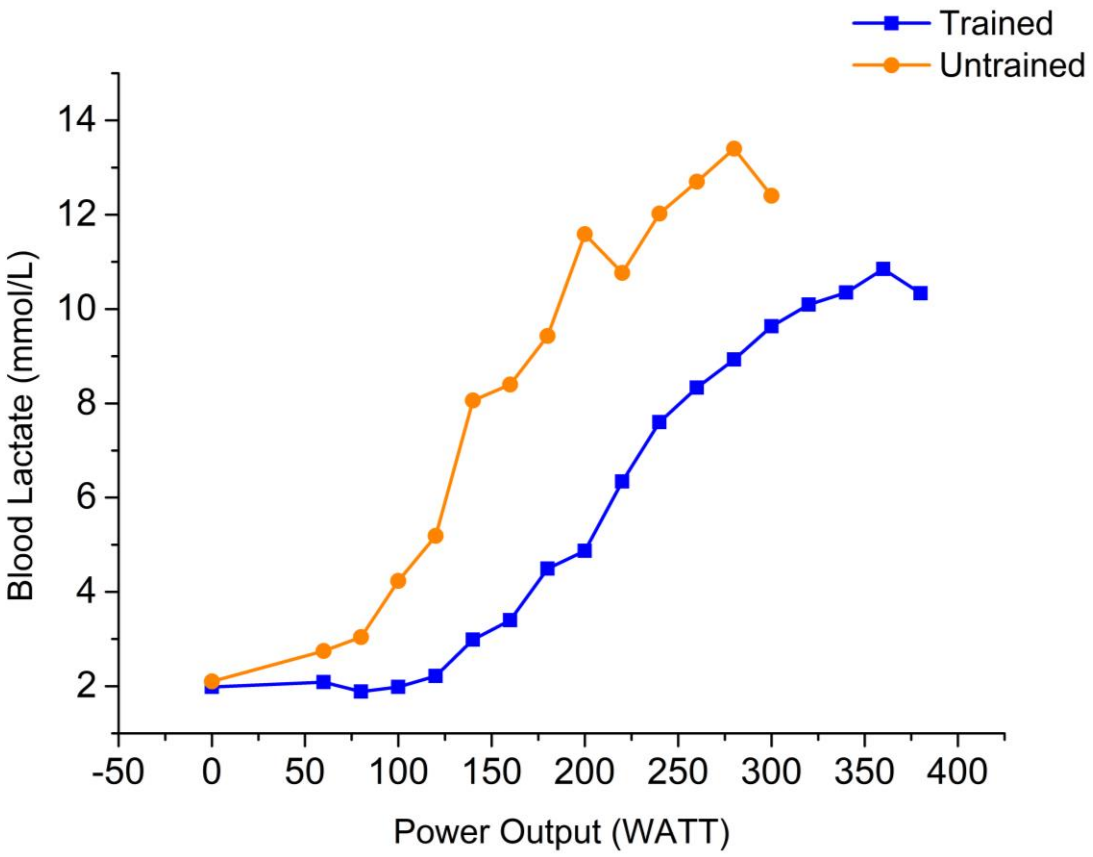

Figure 6.4 - Direct comparison of both sets of participants' average blood lactate response during bout of incremental exercise to voluntary exhaustion

6. 3. 4 **Microwave measurement of blood lactate in untrained participants**

Results corresponding to each mode of measurement (S_{11} and S_{21}), location (arm and leg), and the frequency ranking (10, 20, 50, 100, and 500) are shown in Table 6.1 On average the best performing measurement was achieved with the sensor located on the arm, and with the S_{11} mode of measurement; this consistently achieves a $R_{test} > 0.78$ once the number of discrete frequencies used for training approaches or exceeds 100.

Table 6.1 - Neural network training and test R and RMSE values for each model across the measuring modes of the top ranked frequencies

No. Freq	Data Type	S_{11} Arm	S_{21} Arm	S_{11} Leg	S_{21} Leg	Ave.
10	$R_{training}$	0.8719	0.7191	0.7669	0.7252	0.7708
	R_{test}	0.5543	0.4268	0.5936	0.3682	0.4857
	$RMSE_{training}$	2.0721	2.9301	2.7149	2.9213	2.6596
	$RMSE_{test}$	4.4068	4.1791	3.7223	4.7677	4.2690
20	$R_{training}$	0.8311	0.8897	0.8140	0.7998	0.8337
	R_{test}	0.5267	0.2213	0.6107	0.2734	0.4080
	$RMSE_{training}$	2.3543	1.9321	2.4589	2.5413	2.3217
	$RMSE_{test}$	4.775	5.6949	3.7047	5.4118	4.8966
50	$R_{training}$	0.9529	0.8424	0.9245	0.8016	0.8804
	R_{test}	0.6456	0.50900	0.7060	0.5160	0.5942
	$RMSE_{training}$	1.2949	2.2721	1.6149	2.5297	1.9279
	$RMSE_{test}$	4.0165	4.0477	3.8603	4.278	4.0506
100	$R_{training}$	0.9653	0.9225	0.8571	0.9469	0.9230
	R_{test}	0.7827	0.3274	0.7270	0.2575	0.5237
	$RMSE_{training}$	1.1087	1.6316	2.1857	1.3698	1.5740
	$RMSE_{test}$	2.8786	5.1848	3.081	8.7872	4.9829
250	$R_{training}$	0.9486	0.9607	0.9247	0.9765	0.9526
	R_{test}	0.8047	0.4747	0.5700	0.3718	0.5553
	$RMSE_{training}$	1.3589	1.1724	1.625	5 0.9148	1.2678
	$RMSE_{test}$	2.7426	4.8635	4.6707	5.4387	4.4289
500	$R_{training}$	0.9163	0.9589	0.7968	0.9606	0.9082
	R_{test}	0.7632	0.5449	0.6871	0.3945	0.5974
	$RMSE_{training}$	1.7621	1.228	2.5578	1.2061	1.6885
	$RMSE_{test}$	3.242	5.0805	3.2657	5.743	4.3328
Ave.	$R_{training}$	0.9144	0.8822	0.8473	0.8684	-
	R_{test}	0.6795	0.4174	0.6491	0.3636	-
	$RMSE_{training}$	1.6585	1.8611	2.1929	1.9138	-
	$RMSE_{test}$	3.6769	4.8418	3.7175	5.7377	-

Typically speaking, the results produced from the NN modelling indicate that once 100 frequencies of interest are exceeded, there is little relative improvement in model performance with further increase in the number of frequencies – this is evident in the

plateau effect for both R and RMSE shown in Figure 6.5. The measurements conducted on the leg, also in the S_{11} mode, tend to give next best performance, achieving an R value of approx. 0.7 with 100 frequencies of interest fed into the training model.

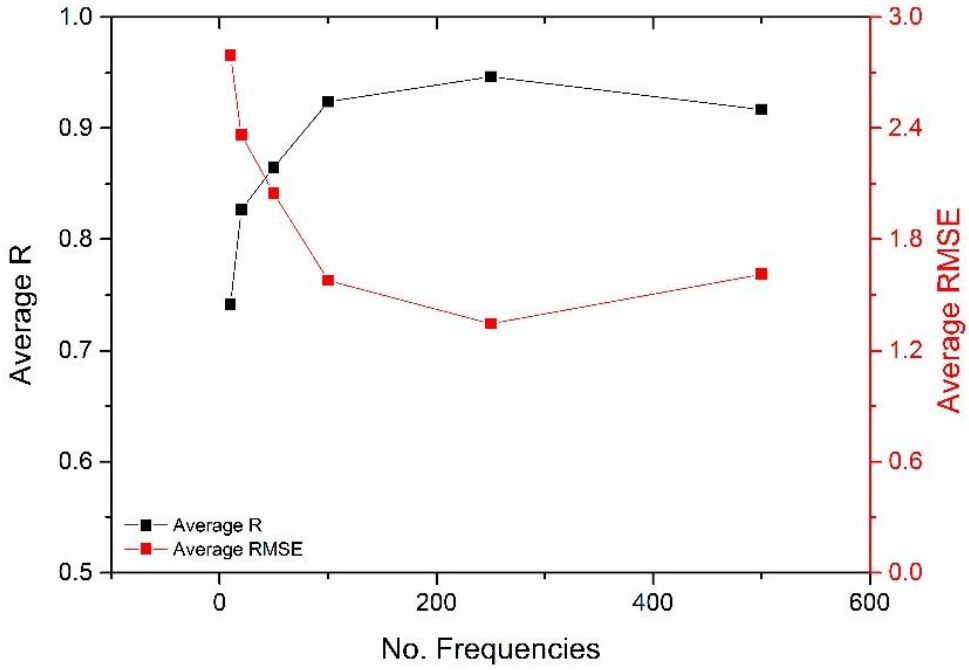

Figure 6.5 - Average R and RMSE values for all modes of measurement, illustrating the impact of increasing the number of frequencies used in the training models.

The training model, created for the top 100 ranked frequencies of interest using the S_{11} arm combination, which tended to be most significantly concentrated in the 3.4-3.6 GHz region of the measured spectra, is shown in Figure 6.6. It is noted that the RMSE reported for the test data is typically higher than that for the training model. However, it is thought that the reported error for the test data in this work is higher than might be desirable. To understand why this is the case, one should compare the model test representations shown in Figure 6.6C and Figure 6.6D. In (C), the model has shown high variation at the high lactate value extremity (i.e. > 14 mmol/L).

Looking back at Figure 6.6A, the data available showed that this concentration is limited, which reflects on the model. The impact of removing these extremity regions is highlighted in Figure 6.6D.

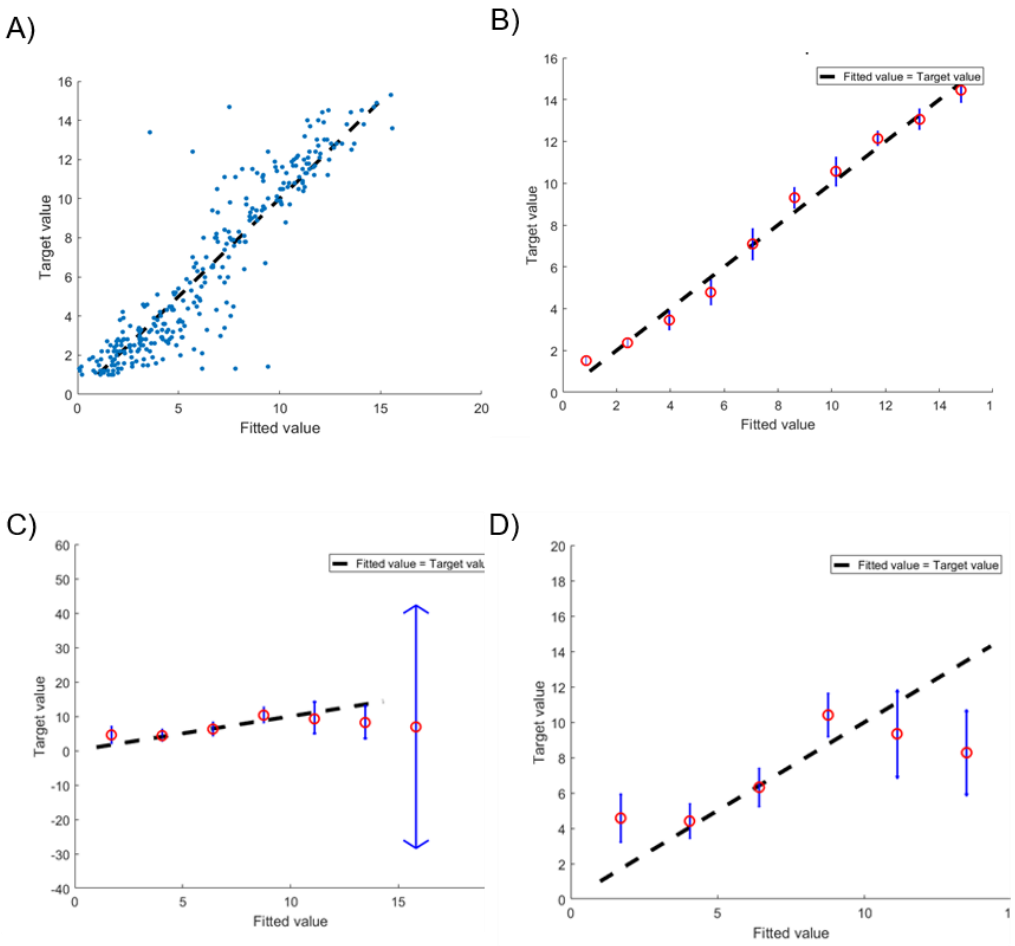

Figure 6.6 Example of the data produced for each model created; this example is using the top 100 frequencies selected via the PMI method for the S_{11} combination; (A) training model fitting, (B) training model calibration, (C) full test data calibration and (D) test data calibration with extremities removed.

It is therefore suggested that the RMSE values produced for the test data should be considered as absolute worst case, and that the actual error in practical use (and with more data to feed to the model in these extremity regions) would be lower. For example,

in the case of the S_{11} arm combination, using the top 100 ranked frequencies, the reported training model error is 1.11 (7.4%), and the test error 2.88 (19.2%).

The tracking capability of the sensor to do this is illustrated in Figure 6.7, where all of the collected data from the 34 participants is overlaid with the predicted data from the NN model, trained using 100 discrete frequencies.

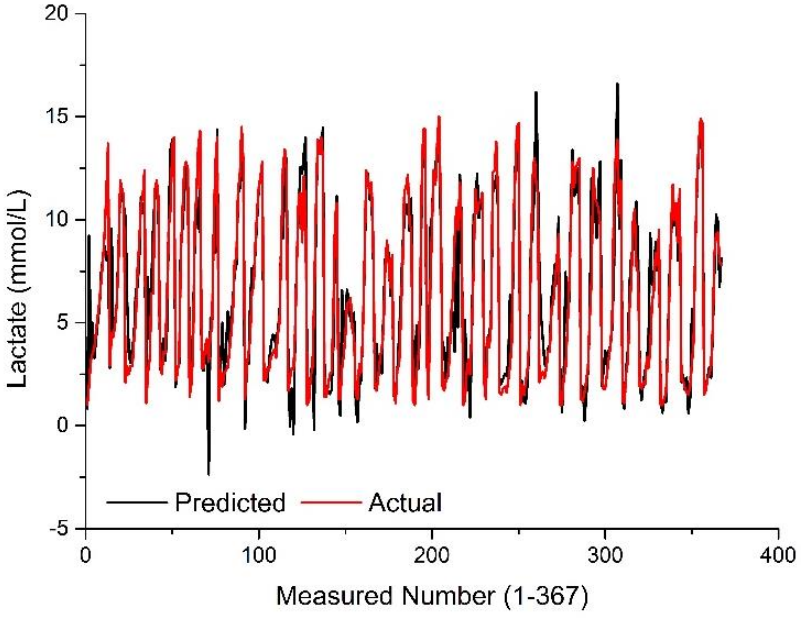

Figure 6.7 - Demonstrates the actual data measured versus the model predicted based upon the neural network model, highlighting the capability of the model to predict the lactate profile, not only absolute value.

During this study, it was noted that the average absolute skin temperature variation between the end of phase 1 and beginning of phase 4 was 2.28 ^{o}C (min 0.92 ^{o}C, max 4.30 ^{o}C). It was also noted that the temperature recorded by the thermocouple sensors tended to fall during exercise, most likely due to participant perspiration (Torii et al., 1992). Heart rate on the other hand, tended to rise as work output increased from a resting average of 85 bpm to a maximum of 172 bpm as the participants reached their

maximal efforts during the trial. Notably however, whereas heart rate tended to fall almost immediately post-exercise, lactate level would continue to rise due to the latency inherent in lactate metabolism. As a result, both temperature and heart rate failed to yield a significant correlation with the microwave wave sensor measurements, with $R < 0.4$ in both cases.

6. 3. 5 **Microwave measurement of blood lactate in endurance trained participants**

Analysis of the 155 combined lactate measurements collected from the combined total population of 10 participants showed no clear correlation between S_{11} spectre measurements and the collected blood lactate concentrations across the entire recorded frequency range, illustrated in Figure 6.8.

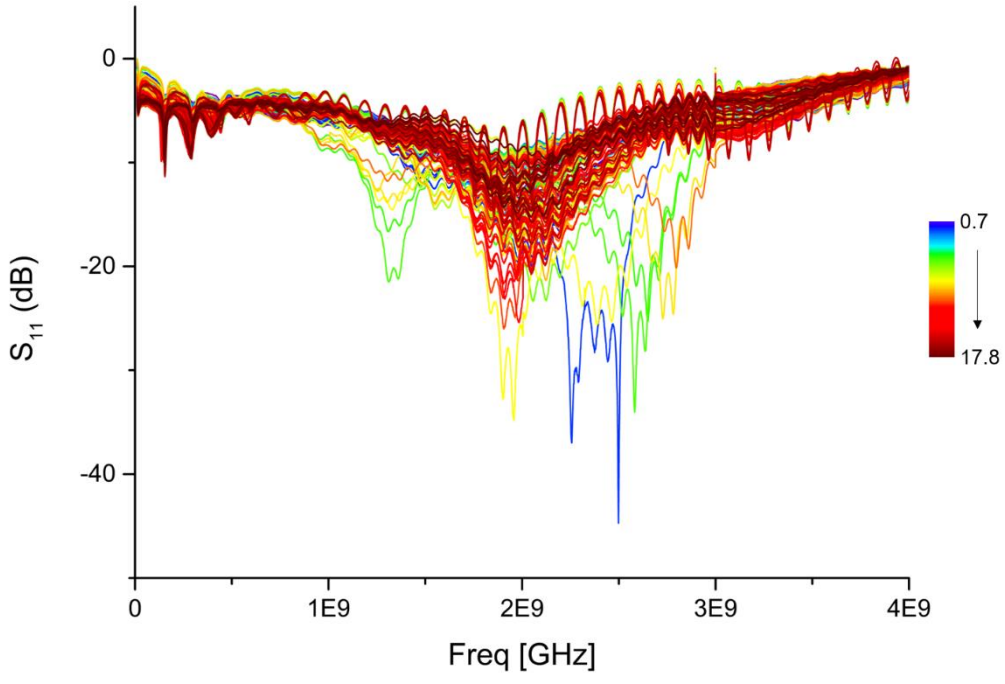

Figure 6.8 - S_{11} (dB) reflection coefficient signal distribution between 1.0 MHz and 4.0 GHz frequency range corresponding to the measured blood lactate samples (mmol/L) shown within the legend

However, assessment of how the microwave sensor interacted with each individual participant was key to understanding the EM waves, and to assess any frequency shifts was an essential part of study 2. 10. Analysis of S_{11} amplitude measurements when compared with blood lactate samples identified a significant linear relationship between all participants as shown in Table 6.2. Individual analysis of each participant and the blood lactate samples collected prior the full duration of the exercise protocol showed a strong linear relationship with an R^2 greater than 0.70 was observed in 7 out of 10 participants. Highest correlation obtained was $R^2 = 0.927$, $P = 0.000$ observed at 3.25 GHz, and the lowest correlation being $R^2=0.583$, $P= 0.001$ observed at 3.75 GHz.

Table 6.2 - Correlation for each participant between blood lactate values and S_{11} (dB) for each participant and the frequency which highest correlation this was obtained

Participant	R^2	Freq (GHz)
P1	0.651**	3.20
P2	0.865**	3.21
P3	0.927**	3.25
P4	0.717**	3.80
P5	0.712**	3.22
P6	0.887**	3.63
P7	0.594**	3.20
P8	0.884**	3.47
P9	0.763**	3.20
P10	0.583**	3.75
Average R^2 / Freq Range	**0.758**	**(3.2 – 3.8)**

** Correlation is significant at the 0.01 level (2-tailed)

S_{11} scattering parameters for each individual participant are shown in Figure 6.9 and Figure 6.10. For each participant, each individual spectrum represents the EM signal corresponding to each blood lactate value collected during progressive incremental exercise. Observations of Figure 6.9 show a linear negative correlation as dB values decrease and lactate concentration (mmol/l) increases, these findings show that in eight

out of the ten participants, as blood lactate concentration increases, the microwave scattering parameter S_{11} (dB) is reduced.

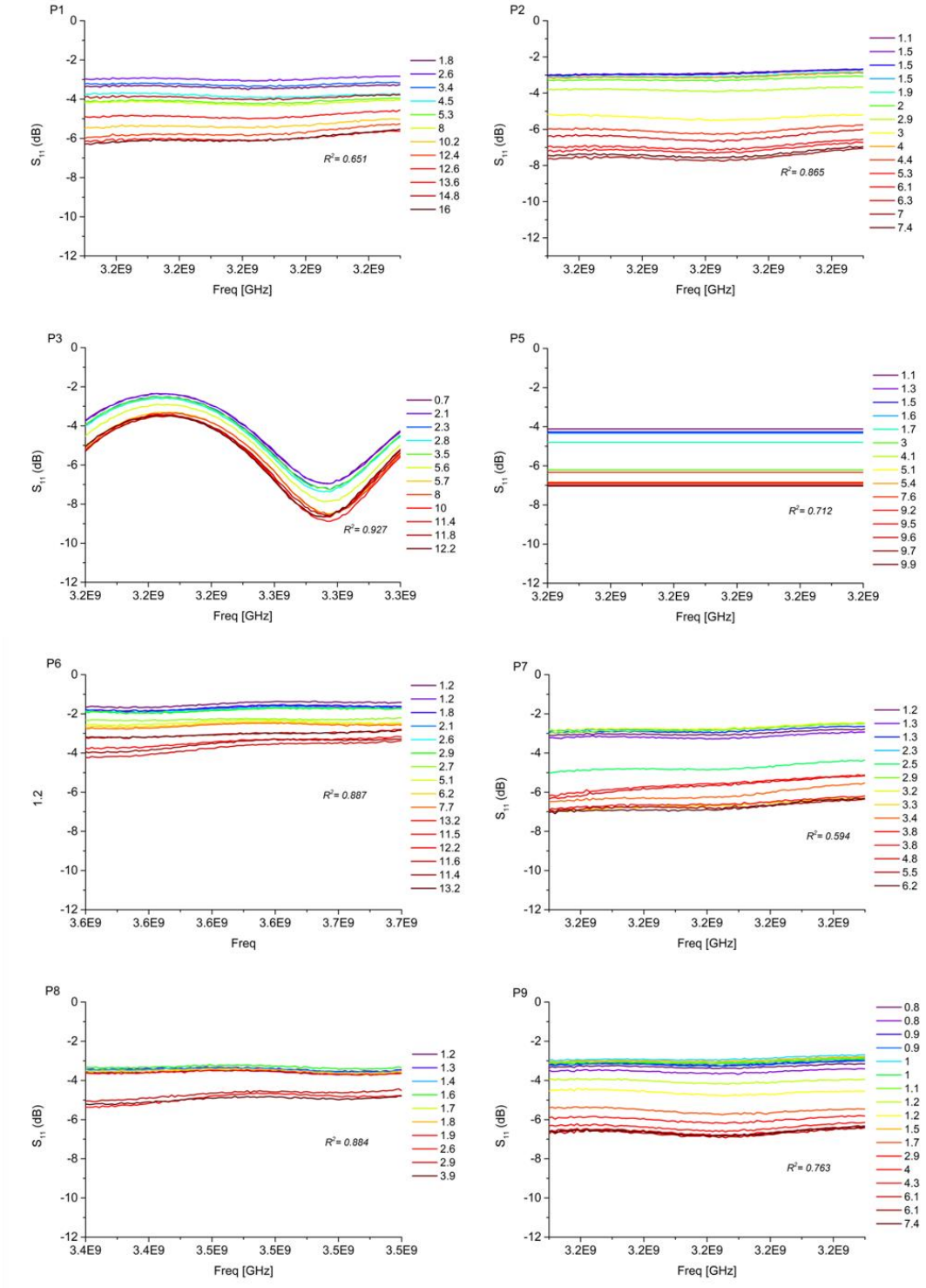

Figure 6.9 - Highest correlating S_{11} (dB) reflection coefficient signal distribution within a 10GHz range for each participant (P1, P2, P3, P5, P6, P7, P8, P9) corresponding to the individual blood lactate concentration shown within the legend (mmol/L).

However, observation of the data showed that the correlation was in the opposite direction of travel, showing as when blood lactate values increased S_{11} (dB) was reduced. As shown in Figure 6.10 , there is a positive correlation between blood lactate concentration (mmol/L) in participants P4 and P10. Further examination is needed to understand the mechanisms involved in this observed change as discussed within section 6.4.2.

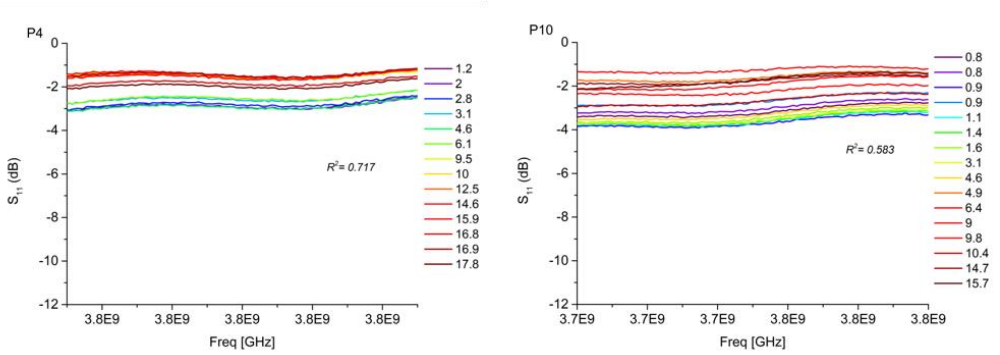

Figure 6.10 - Highest correlating S_{11} (dB) reflection coefficient signal distribution within a 10GHz range for each participant (P4, P10), corresponding to the individual blood lactate concentration shown within the legend (mmol/L).

6. 4 **Discussion**

The research within this chapter reports a novel technique to non-invasively detect blood lactate in humans continuously during exercise using a microwave sensor. This work demonstrates that electromagnetic wave sensors can determine blood lactate levels as correlated with the current best practice technique (Lactate Pro V2 electrochemical sensor) which requires the use of blood sampling via a finger prick. Non-invasive measurement of blood metabolites, such as blood lactate, both continuously and in real-time in a medical setting will reduce the risk of infection, increase the frequency of measurement, and ensure timely intervention only when necessary. Within a sporting

domain, it will be a great advantage to athletes and coaches, enhancing training protocols based on the data output, and enable more effective training regimes, overall improving physical performance and reducing the risk of injury.

6. 4. 1 **Blood lactate study 1: Untrained participants**

The first study in this chapter assessed a total of 34 healthy untrained participants who completed a bout of progressive incremental exercise designed to promote a traceable blood lactate profile. During the study, 345 blood lactate measurements were measured and used for a direct comparison with the microwave sensor. To assess the correlation between the microwave wave sensor and the large number of blood lactate samples collected for this individual study, it was decided to apply the approach of PMI, combined with NN. PMI is a useful method for establishing the relationship between datasets and their supposed target data, and producing rankings which indicate the prominence of relationships, rather than a typical linear model. In this study, PMI was used to consider the relationship between the lactate value measured with the Lactate Pro V2, and the corresponding spectral data captured using the microwave wave sensor. By doing this, it was possible to rank the spectral data by frequency in order of its relevance, and therefore reduce the spectral dataset being provided to the NN. This has significance for two reasons since: 1) it reduces the amount of irrelevant information being provided to the NN, thereby improving the likelihood of a suitable model being generated and 2) it assists in the commercial objectives of the work since limiting the frequency of operation reduces cost, size, and power requirements, all of which are barriers to implementing a wearable system.

Using a PMI method to reduce the necessary dataset required from the sensor, and an NN machine learning algorithm, to create a predictive model, it was demonstrated that there was a reliable correlation (R = 0.78) obtained when the sensor was operating in the S_{11} configuration and placed upon the arm operating at a frequency range of 3.4-3.6 GHz. Furthermore, it was shown that this model has relevance not only measuring absolute lactate values, but also shows tracking capabilities, this is very promising as it would allow for prescribing patient interventions, as well as providing strategic and tactical information to athletes during exercise both during competition.

In the case of the S_{11} arm combination, using the top 100 ranked frequencies, the reported training model error is 1.11 (7.4%), and the test error 2.88 (19.2%). Therefore, in practical use, the error is likely to lie somewhere between these two values. Measures which could be employed to favour lower error can include a software-based threshold, so that values suspected to be within the extreme regions are excluded, or additional data acquisition to target enhancement of data collected within these extremity regions. Ideally these improvements would be coupled with the earlier suggestions regarding improved mechanical fit of the sensor to the skin. It is noted that the error in this case is reported to be higher, which is thought to be a result of the sensor (and particularly the cables) moving during the exercise, which increases noise apparent in the acquired data. A better mechanical fit of the sensor to the skin might resolve such issues, as might the future integration of the electronics into an all-in-one wearable device which would completely remove the need for cables.

A major benefit of real-time on-patient monitoring is the potential to be able to monitor live patient information. It was demonstrated that this model has relevance not only in

predicting absolute lactate values, but also tracking their direction for the purposes of, for example, prescribing patient interventions. Current blood sampling does not give enough resolution to understand whether a patient's lactate level is rising or falling, and therefore deciding on an intervention strategy can be challenging. Thus, being able to track the direction of lactate change is perhaps as important as knowing its absolute value. Furthermore, it was shown that participant temperature and heart rate did not have a significant influence on the results. This work therefore shows the potential for microwave sensors as PoC systems. Future work in this area will focus primarily on reducing the size, cost, and complexity of the system to ensure that the devices can be worn on patients without the need for cables and expensive laboratory equipment.

6. 4. 2 **Blood lactate study 2: Endurance trained participants**

In the untrained study, it was demonstrated that a good correlation (R = 0.78) could be obtained when the sensor was configured in the S_{11} measurement mode and located on the arm of the untrained test subjects. For the current study, the leg sensor measurement was eliminated due to previous findings, this enabled more consideration to be given to the set-up of the single microwave sensor set-up.

Identifying the individual blood lactate response was conducted to improve further understanding of how the microwave sensor interacted with the induvial and help improve any future predictive modelling. Overall, it was shown there was a poor correlation between blood lactate and all the blood samples collected at any one frequency shown in Figure 6.8. However, when peak analysis was undertaken on an individual basis it was reported that between a frequency range of 3.2GHz to 3.8GHz, blood lactate showed a positive significant correlation between S_{11} (dB) and blood lactate

values in every participant with an average R^2 = 0.758. This result is promising as it shows that lactate can be detected using a microwave sensor, however further investigation is needed to understand the cause for the direction of the frequency observed in Figure 6.10.

The microwave sensor was calibrated to the manufacture's specifications prior to each trial and the room temperature remained consistent throughout for each participant. Although microwave signals can be linked to the composition of the MUT within specific frequency ranges, due to the sensitive nature of microwave spectroscopy, environmental factors can cause subtle shifts in the microwave signal measurement. There are a number of likely possibilities for this, as discussed within earlier chapters, the permittivity of a material is derived from several characteristics (e.g., temperature, chemical structure, molecular composition, etc.) (Blakey and Morales-Partera, 2016). In this instance, the discrepancies in skin and body temperature between participants, the skin surface condition, skin and fat depths variation, and possibly other unknown environmental and noise conditions that could cause unforeseen frequency shifts. These factors need to be explored and investigated individually. These results demonstrate that blood lactate was correlated within a frequency range rather than one specific frequency across all participants, indicating a need for personal calibration prior to exercise.

The strong correlations observed in this study which also matched the frequency ranges observed in untrained healthy adults leads us to suggest that a microwave sensor can successfully monitor blood lactate non-invasively during exercise at a frequency range between (3.2 – 3.8 GHz) dependent on individual responses. This study has shown evidence of a microwave sensor successfully predicting blood lactate in human subjects

using a non-invasive technique positioned on the forearm during progressive incremental cycling. Current blood sampling does not give enough resolution to understand whether a subject's lactate level is rising or falling, and therefore deciding on an intervention strategy can be challenging. Thus, being able to track the direction of lactate change is perhaps as important as knowing its absolute value. Previous research, by regarding this technology, has demonstrated the ability to detect a variety of different substances such as, lactate in water and types of oils and to successfully detect lactate in cerebrospinal fluid (Blakey et al., 2012; Korostynska et al., 2013; Mason et al., 2013b). This shows application of this technology could provide masses of benefits to a variety of different practitioners within the world of sports and performance who want to be able to quantify previously difficult performance markers both in the laboratory and out on the field.

The use of a non-invasive sensor will allow for durability, portability, and real time analysis of blood lactate levels. Further research will aim to measure the sensor's capability in detecting parameters such as muscle glycogen and hydration levels non-invasively, which will have applications both in the lab and out in the field by improving the accuracy of research protocols and tracking the physiological adaptations to exercise. Although this technique is a promising concept, further research and development is needed before the sensors will influence the world of sports and exercise science. This research aims to work towards developing sensors which are more flexible in structure to manipulate to the surface of the skin and the anatomy they are placed upon and measuring lactate values about the 16mmol range using endurance trained participants due to their physiological adaptations and increased lactate threshold.

As mentioned within the methodology, data was excluded where the S_{11} measurement is indicative of lost connection with the skin. This occurs when the sensor has moved enough to create a small but notable gap away from the participant and this caused the S_{11} signal to significantly drop and peak. This was common during this study due to the use of the medical tape used throughout the study which was not appropriate for high intensity bouts of exercise and appeared to loosen as the test progressed and this is again shown within the sample range measured, it was usually the later samples which needed to be excluded, this was a stage when the participants' sweating rates were highest and the participant was reaching a state of physical exhaustion which commonly results in more erratic movement on the stationary exercise bike.

6. 5 **Summary**

These findings within this chapter show that a microwave sensor is well suited to detecting blood lactate non-invasively and during progressive incremental exercise on a cycle ergometer. The results indicate that an acceptable correlation can be observed between all lactate samples (R=0.78) when using a predictive model. Additionally, when assessing endurance trained cyclists, blood lactate can be monitored to a high level of accuracy (average $R^2 = 0.758$) when monitoring blood lactate on an individual basis at a frequency range between 3.2 – 3.8 GHz. This research shows promise and has potential for microwave sensors to enable non-invasive continuous monitoring of blood lactate during exercise.

This research has investigated two separate population groups and identified a suitable frequency range to monitor blood lactate during exercise within a laboratory

environment. Future research should be conducted within a real-world environment, such as during a cycling endurance event to assess factors such as increased vibrations and other environmental exposure. However, to get to that stage, additional development of the microwave sensor will be needed to provide an all-in-one wearable device. The results in this chapter have shown that blood lactate can be detected in healthy adults at a frequency range between 3.2 – 3.8 GHz in the S_{11} measurement mode. This information can now enable engineers to reduce the number of cables (and the associated electronics) which can reduce the overall size and increase the robustness which is required for a wearable solution.

CHAPTER 7 - PARAMETER 3: NON-INVASIVE MEASURMENT OF SKELETAL MUSCLE GLYCOGEN

This chapter will detail and report finding from two studies. The initial study was used to assess a microwave sensors capability to detected glycogen under in-vivo conditions. To determine if a microwave sensor has practical application to monitor glycogen concentration during exercise, the second study examines the sensing capability during human trials. The methodology, analysis techniques and findings will be detailed for each study in the corresponding chapters.

7. 1 **Introduction**

It is widely acknowledged that skeletal muscle glycogen is a primary fuel and source for ATP production for sustaining contractile activity in human skeletal muscle (Bergstrom et al., 1967). There is a clear positive association between glycogen concentration and exercise capacity both for endurance and high-intensity intermittent exercise (Bergstrom et al., 1967; Hermansen, Hultman and Saltin, 1967b; Balsom et al., 1999b). Glycogen synthesis is mainly dependent upon daily CHO consumption, training status, and enzymatic profile (Burke, van Loon and Hawley, 2017). Therefore, from an athletic perspective, ensuring athletes have sufficient CHO availability to meet the demands and energy expenditure of their activity is fundamental (Morton et al., 2009b; Bartlett, Hawley and Morton, 2015a). The benefits of high CHO availability on prolonged

exercise performance have been reported in a large body of literature (van Loon et al., 2000; Bussau et al., 2002b). Recently, there has been a growing body of evidence indicating alternative approaches in CHO manipulation (Yeo et al., 2008b; Morton et al., 2009b; Hulston et al., 2010b), showing the potential benefits of low CHO availability. Moreover, the latest recommendations of "fuelling for the work required"(Impey et al., 2018) suggest that CHO availability should vary depending on the demands and targets of selected training sessions and competition, in a bid to gain the most benefits whilst avoiding any adverse consequences of sustained low glycogen stores (Jeukendrup, 2017; Impey et al., 2018).

The importance of monitoring glycogen regularly, therefore, is critical to allow athletes and practitioners to implement these different CHO strategies effectively and efficiently (Hearris et al., 2019). Doing so can ensure athletes take on sufficient CHO to meet the requirements of their sport (Bartlett, Hawley and Morton, 2015a; Hearris et al., 2018). However, there is no current technique to non-invasively monitor and track muscle glycogen during exercise. Currently, the gold standard method to measure skeletal muscle glycogen requires a muscle biopsy. This procedure is common practice in biological science for research purposes, however, due to its invasive nature, is not performed regularly on athletes to assess glycogen stores. The delay in time and resources is a hindering factor associated with the biopsy method (Shanely et al., 2014a; Bradley et al., 2016c; Greene et al., 2017). Although biopsies are a relatively safe procedure, using them as a tool to assess muscle glycogen stores for individual athletic performance monitoring is not recommended due to the practicality and aftercare required. This study is the first human trial assessing whether a microwave sensor can

detect monitor skeletal muscle glycogen concentration during exercise in endurance-trained athletes.

7. 2 **Methodology**

To determine if a microwave sensor can measure glycogen within human skeletal muscle, this chapter details two measurement approaches. The first section of methodology details in-vitro laboratory analysis of a pre-made glycogen solution. The second section describes the collection of human muscle samples before and after a bout of exercise using a muscle biopsy to measure muscle glycogen.

7. 2. 1 **Glycogen study 1: In-vitro measurement of glycogen**

This study was used as a preliminary trial before advancing human trials, ensuring that the electromagnetic sensor is sensitive to changes in glycogen under in-vitro conditions. The study was conducted within a controlled laboratory environment within LJMU research facilities, ensuring true glycogen measurements. To determine repeatability/error, seven different concentrations were used, each measured five times to measure any variation within the measurement.

7. 2. 1. 1 **Glycogen from oyster**

Glycogen is a branched polymer of glucose synthesized by animal cells for energy storage and release. The glycogen used in this experiment was glycogen (Sigma, G8751) from oyster (type II). The glycogen was dissolved in water by stirring continuously for 2 hours. Glycogen concentration was as follows: 400mmol/L, 200mmol/L, 100mmol/L, 50mmol/L, 25mmol/L, 12.5mmol/L, and 0mmol/L. The glycogen concentration range

was carefully selected to mimic glycogen quantities in average healthy humans under varying nutritional conditions.

7. 2. 1. 2 **Experimental set-up**

The 2-port EM sensor used frequencies sweeps between 10MHz and 4GHz. For data acquisition, the sensor was connected to a Rohde and Schwarz ZVA24 VNA via two coaxial cables detailed in Chapter 4. The VNA was calibrated to manufacturer specifications and the measurement conditions are show in Table 7.1. A custom-made plastic reservoir was engineered to give stability and even surface, ensuring no movement once testing had begun. A plastic slot is placed directly over the sensor, this allows for the 200µl of glycogen solution to be inserted consistently during each new sample. The slot was cleaned with water and dried prior to each new sample.

Table 7.1 – In-vitro measurement specifications / storage conditions

No. of Measurements	7 Samples x 5 Repetitions = 35
Microwave Sensor	2 Ports (S_{11}-S_{21})
Volume of Samples	200ml
Temperature	20 °C
Frequency Sweep	10MHz – 4GHz
Channel Base Power	0 dBM

7. 2. 1. 3 **Statistical Analysis**

Enterprise IBM SPSS 22 Statistical analysing package was used to determine the significance of the data. Pearson's correlation was used to report the S-parameters of glycogen and correlation was significant at the 0.01 level. Origin Pro9 was used for the visual interpretation of the data.

7. 2. 2 **Glycogen study 2: In-vivo measurement of skeletal muscle glycogen during exercise**

To determine if skeletal muscle glycogen could be detected non-invasively by a microwave sensor during exercise. A study was designed to enable varying glycogen availability in endurance trained participants via diet manipulation and a controlled exercise protocol. The study was conducted within a controlled laboratory environment within LJMU research facilities.

7. 2. 2. 1 **Participants**

14 skeletal muscle biopsies from the vastus lateralis were obtained from 7 experienced male cyclists (mean ± SD: age, 18 ± 6 years; body mass, 65 ± 15 kg; height, 175 ± 7cm; peak oxygen uptake, VO_{2peak}, 60.5 ± 3.6 $ml.kg^{-1}.min^{-1}$; peak power output, PPO, 380.0 ± 50.2 W). None of the participants had a history of neurological disease or musculoskeletal abnormality and none was under pharmaceutical treatment during the study. Participants were asked to refrain from any form of physical activity for 24h prior to each testing session and to follow standard dietary guidelines to insure they all started the protocol well rested and not glycogen depleted. All participants provided written informed consent and all procedures conformed to the standards of the Declaration of Helsinki (2008). The study was approved by the University Research Ethics Committee (UREC, Liverpool John Moores University).

7. 2. 2. 2 **Experimental design**

After being initially assessed for VO_{2peak} and PPO, participants reported to the laboratory at ~17.30 h, then performed a glycogen-depleting bout of intermittent exhaustive cycling. Upon completion, a recovery diet consisting of varied CHO amounts, was randomly

provided for the following 12 h. Participants returned to the laboratory after the subsequent dietary intervention and an overnight fast to complete a bout of high-intensity intermittent exercise (~50 min; 8 x 5 min intervals at 80 % PPO, interspersed with 1 min active recovery). Muscle biopsies were taken pre- and immediately post-high-intensity training session, and the microwave sensor was positioned onto the participants' active muscle during the exercise. An overview of the experimental protocol is shown in Figure 7.1.

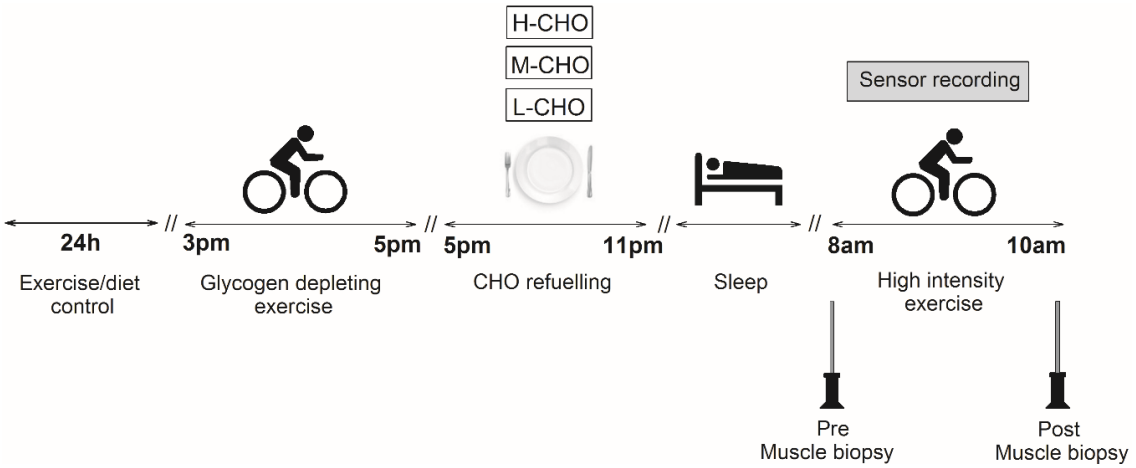

Figure 7.1 - Schematic overview of the experimental protocol. After 24 h of standardised conditions, participants completed an evening bout of glycogen-depleting exercise. Upon completion, subjects received 3 graded levels of carbohydrate [high (H-CHO), medium (M-CHO), low (L-CHO)]. After an overnight fast, subjects completed a high-intensity intermittent cycling exercise.

7. 2. 2. 3 **Assessment of peak oxygen uptake**

Prior to the main experimental trials, participants came for an initial visit to the laboratory where peak oxygen uptake (VO_{2peak}) and PPO was determined using an incremental cycle test to exhaustion on a cycle ergometer (Excalibur Sport, LODE, Netherlands). This visit also acted as a familiarisation session for cycling exercise at high

intensities. The test commenced with a 5 min self-paced warm-up, followed by 2 min cycling at 90 W, at a cadence corresponding to ~70 revs.min^{-1}. The intensity was increased by 30 W every 2 min until volitional exhaustion, for a total testing duration of up to 30min, depending on the level of participants. Breath-by-breath measurements were obtained throughout exercise using a CPX Ultima series online gas analysis system (Medgraphics, MN, USA). VO_{2peak} was stated as being achieved by the following end-point criteria: (1) heart rate within 10 beats.min^{-1} of age-predicted maximum, (2) respiratory exchange ratio >1.1, and (3) plateau of oxygen consumption despite increased workload. PPO was defined as the highest power output that was able to be maintained for 2 mins.

7. 2. 2. 4 **Glycogen-depleting protocol**

To deplete muscle glycogen, a classical intermittent exercise protocol was used (Impey et al., 2016). Participants arrived at the laboratory in the evening (17.30 h) of the initial experimental trial. Height and body mass were recorded and a heart rate monitor (Polar FT1, Kempele, Finland) was fitted. Participants performed an intermittent bout of cycling until volitional exhaustion on the same cycle ergometer previously described. Following a self-determined warm-up, participants cycled for 2 min at 90 % PPO, proceeding with an immediate 2 min recovery period at 50 % PPO. This work to rest cycle was repeated until participants could no longer complete 2 min cycling at 90 % PPO, determined as an inability to maintain a cadence of 60 revs.min^{-1}. At this stage, the duration of cycling performed at 90 % PPO was shortened to 1 min, while the 2 min recovery periods continued. Upon failure of 1 min of cycling, work time was raised back to 2 min, however, exercise intensity was reduced to 80 % PPO. This cycle continued

with 10 % reductions in PPO (recovery maintaining at 50 % PPO for 2 mins throughout) until the inability to perform 60 % PPO for 1 min, thereby resulting in termination of the exercise trial.

7. 2. 2. 5 **Dietary intervention**

Post glycogen depletion trial, participants were randomly selected to consume three varying non-isocaloric CHO diets before the high-intensity exercise trial performed the next morning. The CHO diets consisted of a mixture of food, fluids, and sports supplements. To maximise glycogen availability, the participants were given a high CHO diet (H-CHO, 1 participant) containing 10 $g.kg^{-1}$ of body mass, BM of CHO, a medium CHO diet (M-CHO, 4 participants) containing 5 $g.kg^{-1}$ BM to partially restore glycogen availability and a low CHO diet (L-CHO, 2 participants) containing 2 $g.kg^{-1}$ BM diet to minimise glycogen availability. Additionally, in all three diets, participants consumed 2 $g.kg^{-1}$ BM of protein and 1 $g.kg^{-1}$ BM of fat.

7. 2. 2. 6 **Experimental trial**

Following an overnight fast to ensure glycogen storage is consistent and not impacted by carbohydrate consumption prior to the exercise trail in the morning, participants returned to the laboratory at ~ 09.00 h to commence a bout of high-intensity intermittent exercise. Prior to the trial, participants undertook a skeletal muscle biopsy. Muscle biopsies (~ 30 mg wet weight) were obtained from the vastus lateralis under local anaesthesia (0.5% Marcaine) using a Bard Monopty Disposable Core Biopsy Instrument (12-gauge x 10 cm length, Bard Biopsy Systems, Tempe, AZ, USA). All biopsies were taken by qualified personnel, immediately frozen in liquid nitrogen and stored at (-80℃) for later analysis of muscle glycogen content.

Following the pre-exercise biopsy, the microwave sensor could be applied. Prior to placement, the sensor and area under test were sterilised with alcohol wipes and then allowed to dry. Immediately following the pre-exercise biopsy, the microwave sensor was attached to the participants as close to the biopsy incision site as possible directly above the vastus lateralis muscle (Figure 7.2A). Participants were set up on the cycle ergometer (Figure 7.2B) and instructed to limit movement until the trial began. The sensor was fixed to the participant using kinesiology tape, providing a flexible and stable attachment to the participant throughout the exercise protocol. SMA cables were also taped to the participant, relayed to the ZVL13 VNA on a nearby table.

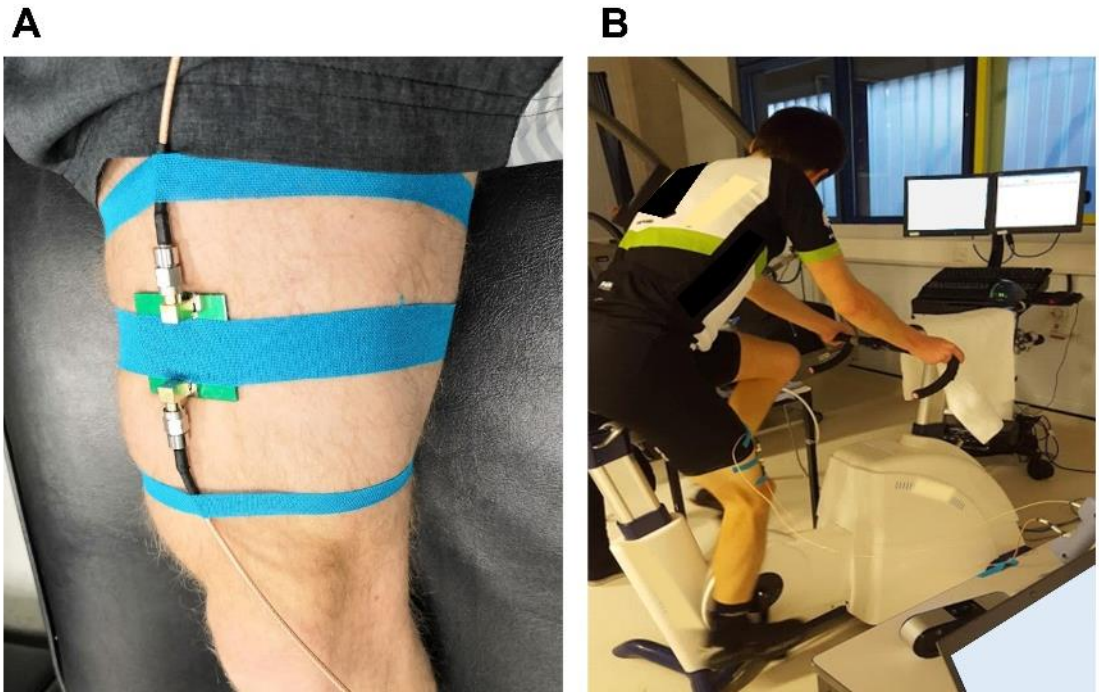

Figure 7.2 - A. Microwave sensor attached to the participant on vastus lateralis using kinesiology tape during exercise trial. B. Experimental set-up, showing participant on the ergometer with the microwave sensor attached during exercise trial.

Once set up, the sensor began recording data and participants performed the previously self-determined warm-up. Participants performed 8 x 5 min bouts of high intensity

cycling at 80 % PPO, interspersed with 1 min active recovery. Heart rate and rate of perceived exertion (Borg, 1970) were measured upon completion of each 5 min stage. Following the final stage, participants dismounted the cycle ergometer and immediately had a post-exercise muscle biopsy to complete the exercise trial.

Muscle glycogen concentrations were determined according to the acid hydrolysis method described by Van Loon et al. (van Loon et al., 2000). In brief, approximatively 2-5mg of freeze-dried tissue was powdered, separated from all visible blood and connective tissue, and subsequently hydrolysed by incubation in 500 μl of 1 M HCl for 3 h at 95ºC. After cooling to room temperature, samples were neutralised by the addition of 250 μl of 0.12 mol. L^{-1} Tris/2.1 mol. L^{-1} KOH saturated with KCl. Following centrifugation, 200 μl of supernatant was analysed in duplicate for glucose concentration according to the hexokinase method using a commercially available kit (GLUC-HK, Randox Laboratories, Antrim, UK). Glycogen concentration is expressed as $mmol.kg^{-1}$ dry weight and intra-assay coefficients of variation were < 5%.

7. 2. 2. 7 **Data management**

For this experiment, S_{11} measurement was monitored due to this being the strongest correlating and more stable scattering parameter in previously reported studies (Mason et al., 2017; Greene et al., 2019). S_{11} data was recorded continuously a every minute. The average reading for the opening and closing three minutes of each trial was used for the pre- and post-trial glycogen results.

Determining tracking capabilities of the sensor, required the use of timestamps to determine the mid-point of the trial and the average of three minutes was used. Initially, the sensor measurements for S_{11} (reflection co-efficient) were carried out on the full range

of the chosen spectra (1 – 4 GHz). This was to identify and highlight all the resonant peaks and to determine any correlation with glycogen that required additional experimental analysis. The frequency range was then narrowed down to a given range depending on the response of the material to the S_{11} signal. Within this given frequency range, only the lowest S_{11} sensing peak (minimum value) was selected for analysis, a common technique amongst microwave sensing analysis (Choi et al., 2014; Choi et al., 2015; Mason et al., 2017). For the unique purpose of this study, the 14-glycogen samples (7 pre- and 7 post-exercise) were then categorised into four distinct ranges (very low, low, medium, and high) based on the glycogen concentrations obtained using the muscle biopsy technique. All electromagnetic data were collected by the same experienced researcher to ensure consistency in skin preparation and sensor placement.

7. 2. 2. 8 **Statistical analysis**

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, Version 25, IBM, New-York, USA). A paired sample t-Test or its non-parametric equivalent (Wilcoxon test) was used to determine the reduction in glycogen values from pre- and post-trial muscle samples. Origin Pro - Data Analysis and Graphing Software (OriginLab, Northampton, USA) was used to perform a 5-point adjacent-averaging and was used for peak analysis on all S_{11} scattering parameter data sets.

A bivariate (Pearson) analysis or its non-parametric equivalent (Spearman) was used to report the correlation between the glycogen values and the S_{11} S-parameters. All data in text and figures are presented as means ± standard deviations (SD), unless otherwise indicated, with P values ≤ 0.05 indicating statistical significance.

7. 3 **Results**

7. 3. 1 **Microwave measurement of in-vitro glycogen samples**

When observing the S_{11} data obtained from the sensor at 10MHz to 4GHz, there were two distinct frequency shifts across this spectrum, namely at 1.6GHz and 2.1GHz as shown in Figure 7.3.

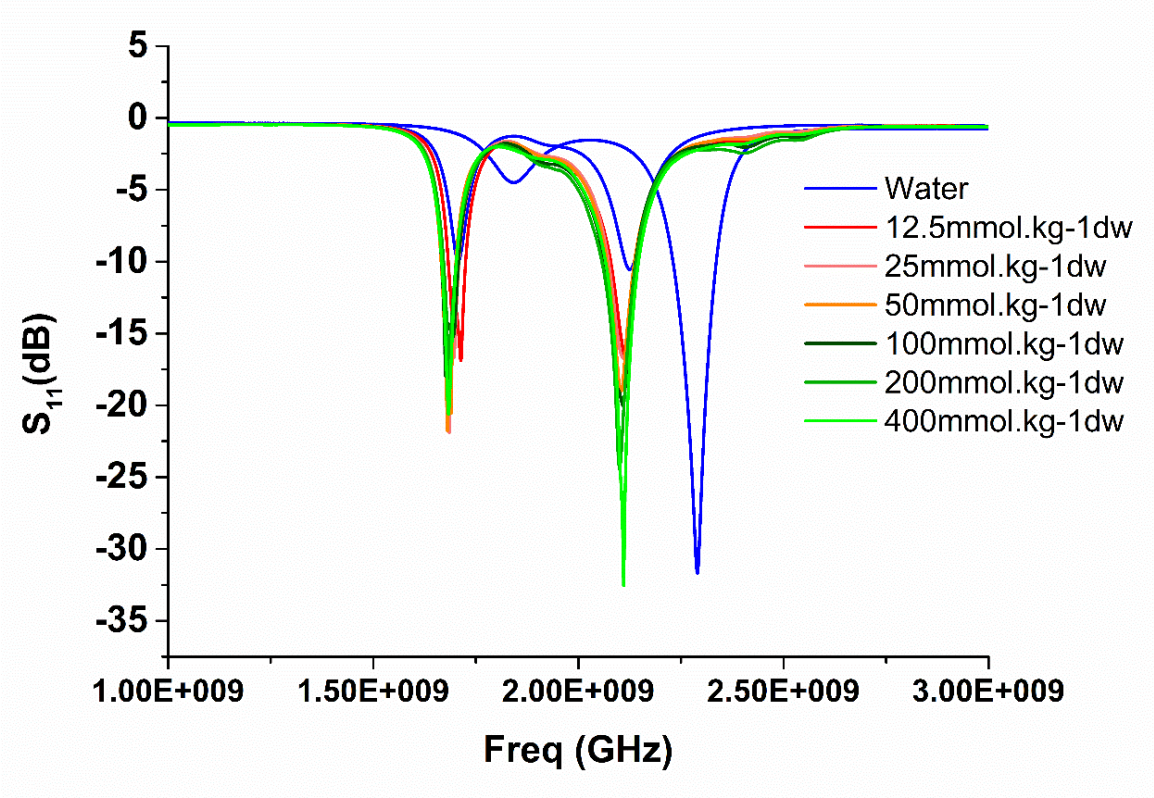

Figure 7.3 – S_{11} signal distribution of microwave sensor between 10MHz - 4GHz frequency ranges under varying concentrations of glycogen in water (mmol/L)

Further analysis identified that there was a strong linear correlation ($R^2 = 0.87$, $p \leq 0.002$) detected between S_{11} (dB) and glycogen (mmol/L) at 2.11 GHz, shown in Figure 7.4B. This correlation can be observed in Figure 7.4A. There is a clear frequency shift between the ranging concentrations in the order of weakest through to the highest concentration in the correct ascending order.

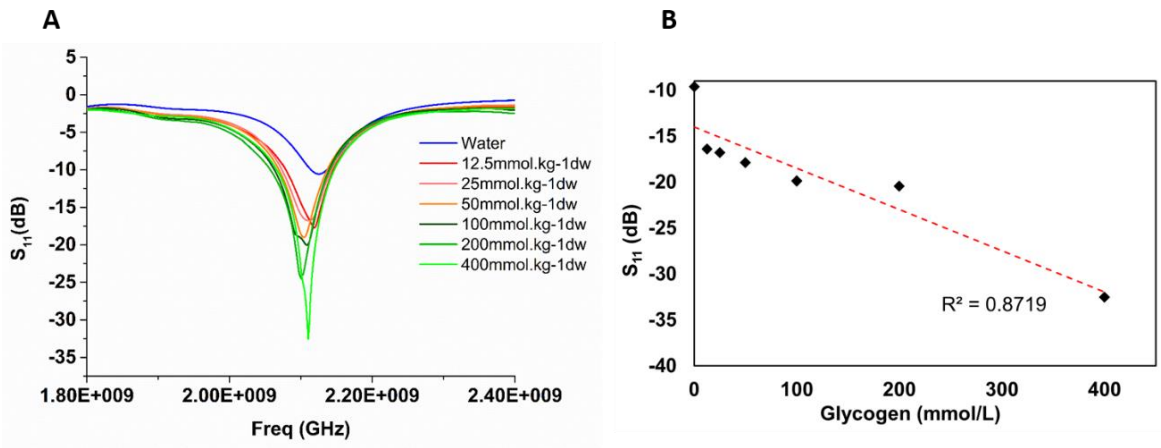

Figure 7.4 - A) S_{11} signal distribution of microwave sensor between 1.8 -2.4 GHz frequency ranges under different concentrations of glycogen in water (mmol/L). B) The linear relationship between S_{11} variations (mean ± SD) and the response to the 7 varying glycogen concentrations at a 2.11 GHz

When observing the S_{21} data obtained from the sensor across 10MHz to 4GHz, there were two resonant peaks as seen in Figure 7.5. However, further analysis showed correlation with glycogen concentration was poor ($R^2 < 0.5$).

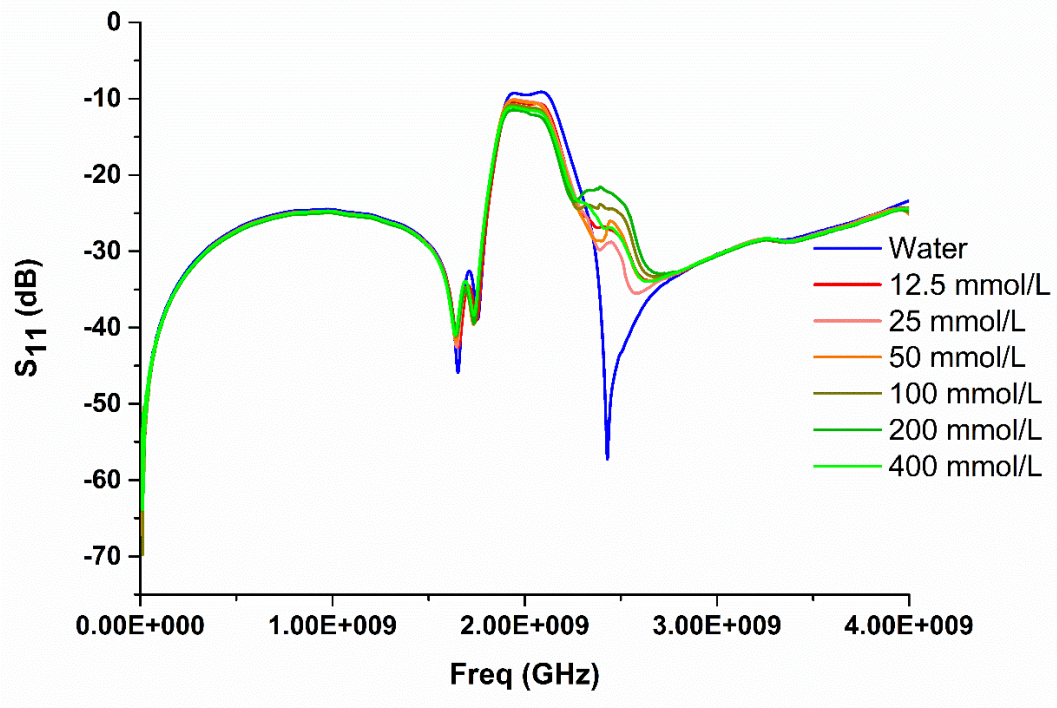

Figure 7.5 - S_{21} signal distribution of microwave sensor between 10MHz - 4GHz frequency ranges under varying concentrations of glycogen in water (mmol/L)

7. 3. 2 **Measurement of skeletal muscle glycogen in humans during exercise**

7. 3. 2. 1 **Skeletal muscle glycogen concentration**

Anaysis of skeletal muscle glycogen, determined by the gold standard muscle biopsy technique indicated that each participant started the exercise trial with varied muscle glycogen concentrations (from 124 to 413 mmol.kg-1 dw) inline with the different CHO diets given (L-CHO, M-CHO and H-CHO). Figure 7.6 shows the muscle biopsy procedure and glycogen concentration determined using the acid hydrolysis method.

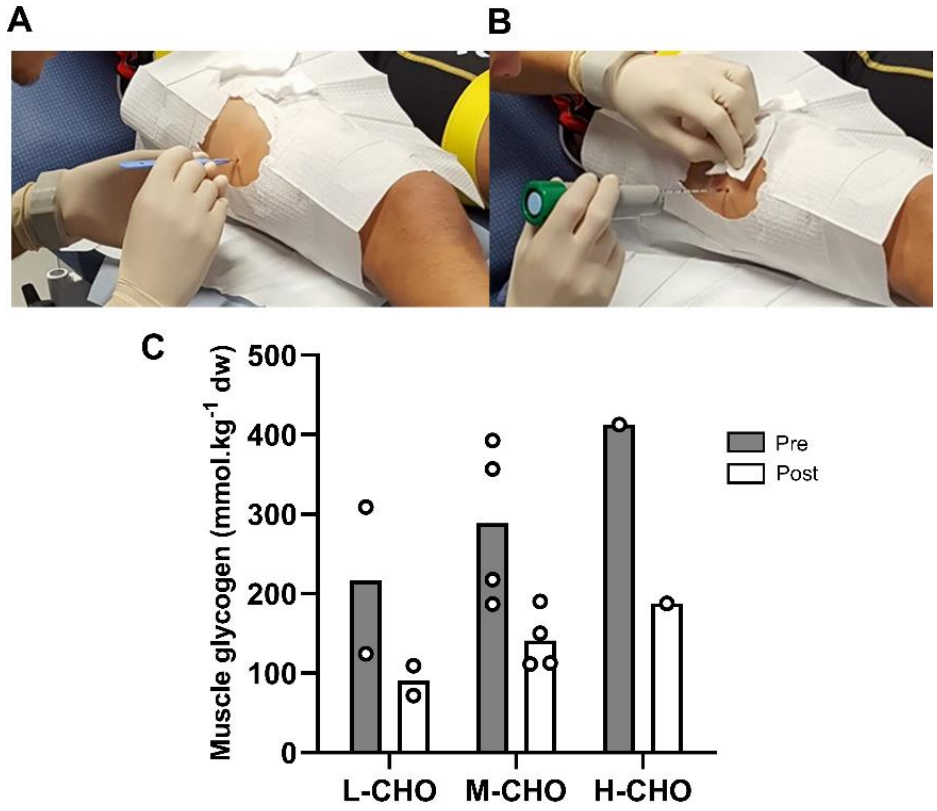

Figure 7.6 –A) Photograph of the scalpel incision of athlete's vastus lateralis in preparation for biopsy procedure. B) Removal of muscle sample using the disposable core biopsy instrument. C) Skeletal muscle glycogen concentration determined Pre- and Post- exercise for each CHO condition. Bars represent mean glycogen concentrations, while the white points represent individual values.

There was a significant reduction in muscle glycogen concentration from pre- to post-trial samples in all participants by 41 ± 30%, P = 0.01, during the completion of the high-intensity exercise session irrespective of the CHO diet consumed.

7. 3. 2. 2 **Microwave measurement of skeletal muscle glycogen**

Full S_{11} spectra observations of all 14 S_{11} scattering parameters can be observed in Figure 7.7. Each spectra represents the electrical signal corresponding to each glycogen value measured for pre- and post- exercise muscle samples for each participant. Peak observations show a distinct drop of S_{11} amplitude in the frequency range of 2.0 – 2.25 GHz as indicated within Figure 7.7.

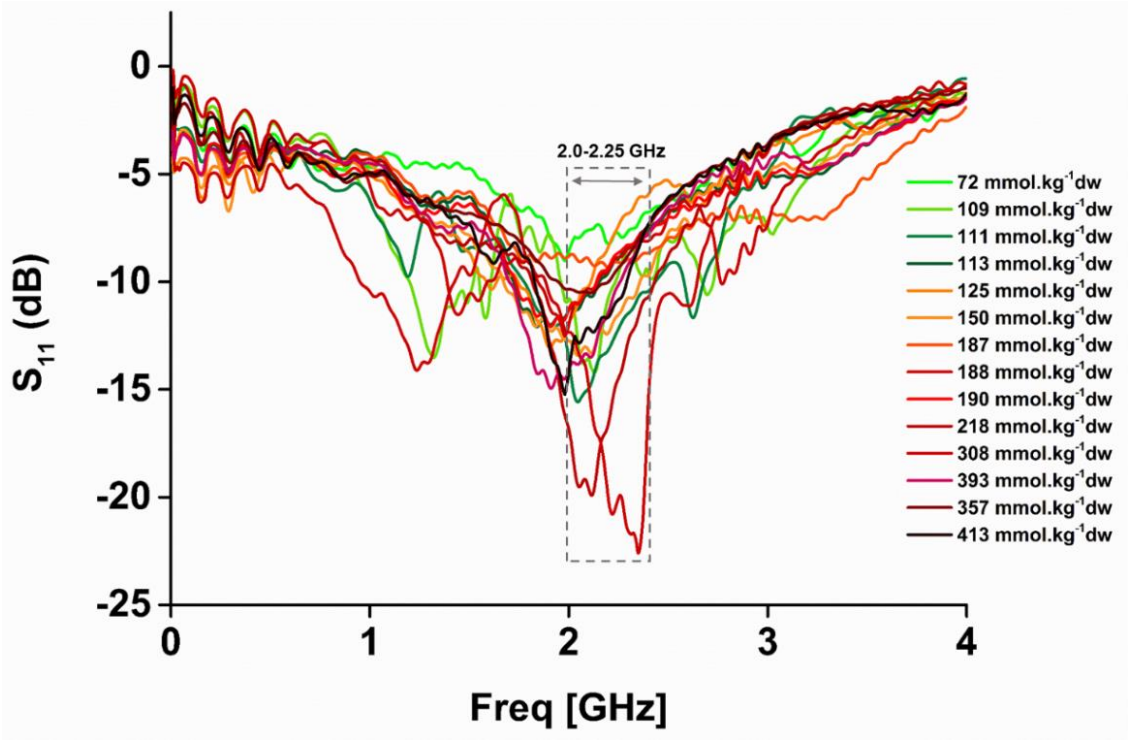

Figure 7.7 – S_{11} (dB) reflection coefficient signal distribution between 10MHz and 4GHz frequency range using a microwave sensor monitoring all 14 pre- and post-exercise glycogen concentrations (mmol.kg^{-1} dw).

Further analysis identified a poor linear relationship between the measured glycogen concentration (mmol.kg^{-1} dw) and S_{11} (dB),($R^2 = 0.13$, $P = 0.21$), observed between 2.0 and 2.25 GHz for the 14 glycogen sample data shwon in Figure 7.8A. The glycogen data was then grouped into four distinct ranges of muscle glycogen concentration (i.e. very low, low, medium and high) as defined in the literature (Impey et al., 2018; Hearris et al., 2019). These grouped ranges of glycogen along with the corrresponding microwave signal are show in Figure 7.8B. The red, orange, yellow and green points, represent the very low (72 mmol.kg^{-1} dw, -7.52 dB), low (121 mmol.kg^{-1} dw, -12.02 dB), medium (198 mmol.kg^{-1} dw, -14.22 dB) and high (350 mmol.kg^{-1} dw, -18.13 dB) mean glycogen concentrations showed a strong linear relationship between measured values and S_{11} ($R^2 = 0.91$, $P = 001$). There was a distinct frequency shift between the ranging concentrations of glycogen in the order of lowest through to the highest concentration in the correct descending order.

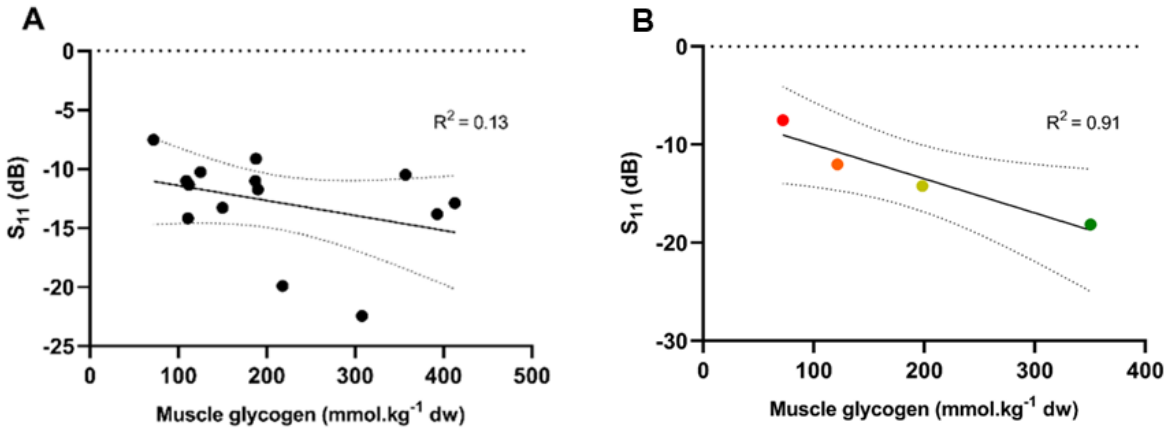

Figure 7.8 – A) The linear relationship between S_{11} (dB) and the 7 pre- and post-exercise glycogen concentrations measured with the biopsy technique. B) Relationship between S_{11} (dB) and the 7 pre- and post-exercise glycogen concentrations measured with the biopsy technique when grouped in four ranges. Dotted lines correspond to 95% CI around the mean (solid line).

7. 3. 2. 3 **Tracking individual S_{11} scattering parameter responses to predict glycogen concentration during exercise**

Additional observational analysis can indicate individual responses to the microwave sensor. The S_{11} reflection coefficients for all three time points (pre-, mid-, post-) for each participant are shown in Figure 7.9 and Figure 7.10. Peak amplitude was taken from a frequency range of 2.0-2.25 GHz in all conditions for each participant. Figure 7.9 shows that there was a good agreement between all three conditions with the Mid-trial S_{11} measurement placing between the pre- and post-trial values for participants 1 to 4 (P1 to P4). This agreement is based on glycogen concentration being reduced in all participants due to the demanding nature of the exercise protocol.

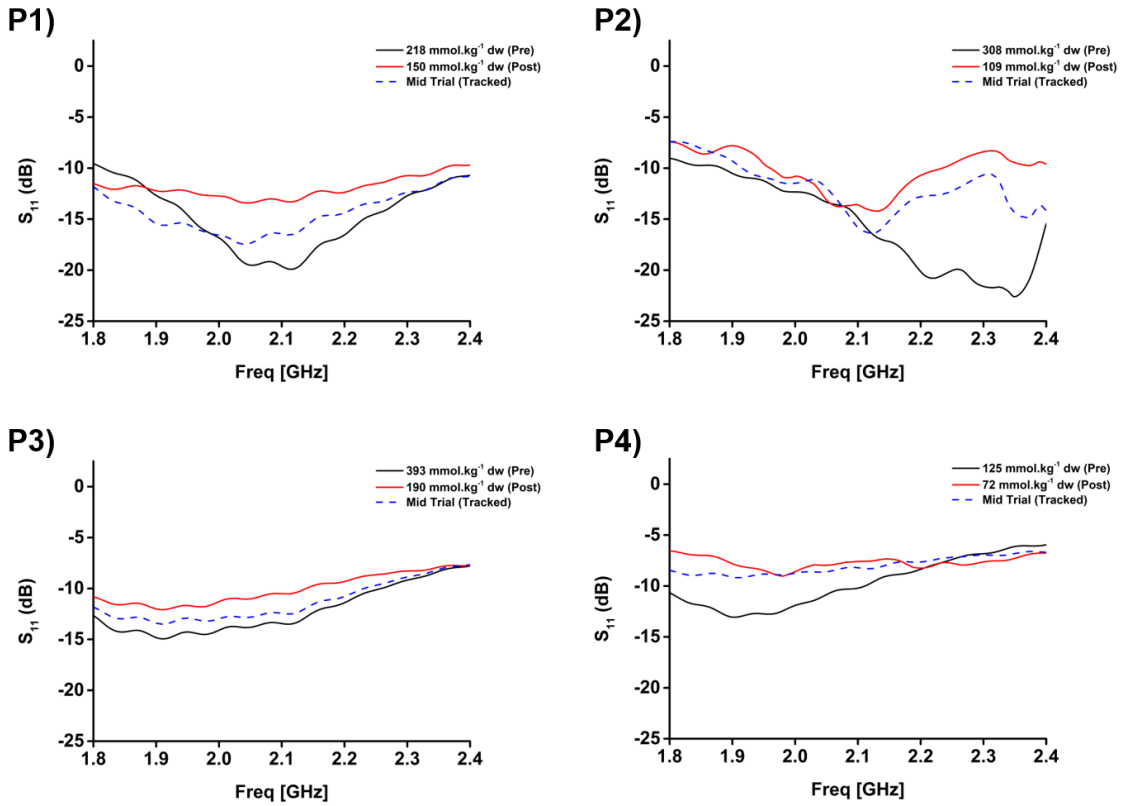

Figure 7.9 - S_{11} (dB) reflection coefficient signal distribution between 1.8 GHz and 2.4 GHz frequency range using the microwave sensor monitoring each individual participant (P1 to P4 only) pre-, mid- trial and post-exercise glycogen concentrations.

Observations of participants 5 to 7 (P5-P7) show post- and mid-trial S_{11} (dB) amplitudes to place in the correct order when matching S_{11} to glycogen concentration. However, as illustrated in Figure 7.10, pre-trial sensor measurements for P5-P7 generated low S_{11} amplitude in comparison to their glycogen values.

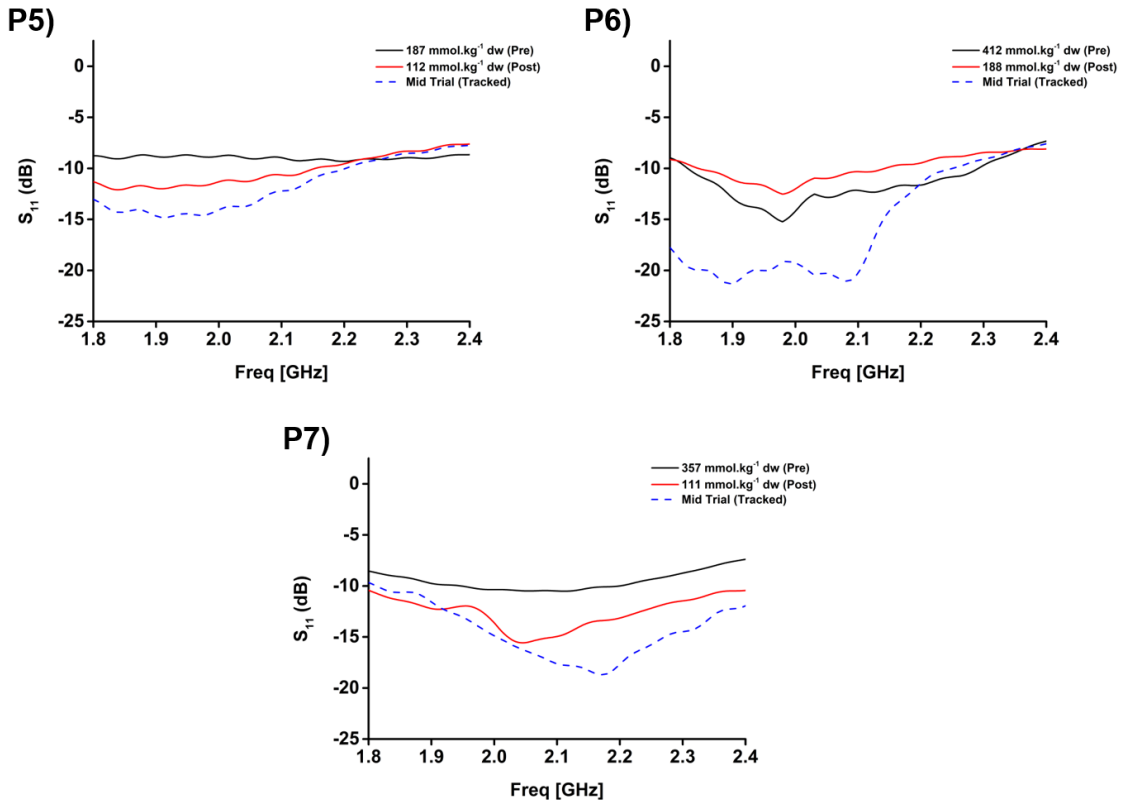

Figure 7.10 - S_{11} (dB) reflection coefficient signal distribution between 1.8 GHz and 2.4 GHz frequency range using a microwave sensor monitoring each individual participant (P5 to P7 only) pre, mid-trial and post-exercise glycogen concentrations.

Assessment of microwave sensor tracking was then completed using a predictive trend analysis ($y = mx + b$) to forecast the unknown glycogen values using the linear relationship observed between the pre- and post- trial glycogen values when grouped together in Figure 7.8B. As muscle biopsies were not taken during the exercise protocol, true glycogen concentrations at the mid stage of the exercise trial are unknown, current

forecasting data, therefore, can only provide an estimate and proof of concept for future work. As the exact glycogen concentrations are unknown, S_{11} spectra measurements for mid trial should therefore be expected to be positioned anywhere between pre- and post-exercise values at a frequency range of 2.0-2.25 GHz for each participant. Further analysis combined the 14 pre- and post- glycogen measurements and the seven mid-trial predicted glycogen concentrations which were calculated during linear regression analysis using trend forecasting to predict mid-trial muscle glycogen concentration. There was a small linear relationship between glycogen concentration (mmol.kg^{-1} dw) and S_{11} (dB), with an $R^2 = 0.337$, ($P = 0.04$) observed between 2.0 - 2.25 GHz using all the pre-, mid-, and post- trial measurements as illustrated in Figure 7.11.

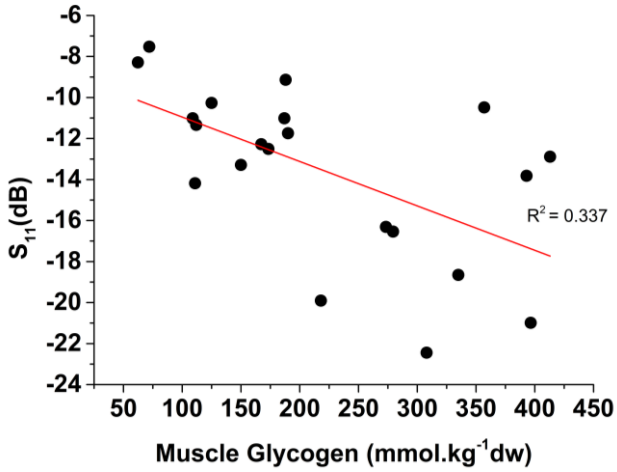

Figure 7.11 - The linear relationship between S_{11} (dB) and pre-, mid-, post-trial glycogen concentrations.

7. 4 **Discussion**

7. 4. 1 **In-vitro measurement of glycogen**

This study was conducted as a preliminary examination, analysing which frequencies in the microwave range best matched the electromagnetic footprint of glycogen in a water

solution before advancing to human trials. Identifying the optimum frequencies allows future development of a portable sensor device, eliminating the need for bulky apparatus such as a VNA by incorporating wearable wireless monitoring. A strong linear correlation ($R^2 = 0.87$, $p \leq 0.002$) was found in the S_{11} data collected at approx. 2.1 GHz. Since the in-vitro measurement of glycogen concentration was to determine whether the sensor could monitor and detect changes, the results are considered sufficient as the impetus for further study. The microwave sensor used in the research demonstrated that S_{11} data at 2.11GHz is sensitive to changing concentrations of glycogen in water, equivalent to the concentrations naturally observed in healthy humans.

In S_{21} data, variation exists at approx. 1.7 and 2.1-2.6 GHz, but correlation is poor ($R^2 < 0.5$). This outcome is not too disappointing however for two reasons: Firstly, the poor repeatability is likely attributable to the measurement method used in this experiment and secondly, 2-port measurements are viewed as less interesting than 1-port for practical reasons of cost and system complexity in the eventual effort to make the system wearable in some form. Similar results were observed in a study using the same technology detecting blood lactate in humans within Chapter 6, where S_{11} was the best performing measurement. However, although S_{21}, does not produce a strong correlation to glycogen within water, it is unknown if it will react the same to skeletal muscle so further examination is needed.

A major benefit of a non-invasive continuous device is the potential to be able to monitor live patient information. Current techniques, as noted earlier do not give enough resolution to understand whether a patient's glycogen levels are rising or falling, therefore, providing an intervention strategy can be challenging for coaches and

nutritionists. Thus, being able to track the direction of glycogen during exercise is perhaps as important as knowing its absolute value. This study has shown that microwave sensors, operating at microwave frequencies, are able to detect molecules such as glycogen. Thus, with further research, it has the potential of becoming a novel approach as an alternative non-invasive method to monitor changes in glycogen levels during exercise. Despite many recent technological advances, muscle biopsies remain the preferred method of choice to measure muscle glycogen despite their invasive method. This will remain so until an alternative device is available which reaches a high standard of accuracy, allowing for portable, real-time, and non-invasive assessments. Another technical challenge is the personal calibration of wearable devices. Every person is uniquely different, and various factors could affect the EM waves under in-vivo conditions.

In the pursuit of a non-invasive device to detect skeletal muscle glycogen in athletes, this study provides the first piece of evidence that an electromagnetic sensor can detect and track changes in glycogen under in-vitro conditions. This technique can be further optimised with additional support and development of online data collection and processing tools driven by software like LabVIEW to operate in conjunction with the microwave sensors. The data produced from this study allows us now to focus on a specific range for better optimisation of the equipment.

7. 4. 2 **In-vitro measurement of glycogen in humans during exercise**

This research provides novel data showing that using a non-invasive electromagnetic sensor is not yet a valid solution to monitor glycogen concentration in human skeletal muscle in-vivo in comparison to the gold-standard biopsy and biochemical assay. The

analysis presented in this study has additionally shown real-time tracking capabilities for a microwave sensor to estimate and monitor glycogen utilisation throughout exercise in endurance athletes, though the correlation with actual glycogen value was small. This study can be considered as the first step to verify whether this design and implementation of a microwave sensor could provide a wearable solution to real-time, continuous monitoring of glycogen during exercise. In addition, this is the first attempt to track glycogen molecules non-invasively using a microwave sensor rather than using algorithms to correlate parameters such as relationships between water storage and glycogen.

Comparison with gold standard muscle biopsies and biochemical assay allowed us to assess true glycogen concentrations at pre- and post- exercise, this also gave us a referencing point to evaluate the microwave sensor's tracking capabilities. Dietary intervention and high-intensity exercise played a key role in providing a wide range of glycogen values. Analysis of the muscle samples showed glycogen concentration ranged from 72 to 413 $mmol.kg^{-1}$ dw and all participants presented a significant reduction in muscle glycogen from pre- to post- exercise with a mean reduction of 41 ± 30%, $P = 0.01$. Although the sample size was small, this large variation in glycogen allowed us to understand the microwave sensor's capabilities in terms of sensitivity over a range of values observed in healthy adults.

Full spectra analysis of the S_{11} reflection coefficient showed that the best-operating frequency for the microwave sensor to monitor glycogen was from 2.0 to 2.25 GHz due to the corresponding linear drop in amplitude between S_{11} and glycogen values shown in Figure 7.7. These results demonstrate that as the concentration of glycogen increases

within the muscle the permittivity of the measurement causes the amplitude of the S_{11} measurement to change, therefore the increases of glycogen molecules can be detected within this unique microwave frequency. Furthermore, this frequency range is in also line with prior in-vivo experiments conducted with oysters' glycogen shown in Figure 7.3.

In the latter study, the average coefficient of variation for five repeated measures was 8.5% indicating a good reliability of the sensor to predict oysters' glycogen in a water solution. More specifically, the CV was 12%, 9%, 8%, 7%, 9% and 6% for the microwave detection of oysters' glycogen concentrations of 12.5, 25, 50, 100, 200 and 400 $mmol.L^{-1}$, respectively. This was expected due to the high water content within skeletal muscle, as glycogen is assumed to share similar dielectric properties to those of aqueous glycogen solutions. On-patient monitoring can explain the changes observed in amplitude either side of this range, as the sensor is interacting with a variety of other naturally occurring parameters. Considering the small sample size in the present study, it is worth noting that due to the individual biological makeup of each participant we could expect to observe slight differences in frequency shifts and peaks within our selected frequency range.

In addition, grouping glycogen concentrations in categories (from very low to high) as classically reported in the literature showed that the sensor was able to provide coherent decreasing values of glycogen concentration from pre- to post- exercise. However, it must be stressed that the approach did not allow for a correlation analysis but instead provided a proof of concept for future studies. With this in mind, further development is needed to improve the sensitivity of measurements. Future studies should include

more data points, creating a larger network to provide insights into the current sensor's capabilities. Sensor design also plays a key role in functionality as sensor's size, length, substrate and patch type are all susceptible to altering the electromagnetic field produced (Daliri et al., 2012; Balanis, 2016).

A major advantage for real-time on-patient monitoring as mentioned earlier is the potential to be able to track live patient information, however, current muscle glycogen methods do not allow for this (Greene et al., 2017), failing to monitor muscle glycogen availability throughout physical activity, and therefore implementing intervention strategies can be difficult, dependent on many nutritional and physiological variables. Therefore, having a device that can track the amount of glycogen stored within the muscle throughout exercise is useful for athletes and practitioners, acting as a "fuel gauge".

To predict glycogen concentration during exercise, and identifying the unknown glycogen values, initially trend analysis was performed with the known observed pre- and post- trial results and the correlation with the S_{11} measurements. Determining the mid-point of the trial for each participant gave us an ideal range to observe whether the microwave sensor measurements fell within expected glycogen concentration ranges. As glycogen values decreased in all participants, correct measurement should place the predicted mid-trial S_{11} value anywhere between the pre- and post- glycogen values. Secondly, using the predicted mid-trial concentrations, enabled observations of individual S_{11} responses also fell between the expected pre- and post- trial ranges. Participants 1-4 showed a strong agreement throughout with all three condition (pre-, mid-, post-exercise) peaks placing in the correct descending order. However,

participants 5-7 displayed different alignments. For these participants, it was observed that pre-trial glycogen measurements generated low peak resonance whereas the mid and post-trial concentrations fell within the expected amplitude range.

The initial low peak amplitude observed in pre-trial conditions can be attributed to the surface conditions of the skin as this is the interface upon the microwave sensor is placed. More specifically, this could be explained by exercise induced change in skin temperature which microwaves have been known to be sensitive to or the change in skin surface conditions (Nelson and Trabelsi, 2008). For instance, it is well known that an increase in skin temperature decreases its permittivity, namely, its ability to transmit an electric field (Srivastava and Varshni, 1956). However, neither the skin nor muscle temperature were measured in this study, so it is difficult to know the exact influence on the S_{11} signal. Increases in moisture content and water activity brought on by sweat could also have impacted sensor measurements as currently there is not a wicking system in place and the sensor is flushed with the skin (Al-Kizwini et al., 2013; Bjarnadottir et al., 2015).

The skin, as mentioned, was cleaned, and dried prior to testing, allowing for normal and consistent conditions. However, skin is also the barrier that the external field couples to the body and changes in testing conditions may result in the observed differences in pre- and post- exercise results. As the participant begins to exercise, the area directly under the sensor is susceptible to perspiration. Previous research has reported the use of a conductive gel to be smeared over the measurement area prior, and that a dry skin surface was not favoured due mainly to the need for consistent pressure to be applied (Gabriel, Lau and Gabriel, 1996). To improve the accuracy of the microwave sensor,

future considerations will be to either wet the surface of the skin prior to sensor placement with water or add an embedded conductive gel or silicon type body between the sensor and the surface of the skin. This could ensure a constant and reliable surface throughout exercise. Additionally, once exercise commenced, all mid- and post- trial measurements for P5-P7 fell into the appropriate amplitude ranges. When combining the predicted values (7 values) with the known glycogen samples (14 values) for the seven participants, a small linear relationship between glycogen (mmol.kg^{-1} dw) and S_{11} (dB), with an $R^2 = 0.337$ ($P = 0.04$), observed between 2.0 - 2.25 GHz.

The sensor used in this research has already demonstrated the ability to monitor blood lactate non-invasively during exercise (Mason et al., 2017). This is due to blood lactate residing in the blood vessels close to the surface of the skin. The electromagnetic field of the hairpin sensor is shown to be optimally concentrated at a distance of up to 10mm from the sensor as illustrated in Ansys HFSS modelling. Muscle tissue is located below the subcutaneous adipose tissue layers. By measuring 216 anatomical sites, it was recently reported that the thickness of adipose tissue layers ranged from 1.9 to 6.1mm in the general population. Furthermore, it was reported that for competitive male athletes, thickness generally ranges from 2.5 to 3.8mm (Störchle et al., 2018). The microwave sensor could therefore penetrate into the muscle tissue as long as the subcutaneous adipose tissue layers did not exceed 10mm in thickness.

As this study only involved healthy endurance-trained males who regularly participated in cycling (> 3 times 1 h./week^{-1}), with the data available it can reasonably be estimated that the thickness of subcutaneous adipose tissue above the vastus lateralis muscle was below 10mm for the participants in this research. In addition, Gabriel et al. (Gabriel,

1996) produced parametric tissue models to predict the penetration depth of radio and microwaves. They estimated at 1MHz that the penetration depth through dry skin, fat and muscle was 2.5mm, 1.3mm and 0.8mm respectively; at 20 GHz these depths were reduced to 1.4mm, 3.9mm and 1.3mm. Melia (Melia, 2013) also experimentally demonstrated microwave penetration in the 1-5 GHz range through fat to range from 1.5cm to 2.5cm, and for both skin and muscle in the same frequency range from 2cm to 8mm. Furthermore, a recent paper from Moghadas and Mushahwar (Moghadas and Mushahwar, 2018) reported detection of lesion phantoms through > 140mm of human tissue (at 1142 MHz). Even though, in our study, the penetration depth of the microwaves through the tissues and the thickness of the tissues were not directly measured, the evidence reported in the literature suggests that microwaves did penetrate through the muscle tissue.

As this was the first study of its kind to use an electromagnetic sensor to monitor muscle glycogen in male subjects during exercise, the results must be considered in light of the limitations of the protocol. Before the exercise trials began, the microwave sensor was tested under two varying conditions to ensure the sensor output provided stable sweeps of the S-parameters. Air and water samples were measured under the same sensing conditions to allow us to calculate the variation between five repeated measurements for both samples. Similar good reliability values were obtained when testing the sensor with oysters' glycogen (Greene et al., 2019). However, acknowledgment that further research with a larger sample size with both male and female participants with varying body compositions is needed to any limiting factors not identified within these results.

Furthermore, more integrated solutions to reducing cable movement must be developed; this possibly lies with the improvements seen in VNA size in recent years and development of an all-in-one wearable device. This is supported with data from a previous blood lactate study where sensor data was more stable when the sensor was placed on the forearm of the participant rather than the leg whilst cycling due to lower movement of the cables (Mason et al., 2018). The nature of microwave detection also generally requires additional support and development of online data collection and processing tools driven by software with machine learning and big data capabilities to optimise the real-world application and functionality. To further understand the individual responses though S_{11} peak analysis, we propose additional data collection of individual participants to enable accurate prediction models to be created. Further understanding of glycogen utilisation during training and competition by elite athletes requires tools to assess glycogen regularly and is ultimately hindered by current invasive techniques. Continuous and non-invasive measurement of skeletal muscle glycogen is necessary if practitioners are to gain the theoretical knowledge of glycogen utilisation within elite athletes outside of controlled laboratory protocols and uncommon organised field research (Greene et al., 2017).

7. 4. 3 **Summary**

In summary, this chapter details research, the first of its kind providing the initial piece of evidence that microwave sensor technology may non-invasively detect and monitor changes in skeletal muscle glycogen concentration during exercise. The study involved endurance-trained participants and assessed a purpose-built microwave sensor

operating at frequencies between 2.0-2.25 GHz, comparing measurements with gold standard muscle biopsy samples. Further development needs to be considered before an all-in-one wearable solution is achieved, with the addition of machine learning and predictive algorithms to convert S_{11} (dB) into biological units (mmol.kg^{-1} dw) or a more user-friendly alternative. To improve sensor accuracy, future research will look to either wet the surface of the skin, use a conductive gel, or integrate a silicon body to the sensor prior to attaching the sensor to allow for a consistent surface for monitoring. This research is the first step in the pursuit to develop a real-time non-invasive tool, allowing athletes and practitioners to accurately monitor skeletal muscle glycogen availability during and throughout exercise. To conclude, monitoring glycogen levels in real-time enables accurate timing of refuelling of CHO which enables sustained bouts of high-intensity exercise, fundamental to success during endurance-based events.

CHAPTER 8 – DISCUSSION, CONCLUSIONS, AND FUTURE WORK

This chapter will present an overview of the experimental findings of this thesis in relation to the original aims and objectives set out in Chapter1. A general discussion is then given which focuses on the critical evaluation of the non-invasive electromagnetic sensor's capabilities at detecting each parameter. Additionally, specific attention is given to the use of the microwave sensor to detect each parameter during exercise in professional sport. Finally, the chapter will close by concluding and outlining the potential direction for future research.

8. 1 **General discussion**

The overall aim of this thesis was to examine the novel use of a non-invasive microwave sensor to detect and monitor the physiological responses of three chosen parameters. The proposed hairpin sensor is simple in design, has low cost of manufacturing and is compatible for integration into an all-in-one electronic solution. The results within this thesis demonstrate promise for microwave sensors to detect physiological parameters via non-invasive continuous sensing. The ability to monitor sweat sodium, blood lactate and skeletal muscle glycogen using microwave sensors would inform sport-specific guidelines by assessing real-time continuous physical activity aiding in refuelling strategies, improving performance monitoring, and reducing associated risks.

It was demonstrated that a microwave sensor along with mathematical modelling has the capability to not only measure each parameter but also to track the direction of the values in real time. This capability has significant relevance in both medical and sporting use cases. As such, this solution may interest physiologists, researchers, and sports scientists, but also biomedical engineers and developers who are looking for solutions for wearable technology to optimise sports performance and online patient monitoring.

Tracking and predicting sodium loss will ensure sufficient refuelling strategies are in place for patients and endurance athletes who could suffer serious associated risks with sodium loss and dehydration (Bates and Miller, 2008). Being able to non-invasively track blood lactate within a healthcare setting will enable better monitoring of critically ill patients, and allow more accurate adjustments to treatment (Bakker, Nijsten and Jansen, 2013b). Finally, being able to track muscle glycogen levels and forecast when levels are starting to drop below optimal levels will allow endurance athletes to refuel or avoid unnecessary refuelling.

The latter strategy is key in cycling endurance events where the combination of weight and energy is fundamental during events, especially on hill climbs where reducing weight and maintaining power is the key to success (Ebert et al., 2007). Additionally, being able to monitor all three parameters simultaneously in real-time would provide invaluable insights into the athlete's physical condition. Tracking sodium loss could ensure the athlete avoids dehydration, monitoring blood lactate levels would allow the athlete to remain in the optimal exercise intensity for the duration of the event, and monitoring glycogen levels would enable the energy for the chosen intensity to be sustained.

8. 1. 1 **Achievements**

This research was the first of its kind at applying a microwave sensor to human participants during exercise. A hairpin microwave sensor was adapted so it could be placed directly onto the surface of the skin where it generated an EM field. The EM field then was shown to penetrate through human tissue non-invasively to monitor the electromagnetic footprint of sodium produced in sweat, lactate produced in the blood and glycogen which was stored in the skeletal muscle.

Research throughout this thesis has shown that a single hairpin microwave sensor operating at microwave frequencies between 10MHz and 4 GHz has been able to detect sodium concentration in sweat, blood lactate and skeletal muscle glycogen as shown below.

- **Sweat Sodium:** 1.6GHz
- **Blood Lactate:** 3.2 - 3.8GHz
- **Skeletal Muscle Glycogen:** 2.0 - 2.5GHz

Importantly, each individual parameter was detected within its own unique frequency range, potentially offering a solution to monitor all three parameters at once using one sensor. This would depend heavily on software capabilities, regarding data management, analysis, and modelling. The advancements in machine learning, artificial intelligence, and big data analysis hold potential in effectively managing and processing this amount of data. This technique can be further optimised with additional support and development of online data collection and processing tools driven by software to operate in conjunction with the microwave sensor. The data produced from this study

allows further research to now focus on a specific frequency range for better optimisation.

The combination of in-vitro and in-vivo analysis has been a useful method of allowing further insight into how the sensor reacts to changes in concentrations under an array of conditions. Having supporting data from both controlled test tube measurements and exercise protocols with human subjects has allowed for confirmation of the accuracy of the sensor. The research within this thesis hopes to lead the effort in determining if a microwave sensor can provide a feasible option to generate reliable non-invasive measurement of biological parameters. If so, this novel approach could improve research protocols and allow for further examination into the physiological adaptations to exercise and a better insight into the human anatomy. This ability undoubtedly will lead to a more individualised approach for the athletes, which is vital at the elite end of the performance spectrum.

This technique of analysis was shown to be useful when assessing sodium concentrations in human sweat. Initial data generated from the sensor whilst it was being worn on the subject seemed to suggest that the microwave sensor was unable to correctly correlate sodium concentrations with the commercially available device (R^2 = 0.149). However, further investigation into microwave sensors, using the same 140 time stamped sweat samples within a laboratory setting demonstrated that in fact, the microwave sensor was able to detect sodium concentration when isolated to a high level of accuracy when compared to the same commercially available device at 1.6GHz (R^2 = 0.862).

Furthermore, this approach led to a similar approach for discovering glycogen in human subjects. Prior to human trials, laboratory-based experiments were conducted to determine if the sensor was able to detect glycogen in a pre-made solution using glycogen from oysters, there was a strong linear correlation identified ($R^2 = 0.87$). This approach was appropriate from an ethical perspective also, to determine that glycogen was possible to detect using a microwave sensor before human trials because the current gold standard technique of measuring glycogen uses an invasive muscle biopsy. However, human trials naturally present more variables than a controlled in-vitro experiment, so it was still necessary to identify if the microwave sensor could detect glycogen stored within the skeletal muscle when placed upon the skin. Results showed that when monitoring glycogen samples across all participants, there was a low correlation ($R^2 = 0.13$). However, when grouping glycogen concentrations into ranges readily used within sports and exercise science there was a strong linear correlation ($R^2 = 0.91$), however, further work is needed to validate these results. As discussed previously, it was shown that glycogen was detected between 2.0-2.25 GHz during both in-vitro and in-vivo analysis.

8. 1. 2 **Challenges**

To fully assess each parameter, it was critical to analyse the sensor under varying environmental and physical conditions. Although all our human exercise trials were completed within a laboratory, many of the challenges which would be faced in the real world were encountered. The research methodology was designed to allow for optimal physiological performance and activation of our chosen parameter. To monitor sodium

loss, the environmental temperature was increased to 30°C to stimulate sweat. To monitor changes in blood lactate levels, exercise intensity was increased progressively until maximal voluntary exhaustion and consequently increasing the movement of the subjects as they approached the latter stages. Depleting muscle glycogen stores involved repetitive bouts of high-intensity exercise over a long duration. This duration presented challenges regarding the attachment of the sensor, as the sensor was positioned using commercially available sports and medical tape rather than a built-in customisable strap.

Analysing a different parameter in the three key physiological layers of the skin, each layer of the anatomy had its own unique challenge. As mentioned throughout this thesis, sweat generation presented us with a number of challenges, initially, sweat would cause the tape which attached the sensor to the participant to come loose, the purchase of sports tape addressed this issue, which was more breathable and longer lasting. Moving forward, the use of an integrated custom strap to provide a secure fit would further improve measurements. Sweat monitoring was also a challenge, this was a sensor design limitation due to the sensor being made of non-breathable materials and was unable to wick away sweat, therefore unable to accurately monitor sweat upon the surface of the skin.

The measurement of blood lactate was completed by taking blood fingertip measurements. A challenge faced during the experimental procedures was to ensure the sensor was in close enough proximity to the finger extremities to ensure a valid measurement. Results showed that placing the sensor on the forearm rather than on the quadricep provided us with more accurate results and this could have been down to a reduction in movement and being closer to the fingertip measurements.

When monitoring skeletal muscle glycogen, it was initially uncertain if the microwave sensor would generate sufficient penetration depth to reach the muscle tissue. However, an issue which was not considered was the changes in skin conditions during exercise brought on by sweat; throughout exercise, the challenge becomes providing more intelligent, real-time, accurate information, making it user friendly and offering coaches and athletes actionable insights. In the experimental chapter for glycogen, it was detailed that a key limiting factor between pre- and post- exercise measurements was the change in the surface of the skin and the sensor.

Throughout all the human experiments the microwave sensor was recording post warm up. This means that the participants had time to warm up effectively and raise their heart rate and induce sweating. However, for the glycogen experiment, it was critical to start microwave sensor measurements as soon as the muscle biopsy was taken to ensure a pre-exercise reading, the issue with this was that the skin conditions would change as soon as the participant would begin to sweat, causing a barrier between the sensor and the skin. The dry surface conditions appear to change the properties of the microwave wave when compared to that of a wet surface. Future application would be to incorporate a gel of some kind prior to exercise to provide a more stable base for the sensor.

8. 1. 3 **Technical limitations**

To enable a large frequency sweep to examine which frequency range correlates to the three chosen parameters, it was necessary to use a VNA capable of producing this capability. Current VNA technology requires the use of relatively bulky equipment to be attached to the microwave sensor away from the participant. Due to this, the cables

which attached the VNA to the microwave sensor experienced some form or movement during the experiment as the sensor was placed directly on the surface of the skin. The noise created by this movement, will be reduced as the frequency range needed becomes smaller due to the results throughout this thesis, leading to a smaller device and with further development into electronic integration could provide an all-in-one wearable solution, completely removing the need for cables.

The development of the microwave sensor was designed for in-vivo application, the size and shape of the sensor ensured ergonomic fit when placed directly over the skin on either the forearm or the quadricep. As the design materials have since advanced, it is now possible to develop a sensor using more flexible materials enabling a more custom fit and even embed the sensors within athletic style garments. With the help of advanced integrated circuit technology, the next-generation microwave sensor system would incorporate wireless and compact sensors which would easily be embedded within such garments. This would be both convenient and ensure highly accurate localisation providing limited movement. The improvements in garment and sensor design will aid in the collection of real-time continuous data, giving it many use applications.

Furthermore, the research in this thesis shows the multiparameter potential of an all-in-one device which is able to monitor key exercise related parameters all at different electromagnetic frequencies. This body of work highlights the capability of microwave sensors at becoming a method of allowing athletes to wear just one device to measure many different parameters. However, to utilise the data generated via a VNA, advancement would need to be made in data analytics and machine learning capabilities

to enable real-time interpretation during physical activity as well as accounting for any environmental noise in more physically demanding conditions.

8. 2 **Conclusion**

In summary, this research presents an accurate, cost effective, efficient method to detect skeletal muscle glycogen, blood lactate or hydration status non-invasively and continuously either using in-vitro or in-vivo methods. The findings of this thesis demonstrated that a hairpin microwave sensor can monitor changes in sodium concentration in human sweat samples at 1.6GHz ($R^2 = 0.862$), blood lactate in untrained participants at 3.4-3.6 GHz ($R^2 = 0.78$), blood lactate in endurance trained participants at 3.2-3.8 GHz ($R^2 = 0.757$) and can monitor changes in glycogen samples at 2.11 GHz ($R^2 = 0.87$), as well as skeletal muscle glycogen measurements when grouped into exercise specific ranges at 2.0-2.25 GHz ($R^2 = 0.91$). However, further research is needed before being able to quantify glycogen effectively as there was a low correlation reported when assessing glycogen without any grouping method ($R^2 = 0.13$). Additionally, the design of the sensor needs to be considered for real-time detection of sweat sodium during exercise, as results showed poor correlation ($R^2 = 0.149$).

8. 3 **Future research**

The research contained within this thesis has presented a unique solution to monitoring three different parameters that have significant relevance to sport and exercise. With further research and development, a single microwave sensor could provide greater insights into human performance than is currently unattainable with traditional techniques. Ultimately, this may lead to a more individualised approach for athletes and

could be utilised during competition and training to enhance performance and reduce injury.

As detailed, each parameter measured within this thesis was identified via the unique electromagnetic frequency range specific to each parameter. Future research should now seek to further understand and explore if it is possible to measure each of these three parameters during one exercise trial. Additionally, as this research is the first of its kind, further investigation is required to test the reliability and validity of the results found for each parameter when monitoring human subjects via a test-retest method. This will need to be done with additional subjects from multiple population groups. The work within this thesis used healthy and active participants during exercise to induce specifical physiological changes in each parameter's concentration.

Future investigations are necessary to validate the frequency ranges identified to monitor each parameter on a more diverse population to assess whether variables such as BMI and body fat percentage affect microwave interactions as little is known thus far. This could possibly be achieved using dual-energy X-ray absorptiometry (DEXA) scans and the use of meat samples to assess the impact of ranging tissue properties and thicknesses. Finally, the use of this technology within a real-world setting/environment would need to be conducted. Effects of factors such as noise, vibrations and long-term use on the microwave sensor would need to be studied in order to make this technology a feasible solution outside of a controlled laboratory setting.

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