Brain and plasmatic lactate and glucose metabolism during exercise: Experimental protocols and data analysis framework

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Abstract

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Lactate has long been thought to be a waste product of anaerobic glycolysis that causes muscle fatigue, pain, or damage. Later studies clarified that lactate is not a waste product but a fuel created by skeletal muscles and brain astrocyte cells during moderate to intense physical exercises and is one of the primary fuels of the body and brain for energy production. Overall, it has been shown that lactate is a mysterious chemical that plays a more important role than previously thought.

Although there have been significant advances in our knowledge of the metabolites involved in neuronal energy metabolism during recent years, there are still many unknowns about brain energy metabolism due to its complexity.

This study evaluates the effect of exercise on brain and body energy metabolism, especially the fate of lactate and glucose in two regions of the brain (including the posterior cingulate cortex and the supplementary motor area) and plasmatic level.

Previous studies are limited to animal experiments or a result of measuring the difference in arterial lactate concentration in humans, which do not give us a clear understanding of the fate of brain lactate and glucose. In this study, non-invasive imaging techniques, including Magnetic Resonance Spectroscopy and Positron Emission Tomography, were used to understand the concentration of lactate and the rate of cerebral glucose absorption after exercise.

The analyses performed on 29 healthy subjects (between 25 and 45 years of age) indicate an increase in brain lactate concentration and a decrease in brain glucose absorption due to exercise, which once again confirms the claim of replacing lactate instead of glucose as fuel for neurons. In addition, our analysis shows a direct correlation between plasma lactate and brain lactate, from which it can be concluded that plasma lactate is the primary source of brain lactate.

Most importantly, based on the original findings, the increase in lactate and decrease in glucose observed in the brain are considered two independent phenomena occurring in parallel following intense exercise. Although it should be noted that there is a strong relationship between the changes of measured quantitative metabolites and the states of rest or exercise, so with the changes in the concentration of metabolites, we can significantly predict the presence in the state of exercise or rest.

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Lastly, I would be remiss in not mentioning my husband, Sobhan. His faith in me has inspired me to be an independent thinker and have credence in my talents to pursue new things that interest me. I'd also like to thank my parents for teaching me that it is vital to leave the world a bit better than I found it and that a career in research may be an important part of that. And, of course, thank you for your unwavering support throughout my academic career's ups and downs.

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Chapter 1

Introduction

This chapter gives a concise summary of the thesis's fundamental components. A detailed assessment of the literature on this subject is presented in chapter 2.

1.1 Energy metabolism of the brain

The brain is one of the most sensitive and complicated organs of the body; although it makes up only 2% of total body weight, it has the highest amount of received energy, and according to studies, it accounts for roughly 20% of the total energy [1,2]. The brain's energy metabolism is a continuous and tightly controlled process regulated mainly by juncture interactions between brain cells that process information and coordinate body activities [3]. We will notice increased energy expenditure with increased nerve activity, contributing to improved neuron function [4]. Therefore, neurons are expected to utilize about 80 percent of the energy created in the brain [5], with a considerable portion of it being needed to restore neuronal membrane potentials following depolarization [6]. Other neural activities, including neurotransmitter production and vesicle recycling, also donate to the necessity for neurons to have a high metabolic rate [7–9]. Therefore, energy requirements are not uniform across the brain, and increased blood flow is required by neurovascular and neurometabolic coupling mechanisms to compensate for the different energy demands across the brain.

Currently, our knowledge about the regulation of energy metabolism in the brain has dramatically advanced over the past few decades, relying on various approaches from molecular biology to

in vivo animal and human imaging. This has led to the discovery of a surprisingly important role for key brain metabolites, including glucose and lactate, which also have more complex effects than those involved in energy production [10–12].

1.2 Fueling brain work: Glucose and Lactate metabolite

To lead a healthy life, an individual's daily brain metabolism is supposed to be above a standard level. This helps us to prevent diseases of the central nervous system, such as depression and neurodegenerative conditions. To do so, the brain demands energy as fuel. Energy for the brain is mainly provided by glucose during non-activated states [1, 2]. Glucose is a crucial substrate for the brain and can supply its metabolic needs. At rest, brain glucose metabolism shows considerable regional variability and is significantly impacted by local brain activity [13–15], indicating a close relationship between energy metabolism and brain function. Despite significant metabolic variations across the white and grey matter, brain glucose content remains constant throughout brain regions, showing a strong relationship between glucose supply and demand. However, since glucose transport is facilitative, brain glucose concentrations can alter drastically and becomes more pronounced when metabolism slows down [16].

Although glucose is the primary fuel source for the brain, it has been shown that the brain can use other substrates than glucose, including lactate, for metabolism in an active state. Lactate is generated by astrocytes in the brain under aerobic conditions through the activation of glycolysis, which is initiated by the astrocytic uptake of neuron-released glutamate. The brain can also use lactate from the blood [17, 18]. Under normal conditions, blood lactate cannot maintain normal brain function because the blood-brain barrier transfer of lactate is low in adults [19]. However, studies focusing on post-exercise lactate levels showed that intense exercise gradually increases lactate uptake as workload and plasma lactate levels increase [20]. It has been shown that glucose metabolism might be spared during exercise because lactate may be preferred over glucose [21, 22].

Therefore, as mentioned, there is a connection between physical activity and the way the brain works, which is less known due to the complexity of the brain. This study focuses on the effect of exercise on energy metabolism to find out how brain function is probably related to physical activity

and exercise.

1.3 Problem statement

The research on the effects of exercise on the brain is extensive, covering both acute and long-term impacts and examining changes from the cellular to the system/cognitive levels in animals [23, 24]. The capacity of exercise to modify brain function parameters from the physiological [25] and pathophysiological [26] to the functional, with experiments testing the response to exercise to mood disorders [27, 28] and cognition, particularly memory [29], is now well established. However, the mechanisms involved are frequently unknown [30, 31]. We lack even a fundamental grasp of the sort of effort regimen that may have a demonstrable influence on normal function, let alone different disorders, due to that lack of information [32].

Although there have been reports of a decrease in brain glucose utilization during exercise as measured by PET imaging with 18FDG, which has been hypothesized to be secondary to utilization instead of lactate taken up from the periphery [33], this hypothesis has not been tested in detail. It is cast into doubt by results showing an increase in lactate generation from glycogen stocks found in astrocytes involving adrenergic stimulation during exercise [34, 35]. Measurements of alterations in brain lactate after exercise have also been made in several investigations [18, 36, 37]. However, these studies are restricted to invasive venous blood measurements from the dominant jugular vein, and technology was only able to assess metabolites present in blood exiting the brain.

To fully understand the changes in brain metabolism, it is crucial to gain a complete picture of the glucose, especially the fate of peripherally derived lactate, which is generated during exercise. Therefore, we propose using non-invasive medical imaging techniques to measure regional brain glucose utilization and brain lactate concentration.

1.4 Objectives of the Thesis

The objectives of this study are twofold:

(i) Data acquisition and analysis framework

- To investigate imaging technics to quantify cerebral and plasmatic lactate and glucose in human
- To develop a data analysis framework to investigate brain energy metabolism

(ii) Experimental protocol to investigate the following hypotheses

- Glucose uptake by the brain is reduced during exercise, leading to a decrease in 18FDG uptake
- Brain lactate concentration increases after exercise compared to its resting value

As a consequence, an experimental approach that allows measuring direct glucose metabolism and lactate concentration in the brain is required to understand better the mechanisms involved in the observed changes in the energy metabolism of the brain during exercise, which can lead to better-defined interventions with exercise or other approaches in patients with various neurological disorders.

Therefore, this thesis proposes measuring brain glucose utilization using positron emission tomography (PET) of 18FDG and brain lactate concentration using magnetic resonance spectroscopy (MRS) under conditions of rest and exercise-induced lactate concentration elevation in the plasma. For this purpose, data were collected from several healthy volunteers under two conditions of rest and exercise and in two regions of interest in the brain: the Posterior Cingulate Cortex (PCC) and the Supplementary Motor Area (SMA). After performing the necessary pre-processing of the data, the behavior of brain glucose and lactate were analyzed before and after exercise. We also investigate the association between these brain metabolites and the equivalent parameters in the blood. We explicitly attempt to uncover a link between the reduction in brain glucose and increased brain lactate following exercise after directly proving the hypothesis using non-invasive imaging methods.

1.5 Organization of the Thesis

The remaining sections of this thesis are organized as follows: The most pertinent studies are covered in Chapter 2, together with a critical evaluation of their contribution, and an explanation of why the resources offered are important for addressing the research problem. then illustrates how

our work is unique compared to others. Chapter 3 briefly describes the study variables and their relationships with each other. The last section in Chapter 3 depicts the relationship between variables with a diagram. Chapter 4 provides our proposed method for directly measuring glucose and lactate using non-invasive medical imaging techniques. Chapter 5 shows that the hypotheses were correct, and the glucose uptake in the brain decreases after exercise, while we see an increase in lactate concentration. We also interpret the association between decreases in glucose and increases in lactate. Chapter 6 highlights the most important findings of this study and discusses future research prospects. Lastly, Appendix A, B, and C offers detailed explanations of specific concepts and approaches taken in the data collection protocol.

Chapter 2

Literature Review

2.1 Introduction

Energy is one of the essential factors for the normal functioning of body organs. Many tissues can use fat or protein as an energy source, while others, such as the brain, rely on glucose. The brain is the most energetic organ and the most active part of our body in terms of metabolic activity. Neurons use a large portion of this energy, and as brain activity increases, so does energy consumption [38].

Brain energy metabolism is a complicated process, and according to our current understanding of the relationship between brain activity and energy metabolism, it is expected that several complex pathways and mechanisms have yet to be discovered. However, it is well known that in the inactive state, the first vital substance, the brain's primary fuel, is glucose since it supplies the energy required for neuron activity. The importance of this sugar for brain functioning is so great that if the glucose does not reach the brain within 3 minutes, the neurons will be destroyed. Although glucose is the primary fuel source for the brain, many recent studies have demonstrated that high-energy metabolites such as lactate are generated during high-intensity exercise, which can be preferentially used as an energy source for the brain instead of glucose [37].

Much research has been conducted on the effects of exercise on brain metabolism, especially lactate metabolism, which is limited to invasive methods and focused on jugular vein blood measurement. They were also not able to measure brain lactate independently and directly [18, 33].

Therefore, to better understand the mechanism of the brain, it is necessary to examine the lactate metabolism in the brain during physical exercises directly and in a non-invasive way. This allows us to more thoroughly investigate its interaction with other brain metabolites, such as glucose and blood parameters.

In this chapter, we start the literature review by discussing the relevance of physical exercise on overall health, particularly brain and neuron function. The following sections will go through the essential theories and evidence on the effects of exercise on brain metabolism. Another point of contention is the fate of lactate produced after intense activity in the muscle and brain. This review will concentrate on different techniques for detecting brain lactate and glucose. Finally, this study develops a framework by including the most critical existing ideas and methodologies; it also fills gaps in previous research and contributes to new knowledge.

2.2 Effect of physical activity on public health

Physical activity is any activity or movement of the body caused by the contraction and expansion of skeletal muscles and leads to energy consumption [39]. Examples of daily life include doing diligent work at home, playing and traveling, fast walking, light weight-lifting, or even gardening are examples of them [40]. Therefore, physical activity in terms of intensity can be categorized into three levels: light, moderate, and intense [41]. A general surgeon in the United States has traditionally recommended moderate to vigorous physical activity in his report and emphasized that these activities should be done continuously [39] because certain benefits can only be achieved by engaging in more intense, structured, and repetitive physical activity. In this way, we call exercise a particular type of physical activity planned and purposeful to achieve physical fitness or other therapeutic properties [42]. Exercising at the gym, swimming, biking, running, and other sports such as golf and tennis are many different types of exercise. Although exercising increases fitness, it is not only related to the type of exercise but generally depends on its dose. Exercise dose has different definitions in the literature, but it is usually considered a product of the type of exercise, the intensity of exercise, and the duration of exercise [43, 44].

Research has shown that sports activity has beneficial effects, can control or delay the onset

of age-related diseases, and help improve health. For example, exercise has a protective effect on cardiovascular diseases. Data from several sources have shown that more physical activity can reduce the risk of cardiovascular diseases even more [45–49]. Physical activity regularly can also help lower blood pressure and enhance cholesterol levels [50]. Therefore, there is an inverse relationship between the level of exercise and cardiovascular diseases.

Another benefit of physical activity is controlling blood glucose and body weight, which is one of the effective ways to improve and treat type 2 diabetes [51, 52]. Exercise also prevents common cancers among adults such as colon cancer [53, 54], breast cancer [55], and lung cancer [53, 56].

2.3 Effect of physical activity on brain function

In the preceding section, we showed that many scientific investigations had identified exercise as an essential health parameter, from avoiding cardiovascular disease to battling obesity and preventing diabetes or other malignancies. But there is one area that is less well understood due to the intricacy of this human organ, which is the connection between physical exercise and how the brain operates. However, in recent years, several researchers have underlined how physical activity and exercise are related to brain health and function.

2.3.1 Depression and Anxiety

Together, these studies show that exercise training affects brain tissue in several ways, one of which is the reduction of symptoms of depression and anxiety [57–59]. Stress creates a destructive substance in the blood and brain and naturally destroys brain neurons. Performing various sports exercises causes more adrenaline (stress hormone) to be burned, increasing relaxation [60]. Also, a hormone called endorphin - which is referred to as a happy hormone - is released in the body, which naturally reduces stress and ultimately reduces the rate of brain cell loss [61, 62]. On the other hand, by relying on the concept of body composition measurements, Zimmermann and her colleagues have demonstrated a positive correlation between the severity of depression and body fat mass. In contrast, it has a negative correlation with body muscle mass [59]. Therefore, considering that doing sports activities reduces body fat and strengthens muscles, it is reasonable to assume that

exercise is a barrier to depression.

2.3.2 Alzheimer's

According to researchers, another protective impact of exercise on brain health is the prevention of Alzheimer's disease, the most frequent form of dementia [63–65]. Alzheimer's is a type of neurodegenerative disease that affects problems in memory, thought processes, and behavior [66]. Prevention of Alzheimer's disease is one of the biggest challenges facing modern science. The consequences of this disease can be devastating, and health experts worry that the condition will also appear in younger people. However, one thing that helps prevent Alzheimer's is exercise [67]. Exercising can avoid factors that increase the risk of dementia. Obesity, diabetes, high blood pressure, and depression are among these risk factors [67, 68]. On the other hand, studies show that people with Alzheimer's have much less grey matter in their brains than healthy individuals [69]. Additionally, Lucas and his colleagues demonstrated that active older adults had brains with more significant amounts of grey matter [70], supporting the idea that exercise can ward off dementia.

2.3.3 Neuroplasticity

Exercise's enormous impact on cognitive function and the plasticity of the brain is another factor in the human need for exercise [71–73]. The concept of brain plasticity, commonly referred to as neuroplasticity, is founded on the notion that the brain has the capacity to be plastic and is ready to change and expand at any age. Although it is often assumed that brain growth slows down with age, current research has revealed that the brain releases neurotrophic substances like brain-derived neurotrophic factor (BDNF) in response to physical exercise [72–74], which plays a vital role in neuroplasticity [75, 76]. Therefore, by strengthening the power of neural flexibility, exercise shows that brain growth is not confined to childhood and can be boosted in adulthood.

2.3.4 Cerebral Metabolism

Exercise is also one of the oldest, best, and most important factors in increasing body metabolism, which few can deny its role [77–79]. Metabolism is a complicated chemical process in the body's cells. During this process, the calories in food and beverages combine with oxygen to generate the

required energy for cell and body function [80]. Unlike other parts of the body that get most of their energy from fats, carbs, and proteins, the brain absorbs most of the energy required to activate neurons from carbohydrates. Therefore, cerebral metabolism is defined as the intra-cerebral exchange of primary metabolites such as glucose and lactate throughout the arterial and venous blood circulation in the brain [38].

Extensive studies have been conducted on brain energy metabolism and the quantities involved, often under resting conditions [81–84]. However, considerable advancement has been made in our knowledge of regulating energy metabolism in the brain during the last several decades. As a result, efforts have been made to measure and investigate brain glucose and lactate concentrations under the influence of intense exercise using various methods, including molecular biology and in vivo animal and human imaging.

According to the findings of recent studies, different forms of moderate to vigorous exercise can affect brain metabolism in addition to increase skeletal muscle metabolism. Scheinberg and his colleagues were the first to measure the cerebral glucose metabolism rate during exercise [85]. According to their studies, no significant changes in the rate of cerebral glucose metabolism were observed during exercise, which led researchers to focus less on this topic, and not much information was available about cerebral glucose metabolism. After five decades, Ide et al., by measuring the difference in arterial and venous glucose concentration, reached contradictory results with what was observed before. They found that the arterial-venous concentration difference of glucose decreased during light exercise (30% Vo2max) compared to the resting state, increased during moderate-intensity activity (60% Vo2max), and remained unchanged during intense exercise (Above lactate threshold) [37, 86].

The discovered variations in glucose behavior during exercise drew the attention of other researchers to this subject, and we have seen a plethora of studies investigating the effects of exercise on brain metabolism. Considering that the dose of exercise may influence the results, most studies have quantified brain glucose uptake during various intensities of exercise. Figure 2.1 depicts the average cerebral oxygen, glucose, and lactate consumption extracted from seven studies during exercise [87–92]. Collectively, these findings reveal a considerable decrease in cerebral glucose absorption with increasing exercise intensity. Similar investigations have also been conducted to

compare the quantity of brain glucose in two states—rest and extreme exercise— which are consistent with the presented results and demonstrate that intense exercise reduces global glucose absorption in the brain [18,33,93,94]. However, few studies provided the opposite conclusion, stating that glucose levels did not significantly alter during exercise and that glucose absorption remained steady [95,96].

However, a dilemma arises: how are neurons supplied with fuel after an increase in the requirement for energy due to strenuous activity and a reduction in brain glucose absorption?

AAlthough it is generally established that glucose serves as the sole source of brain fuel at rest, glucose consumption declines as exercise intensity rises, indicating that other substrates may also fuel neurons. The first demonstration of lactate's potential as a source of energy for the human brain under specific circumstances was made by Gallagher and colleagues [97]. Even though lactate plays a minor role in cerebral metabolism at rest, it has been demonstrated that following incremental exercise, the concentration of lactate in the brain multiplies [37, 92, 98–100]. This concentration increase is generally achieved in two ways, raising blood lactate concentration and increasing lactate production by astrocytes in the brain during glycolysis [101–103].

Numerous research in this area has attempted to explain the cause of the rise in brain lactate

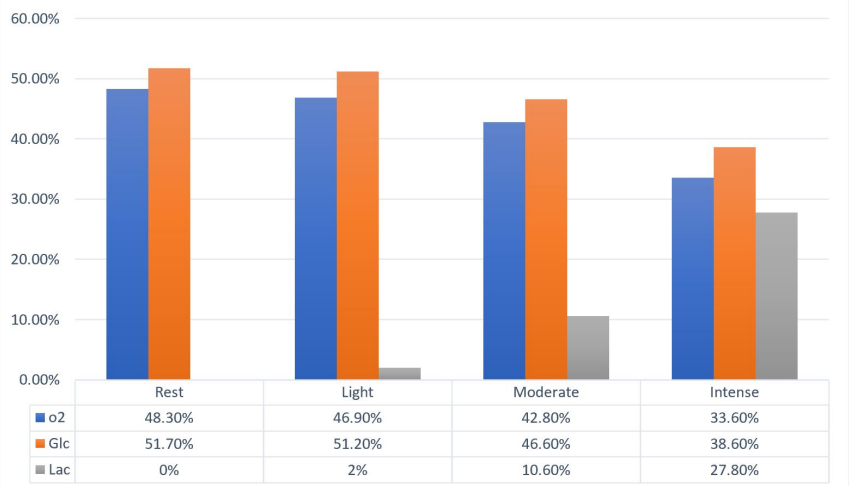


Figure 2.1: This figure shows the average consumption of cerebral oxygen (Blue), glucose (Orange), and lactate (Gray) extracted from seven studies during different exercise intensities. The bar graphs show that with the intensity of exercise (from rest to intense), the average consumption of brain oxygen and glucose decreases, while the average consumption of lactate increases.

concentration during exercise by measuring blood lactate and demonstrating the difference between arterial and venous lactate concentration. Cumulative findings from these studies suggest that intense exercise increases blood lactate produced by muscles [18, 33, 103–105]. On the other hand, by observing the increase in cerebral arterial-venous lactate difference [33, 91, 92], they were able to show that the exponential growth in cerebral lactate absorption is related to the increase in its arterial concentration [38, 98]. Thus, one explanation for the rise in brain lactate concentration is that blood lactate generated by muscles crosses the blood-brain barrier and enters the brain, which is utilized as a source of energy for neurons. Another cause of elevated brain lactate is lactate derived from astrocytes. It has been shown that when energy demand increases, astrocytes break down the stored glycogen into lactate and provide an energy source for neurons [103, 106]. With all of these interpretations, the relationship between exercise intensity, reduction in brain glucose absorption, and increase in brain lactate concentration have become a challenging issue due to their inability to directly measure brain lactate and the unknown and complex nature of the brain.

In this review, we highlighted the importance of exercise for health and emphasized the relation between physical activity and exercise and brain health, performance, memory, and metabolism. Of course, exercise's influence on brain health is not limited to these few cases, and this medicine affects many situations both directly and indirectly. The significance and function of glucose and lactate in brain metabolism after incremental training were briefly discussed here. We go into further depth on the generation of glucose and lactate as well as their fate in the muscle and brain after exercise in chapter 3.

2.4 Previous research: methodological approaches

As demonstrated in the previous section, our understanding of how the brain regulates its energy metabolism has significantly advanced over the past few decades. As a result, extensive research has been done on the impact of exercise on brain metabolism using various molecular biology techniques to in-vivo imaging in animals and humans. This has led to discovering a surprisingly important role for lactate alongside glucose. However, the critical distinction between these studies is the adopted methodological approaches.

Because the design of the data collection protocol in these research fields will have a significant impact on the statistical analysis, interpretation of the extracted data, and, ultimately, the rejection or confirmation of the hypotheses, a slight change in the framework results in significant changes in the results. Therefore, in this section, we review some previous works in light of the data collection framework and its limitations.

What we know about the brain and plasmatic lactate and glucose is largely based on empirical studies investigating how these metabolites contribute to energy metabolism in the body and brain. Several extensive cross-sectional studies have employed invasive techniques to assess brain and plasmatic lactate and glucose in animals and humans. Some of these studies attempted to increase blood lactate levels through intravenous lactate injections and intense exercise. Finally, by measuring the difference in arterial-venous lactate concentration and glucose, they have shown a decrease or increase in the absorption of these metabolites after exercise [26, 91, 92, 95, 99]. By adding transcranial Doppler ultrasound of the middle cerebral artery to this protocol, van Hall and colleagues were able to examine cerebral perfusion more precisely [18].

Researchers have been working on indirect ways to represent the behavior of brain lactate in the interim. Lactate dehydrogenase (LDH) levels, which are primarily in charge of lactate processing for energy in a low oxygen environment and play a role in the conversion of lactate and pyruvate, were measured in an experiment performed by Kinni et al. on male rats after three weeks exercise [107]. The results of this study following brain dissection after euthanasia indicate an increase in LDH due to exercise, which indirectly verifies the notion of elevated lactate in the brain after exercise. In a different research, Matsui and his colleagues used a capillary electrophoresis mass spectrometry-based approach on endurance-trained rats to assess the level of monocarboxylate transporters (MCTs), which are responsible for transporting astrocyte-derived lactate as energy to neurons [35]. They demonstrate that MCT2 levels rise, indicating that the brain uses astrocyte glycogen-derived lactate as fuel during intense exercise.

Wyss et al. measured both brain lactate and glucose using voltage-sensitive dye imaging (VSD) and [18F] fluorodeoxyglucose (FDG) with sensory stimulation in unconscious rats [93]. Several studies have also used positron emission tomography (PET) of 18FDG to measure brain glucose uptake [33, 108, 109].

There are drawbacks to each method suggested for measuring brain lactate and glucose levels. Most investigations need invasive procedures, and in some of them, a radiotracer injection is required for imaging. The inherent risks of this procedure are numerous and not appealing to everyone. Furthermore, the techniques mentioned earlier cannot assess brain lactate directly and do not provide a precise picture of what happens to lactate generated after exercise. Despite the fact that several studies have been conducted on animals and, in general, cannot be implemented on humans (since some of them are ex vivo), the rest have been conducted in vivo on humans, but because the majority of them have only included men, it can be argued that the findings of these tests cannot be applied to society. Another problem of this study is the small sample size (usually $n < 10$),which does not yield statistically significant findings.

Therefore, an experimental strategy that enables direct and non-invasive monitoring of brain glucose and lactate levels is required to understand brain energy metabolism after exercise better. In this thesis, we propose the methods of magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) of 18FDG to evaluate brain lactate and glucose, respectively.

As we mentioned, PET-18FDG is a well-known technique in the field of brain glucose measurement, which has also been used in previous studies. MRS is also a relatively new technique that has recently attracted the attention of researchers. This technique is used to measure the concentration of metabolites in the region of interest, which is used here for the first time to record brain lactate after exercise.

2.5 Conclusion

Everyday tasks that we perform throughout our lives depend on our brains. Whatever we are doing, it doesn't matter; what matters is that our brain is constantly at work, even while we are asleep. Today, regular physical activity is considered an essential component of a healthy lifestyle, which will benefit not only the physical but also the brain health of an individual.

Scientists have been looking for the effects of daily exercise on the brain for years; although today this research is still not complete, some recent studies have shown that regular intense or

moderate exercise activities affect the brain tissue in different ways. More production of brain-building hormones, fighting stress and depression, and preventing and reducing the symptoms of dementia, are small sets of effects of exercise on the brain. One of the most recent studies that have piqued the interest of experts is the shift in brain energy metabolism after exercise.

Numerous studies have demonstrated that lactate, once thought to be a redundant product with no particular function in the brain, is now recognized as a replacement for glucose in the fuelling of neurons. It has even been demonstrated to be preferable to glucose during vigorous exercise.

Finding the link between the energy metabolism of the muscles and the brain after exercise is an issue that currently interests academics. However, because of the brain's complicated and unknown structure, they are limited in detecting and understanding the destiny of brain lactate and glucose. Therefore, they have inevitably used aggressive and indirect methods for this purpose. Risks associated with invasive procedures are often unpleasant. On the other hand, indirect assessment of metabolites involved in energy metabolism raises the likelihood of mistakes in findings since there are several unknown pathways in brain metabolism, and our knowledge about them is restricted. Another gap that prevents statistical analyses from being valid is small sample sizes or unisex samples.

As a result of these constraints, we propose a data collecting framework to investigate MRS and PET imaging techniques for the quantification of cerebral and plasmatic lactate and glucose in humans following moderate-high intensity exercise, as well as a data analysis framework to develop brain energy metabolism analyses. Chapter 4 deeply discusses the aforementioned imaging methods, statistical tests, and models for examining brain energy metabolism.

Chapter 3

Conceptual Framework

This chapter presents a planned arrangement of research components to highlight the strategy that lends significance to our research. This chapter depicts the expected relationship between the variables or characteristics we wish to explore. For this purpose, we first examine the most relevant variables in this study and discuss their central role in different aspects of the research. After developing a practical understanding of each variable and its applications, we focus on relationships and how communication breaks, overlaps, and tensions between them based on the literature review completed on the research in chapter 2, as well as our expertise. Finally, by connecting all the parts, we provide a scheme that summarizes the details of the research and helps draw a coherent conclusion.

3.1 Glucose

For metabolic life to continue, every cell in the human body needs the energy to perform metabolic tasks. Glucose is a cellular fuel that serves as the primary energy source, especially for the brain, muscles, and several other body organs and tissues [110]. In fact, the term ”blood sugar,” which we are all familiar with, indicates glucose concentration in our blood. Many body cells use glucose with compounds such as amino acids and fats, while brain cells or nerve cells recognize glucose as their primary food source.

3.1.1 Fate of glucose in muscles

This chemical is one of the essential substances needed by the body [111]. Usually, most of it is absorbed by the muscles and burns immediately. After the cells of the body consume all the glucose they require, the remaining glucose is stored in the form of small packages called glycogen in the liver and muscles of the body [112]. In this way, our body can hold the amount of glucose it needs to release in the blood to maintain the average blood sugar level when we exercise or sleep [113].

Glycolysis

When the cells have exhausted all their glucose and do not receive more glucose from the body, they can turn to the cell's glycogen reserves for the energy they need through glycolysis. Glycolysis is the process in which glucose is broken down into pyruvate to produce energy. This process is used by all body cells to produce energy, which has two pathways: aerobic glycolysis and anaerobic glycolysis.

If glycolysis is done in the presence of oxygen, it is called aerobic glycolysis, and if it is done in the absence of oxygen, it is called anaerobic glycolysis. Based on figure 3.1, the final product of aerobic glycolysis is pyruvate, which enters the Krebs cycle to make additional energy, which produces carbon dioxide and water as side products in addition to Adenosine triphosphate (ATP). In contrast, anaerobic glycolysis produces lactate [111, 114].

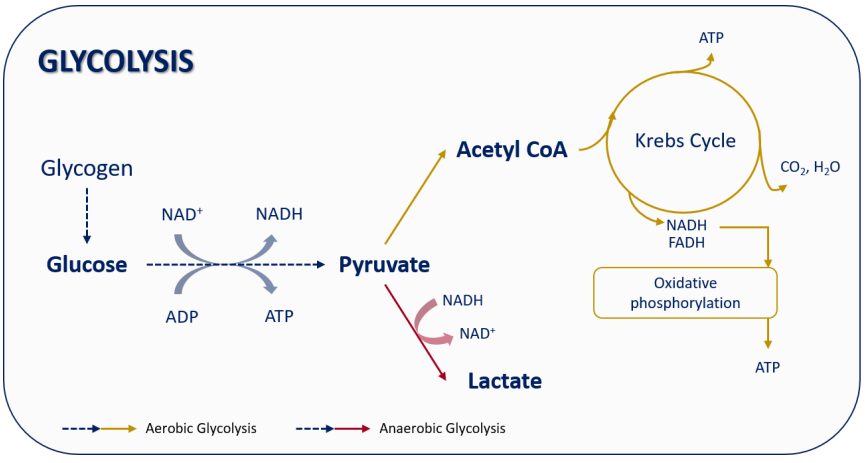


Figure 3.1: This diagram shows the two aerobic and anaerobic glycolysis pathways as well as the Krebs cycle.

3.1.2 Fate of glucose in the brain

The brain relies exclusively on glucose to meet its energy needs during the non-active state. Because the central nervous system, of which the brain is the most important component, cannot store glucose and has a high energy need, it is extremely dependent on the quantity of glucose found in circulation. Blood glucose enters the brain as a nutrient passing through the blood-brain barrier (BBB). The metabolic destiny of glucose in the brain is determined by cell type and the selective expression of metabolic enzymes. While astrocytes are mostly glycolytic, neurons are primarily oxidative [115].

Neurons cannot store glucose in the form of glycogen, so according to Figure 3.2a, each glucose molecule undergoes glycolysis to become pyruvate, which then enters the Krebs cycle to produce carbon dioxide, water, and ATP molecules. In astrocytes, glucose is preferentially stored as glycogen or processed by glycolysis, where the resulting pyruvate is then transformed into lactate (Figure 3.2b) [116].

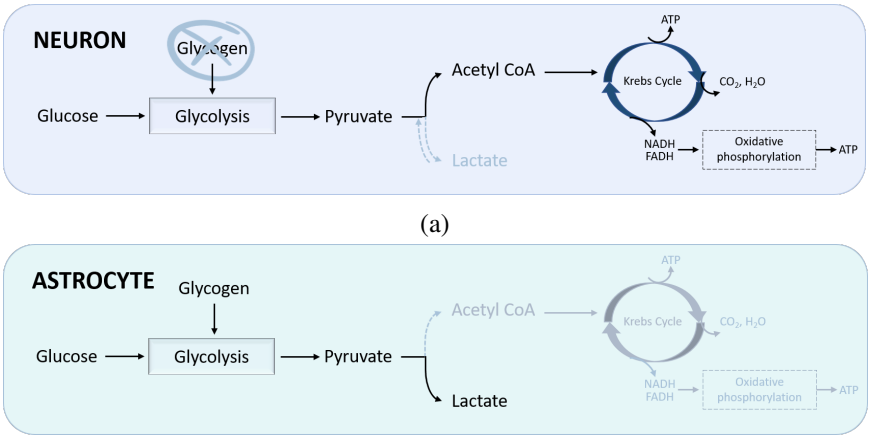


Figure 3.2: Glucose metabolism pathways in the brain. (a) Fate of glucose in neurons. Neurons do not store glucose as glycogen and their metabolic pathway is mainly **oxidative**. (b) Fate of glucose in astrocytes. Glucose can be stored as glycogen in astrocytes or participate directly in the glycolysis process. Unlike neurons, the metabolic pathway in astrocytes is mainly **glycolytic**. (The gray paths are not the main and preferred paths of the cells.)

3.2 Lactate

When the body is engaged in physical exercise, it prefers to receive its energy from aerobic activity (oxygen). However, when the exercise's intensity surpasses its capacity to carry oxygen, and the body is unable to deliver enough oxygen to fulfill its demands, anaerobic glycolysis takes over as the primary source of ATP and energy generation [116]. Following anaerobic glycolysis (Figure 3.1), much lactate is released into the body. Lactate has long been thought to be a waste product of glycolysis that causes fatigue, pain, or muscle damage [117]. Later studies clarified that the primary reason for muscular soreness following exercise is the accumulation of lactic acid and the inability to eliminate it instead of lactate. Dr. George Brooks deserves credit for influencing people's perceptions about lactate. Brooks and his students demonstrated in the 1970s that lactate is not a waste product but a fuel created by muscle cells, which are one of the body's primary fuels for generating energy. The brain and heart will function better if they receive their energy from lactate rather than glucose. Additionally, according to Brooks, the body uses lactate for three fundamental purposes: as one of its major fuel sources, to control blood sugar levels, and as a reliable marker of stress tolerance [118]. Studies on lactate generally demonstrate that lactate plays a significantly more prominent role than previously believed. Lactate is a mystery chemical that requires further investigation to understand its purpose in the body.

3.2.1 Fate of lactate in muscles

Lactate enters the bloodstream after being created by skeletal muscle or other tissues and is eventually absorbed by the liver. In the liver, lactate is converted to glucose under the Cori cycle. In Cori's cycle, lactate is first converted to pyruvate and then back into glucose under the process of gluconeogenesis [119], which is given to the muscle and can assist in maintaining blood glucose levels (Figure 3.3). According to another theory known as the lactate shuttle, when lactate leaves the muscle and enters the blood, it can not only enter the Cori cycle in the liver but can also be used in other tissues, including inactive skeletal muscle, the heart, and kidneys, to produce glycogen or be converted into pyruvate, which can then enter aerobic metabolism [118].

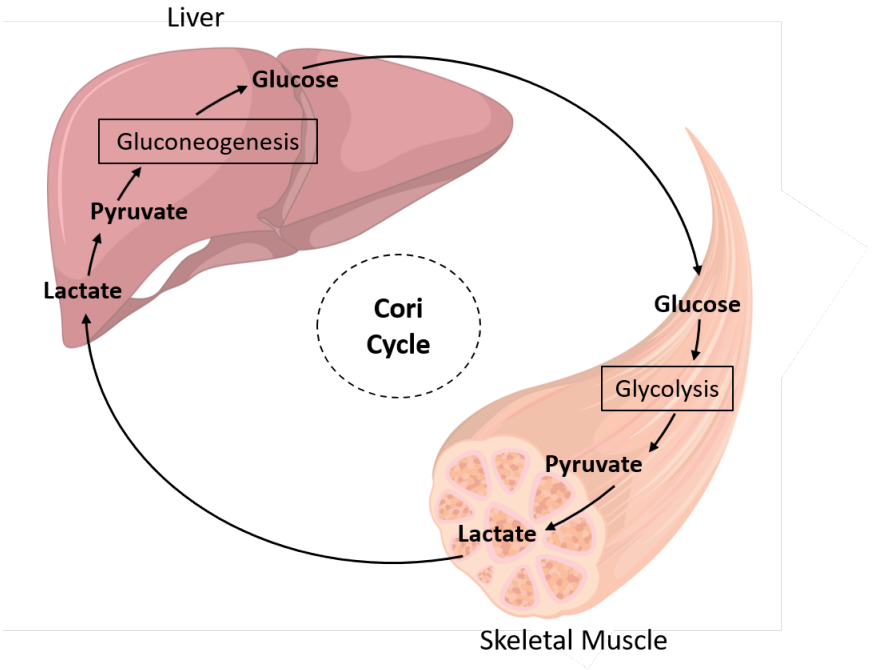


Figure 3.3: Cori Cycle

3.2.2 Fate of lactate in the brain

Brain lactate levels generally increase in two ways: from peripheral skeletal muscle and the central nervous system.

Specifically for lactate derived from skeletal muscle, we demonstrated that lactate, after production, leaves the muscle and enters the bloodstream. Under the action of MCT, a portion of blood lactate can cross the BBB, enter the brain, and disseminate in the extracellular space. Previously, the buildup of lactate in the brain was thought to be the source of exercise-induced central tiredness. However, studies evaluating the role of lactate confirmed that circulating blood lactate is the primary energy substrate during exercise, as evidenced by increased lactate utilization and decreased glucose absorption. On the other hand, in Figure 3.2b, we showed that circulating glucose may be taken by astrocytes and even stored as glycogen. Following the increase in the intensity of sports activities and a rise in the energy demand by neurons, the glycogen in astrocytes is quickly broken down into lactate under glycolysis and released into the extracellular space [103, 120]. Pellerin and

Magistretti described this mechanism as the astrocyte-neuron lactate shuttle (ANLS), in which astrocytes produce lactate and neurons take it as a necessary supplement in the substrate of energy metabolism [121]. Therefore, exercise raises extracellular lactate levels in the brain by increasing blood lactate and lactate produced by astrocytes (Figure 3.4). According to research by Schurr et al., lactate can take the place of glucose as the only substrate for neurons' energy metabolism [122]. Therefore, lactate in the extracellular space is carried by MCTs into neurons, where it is transformed into pyruvate by the activity of lactate dehydrogenase 1 (LDH1). Finally, pyruvate is converted into acetyl-CoA, which enters the Krebs cycle and results in the creation of ATP [120], as shown in figure 3.2a.

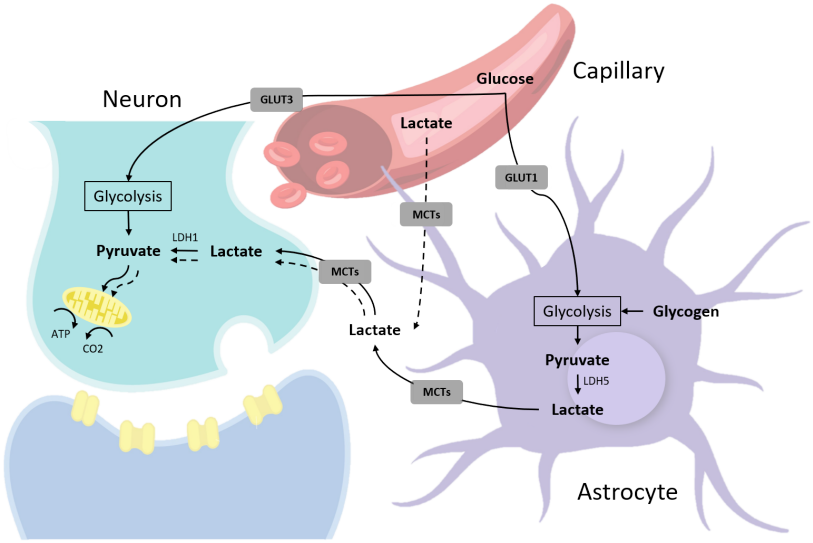


Figure 3.4: The hypothesis of astrocyte-neuron lactate shuttle

3.3 Conclusion

Although there has been substantial progress in recent years in our knowledge of lactate's role and function as a substrate for neurons' energy metabolism, there is still a great deal of mystery surrounding the lactate metabolism system. We addressed the impact of exercise on the body's and brain's energy metabolism from various perspectives in light of the literature review. Although the effects of exercise on skeletal muscles are practically understood, there are still many unknowns

about the brain's energy metabolism due to its complexity. Previous studies have had several limitations in recording and measuring brain glucose and lactate in humans following exercise; hence most conclusions concerning energy metabolism were either based on animal experiments or as a result of measuring the difference in arterial lactate concentration in humans. Since it cannot be confidently claimed that animal results can be extended to humans and that human measurements are not accurate enough, we still do not have a clear understanding of the astrocyte-neuron lactate transport system.

Figure 3.5 depicts a conceptual framework of the predicted relationship between the variables under consideration based on information gained through reviewing the literature and assumptions established in our minds. In summary, this framework demonstrates that body energy consumption rises in skeletal muscles and the brain after exercise, which can be linked. Skeletal muscles enter the anaerobic pathway to provide energy after heavy activity. Lactate is generated and delivered into the bloodstream via the anaerobic route. Some of the blood lactate reaches the liver and is converted back into glucose, while the remainder is utilized as fuel for other organs such as the heart and brain. It is released into the extracellular environment after entering the brain and used as fuel by neurons. We anticipate that the brain has a second lactate generation mechanism operating in parallel. In this manner, astrocytes absorb glucose from the circulation, convert it to lactate, and then spread it into the extracellular space, where it is taken up as neuron fuel.

Therefore, according to the mentioned situations, we expect a decrease in brain glucose absorption and an increase in brain lactate concentration after exercise compared to rest. It is also hypothesized that there would be a link between decreased glucose absorption and increased lactate use in the brain.

In order to evaluate the proposed hypotheses, in this study, we have designed an experiment in which we investigated imaging techniques to determine the amount of brain and plasmatic lactate and glucose in humans and developed a data analysis framework to investigate brain energy metabolism.

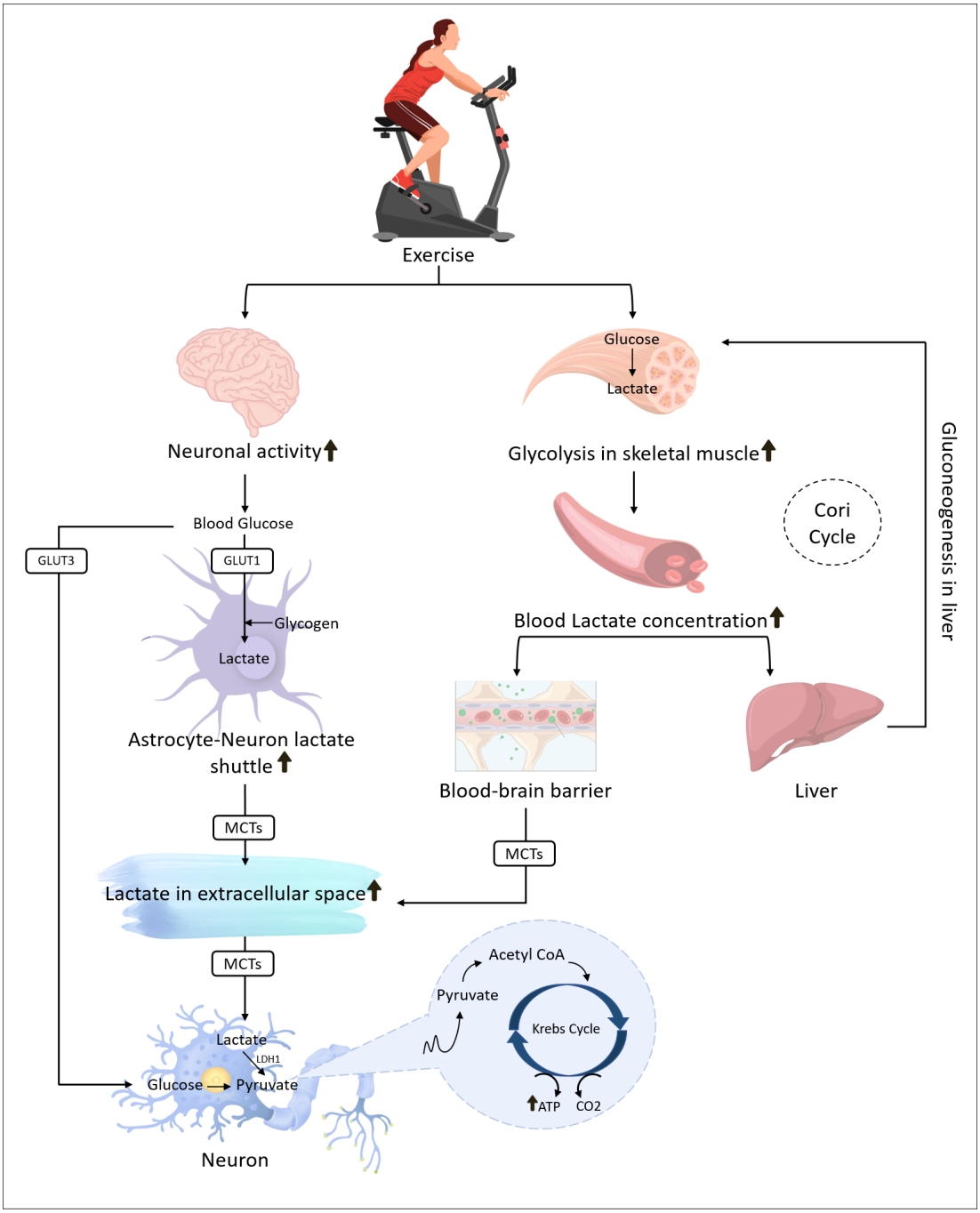


Figure 3.5: Schematic diagram of how exercise increases brain lactate concentration in two ways: skeletal muscle-derived lactate and astrocyte-derived lactate. In the first pathway, the glycolysis process increases following exercise, which leads to more lactate production in skeletal muscles and an increase in its concentration in the blood. Then part of the lactate released in the blood is converted into glucose again in the liver through the Cory cycle. Another part of it passes through the blood-brain barrier and is released into the extracellular space and becomes available to neurons. In the second path, according to the Astrocyte-Neuron lactate shuttle theory, the lactate produced in the extracellular space is released and becomes available to the neurons.

Chapter 4

Data acquisition and Analysis Method

Following the definition of the research topic in Chapter 1 and the examination of the literature in Chapter 2, I will address the methodological section of the thesis. This chapter provides a chance to legitimize the research and highlight the philosophical foundation of the study. We first introduce the research objectives. Then we share complete details about our data collection methods. Then we describe how to process and analyze the research data. Finally, and most importantly, we clearly state the reason for choosing the methods that we have done, and we emphasize the superiority of the strengths of this approach over other methods.

4.1 Methodological Approach

A growing body of research has documented the many advantages of exercise, including the function of physical activity in promoting brain health. Now, researchers are looking to make important discoveries about how exercise fuels the body, especially the brain.

Decades of research in the field of metabolic regulation in sports led to the claim that lactate is the primary energy source, nicknamed ”lactormone” [123]. Lactate was once assumed to be a waste product of anaerobic metabolism, which is the leading cause of pain and fatigue after exercise. Contrary to the initial idea, lactate is known as a preferred substrate over glucose as a fuel for the brain and other organs [117].

In the studies conducted on fueling the body, considerable increases in lactate relative to glucose flux are observed in the transition from rest to exercise, which is used as fuel for skeletal muscles, kidneys, heart, and brain [123, 124]. In the early 1950s, Henry McElwain showed that lactate is an efficient fuel for the brain [125], hose findings have supported Schurr and others for decades [126, 127]. In general, it has been well proven that glucose serves as the brain's primary fuel source in people at rest, and its absorption is entirely reliant on cerebral blood flow and the difference in arterial-venous glucose concentration [110]. In most circumstances, glucose transfer to the brain is high compared to the corresponding values of lactate in the resting state, and lactate has a negligible contribution to supplying the brain with fuel. However, the importance of lactate in fueling is discussed following exercise. Studies on healthy individuals and animals have demonstrated that blood lactate levels rise during intense activity and reach the brain across the BBB, which is preferred over glucose and supplies the energy required by the brain. On the other hand, according to the ANLS hypothesis, with the increase in exercise intensity and the activity of neurons, lactate is produced by astrocytes in the brain environment and absorbed and consumed by neurons [103, 120, 128]. As a result, exercise is involved in brain energy metabolism by increasing brain lactate concentration and reducing brain glucose absorption.

Research on lactate aimed to elucidate the pathways and controls of lactate formation and elimination before, during, and after exercise. This work has covered both in vivo and ex vivo research on humans and animals. Most of the tests were indirect and invasive and used classical measurements of arterial-venous difference.

Since the brain is a complex organ, an experimental approach that allows direct and non-invasive measurement of glucose metabolism and lactate concentration in the brain is necessary. This will help us better understand the mechanisms underlying the observed changes in brain energy metabolism during exercise, which could lead to better-defined interventions with exercise or other approaches in patients with various neurological disorders.

In this quantitative experimental study, we aim to generate generalizable knowledge about the effects of exercise on brain energy metabolism by examining imaging techniques to quantify cerebral and plasmatic lactate and glucose in humans. This necessitates a properly planned study under controlled conditions that other researchers can replicate. Also, developing a framework for data

processing and analysis is another goal of this study.

4.2 Data Acquisition Framework

After briefly and clearly stating the research hypotheses in Chapter 1, to test whether exercise alters brain glucose utilization and brain lactate levels, we set out to design a comprehensive experiment with standardized protocols to maximize the reliability and accuracy of our data. In the first place, based on the literature review and the use of current knowledge, we determined the best method to evaluate our hypotheses in a way that removes the limitations of the previous methods. Finally, we considered each approach's feasibility and participants' availability to ensure that we could complete the required data collection. Since this study aims to obtain information that may be important in designing interventions to help older people prevent the progression of serious diseases (neurodegenerative conditions, among others), the recruitment and withdrawal policies of the subjects were designed in such a way that their presence leads to scientific advancement and none of them feel pressured at any point. Appendix A provides comprehensive information on how participants were recruited, including the survey, inclusion criteria, and exclusion criteria. In short, the survey form, which consists of two parts, the adult medical history form and the Get Active questionnaire, is designed to determine the target population. Based on that, we have selected volunteers by considering three criteria: body mass index (BMI), history of regular aerobic exercise, and medical records. In addition, although it is possible for the volunteer to withdraw at any stage of the study, there are also limitations defined by the researchers that prevent some people from entering the project.

Furthermore, despite the fact that all the procedures used in this protocol are well established and safe, it has been approved by the *Research Ethics Committee of the Ministry of Health and Social Services* for the safety of the subjects.

4.2.1 Sample Size

One of the initial phases in the design of a study is determining the sample size. The optimal sample size is an essential component of any research, with the help of which the study's hypotheses

are answered. Since it is impossible to study the whole target population, studies are done on samples, and finally, the results are generalized to society.

Here, to test whether exercise changes brain glucose consumption and brain lactate levels, we studied 29 healthy and physically trained people, including 13 females and 16 males aged 25 to 45 years. A summary of the participants' information is provided in Table 4.1, and more intricate details of each are included in Appendix B.

Table 4.1: Summary of participant information

Study	N	Sex	Age	Height (m)	Weight (kg)	BMI (kg/m2)	Training Hour (min/week)
Brain Energy Metabolism	29	n_{female} = 13 n_{male}= 16	M= 33.07 SD= 5.74 Min= 26.00 Max= 45.00	M= 1.73 SD= 0.09 Min= 1.54 Max= 1.87	M= 70.36 SD= 12.61 Min= 53.20 Max= 95.00	M= 23.32 SD= 2.41 Min= 18.46 Max= 28.09	M= 235.76 SD= 120.97 Min= 60.00 Max= 600.00

4.2.2 Experimental Design

As discussed in detail in previous chapters, glucose and lactate molecules are very important for energy production in the brain. Previous studies have shown how the brain generates energy to operate changes during high-level exercise. According to the presented results, glucose, the brain's primary fuel at rest, is replaced by lactate with the intensity of sports activity. However, with our limited knowledge of the complex structure of the brain and the limitations of previous research in recording data, we need to understand how this happens entirely. If we know the pathways and how the energy metabolism of the brain follows exercise, we may be able to improve the brain metabolism of patients with a wide range of brain diseases by providing an exercise program and, as a result, improving their condition. In this regard, we suggest using specialized PET and MRS imaging methods to measure specific aspects of how the brain uses glucose and lactate.

This project was carried out in the PERFORM research centre of Concordia University with the help of skilled technicians of this complex in two stages rest and exercise.

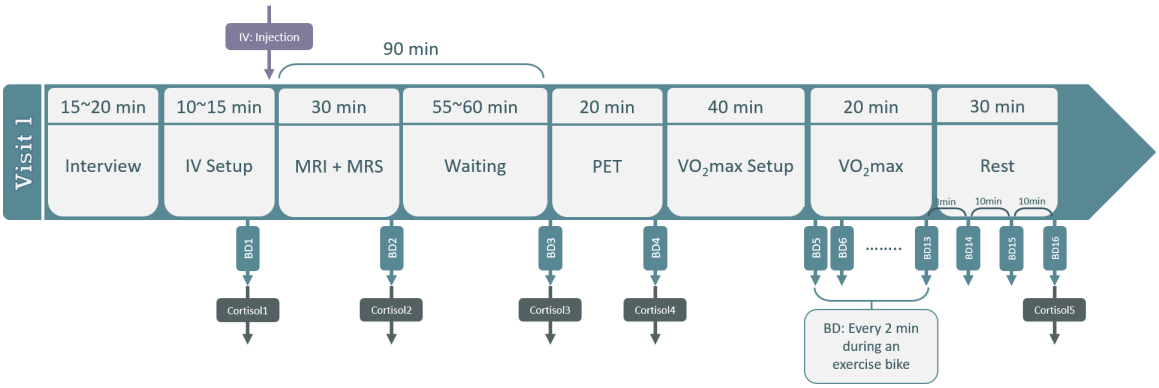
During the first visit, as shown in Figure 4.1a, participants undergo MRS imaging to measure brain lactate and 18FDG PET to measure brain glucose at rest. The MRS study takes approximately 45 minutes, during which two regions of interest (ROI), the supplementary motor area (SMA) and the posterior cingulate cortex (PCC), are imaged, and the order of acquisition for the two ROIs is

also tried to counterbalanced across subjects to avoid systematic order effects. However, a PET study is possible 90 minutes after 18FDG injection and takes about 20 minutes. On the same day, the subject also undergoes an exercise study on a stationary bike, which allows us to measure their exercise anaerobic threshold to determine exercise intensity at the second visit. Also, in order to check plasma lactate and glucose, several blood samples and saliva samples (between 13 to 15 blood samples and five saliva samples) are collected at regular intervals. For blood collection, a plastic catheter is installed in the arm's vein so that only one venipuncture is needed for blood collection. The same catheter is also used to inject the radioactive tracer for PET imaging. Sterilized absorbent swabs are also used for saliva samples, so the subject is asked to keep the swabs on the cheek until they are completely wet and then return them to the particular container. In addition, before any injection or PET scan, a rapid strip pregnancy test is performed on female subjects. If the result is positive, the participant is removed from the project. At different stages of this visit, the participants also fill out four behavioral questionnaires to evaluate the differences in stress and the state of physical fitness (see Appendix C). The first visit lasts about 4 hours in total.

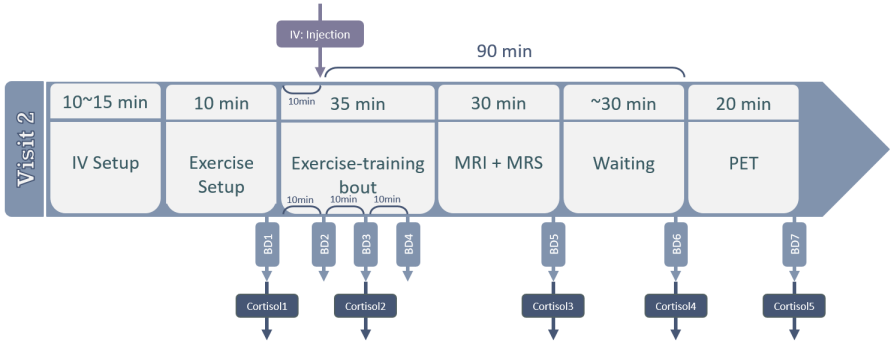
The second visit is done at an interval of 2 to a maximum of 14 days after the initial measurements. Subjects are returned to measure the same parameters, but this time in the training condition. The only significant difference with the previous visit is that before any imaging, the participants participate in a practice test whose protocols follow the results of the first practice test. The parameters of this exercise are adjusted based on the initial measurement of the subjects' ischemic threshold in such a way as to ensure that they reach that threshold relatively quickly (within 10 minutes) and remain there for 25 minutes. Unlike the first visit, the radioactive tracer (18FDG) is injected when the subjects reach their ischemic threshold (10 minutes after the start of exercise) while exercising on a stationary bike (Figure 4.1b). Immediately at the end of the training, other steps related to MRS imaging, PET scan, blood sampling, and a saliva sample are performed the same way as the previous step. Including all these steps, the second visit of this project takes about 3 hours.

The study procedure in this project is summarized as follows:

- Exercise test and determination of ventilation threshold
- Measurement of blood lactate and cortisol levels



(a)



(b)

Figure 4.1: Schematic of the lactate project. (a) Visit 1 is under the rest state and measures the baseline parameter, duration is 4 hours. (b) Visit 2 is under the exercise state and evaluates the same parameters, duration is 3 hours.

- 18FDG PET measurement of cerebral glucose utilization
- MRS determination of brain lactate concentration
- Behavioral questionnaires to assess differences in stress and physical fitness states

Posterior Cingulate Cortex (PCC)

The posterior cingulate cortex (PCC) is one of the unknown areas of the cortex, which is located behind the anterior cingulate cortex and forms part of the posterior middle cortex (Figure 4.2a) [129]. The PCC is a key node in the default mode network (DMN) and is anatomically

highly connected, suggesting that it is a major hub in human connectivity [130]. Non-human studies have documented several structural connections for PCC that are less well documented in humans. Despite its importance in health and disease, there is considerable uncertainty about PCC function. However, studies show that the PCC is involved in various roles and has strong connections with areas involved in learning, emotions and social behavior, and attention [131, 132].

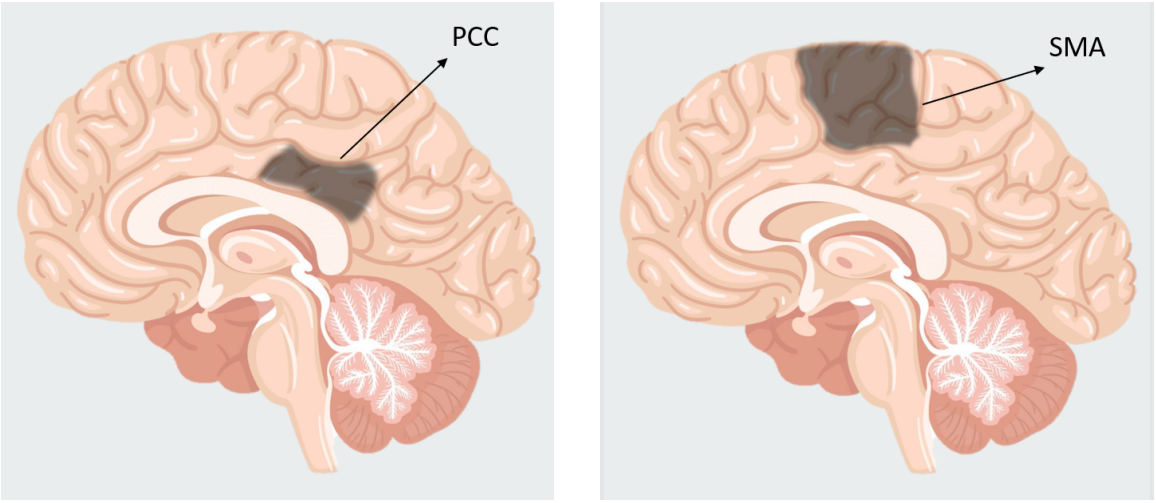
In addition to structural analyses that highlight the PCC's critical role in the cortical organization, imaging studies have shown that the PCC has very high metabolic activity both at rest and during cognitively challenging tasks that are highly dependent on glycolysis. In the PCC, cerebral blood flow and metabolic rate are about 40% higher than they are throughout the entire brain. Almost all other brain regions have lower blood flow levels than PCC, even when PCC activity is considerably reduced [129, 133]. In this study, we have chosen the PCC because, unlike the SMA, it is not involved in the motor cortex to check whether the changes in brain energy metabolism are only limited to the areas involved in the motor system or are the same in the whole brain. In addition, since the PCC is a key node and central hub in human connections, it is a suitable choice to observe changes in energy metabolism compared to other regions in the brain. More information on the structure and function of the PCC is available in the Handbook of Clinical Neurology [132].

Supplementary motor area (SMA)

The supplementary motor area (SMA), which consists of the caudal and rostral sections, is situated on the superior and medial sides of the superior frontal gyrus (Figure 4.2b) [134]. Neurons in the SMA go directly to the spinal cord and are responsible for planning and controlling complex movements. Although the exact role of the SMA is still not fully clear, human and non-human studies show that the SMA appears to be critical in the selection, initiation, and control of some voluntary movements, such as stabilization of body position while walking or standing, coordination of opposite limbs, and control the temporal sequences of movements [135–137].

In addition, the measurements made on cerebral regional blood flow or cerebral energy metabolism in humans confirm that SMA is the largest areas of the motor cortex in such a way that performing complex voluntary movements leads to an increase in blood flow in this area [138–140]. Therefore, the most straightforward interpretation that supports this phenomenon is that the energy demand

in the SMA increases following an increase in neuronal activity. Also, considering that SMA covers a large area, it is a suitable option for data collection compared to other areas involved in the brain's motor cortex. More details on the structure and function of SMA are available in the review provided by Goldberg [141].



(a) (b)

Figure 4.2: (a) Posterior cingulate cortex location: PCC borders the Brodmann's area 24 (anterior), the parietal-occipital sulcus (posterior), the marginal ramus of the cingulate sulcus (above), and the corpus callosum (below). (b) Supplementary motor area location: SMA borders the leg area of the primary motor cortex (posterior), pre-SMA (anterior), part of the cingulate gyrus (inferior), and dorsal premotor cortex (lateral).

Magnetic resonance spectroscopy (MRS)

Magnetic resonance spectroscopy (MRS) can be named the twin method of the famous magnetic resonance imaging (MRI) (Figure 4.3a). MRS, like MR imaging, is a non-invasive analytical technique used to quantify metabolites inside the body, especially the brain [142, 143]. While MRI provides anatomical information, MR spectroscopy provides chemical and quantitative information that is useful in clinical research and increases the understanding of the pathology of the brain, prostate, breast, and other human organs. Meanwhile, most research focuses on the human brain and identifies tissue alterations in Parkinson's and Alzheimer's disease, epilepsy, and stroke [144, 145].

MRS technology is performed on the same MRI scanners capable of single-voxel (SV) and multi-voxel (MV) spectroscopy and is defined as a series of tests that are added to an MRI scan

of the brain or spine. Finally, it produces a ”spectrum” of information about the chemicals present and their relative concentrations [144, 146]. This spectrum can be obtained from chemical elements such as hydrogen ions or protons. However, proton spectroscopy is more commonly used due to its high natural abundance [147]. The acquired spectrum is typically in the Digital Imaging and Communications in Medicine (DICOM) format, containing raw (unprocessed) data. In the raw data, water produces a much larger signal (4.7 ppm) than other metabolites, which makes the spectrum dominated by water and the rest of the spectra invisible. Therefore, removing the water peak is a part of any MRS sequence; if not done correctly, the relative height of the peaks will change [148, 149]. Finally, after performing the necessary pre-processing, the spectra are stored in the defined data format so that they can be processed in the data analysis software to investigate a relevant objective.

(a) (b)

Figure 4.3: (a) Magnetic resonance spectroscopy machine. (b) Positron emission tomography scan Machine. Both devices are in the Perform Center’s imaging unit.

The number of peaks in the spectrum - each of which represents a metabolite - is determined based on the echo time (TE) [150], while the unit of measurement of their frequency is parts per million (ppm) [143] (Figure 4.4). Among the most common peaks in the MR spectrum, we can

mention lipids (at 1.3 ppm), lactate (at 1.33 ppm), glutamine/glutamate (at 2.2-2.4 ppm), creatine (at 3.0 ppm), which are drawn in a graph with different heights [151].

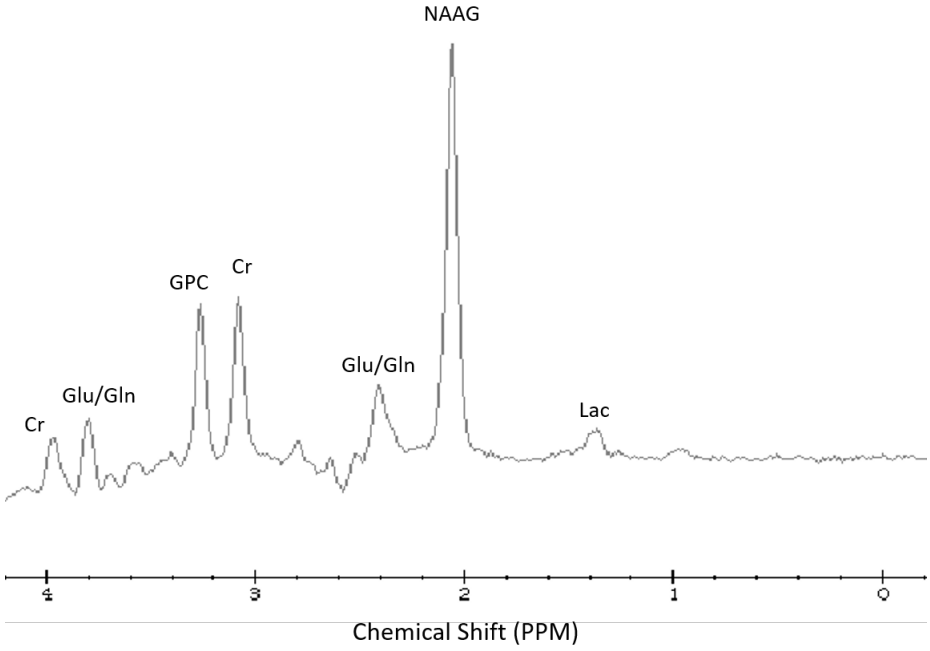


Figure 4.4: Magnetic resonance spectroscopy: The spectrum shows peaks for the following metabolic products: Lactate (Lac), N-acetyl-aspartyl-glutamate (NAAG), glutamate (Glu), glutamine (Gln), Creatine (Cr), and Glycerylphosphorylcholine (GPC).

In addition to being non-invasive and capable of measuring several metabolites simultaneously, this valuable method is very safe, and the magnetic field or radio waves used in the device do not threaten health. However, this imaging technique requires observing some points to guarantee its safety. Some of the essential issues are as follows:

- No pins, metal plates, or other implants inside the body
- No pacemaker or artificial heart valves
- Remove the hearing aid
- Absence of skin tattoos

- No history of diabetes or kidney problems
- Absence of an implanted drug injection device (insulin pump) or an intrauterine device

In general, the presence of any metal material in the body reduces the quality of the images and changes the obtained results. Also, since the basis of the operation of this device is based on a strong magnetic field, the presence of any metal disrupts the work and harms the patient [152]. The FDA has published a new guidance document - *Testing and Labeling of Medical Devices for Safety in the Magnetic Resonance (MR) Environment* - that provides more information on what is and is not allowed in the imaging environment.

Positron emission tomography (PET)

Positron emission tomography, which is also called PET scan for short, is a type of modern imaging study that has research applications in diagnostic sciences in medical physics, especially nuclear medicine (Figure 4.3b). By examining blood flow and body metabolism, this method provides information on the metabolic or biochemical function of tissues and organs, which can be important clues about how diseases occur [153, 154].

Since PET is a type of nuclear medicine method, it requires using small amounts of radioactive substances known as radiotracers to examine the tissue being studied. Radioactive tracers attach a radioactive atom to chemicals naturally used by a particular organ or tissue during its metabolic process [155]. Depending on the purpose of the scan, different tracers are used in imaging, of which F-18 fluorodeoxyglucose (FDG) is the most common, which is widely used in brain PET scans - because the brain uses glucose for its metabolism [156].

The tracer is often injected into a vein in the hand or arm through an intravenous (IV) catheter; then, after about 30 to 60 minutes, it accumulates in areas of the body with higher metabolic activity levels. The tracers can be recognized by the gamma rays they emit when passing through any part of the body (Figure 4.5). The movement speed of these compounds and their absorption rate by different tissues provide valuable information about the physiology and activity level of the tissue, which is a sign of its metabolism change [157]. The gamma rays produced by the tissues are

recorded by the scanner and analyzed by special computers. The final result appears as three-dimensional color images of the tissue on the computer screen.

Therefore, PET is a non-invasive imaging method, except for intravenous injection, which is usually painless. This technique is effective for diagnosing or treating brain abnormalities, types of cancer, and heart disease [156]. Also, PET can detect the onset of the disease before it is seen by other imaging methods such as magnetic resonance imaging (MRI) and computed tomography (CT) [158]. In newer technologies, PET is combined with other imaging techniques, CT and MRI, and is known as PET/CT and PET/MRI, respectively. Combining the images allows the doctor to interpret the results of two tests in a single image containing more accurate information [159].

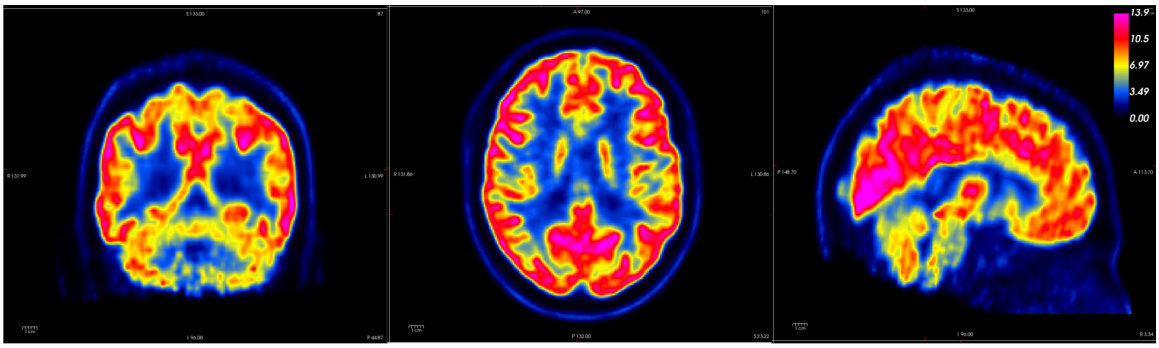


Figure 4.5: 18F-FDG Positron emission tomography scan of a healthy brain

Although PET scan includes radioactive tracers, due to the small amount of radiation in the tracer, this method is almost safe and has few risks and side effects. In addition, it is necessary to refrain from vigorous physical activity, such as exercise 24 to 48 hours before the test, and refrain from eating and drinking alcoholic beverages for a few hours before the scan; otherwise, the distribution of the PET tracer in your body may change, resulting in a suboptimal scan [158].

4.2.3 Data acquisition

Before starting any data collection on the first day of the experiment, a short interview will be conducted in person with the participant (see Appendix C). After the interview and before starting the primary protocol, the heart rate and blood pressure at rest, as well as the height and weight of the subjects, are measured. In addition, the subject must fast for at least 4 hours before the test, and

in order to make sure of this issue, the subject's blood sugar is also measured at the beginning of both visits. Also, a rapid pregnancy test helps us to prevent pregnant women from participating in the project. More information on this is provided in Appendix C.

Exercise test and determination of the ventilatory threshold

As mentioned, this research project consists of two stages: resting and exercise conditions.

Therefore, during the first visit, after recording the required data at rest, a cardiopulmonary exercise test (CPET) with limited symptoms is performed to identify the ventilation threshold (VT) and maximal oxygen consumption (VO2max) in order to determine the training intensity of each subject in the second stage.

VT refers to the point during exercise where carbon dioxide (CO2) extraction occurs at a faster rate than the consumption of oxygen (O2). An individual's threshold is said to reflect anaerobic levels and lactate accumulation. In fact, VT indicates a level of blood lactate accumulation that accumulates faster than it can be cleared, causing the person to breathe more quickly to expel excess CO2. This threshold occurs in a percentage of the VO2max of athletes based on their training status, around 50-60% VO2max in untrained people and approximately 70-80% VO2max in trained people.

During CPET, subjects perform a 3-minute non-resistance warm-up on a stationary bicycle after 3 minutes of rest while connected to a 12-lead electrocardiogram and connected to a respiratory circuit through a face mask (Figure 4.6). After the warm-up, the resistance is gradually increased up to the maximum capacity of the participant. Meanwhile, gas exchange parameters (oxygen consumption, carbon dioxide excretion, minute ventilation), heart rate, and blood pressure are measured and monitored. In addition, every three minutes, the rate of perceived exertion (RPE), which counts breathlessness, dyspnea, chest pain, and musculoskeletal pain, is evaluated using the Borg scale. Also, as shown in Figure 4.1a, every two minutes after pedaling, a blood sample is taken from the subject to check plasmatic lactate and glucose levels.

The protocols of this exercise are adjusted based on the initial measurement of the ischemic threshold of each participant so that first, they are given 5 minutes to warm up; then, in the second 5 minutes, by increasing the pedaling power (Watts), we guide the subject to reach 80% VO2max. During the next 25 minutes, the subjects are expected to pedal at the same power. Still, if the

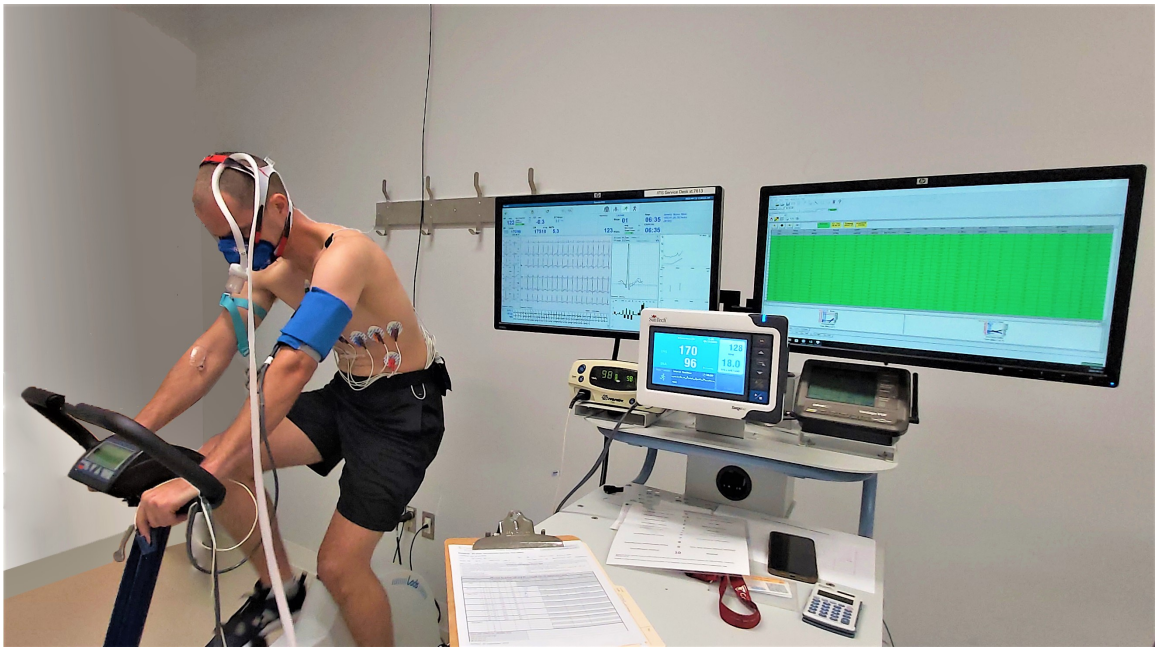


Figure 4.6: Determination of the ventilatory threshold and VO2max during the first visit at the PERFORM Cardiopulmonary Suite

participants cannot continue with such intensity, we will reduce that for a few minutes. If the participant's physical condition allows, we will increase it again. However, throughout the training, the participant is encouraged to continue with all the energy, and every 5 minutes, the power of the bike (watts), intensity and number of pedal revolutions per minute (RPM), and the number of heartbeats per minute (BPM) are recorded. In addition, to evaluate the plasma lactate level, a total of 3 blood samples are taken at 10, 20, and 30 minutes from the start of exercise. Also, in order to maintain the subject's safety and increase accuracy in the data collection process, an accredited sports physiologist supervises all CPET stages and sports training competitions.

Blood lactate and cortisol levels measurements

In order to evaluate blood lactate and glucose levels and changes after exercise, it is necessary to take several blood samples at set time intervals. For this purpose, we use an intravenous (IV) plastic catheter installed in the hand or arm vein so that only one venipuncture is needed to collect blood samples. At each time point, only 2 ml of venous blood sample is taken in a vacuum tube. Samples are centrifuged for 15 minutes, and the collected plasma is immediately stored at -80°C.

The compounds in the plasma are extracted and analyzed by expert technicians.

In addition, cortisol, a hormone effective in measuring stress levels, is collected from saliva several times during the experiment using Salivette™. This saliva collection system has a sterile absorbent swab that participants hold on their cheek until it becomes wet (about 60 seconds) and return the swab to the tube without touching it. It is necessary for the participants to refrain from consuming alcohol the day before the test so as not to interfere with the test. The samples are immediately frozen at -80°C, then thawed at the time of analysis and centrifuged at four °C for 10 minutes. The relevant technicians perform these steps, and the desired parameters are extracted.

18FDG PET measurement of cerebral glucose utilization

Positron emission tomography is used in research to observe the functions of different organs in 3 dimensions. In this study, we use PET to examine the brain metabolism of the participants in the two areas of interest, PCC and SMA. A radiotracer called 18F-FDG is administered through a catheter implanted in a vein in the arm. When used in conjunction with PET, this radiotracer allows researchers to evaluate how various brain regions utilize glucose. For this purpose, the subject must fast for at least 4 hours before the test because the distribution of the radiotracer in the body may change, and the desired results may not be obtained.

For the first visit, the resting part of the study, we inject a dose of 350-400 MBq[1] 18F-FDG in order to keep the overall radiation exposure below 20 mSv while the subject is sitting comfortably in an armchair. Then, for 90 minutes following the injection, the subject is kept in a quiet environment so that the product has enough time to accumulate in the brain. While in the second visit, the training part, the same amount of tracer is injected, but this time when the subject achieves the aerobic threshold (10 minutes after the start of exercise). In both stages, the injection's exact time (hours and minutes) is recorded so that the PET scan can be started 90 minutes later.

The subject lies on a bed for PET imaging and is slowly moved into a short tube. The tube is open at both ends. Also, an intercom system allows the subject to communicate with the technician if needed. During imaging, it is crucial to remain still; for this purpose, the head is fixed with a

[1]MBq (megabecquerel) = 10*6 Bq (Becquerel)
Becquerel (Bq) is a unit of radioactivity in the International System of Units representing a minimal amount of radioactivity. Therefore, megabecquerel (MBq), one of the most common multiples of Bq, is usually used.

holder's help to ensure that it does not move. The exact time (hours, minutes) of the start of acquisition is recorded. First, two short CT scans (less than 2 minutes) are taken to determine the subject's position and to weaken and correct the scattering of PET images; then, the main scan is performed for 20 minutes. Finally, the images are reconstructed using our standard locally implemented iterative procedures (with attenuation, randomness, scattering, decay, and motion corrections). Certified imaging technicians perform these procedures under appropriate conditions with their professional instructions.

T1 MRI and MRS acquisitions

Before each PET session, participants undergo an MR study. MR spectroscopy is a crucial research tool that can measure the concentration of specific molecules in specific brain areas. Lactate is one of these molecules obtained in this study in the two regions of interest, PCC and SMA, during two MRS measurements.

In addition to measuring resting lactate values, the first visit is also used to obtain a high-resolution full-volume T1 image that is used for the co-registration of PET scan images. During both visits, measurements are made in a box measuring 4x4x3 cm3 around the PCC and SMA areas, which are located using the MNI template. Each measurement consists of 304 averages with TE/TR = 270/2000 ms for a total scan duration of 10 minutes per region. Also, the order of acquisition for the two regions of interest is counterbalanced across subjects to avoid systematic order effects.

For this study, no imaging material is injected. Similar to PET scanning, the subject lies on a specialized bed and slowly slides into a large open MRI tube at both ends. Also, at any time during the operation, it is possible to talk to the technician through the intercom system. Since the sound of the MRI is very loud during the process, ear protectors are used to protecting the ears from acoustic noise for the participant's comfort. In addition, it is vital to stay still while the MRI is being performed, so a pillow is placed around the head to help keep the head still.

The technician of the department performs all MR spectroscopy imaging steps. Also, before any imaging, the technician checks the safety screening form filled out by the participant and issues the imaging license if there are no problems. Questions have been asked in the MRA safety screening form to ensure that the participant has no issues during imaging. Some of these concerns are as

follows:

- Having previous MRI experience
- Being pregnant or breastfeeding
- Being claustrophobic
- Being connected to any supportive medical device
- Having any metal implants in the body and around the eyes

The subject answers each of the questions carefully with yes or no and has enough time to ask about each of the questions.

4.3 Data Analysis Framework

The primary data collected and compiled from the participants during two visits is insufficient to confirm the research hypotheses. There is still a need to apply extensive statistical analysis to the data in order to conclude them. In other words, the analysis process refers to processing a large amount of data, which leads to finding the logic behind the emergence of the main findings.

Starting any work and operation in the first stage involves preparations and prerequisites. ”Data Mining” is not exempted from this law and requires preliminary preparation and processing. Therefore, it is necessary to prepare and adjust all the data used for the intended purpose or so-called ”pre-processing” before starting the processing. Data pre-processing includes all transformations performed on raw data to make subsequent processing more straightforward and effective. This operation is performed after data extraction and plays an essential role in the data processing process and its results.

Therefore, we briefly explain the data pre-processing steps and then propose how to analyze the collected data to support the hypotheses.

4.3.1 Preprocessing of PET scan measurements

The PET measurements used in this study are Standardized Uptake Values (SUVs) Ratios (SUVR).

SUVs represent tissue activity within an ROI and remove variability caused by differences in patient size and the amount of FDG injected. Therefore, SUV is used as a relative measure of FDG absorption [160], which is summarized in equation 1:

$$SUV = \frac{c_{img}}{ID/BW} \tag{1}$$

Where c_{img} is the radioactivity concentration measured from the image by PET scan, ID is the injected dose, and BW is the body weight.

SUVR is the ratio of SUV data from two different regions (a target and a reference region) in a PET image that provides a surrogate measure of the amount of tracer available [161]. In this study, we have considered Pons as the reference area used to calculate SUVRs, and finally, we figured SUVR for PCC and SMA interest areas according to equation 2.

$$SUVR = \frac{SUV_{target}}{SUV_{reference}} \tag{2}$$

Another prerequisite for PET image analysis is spatial normalization and image alignment. For this purpose, we have used each subject's high-resolution T1 MR study as a reference for co-registration and spatial normalization in each PET study.

4.3.2 Preprocessing of MRS measurements

As we discussed in detail, MRS enables non-invasive measurement of tissue metabolite levels in vivo and provides valuable information for clinical diagnosis and research. However, it is necessary to filter and process all scans before analyzing the data to remove any inaccuracies. Advanced preprocessing and simulation of MRS data are done using the FID appliance (FID-A) toolkit.

FID appliance (FID-A) toolkit

FID-A *(https://github.com/CIC-methods/FID-A)* is an open-source, MATLAB-based toolbox specifically designed to handle raw MRS datasets. By providing a convenient tool for handling such raw, multidimensional data, this software tool facilitates the implementation and use of higher-level processing and analysis routines such as advanced coil combining, frequency and phase shift correction, and MRS functional time processing. It also removes the degenerate averages of individual motion before further analysis [162].

Using this tool, in brief, the following sequence of operations is applied to the raw outputs of MRS:

I. Coil array reconstruction: Each of the receiver elements of the MRI system detects different signal phases, signal amplitude, and noise depending on the position of the head relative to the coil element and the location of the voxel. Therefore, it is necessary to combine the diagnostic signals by different elements of the coil in such a way as to maximize the signal-to-noise ratio in the resulting spectrum. In the first place, the reconstruction of the weighted coil array was done using the weight of the receiver and the phases, which are determined by the magnitude and phase of the first point in the time domain, respectively.

II. Removal of Motion-Corrupted Averages: The identification of motion-corrupted averages is made by calculating the deviation of each average from the rest of the averages. This step removes averages corrupted by moving with a threshold of 4 standard deviations. This process is repeated until there are no more broken moving averages.

III. Spectral Registration: After removing averages corrupted by motion, estimation and correction of frequency errors and phase drift in in-vivo MRS data are performed. This process continues in a limited frequency range until the frequency estimation, and phase shift reach convergence.

After performing all corrections on the uploaded scan that protect the user from potentially significant errors during data analysis, the final processed spectrum is generated. Finally, FID-A

converts this spectrum to the MRS analytical software format used in this study, Tarquin, in two modes ”water-unsuppressed data” and ”water-suppressed data.”

TARQUIN software

TARQUIN is an open-source application developed to quantify metabolite concentrations in MRS data automatically. In addition to being free, this software is available on computer operating systems. It uses batch and one-time analyses using both a command line interface and a graphical user interface (GUI). A quantum mechanics-based metabolite simulator is packaged with TARQUIN to optimize basis set construction for investigating specific pathology sequence parameters [163].

In this study, after the pre-processing applied by FID-A, a default analysis is always performed on the data by TARQUIN. This analysis uses the metadata in the files modified by FID-A as input to specify the details for TARQUIN. Although TARQUIN can automatically simulate and quickly adapt the basis set used during data analysis, it is not suitable in cases where default options need to be set. For this reason, we use the advanced analysis option included in TARQUIN to adjust our input parameters (Figure 4.7). Unlike the quick fit procedure, in this process, we must determine the main acquisition parameters after selecting the data format and inputting data files. Some of these parameters are automatically read from the input files, but the rest must be applied manually according to our purpose.

The set of input parameters that we considered for MRS data analysis is presented in Table 4.2.

One of Tarquin’s most critical steps in data processing is correcting the eddy current. The eddy current is created following rapid gradient switching and leads to unwanted short-term fluctuations of the B0 field. This unwanted effect leads to the deterioration of the shape of the spectrum lines and affects the results obtained from the spectral analysis [164]. Therefore, it is necessary to apply eddy current correction during the preprocessing of any in-vivo MRS data set. TARQUIN analysis software estimates any nonlinearity in the phase of unsuppressed data in the time domain and then performs eddy current correction for unsuppressed and suppressed water data. TARQUIN, having this capability, eliminates the need to do this step in advance.

Finally, after setting the correct parameters, we extract the analysis graphs from Tarquin and use

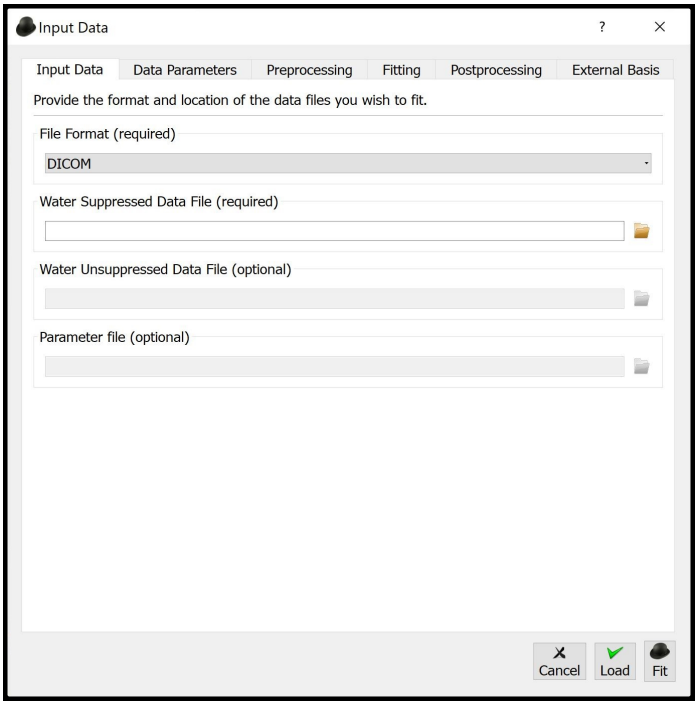


Figure 4.7: The advanced fit window

Table 4.2: The output of logistic regression analysis in PCC with two explanatory variables (FDG, Lac) and a binary categorical variable (V1, V2).

TARQUIN's set of input parameters	
Input Data	File Format: LCModel RAW Input Data Files: Water Suppressed Data and Water Unsuppressed Data
Data parameters	Sampling frequency (Hz): 5000 Transmitter frequency (Hz): 127.700000E6 Echo time (s): 0.2700 Data points: 4096
Pre-processing data	Reference offset (ppm): 4.65 Eddy current correction
Fitting data	Start point: 10 End point: 2048 Maximum iteration: 75

them to see the appropriateness of the analysis, concentration values , and standard deviations for different metabolites. An example of the fitted signal and the concentration of metabolites extracted from Tarquin is presented in Figure 4.8.

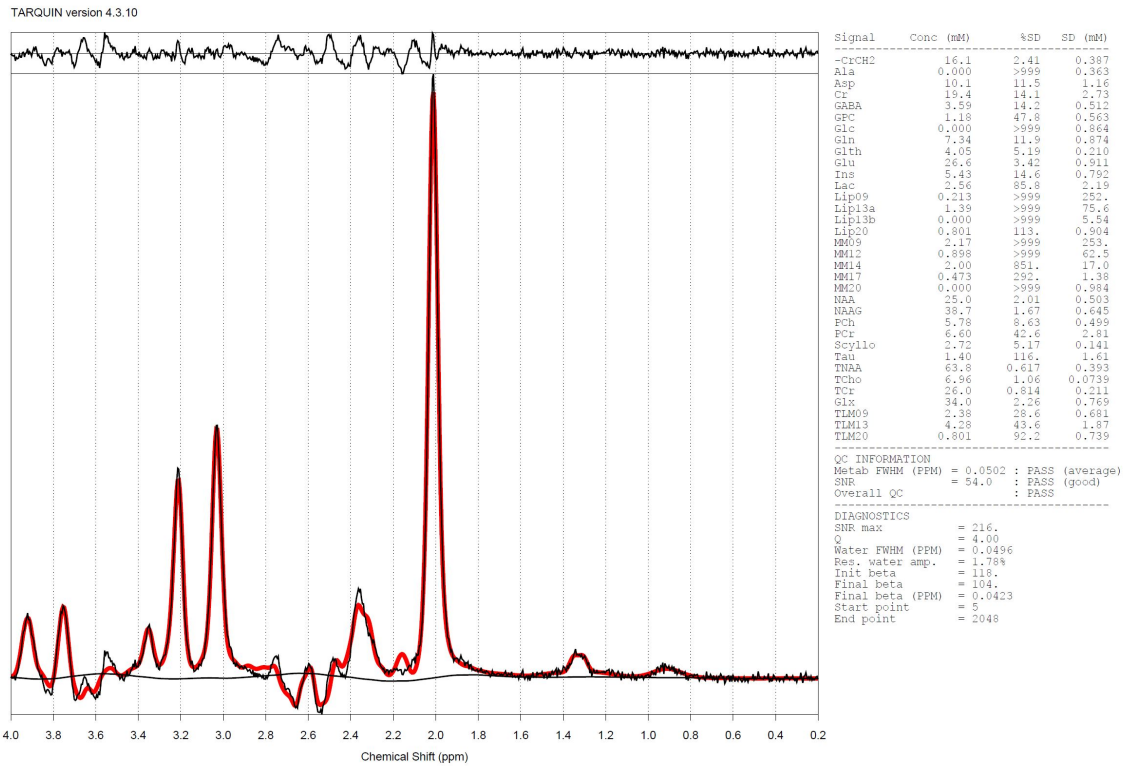


Figure 4.8: An example fit to the brain MR spectroscopy.

4.3.3 Analysis of acquired data

After performing the necessary pre-processing on the measured data from the PET and MRS scans, we checked the dataset for missing data and outliers before starting the primary analysis.

Meanwhile, some plasma measurements (3 blood samples) were missing. Since several blood samples were taken for each subject at multiple time intervals, we have recovered the missing data using the averaging method from the samples whose values are known. Also, we checked all the data using MATLAB's outlier detection function and found that the FDG value of the first visit of one of the subjects is an unusual or outlier compared to the rest of the values. In order to reduce the impact of outlier data on the algorithm's performance, we have removed all the data related to that subject from the data set.

In the following, we briefly explain the statistical tests and methods used in this study in order to extract insight and logical information from the data.

Paired sample t-test

Paired t-test is a subset of parametric statistical tests. Parametric tests are the most effective tests used in cases to generalize the results obtained from the sample group to the statistical population. These tests have high statistical power and the ability to deal with data collected in complex designs. The use of parametric tests is subject to the following assumptions:

- The description of variables should be based on relative or distance scales.
- Each of the observed items must be independent, which means that the selection of one item does not depend on the selection of any other item.
- Data distribution in society is normal or close to normal.

If the collected data comply with the three conditions, paired t-test can be used to analyze the data. Paired t-tests are a subset of student t-tests commonly used in intervention studies. This test is used in studies that aim to investigate the impact of an intervention on a group of people at two different times (before the intervention and after the intervention), and its work is based on evaluating the difference in the averages of two dependent populations (paired with each other). The paired t-test examines each person twice at two different times [165].

In this study, as mentioned, our goal is to observe and investigate the effect of exercise on brain glucose absorption and brain lactate concentration. Therefore, we intend to test the values of a quantitative variable, which we have collected before and after the effect of a factor (exercise) on the same people and evaluate the significance of the difference.

Here are the assumptions related to the paired t-test as follows:

I. Null hypothesis (H0): There is no statistically significant difference between the two groups' averages.

II. Alternative hypothesis (H1): There is a significant difference between the average of the two communities, which is not caused by sampling error or chance.

$$\begin{cases} H0: & \mu A = \mu B \\ H1: & \mu A \neq \mu B \end{cases} \tag{3}$$

The test statistic for the paired t-test is calculated as follows:

$$T = \frac{\overline{d}}{\widehat{\sigma}/\sqrt{n}} \tag{4}$$

In this regard, $\overline{d}$ means the average difference between two variables (before and after the effect). This statistic has a t distribution with (n-1) degrees of freedom. Also, $\widehat{\sigma}$ shows the standard deviation of the differences and n is the sample size. In this case, we have:

$$d_i = x_{i_A} - x_{i_B}, i = 1, 2, ..., n \tag{5}$$

As a result, the value of $\overline{d}$ and $\widehat{\sigma}$ can be calculated as follows.

$$\overline{d} = \frac{1}{n} \sum_{i-1}^{n} d_i \tag{6}$$

$$\widehat{\sigma} = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (d_i - \overline{d})^2} \tag{7}$$

Finally, we examine the p-value (probability value) for the t-statistic to determine whether the difference between the two groups is significant. The p-value expresses the likelihood of seeing the test findings under the null hypothesis. Therefore, a low p-value indicates reduced support for the null hypothesis. Here, we have considered a cut-off value of 0.05 to determine statistical significance.

In addition to the listed presuppositions for parametric tests, another additional condition has been defined for the paired t-test: the normality of the distribution of the measured differences. Therefore, before entering into the analysis, it is necessary to check the normality of the measurement differences. For this purpose, we have used the Shapiro-Wilk test.

Shapiro-Wilk Test

The Shapiro-Wilk test is one of the normal distribution fitting tests. With the help of this test and its statistics, we can determine whether the data follows a normal distribution. Considering this issue, this test can be regarded as part of the group of non-parametric statistics methods. In the original form of this test, a method for estimating the distribution parameter is not considered. Still, the ordinal statistics and their distribution, as well as the main data, are used to calculate the statistics of this test, which is called a non-parametric method. Similar to other statistical hypothesis tests, the Shapiro-Wilk test also has two assumptions, Null Hypothesis, and Alternative Hypothesis, which are summarized as follows:

I. Null Hypothesis: represents the normal distribution of data.

II. Alternative Hypothesis: It represents the non-normality of the data.

If we assume that $x_1, x_2, ..., x_n$ are a sample of a population, then the Shapiro-Wilk Test statistic is defined as follows:

$$W = \frac{\left(\sum_{i=1}^{n} a_i x_{(i)}\right)^2}{\sum_{i=1}^{n} (x_i - \overline{x})^2} \tag{8}$$

where $x_{(i)}$ represents the i-th ordinal statistic and x_i is the random sample value. Also, a_i means a vector defined as below:

$$(a_1, a_2, ..., a_n) = \frac{m^T V^{-1}}{(m^T V^{-1} V^{-1} m)^{1/2}} \tag{9}$$

In this formula, vector m is the mathematical expectation value of ordinal statistics and V is the covariance matrix of ordinal statistics. Also, $\overline{x}$ means the average of observed values from a random sample. That is, we have:

$$\overline{x} = (x_1 + x_2 + ... + x_n)/n \tag{10}$$

The Shapiro-Wilk Test is more powerful than similar tests such as the Kolmogorov-Smirnov test and the Anderson-Darling test and is a more suitable method for small sample sizes (less than

50 samples) [166].

In this test method, to determine whether or not to reject the null hypothesis, we rely on the probability value (P-value) produced in most statistical software. We reject the null hypothesis and conclude that the sample data were not drawn from a normal population if the P-value is less than 0.05.

Generalized Linear Model (GLM)

Generalized linear models, abbreviated as GLM, is an advanced statistical modeling technique that John Nelder and Robert Wedderburn formulated in 1972 to unify different statistical models. GLM models, which are straightforward models appropriate for counting data, allow us to build a linear link between the response and the predictors, even though the underlying relationship is not linear. This approach provides for regression modeling when the responses are distributed as a member of the exponential family, in which the variance is a known function of the mean.

Similar to other statistical models, GLM follows some basic assumptions:

- Data should be distributed independently.
- The response variable does not need to follow a normal distribution, but it should have a distribution of an exponential family.
- The response variable and the explanatory variables do not require a linear connection; nonetheless, the transformed response variable (in terms of the link function) is considered to have a linear dependence on the explanatory variables.
- The error distribution of the response variable does not need to be normally distributed.

In addition, three components are considered for each GLM:

- **Random component:** The only random component in the model expresses the type of probability distribution (Poisson distribution, binomial distribution, or normal distribution) of the response variable.

- **Link function:** It is shown as η or $g(\mu)$ and determines the type of relationship between random and systematic components. Link functions show how the predicted response value relates to the linear combination of explanatory variables.

- **Systematic component:** It is a quantity that specifies the explanatory variables of the model and their linear combination.

Generally, generalized linear models are used in parametric and non-parametric ways to estimate the curve. They include linear, logistic, and Poisson regression models [167, 168].

In the following, we will give a brief explanation of linear regression and logistic regression, which are used in the analysis of this study.

I. **Linear Regression**

Linear regression is a more specific model of GLM and the simplest regression model, which includes two types of simple linear (SLR) and multiple linear (MLR). Linear regression models are a basic and common type of statistical analysis that usually works on continuous data. The basis of this method is to model the target value based on independent predictors, which is used to find the cause-and-effect relationship between variables.

SLR includes only one predictor variable, while MLR seeks to predict changes in more than one independent variable on a dependent variable. The equation of MLR, which is a more comprehensive form of SLR, is modeled as follows:

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + ... + \beta_p x_{ip} + \varepsilon_i \tag{11}$$

where y_i is the dependent variable and x represents the independent variables that have p dimension. Also, the i=1,2,...,n index shows the observation number of the independent variable. β_0 is the width from the origin and represents a fixed value in the model, while β_p is called partial regression coefficients for each independent variable; the calculation of these coefficients is done using the minimization of "ordinary least square" and based on the calculation of "partial deviation". Finally, ε is the regression model's error term, which represents the difference between the observed and fitted values of the dependent variable [169]:

$$\varepsilon = y_i - \widehat{y}_i \tag{12}$$

As we mentioned, one of the methods of estimating the parameters of this model is to use the "Ordinary Least Square (OLS)" technique. In this case, if we introduce the sum of squared error (SSE) function ($S(\beta)$) as below, the purpose of estimating the parameters of the linear regression model is to calculate the β vector according to the value of the observations so that $S(\beta)$ is as minimized as possible.

$$S(\beta) = \sum_{i=1}^{n} \mid y_i - \sum_{j=1}^{p} X_{ij}\beta_j \mid^2 = \parallel Y - X\beta \parallel^2 \tag{13}$$

where X is the matrix of multivariate inputs whose dimensions can be considered as (p+1)×n. On the other hand, the Y matrix is a set of dependent data variables, which is an n-line vector, and β represents the parameters vector. It is clear that $\parallel Y - X\beta \parallel^2$ means square distance or Euclidean Norm.

If we denote the estimation of the parameters vector by $\widehat{\beta}$, the parameters of the model are the values that apply in the following equation:

$$\widehat{\beta} = argmin_{\beta} S(\beta) \tag{14}$$

To get the $\widehat{\beta}$, we take the gradient from the $S(\beta)$ function with respect to vector β and set the gradient to zero, and finally, with the help of derivation and solving the matrix equations, we get the estimation of the parameters as follows:

$$\widehat{\beta} = (X^T X)^{-1} X^T Y \tag{15}$$

This estimation is possible when the matrix $(X^T X)^{-1}$ is invertible. This means that its determinant must be opposite to zero to indicate the absence of linear dependence between the rows or columns of the matrix [170]. For this reason, the linear regression model has

conditions that are summarized below:

- Independence of observations: descriptive variables are independent of each other.
- Homogeneity of variance or homoscedasticity: the size of the error does not change significantly in the values of the independent variable.
- Normality: The data has a normal distribution.
- Linearity: there is a linear relationship between independent and dependent variables [171].

Building on all the concepts described, multiple linear regression calculates four things to find the best fit for each independent variable:

- Estimate: reports the estimated value of the regression coefficients that lead to the smallest overall error in the model.
- Standard Error (Std. Error): The standard error of the estimation of the regression coefficients is reported, which indicates the accuracy of the coefficients. The larger the standard error, the lower the confidence in the estimate.
- Student's t-statistic: This statistic is obtained by dividing the estimated coefficients by the standard error.
- P-value: p-value corresponds to the t-statistic. The smaller the p-value, the greater the significance of the estimated coefficients (if the p-value is less than 0.05, the assumption of the equality of the coefficients with zero is rejected, and the desired coefficient is significant.)

II. Binary Logistic Regression

Binary Logistic Regression (BLR) is a statistical regression model to show the effect of quantitative or qualitative variables on the bivariate dependent variable. BLR is a generalized linear model with a polynomial distribution of its error and the logit function as the link function. Therefore, this model is one of the classification algorithms used to assign data to a set of classes.

Logistic regression analysis is similar to linear regression analysis, but the important difference between these two models can be seen in two features of logistic regression:

I. The conditional distribution of $(y \mid \overrightarrow{x})$ is a Bernoulli distribution instead of a Gaussian distribution because, unlike linear regression, the dependent variable is a qualitative, binary variable represented by zero and one codes.

II. Predicted values are probabilities and are limited between zero and one and are obtained with the help of the logistic distribution function.

In fact, two main goals are desired in a BLR model:

- Studying how the relationship and the impact of independent quantities on the dependent quantity.
- Predicting the probability of occurrence of dependent quantity by having independent quantities.

The statistical model of binary logistic regression is as follows:

$$logit(p) = ln(\frac{p}{1-p}) = \beta_0 + \beta_1 x_{1,i} + ... + \beta_k x_{k,i} \tag{16}$$

where p is the probability of occurrence of the dependent quantity. Therefore, as we mentioned, what is predicted in logistic regression is probability. Therefore, we can present the above relationship as follows:

$$p = Pr(y_i = 1) \tag{17}$$

$$p = Pr(y_i = 1 \mid \overrightarrow{x}_i; \overrightarrow{\beta}) = \frac{e^{\beta_0+\beta_1 x_{1,i}+...+\beta_k x_{k,i}}}{1 + e^{\beta_0+\beta_1 x_{1,i}+...+\beta_k x_{k,i}}} = \frac{1}{1 + e^{-\left(\beta_0+\beta_1 x_{1,i}+...+\beta_k x_{k,i}\right)}} \tag{18}$$

This model can well measure the impact of each x on the probability of occurrence of the response quantity and evaluate their significant or non-significant impact, where e, Nepper's

constant, is the base of the natural logarithm (approximately equal to 2.718281828459047) and x_j is an independent variable and β_j are the logistic regression coefficients of the independent variable and β_0 is the width from the origin.

Logistic regression can be defined by the logistic function. This function is represented by $\sigma : R \to (0, 1)$ and is calculated as follows:

$$\sigma(t) = \frac{e^t}{e^t + 1} = \frac{1}{1 + e^{-t}} \tag{19}$$

Including the logistic function, we can rewrite the logistic regression as follows:

$$Pr(y_i = 1 \mid \overrightarrow{x}_i; \overrightarrow{\beta}) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 x_{1,i} + ... + \beta_k x_{k,i})}} = \sigma(\beta_0 + \beta_1 x_{1,i} + ... + \beta_k x_{k,i})) \tag{20}$$

As the linear regression function based on the least squares method predicts the dependent variable with the least error, in the logistic regression, the exponential function based on the "Maximum Likelihood Estimation (MLE)" method estimates the probability of the event with the least error. In this case, if we introduce the logarithm of the likelihood function ($L(\beta)$) as below, the goal of estimating the parameters of the logistic regression model is to calculate the vector β according to the value of the observations to maximize $L(\beta)$ as much as possible.

$$\begin{aligned} L(\beta) &= log(\prod_{i=1}^{n} Pr(y_i = 1 \mid \overrightarrow{x}_i; \overrightarrow{\beta})^{y_i} \times Pr(y_i = 0 \mid \overrightarrow{x}_i; \overrightarrow{\beta})^{1-y_i}) \\ &= \sum_{i=1}^{n} y_i log Pr(y_i = 1 \mid \overrightarrow{x}_i; \overrightarrow{\beta}) + (1 - y_i) log Pr(y_i = 0 \mid \overrightarrow{x}_i; \overrightarrow{\beta}) \end{aligned} \tag{21}$$

where if $y_i = 1$ for data i, the goal is to increase $Pr(y_i = 1 \mid \overrightarrow{x}_i; \overrightarrow{\beta})$, and if $y_i = 0$, the goal is to increase the value of $Pr(y_i = 0 \mid \overrightarrow{x}_i; \overrightarrow{\beta})$.

As we said, the optimal parameter is the parameter that maximizes the value of $L(\beta)$. For this purpose, we use the Stochastic Gradient Ascent method. In this method, as the name suggests,

we randomly select an example from the data samples, then we calculate the gradient of the exponential and move the parameter a little in the direction of the gradient to reach a new parameter. The gradient of the local direction shows us the greatest increase in the function, so we move a little in that direction to reach the greatest local increase of the function. We repeat this process until the gradient gets close enough to zero:

$$\begin{cases} Initialize\ \overrightarrow{\beta^{old}}\ randomly \\ loop\ until\ convergence: \\ for\ i = 0\ to\ n; \\ \quad for\ j = 0\ to\ m; \\ \quad\quad \overrightarrow{\beta_j^{new}} = \overrightarrow{\beta_j^{old}} + \alpha(y_i - \frac{1}{1+e^{-\left(\beta_0^{old}+\beta_1^{old}x_{1,i}+\ldots+\beta_k^{old}x_{k,i}\right)}})\overrightarrow{x_{i,j}} \\ \quad \beta^{old} = \beta^{new} \end{cases} \tag{22}$$

Here, α is the value we move in the direction of the gradient each time, and $(y_i - \frac{1}{1+e^{-\left(\beta_0^{old}+\beta_1^{old}x_{1,i}+\ldots+\beta_k^{old}x_{k,i}\right)}})$ $\overrightarrow{x_{i,j}}$ is the partial derivative of data i in dimension j [172, 173].

In logistic regression, like other regression models, the importance of the final result can be evaluated with the p-value. Suppose the significance level of the logistic regression coefficient of one or more independent variables assumed by the researcher is greater than 0.05. In that case, the researcher's assumptions about the effectiveness of all independent variables assumed on the dependent variable are not confirmed. In other words, in this case, the assumed theoretical model of the researcher, which is the same set of independent variables that the researcher assumed as the effective factors in the dependent variable, is rejected.

Pearson Correlation Coefficient

One of the most famous ways to measure the linear correlation between two quantitative variables is to calculate the Pearson Correlation Coefficient. This index was introduced by the English statistician Karl Pearson in 1900. Pearson's correlation coefficient is a parametric approach for data with a normal distribution or a large number of observations [174].

Pearson's correlation coefficient between two random variables is defined as equal to their covariance divided by their standard deviation. This coefficient, when applied to a statistical population, is usually indicated by the Greek letter ρ and is called the correlation coefficient of the population:

$$\rho_{X,Y} = \frac{cov(X,Y)}{\sigma_X \sigma_Y} = \frac{E[(X-\mu_X)(Y-\mu_Y)]}{\sigma_X \sigma_Y} \tag{23}$$

where cov is covariance, σ_X is the standard deviation of X, μ_X is the mean of X, σ_Y is the standard deviation of Y, μ_Y is the mean of Y, and E is the expectation.

Also, when the Pearson correlation coefficient is applied to a sample, it is indicated by r and it is called the sample correlation coefficient:

$$r_{X,Y} = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \overline{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \overline{y})^2}} \tag{24}$$

where n equals to sample size, x_i, y_i are the individual sample points indexed with i, and $\overline{x}$, $\overline{y}$ are the average of the sample, which are similar quantities and are defined as follows:

$$\overline{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \quad and \quad \overline{y} = \frac{1}{n}\sum_{i=1}^{n} y_i \tag{25}$$

This coefficient, which ranges from -1 to 1, quantifies the nature, direction, and intensity of the link between two variables. A direct or positive connection means that if one of the variables rises (or decreases), the other also increases (or decreases), and $r = 1$ denotes a complete direct link between two variables. Additionally, $r = -1$ demonstrates a fully inverse relationship between two variables, whereby if one variable rises, the other one falls, and vice versa. Additionally, a correlation value of 0 indicates no link between the two variables that can be described as linear [175].

One more helpful criterion in the correlation coefficient analysis is the significance threshold; the null hypothesis is refuted, and we conclude that there is a significant connection between the two variables if the p-value is less than 0.05.

Partial Correlation

If X and Y are two random variables and Z is a control variable that is dependent on X and Y variables, then the partial correlation coefficient between X and Y by removing the effect of the control variable Z is denoted by $\rho_{XY.Z}$ and is calculated as follows be:

$$\rho_{XY.Z} = \frac{\rho_{XY} - \rho_{XZ}\rho_{YZ}}{(1-\rho_{XZ}^2)^{1/2}(1-\rho_{YZ}^2)^{1/2}} \tag{26}$$

In this regard, ρ_{XY} means the simple correlation coefficient between X and Y. The range of values of this coefficient, like the correlation coefficient, is in the range of -1 to 1. This means that the closer the partial correlation coefficient is to 1 or -1, the greater the intensity of the relationship between two variables by controlling and eliminating the effect of other variables, and the closer this coefficient is to 0, it indicates the lack of dependence between the two [176].

Linear interpolation

The simplest approach for fitting curves with linear polynomials is linear interpolation. Using this technique, additional data points can be added to the current discrete data range. In other words, it is feasible to infer an unknown value between two data points by traversing a straight line between them via linear interpolation. Based on the presumption that the rate of change between known values is constant and can be estimated from these values using a straightforward slope calculation, the notion of linear interpolation is used to approximate data. The unknown value between two known points may then be estimated using one of the points and the rate of change.

The linear interpolation formula is presented as follows:

$$y = \frac{y_0(x_1 - x) + y_1(x - x_0)}{x_1 - x_0} \tag{27}$$

where (x_0, y_0) and (x_1, y_1) are two known points, and the formula of linear interpolation is in the range of (x_0, x_1) [177].

4.4 Evaluation and justification of methodological choices

In this chapter, we describe in detail the steps and techniques used for data collection. We also discussed in detail how to pre-process the raw data and finally analyze the data in order to extract the logic behind the data.

In this study, we chose MRS and PET scan imaging techniques to measure brain lactate and glucose levels, which are much more accurate than other existing methods. In addition to being non-invasive, the MRS technique provides the possibility of direct measurement of brain metabolites in the area of interest.

Also, in the sports protocol part, although using a treadmill could lead to better results, we considered a stationary bike for this experiment due to the safety of the participants. Obviously, running on a treadmill requires far less energy than pedaling a stationary bike, and the subjects could stay at 80% of their vo2max for longer. Still, over time and given that the subjects have been fasting, it was possible that they would suddenly lose their strength and energy and not be able to continue. In this case, we would not be able to control the situation, and the participant would be injured.

Another thing that could have helped to improve the goal was collecting data in one day instead of two visits. But as I mentioned, the subjects had to be fasting; on the other hand, each visit takes about 4 hours, so it was almost impossible for the subject to have both visits in one day.

In this study, we chose Tarquin software to process the data extracted by MRS, which, besides being free, automatically determines the number of molecules in the MR spectrum.

In addition to all this, it should be considered that the quantitative data collected was from human samples and based on the laboratory, and despite controlling the conditions, there is always the possibility of errors in such samplings. However, we considered the most standard approach in this study, which has sufficient accuracy and can be repeated by other researchers.

We also used the most reliable techniques to model and analyze the variables in a way that leads to valid and reliable results. We also took steps to confirm or reject the study's hypotheses by extracting logical relationships between the variables. Therefore, we believe that the methodology design is consistent with the goals of the project and is the best approach to answer the statement of

the problem and research questions.

Chapter 5

Research Results and Discussion

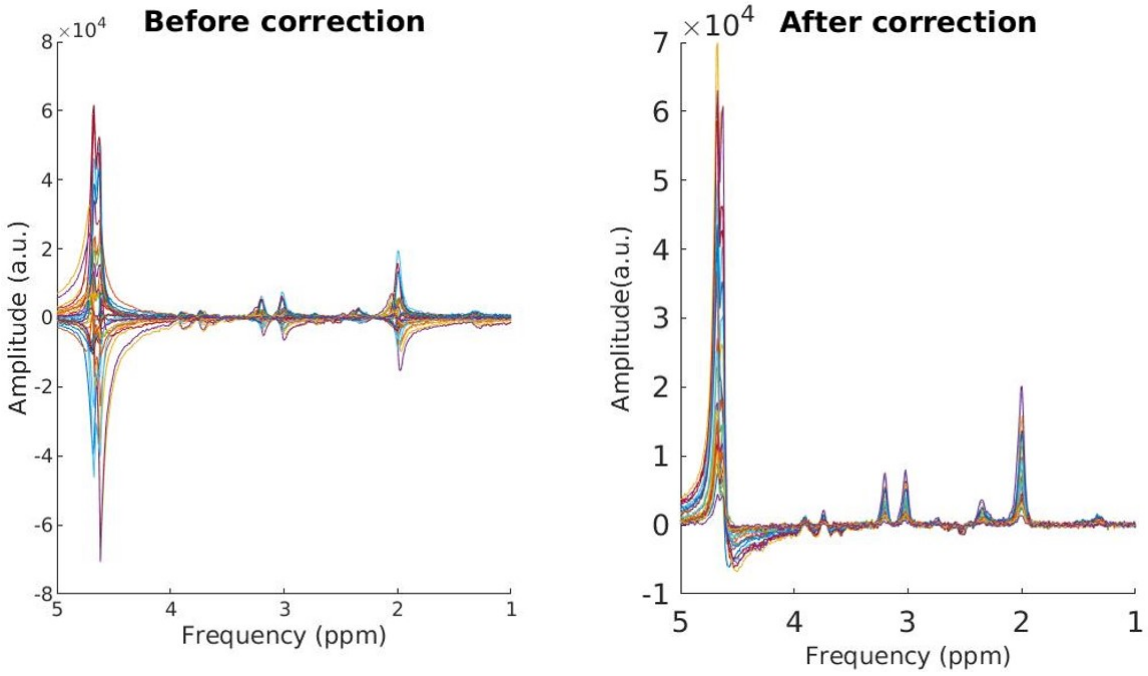
This chapter reports the main findings of data collection and analyses conducted to evaluate the relationships between variables. In this chapter, by presenting a summary of the results objectively and in a logical order, we answer the research questions and state whether the findings were in the direction of confirming the hypotheses or rejecting the hypothesis. We also use the obtained results as a stepping stone for discussion and focus on evaluating the results' meaning, importance, and relevance.

In summary, the findings reported in this chapter were collected to investigate the effect of intense exercise on brain energy metabolism (glucose and lactate) and the behavior of the same metabolites at the plasma level. The data used in these results were recorded with the help of two imaging techniques, MRS (to measure the amount of brain lactate) and PET scan (to measure the concentration of brain glucose) for the two regions of interest, PCC and SMA. Also, we have collected data related to plasma with blood samples taken in two visits (rest and exercise) from 29 healthy subjects (13 females and 16 males, 25 to 45 years old).

In the following, we present the results obtained from the pre-processing performed on the data and the statistical analyses used. In addition, we explain and evaluate the importance and meaning of each of the obtained results and their interpretation.

5.1 FID-A Pre-processing Results

We used the FID-A toolbox to process the raw data from the MRS imaging technique to correct potential errors introduced during imaging (coil array reconstruction, individual motion decay averages, frequency errors, and phase drift) for subsequent analyses. As we can see in Figure 5.1a, each of the receivers of the MRI system has detected a signal with a different amplitude, noise, and phase. The strongest receiver signals have the highest weight, and the weakest ones have the lowest. Therefore, we have taken a step towards optimizing SNR by reconstructing the coil array through phase adjustment and applying amplitude weighting. Figure 5.1b shows the output after making corrections.



(a) (b)

Figure 5.1: Coil array reconstruction [sub-17/V2/PCC]: (a) MRS data, prior to coil combination. (b) MRS data, after coil combination. Each curve corresponds to the signal received by different coil elements during MRS imaging based on head position as well as voxel location.

Figure 5.2 also shows the steps of pre-processing to correct the MR spectrum's defects. Each of the data is firstly corrected in terms of movement to eliminate the effect of small movement values created by the subject (natural physiological movements) on the quality of the spectrum, which leads to small changes in frequency and phase. Figure 5.2a shows the spectrum before removing

motion-corrupted averages, which does not show any difference compared to Figure 5.2b, and it means that the motion correction function did not find the average corruption to remove. In the next step, after removing the averages corrupted by the movement, as seen in Figure 5.2c, the estimation of frequency errors and the phase shift is done on the data. Figure 5.2d shows the data after frequency correction and phase shift, which shows that compared to Figure 5.2b, after Spectral Registration, the alignment of the averages has increased significantly. Finally, FID-A generated the final spectrum after all corrections in two ”water-unsuppressed data” and ”water-suppressed data” modes.

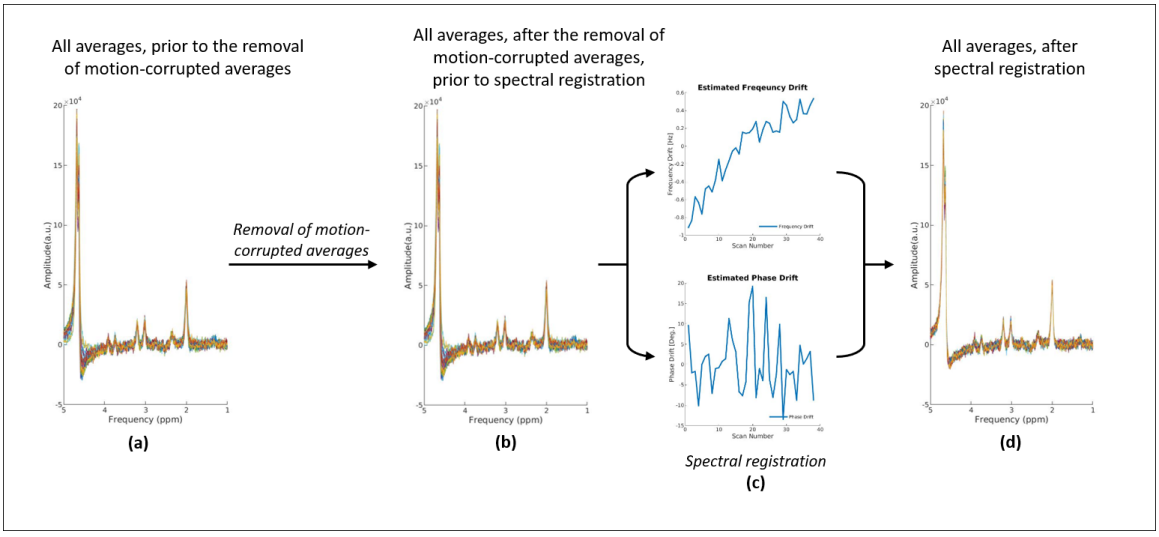


Figure 5.2: Preprocessing operations to remove/correct spectral defects using FID-A toolbox [sub-17/V2/PCC]: (a) All averages before the removal of motion-corrupted averages. (b) All averages After the removal of motion-corrupted averages [Original number of averages: 38.00, Number of bad Averages removed: 0.00, Number of remaining averages in the processed dataset: 38.00]. (c) Estimated frequency and phase drift [Total frequency drift was: 1.448322, Total phase drift was: 32.636935]. (d) All averages after frequency correction and phase shift.

5.2 TARQUIN Pre-processing Results

We have used the TARQUIN software to determine the amount of cerebral lactate in the MR spectrum in two areas of interest, PCC and SMA. As Figure 5.3 shows, we feed Tarquin from the two files ”water-suppressed” and ”water-unsuppressed” produced by FID-A. According to the shown outputs, Tarquin fits the best signal according to the input parameters (Sampling frequency:

5000 Hz, Transmitter frequency: 127.700000E6 Hz, Echo time: 0.2700 s) on the MR spectrum and extracts the corresponding values of its metabolites.

Before delving deeper into interpreting the effect of exercise on brain lactate, with a cursory look at the outputs presented in Figure 5.3, the increase in lactate concentration (for subject No.17) in the second visit (under exercise) is well visible in both PCC and SMA regions.

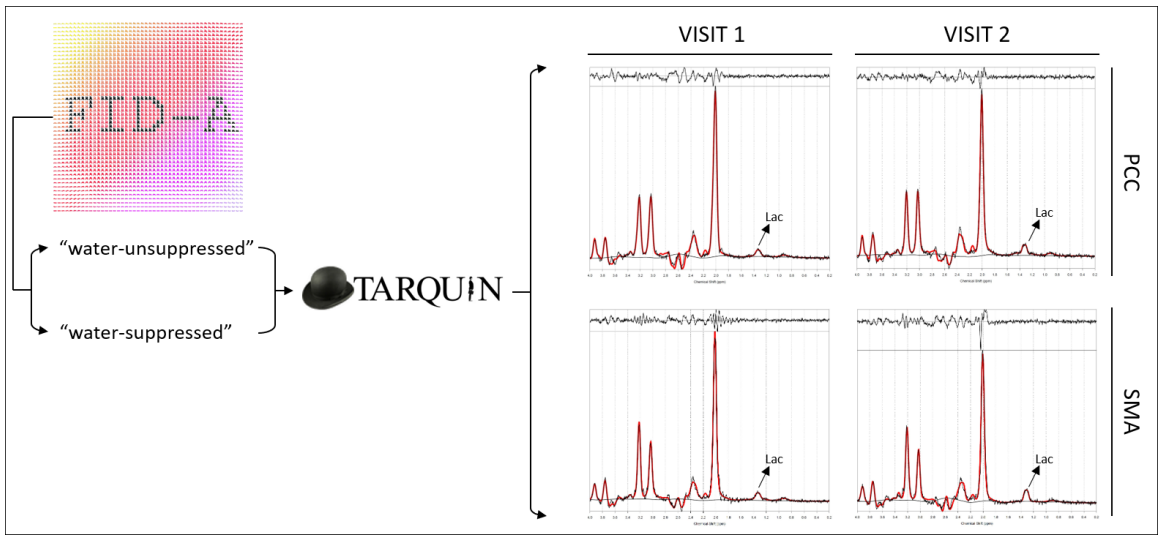


Figure 5.3: Tarquin's final results [sub-17]: The presented spectra are related to the first visit (under rest) and the second visit (under exercise) in two areas of PCC and SMA. The results show an increase in lactate (1.3 ppm) in both areas after exercise.

5.3 The effect of exercise on brain energy metabolism

Our aim of this study was to observe and investigate the effect of exercise on brain glucose absorption and brain lactate concentration. In other words, we intended to test the quantitative variable values of lactate and glucose that we have collected before and after the effect of a factor (exercise) on the same people and evaluate the importance of the difference. As we have mentioned before, our main hypothesis is that lactate concentration increases after exercise and cerebral glucose uptake (from now on we use FDG to represent glucose) decreases. Figure 5.4 shows the visual results of brain lactate behavior following exercise. The amount of lactate in the PCC area in the second visit compared to the first visit (for subject 17) increased by 48% and in the SMA area by

120%. More generally, Figure 5.4e shows the average amount of lactate for all subjects (29 people), in PCC the average lactate increased by 56% and in SMA by 47% after exercise.

Figure 5.5 also graphically shows the amount of FDG absorption under the influence of intense exercise. The amount of FDG absorption in the PCC area in the second visit compared to the first visit (for subject 17) decreased by 49% and in the SMA area by 52%. More generally, Figure 5.5e shows the average absorption of FDG for all subjects (29 people), in PCC the average FDG decreased by 54% and in SMA by 53% after exercise.

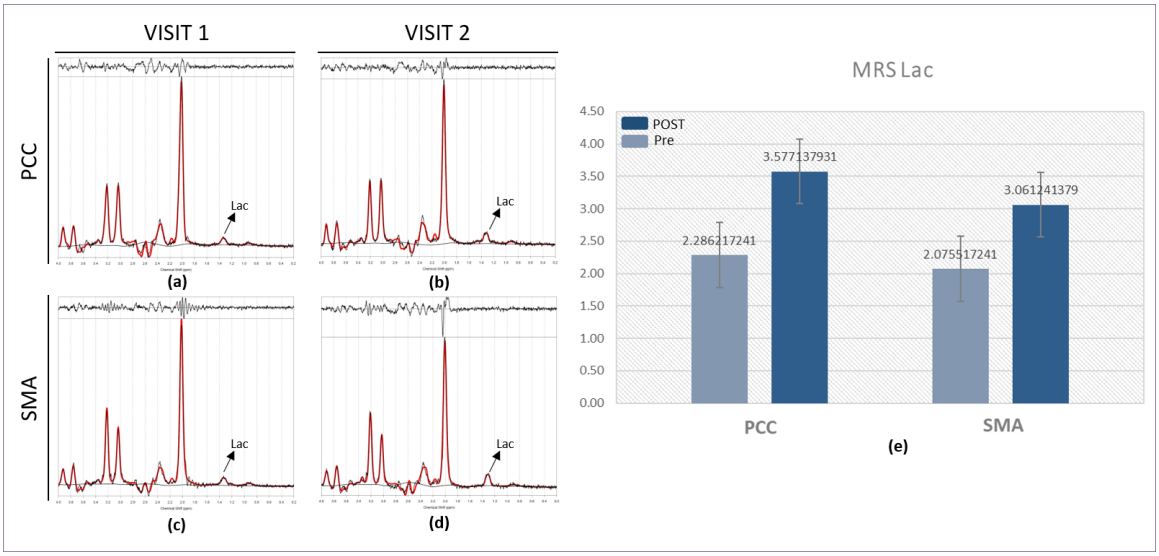


Figure 5.4: Lactate behavior following exercise: (a) It shows the output of Tarquin [Sub17/PCC/V1], in which the amount of lactate is 2.016 mmol. (b) It shows the output of Tarquin [Sub17/PCC/V2], in which the amount of lactate is 2.974 mmol; this amount has increased by 48% compared to (a). (c) It shows the output of Tarquin [Sub17/SMA/V1], in which the amount of lactate is 2.078 mmol. (d) It shows the output of Tarquin [Sub17/SMA/V2], in which the amount of lactate is 4.58 mmol; this amount has increased by 120% compared to (c). (e) It shows the average lactate for all subjects (29 people) in both areas before and after exercise, which increased by 56% and 47% in PCC and SMA, respectively, after exercise.

As the graphical images and bar graphs show us, lactate concentration and FDG uptake in both ROIs increased and decreased, respectively, following the exercise. However, it is necessary to check the significance of the obtained results. Therefore, we have used paired sample t-test to confirm or reject this hypothesis.

As we mentioned in the methodology section, one of the conditions for using this test is the normality of the measurement differences. Therefore, before examining the paired sample t-test, we

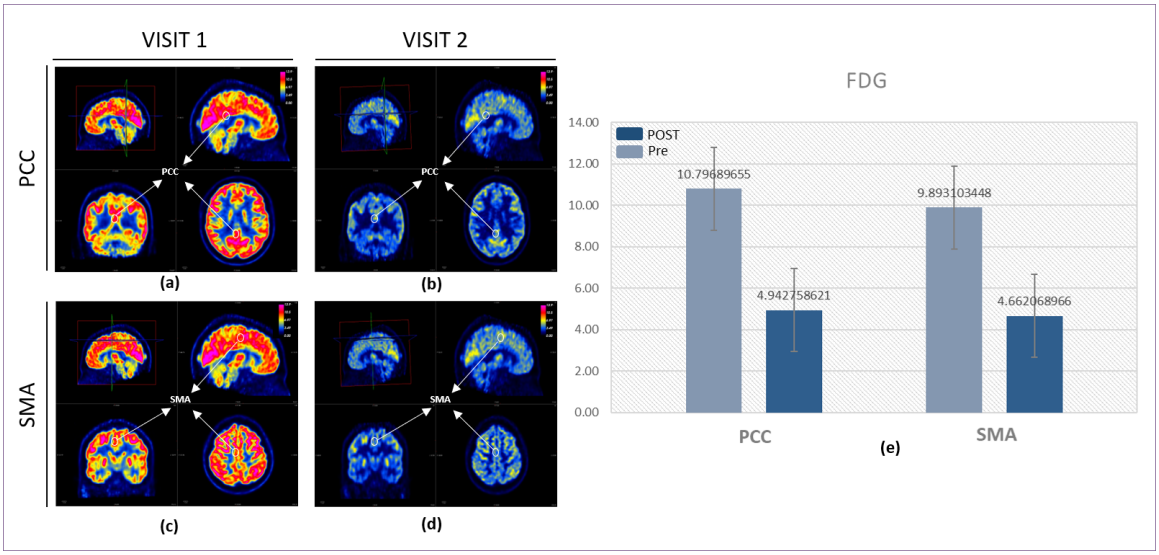


Figure 5.5: Glucose behavior following exercise: (a) It shows the output of PET scan [Sub17/PCC/V1], the red color indicates a high rate of FDG metabolism in which the amount of FDG is 9.5. (b) It shows the output of the PET scan [Sub17/PCC/V2], the blue color indicates a low rate of FDG metabolism in which the amount of FDG is 4.8; this amount has decreased by 49% compared to (a). (c) It shows the output of the PET scan [Sub17/SMA/V1], in which the amount of FDG is 9.4. (d) It shows the output of PET scan [Sub17/SMA/V2], in which the amount of FDG is 4.5; this amount has decreased by 52% compared to (c). (e) It shows the average FDG for all subjects (29 people) in both areas before and after exercise, which decreased by 54% and 53% in PCC and SMA, respectively, after exercise.

examine the normality of the measurement differences through the Shapiro-Wilk test.

5.3.1 Shapiro-Wilk test results

As we said, we have used the Shapiro-Wilk test to check the normality of data differences. Figure 5.6 shows the output of the normality test. The inputs of this test are the difference in lactate measurement in the first and second visit (DiffLacPCC or DiffLacSMA) and the difference in FDG measurement in the first and second visit (DiffGlcPCC or DiffGlcSMA) in the PCC and SMA areas.

In Figure 5.6, the Statistic is the Shapiro-Wilk test statistic (W), which is a measure to show the correlation of ordered and standardized sample quantiles with standard normal quantities. This statistic varies between 0 and 1, where 1 indicates a complete match. Referring to the estimated statistics for each of the input parameters (0.966, 0.962, 0.968, 0.943 respectively), they represent high compatibility. Also, the degree of freedom is marked with the label df and the significance

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
DiffLacSMA	.118	29	.200*	.966	29	.446
DiffGlcSMA	.120	29	.200*	.962	29	.371
DiffLacPCC	.116	29	.200*	.968	29	.511
DiffGlcPCC	.115	29	.200*	.943	29	.117

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Figure 5.6: Shapiro-Wilk test using SPSS [Second table]: DiffLacSMA indicates the difference between SMA lactate value in the first and second visit, DiffGlcSMA indicates the difference between SMA glucose value in the first and second visit, DiffLacPCC indicates the difference between PCC lactate value in the first and second visit, DiffGlcPCC indicates the difference between PCC glucose value in the first and second visit. The sig label in the last column indicates the normality of the distribution of the measurement difference.

level or p-value is marked with the label Sig. The data distribution is considered normal according to the Sig value seen in the last column of the second table. As mentioned before, if the p-value is less than 0.05, the assumption of normality of data distribution is rejected. Therefore, since the p-value for the input parameters (equal to 0.446, 0.371, 0.511, and 0.117 respectively) are greater than 0.05, we consider the data distribution to be normal. In addition, to determine the normality graphically, we can use the output of a normal Q-Q plot provided by SPSS. If the distribution is normal, the points generally follow the diagonal line. As Figure 5.7 shows, our data are clustered around the diagonal line - confirming that their distribution is normal.

5.3.2 Paired sample t-test results

After confirming the normality of the measurement difference, we will present the paired sample t-test results. The output of the test in Figure 5.8 has provided a lot of information, which refers to the differences between the two visits (before and after exercise). The columns of this table are labeled ”Mean”, ”Std. Deviation”, ”Std. Error Mean”, and ”95% Confidence Interval of the

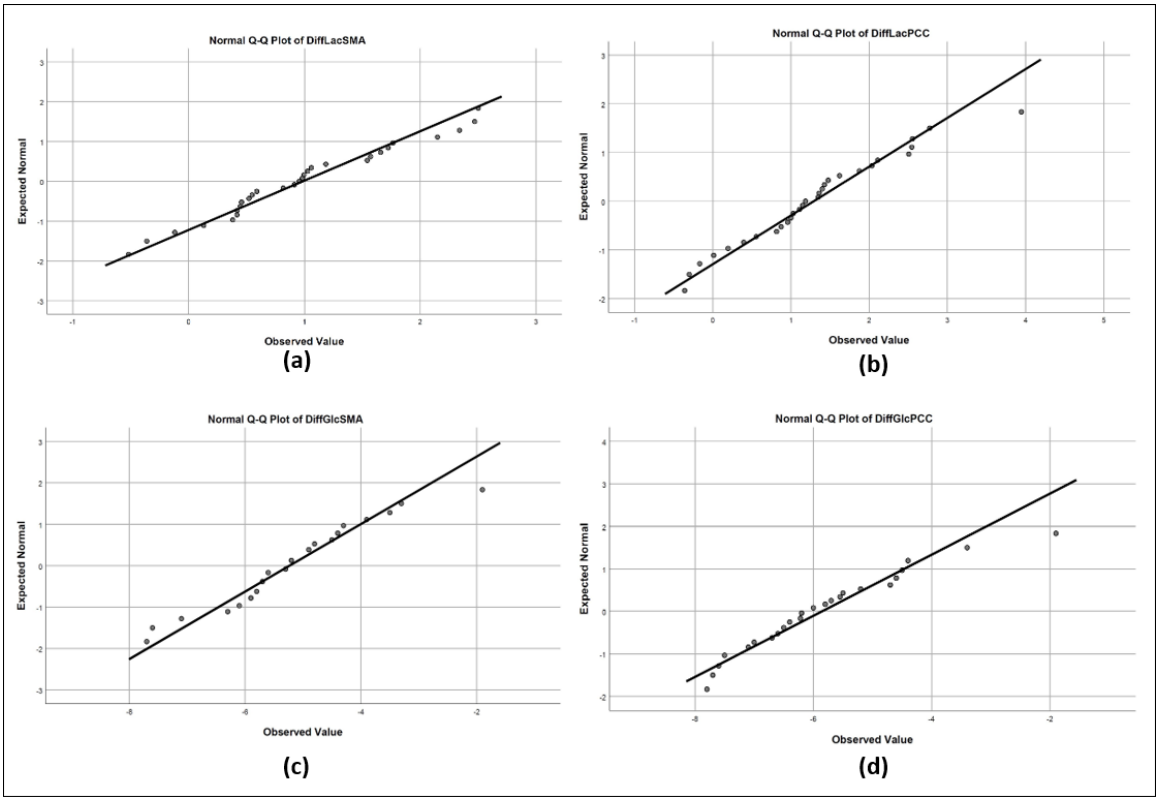


Figure 5.7: Normal Q-Q Plot using SPSS: Following the diagonal line by the points indicates the normality of their distribution.

Difference". The last three columns also show the results of the paired t-test, namely the test statistic (t), degrees of freedom (df), and significance level (Sig. (2-tailed)).

The first pair shows the importance of the lactate difference in the SMA area. In this case t (28) = 6.582, $p < .000$, according to their average and the direction of the t value, it can be concluded that there is a statistically significant increase (0.985 mmol, $p < .000$) to There is an effect on lactate levels following exercise. Also, for the second pair, which shows the difference of lactate in PCC, considering t (28) = 6.962, $p < 0.000$, it can be confirmed that lactate has a significant increase (1.29 mmol, $p < 0.000$) after exercise.

The third pair and the fourth pair arrange the information related to the FDG difference in the SMA and PCC areas in a similar way. For the third couple, t (28) = -22.967, $p < 0.000$, according to the average value, it can be concluded that exercise caused a significant decrease (-5.231, $p < 0.000$) in FDG absorption. Similarly, in the PCC region (t (28) = -22.660, $p < 0.000$) the results show a

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	LacSMApost - LacSMApre	.985724	.806435	.149751	.678973	1.292475	6.582	28	.000
Pair 2	LacPCCpost - LacPCCpre	1.290921	.998607	.185437	.911071	1.670771	6.962	28	.000
Pair 3	FDGSMApost - FDGSMApre	-5.2310	1.2265	.2278	-5.6976	-4.7645	-22.967	28	.000
Pair 4	FDGPCCpost - FDGPCCpre	-5.8541	1.3912	.2583	-6.3833	-5.3249	-22.660	28	.000

Figure 5.8: Paired Sample Test using SPSS: Pair1and Pair2 indicate the statistical information related to the difference of lactate in the SMA and PCC, respectively; according to the p-value and mean, lactate has increased significantly after the exercise in both ROIs. Pair3 and Pair4 indicate the statistical information related to the difference of FDG in the SMA and PCC, respectively; according to the p-value and mean, FDG has decreased significantly after the exercise in both ROIs.

significant decrease (-5.854, $p < 0.000$) in FDG absorption.

These results are consistent with the initial hypothesis that intense exercise increases brain lactate concentration and decreases brain glucose uptake.

Therefore, according to the presented results and relying on the literature review, it can be said that after exercise, glucose is no longer the main fuel of the brain and lactate is its suitable substitute. Currently, this claim is accepted only in PCC and SMA regions and cannot be generalized to all brain regions. It should also be mentioned that no significant difference was observed between males and females in the concentration of brain lactate and glucose after exercise, and the results obtained can be generalized to both populations of society.

5.4 The effect of exercise on plasmatic lactate and glucose

Another of our goals in this study was to investigate the effect of exercise on plasma lactate and glucose. According to the literature review, we expect to see an increase in both metabolites following exercise.

As mentioned in the data collection framework, 7 blood samples were taken during the second visit, the first of which (BD1) was before the start of the exercise test and was considered the baseline value. The second to fourth samples (BD2, BD3, and BD4) were collected during exercise

and 10, 20, and 30 minutes after starting pedaling, respectively. The average values of the samples are shown in Figure 5.9.

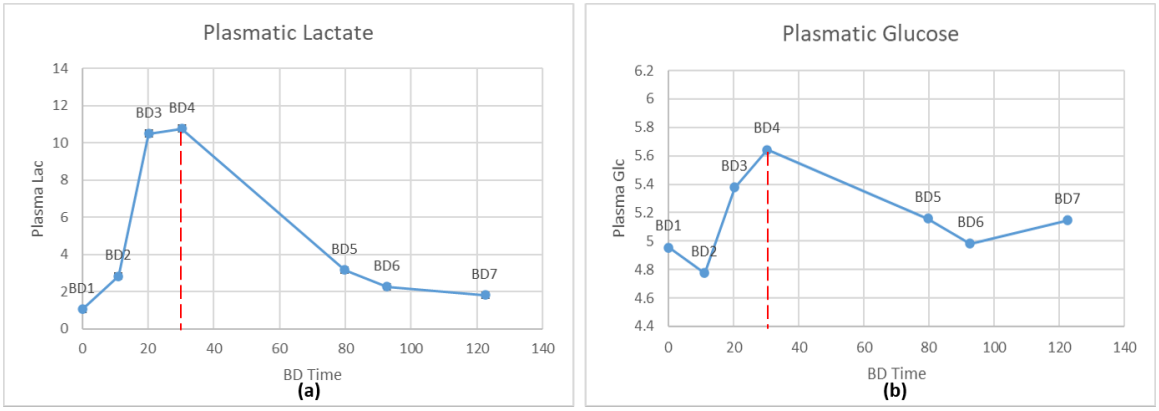


Figure 5.9: (a) Plasmatic lactate behavior following exercise [Average of all subjects]. (b) Plasmatic glucose behavior following exercise [Average of all subjects]. BD1 was collected before the start of exercise and is considered as the base, BD2, BD3, and BD4 were collected during exercise at 10, 20, and 30 minutes after the start of exercise, respectively. BD5, BD6, and BD7 were also collected after finishing the exercise, which correspond respectively to: after completing the MRS imaging, before starting the PET scan, and after completing the PET scan.

In Figure 5.9a, as mentioned, BD4 corresponds to 30 minutes after the start of the exercise and 5 minutes before its completion. As the graph shows, it can be well observed that the amount of plasmatic lactate increases significantly during exercise (BD1=1.05 mmol/L, BD4=10.76 mmol/L). The peak of the graph was in BD4, and after that, with time, the amount of plasmatic lactate decreased to 1.8 mmol/L in BD7. Also, based on Figure 5.9b, we find that the amount of plasmatic glucose also increased during exercise (BD1=4.95 mmol/L, BD4=5.61 mmol/L). Similarly to lactate, plasma glucose decreased after exercise and reached 5.14 mmol/L in BD7.

As we explained in detail in the data collection protocol section, the subjects were not able to complete the exercise test with the same intensity (80% of Vo2max) and performed the exercise test with different intensities for 25 minutes. Therefore, we calculated an ”exercise intensity” value for each subject by calculating the area under the curve (AUC). Then, we divided the subjects into two groups, moderate intensity, and high intensity, based on the cut-off value of 63 ml/kg/min to observe the effect of exercise intensity on the behavior of metabolites.

Figure 5.10 shows the difference between the two groups ”moderate-intensity” and ”high-intensity” at the plasmatic level, which are displayed with blue and orange colors, respectively. As expected, the high-intensity group (14 subjects with an average intensity of 69.92 ml/kg/min) showed a greater increase in plasmatic lactate and glucose during exercise than the moderate-intensity group (15 subjects with an average intensity of 55.77 ml/kg/min). Relying on these results, we can once again confirm the many theories presented that the intensity of exercise affects the production of metabolites, although this difference in groups can affect our desired results!

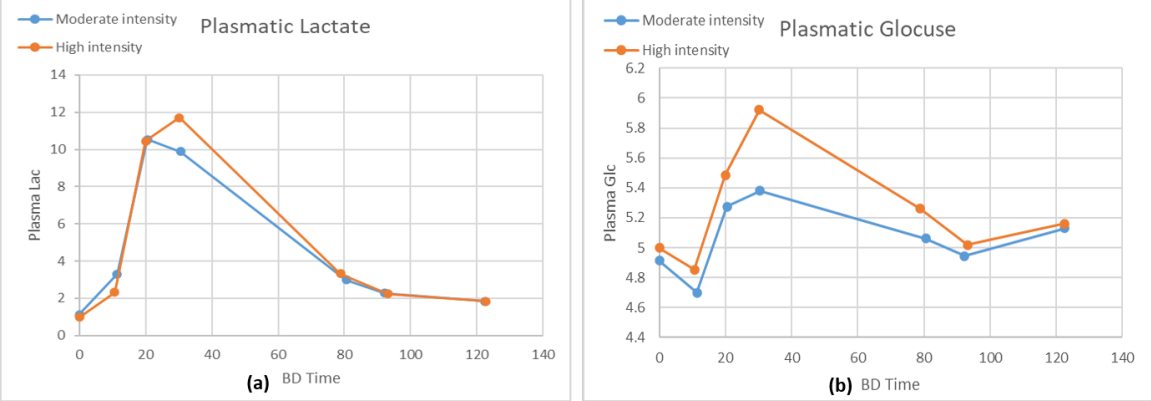


Figure 5.10: The effect of the difference in exercise intensity on plasmatic lactate (a) and glucose(b). The ”medium-intensity” group includes 15 subjects with an average intensity of 55.77 ml/kg/min, which are marked with blue color. The ”high-intensity” group includes 14 subjects with an average intensity of 69.92 ml/kg/min, which is displayed in orange color.

In general, based on the graphs in Figures 5.9 and 5.10, we conclude that although the amount of changes in plasmatic glucose was not as impressive as that of plasmatic lactate during and after exercise, the presented results can confirm our hypothesis. The graphs above show us the fact that although the plasma lactate and glucose values increase due to exercise, when the exercise stops, these values are not stable and quickly decrease and return to their normal value.

This principle can also apply to the amounts of these metabolites in the brain! Because we were not able to measure brain lactate and glucose during exercise. Although it has been tried to start recording the lactate amount through MRS immediately after exercise, it should be considered that there were two areas for imaging, each took about 10 minutes. In other words, the imaging of the second region was between 10 and 20 minutes after finishing the exercise (the effect of the order of

data acquisition in MRS is evaluated in the next sections).

5.5 Is there a relationship between brain Lac and FDG?

In section 5.3, we obtained valuable information about the behavior of brain lactate and glucose after exercise, which shows us that after moderate to intense exercise, lactate is considered the main fuel in the two regions of interest, PCC and SMA. Because the amount of FDG metabolism has decreased sharply, while the concentration of lactate has been increasing. For this reason, after our primary hypothesis was confirmed, a secondary hypothesis was formed whether there is a relationship between lactate increase and glucose decrease or not.

5.5.1 Simple Linear Regression results

We have used simple linear regression (SLR) to find a relationship between lactate and brain glucose. Figure 5.11 shows the results of SLR between FDG and lactate in two areas of PCC and SMA.

We have performed linear regression in two cases in each of the areas: by normalizing the data of the second visit using the values of the first visit (Figure 5.11a, 5.11c), without normalizing the data of the second visit (Figure 5.11b, 5.11d). In the first case, our goal was to investigate the relationship between the differences of metabolites with their base values to analyze what link was formed between them due to the effect of exercise. While in the second case, we are interested in checking whether there is a relationship between lactate and FDG in the second visit, regardless of the increase or decrease of the metabolites compared to the baseline value.

As the scatterplots in Figure 5.11 show, it can be visually concluded that there is no strong linear relationship between any of the states. Although the partial negative slope in both ROIs can be seen during the second visit (Figure 5.11b and Figure 5.11d), which indicates the opposite behavior of FDG and lactate; That is, as one increases, the other decreases, and vice versa. Although the graphs sufficiently show the absence of an SLR between metabolites, it is necessary to check and prove it from a statistical point of view.

Figure 5.12 presents the statistical results of linear regression for PCC in two states of normalization with baseline (Figure 5.12a) and without normalization (Figure 5.12b). The results related to SMA are shown in Figures 5.12c and 5.12d, respectively.

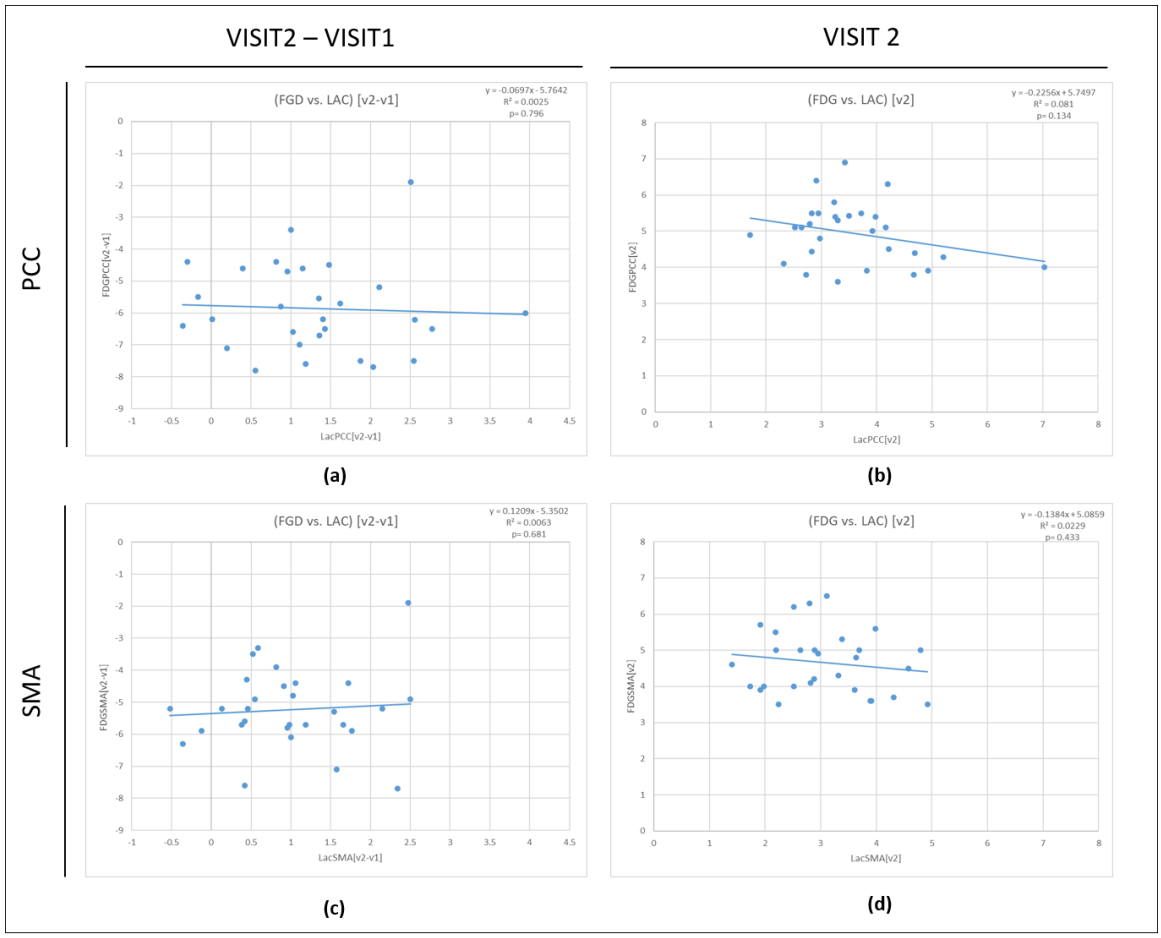
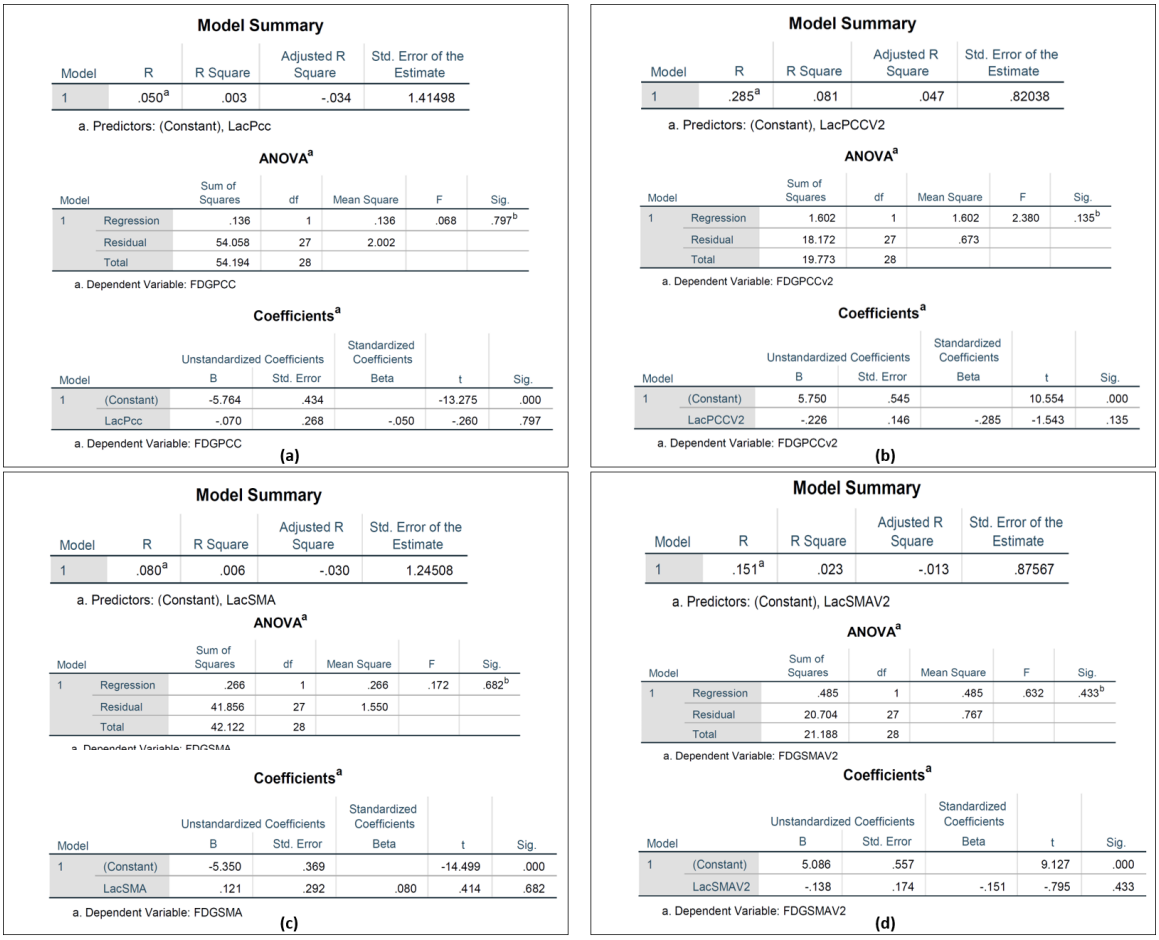


Figure 5.11: Linear regression model fitted to lactate and FDG values: (a) FDG vs. Lac in the PCC with normalization [V2-V1]. (b) FDG vs. Lac in PCC without normalization [V2]. (c) FDG vs. Lac in the SMA with normalization [V2-V1]. (d) FDG vs. Lac in SMA without normalization [V2]. The horizontal axis represents lactate values and the vertical axis represents FDG values. Each point represents the amount of FDG and lactate of each participant.

Each time linear regression analysis is applied, three tables labeled ”Model Summary”, ”ANOVA”, and ”Coefficients” are produced, which contain important information. The Model Summary table shows the features and statistics related to the fit of the regression model. The value of Pearson’s correlation coefficient is indicated by the label (R), the closer it is to 1 (or -1), the more suitable the regression model is. The coefficient of determination is also shown with the label (R Square), which

is the square of the correlation coefficient. Values close to one indicate a better fit. The ANOVA table is the analysis of variance and its most important output is the p-value which is labeled with (Sig.). A value smaller than 0.05 for Sig indicates a suitable regression model. The last table also estimates the regression coefficients or the estimation of the parameters.



Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.050[a]	.003	-.034	1.41498

a. Predictors: (Constant), LacPcc

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.136	1	.136	.068	.797[b]
	Residual	54.058	27	2.002		
	Total	54.194	28			

a. Dependent Variable: FDGPCC

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	-5.764	.434		-13.275	.000
	LacPcc	-.070	.268	-.050	-.260	.797

a. Dependent Variable: FDGPCC

(a)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.285[a]	.081	.047	.82038

a. Predictors: (Constant), LacPCCV2

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.602	1	1.602	2.380	.135[b]
	Residual	18.172	27	.673		
	Total	19.773	28			

a. Dependent Variable: FDGPCCv2

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	5.750	.545		10.554	.000
	LacPCCV2	-.226	.146	-.285	-1.543	.135

a. Dependent Variable: FDGPCCv2

(b)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.080[a]	.006	-.030	1.24508

a. Predictors: (Constant), LacSMA

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.266	1	.266	.172	.682[b]
	Residual	41.856	27	1.550		
	Total	42.122	28			

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	-5.350	.369		-14.499	.000
	LacSMA	.121	.292	.080	.414	.682

a. Dependent Variable: FDGSMA

(c)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.151[a]	.023	-.013	.87567

a. Predictors: (Constant), LacSMAV2

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.485	1	.485	.632	.433[b]
	Residual	20.704	27	.767		
	Total	21.188	28			

a. Dependent Variable: FDGSMAV2

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	5.086	.557		9.127	.000
	LacSMAV2	-.138	.174	-.151	-.795	.433

a. Dependent Variable: FDGSMAV2

(d)

Figure 5.12: Simple Linear Regression analysis in SPSS: (a) It presents the statistical results related to the present graph in Figure 5.11.a. (b) It presents the statistical results related to the present graph in Figure 5.11.b. (c) It presents the statistical results related to the present graph in Figure 5.11.c. (d) It presents the statistical results related to the present graph in Figure 5.11.d.

Therefore, according to the graph in Figure 5.11a and the statistical information presented in Figure 5.12a, it can be concluded that no significant linear relationship (R= 0.05, R^2= 0.003, F (28) = 0.068, p= 0.797) was found between FDG and lactate normalized with baseline in PCC. Similarly, with the reference from Figures 5.11b and 5.12b, we found that without normalizing the data, we

were not able to find a significant linear relationship (R= 0.285, R^2= 0.08, F (28) = 2.380, p= 0.135) between the parameters. In addition to PCC in the SMA area, there is also a significant linear relationship in the normalized state (R= 0.08, R^2= 0.006, F (28) = 0.172, p= 0.682) and without normalization (R = 0.151, R^2= 0.023, F (28) = 0.632, p= 0.433) is not seen.

5.5.2 Multiple Linear Regression results

As we mentioned earlier, the intensity of the subjects' exercise during the exercise test conducted on the second visit was not stable and it is subject to change due to the reasons, the most important of which is that the participants were fasting.

In the previous part, no significant linear relationship was found between lactate and FDG variables. Therefore, since the change in the concentration of these metabolites followed exercise, we decided to repeat the linear regression tests by adding the third variable (exercise intensity corresponding to each of the subjects).

At this stage, the model we considered has two independent variables (FDG and exercise intensity) and one dependent variable (lactate). We know that exercise increases the concentration of lactate, so changes in lactate depend on the intensity of exercise. On the other hand, according to the literature review, we have found that one of the lactate production pathways is the ANLS hypothesis, according to which glucose is converted into lactate. For these reasons, in MLR (multiple linear regression), we have considered lactate as a dependent variable with intensity and FDG.

Figure 5.13 presents a representation of the MLR model in three-dimensional space for PCC with normalization, PCC without normalization, SMA with normalization, and SMA without normalization with baseline, respectively with the labels "a", "b", "c", and "d". For a more detailed analysis of these models, we refer to the statistical information provided in Figure 5.14.

The statistical outputs of the tables in Figure 5.14 are labeled with the same labels as in Figure 5.13 and indicate their compatibility. Therefore, relying on the results of Figure 5.13a and 5.14a, we find that the regression model with two variables is significant (R=0.499, R^2=0.249, F (28) =4. 311, p=0.024). By going deeper into the Coefficients table, the regression model is summarized as follows:

$$Y = -0.086FDG + 0.009Intensity - 0.739$$

This model indicates a positive relationship between exercise intensity and lactate and a negative effect of FDG on lactate. But more carefully, we notice that according to the t-test, the FDG variable in the model does not have a significant effect on Y, because the sig value for this variable is equal to 0.491, which is greater than 0.05. Therefore, the significance of the model depends on the intensity of the exercise (p=0.007).

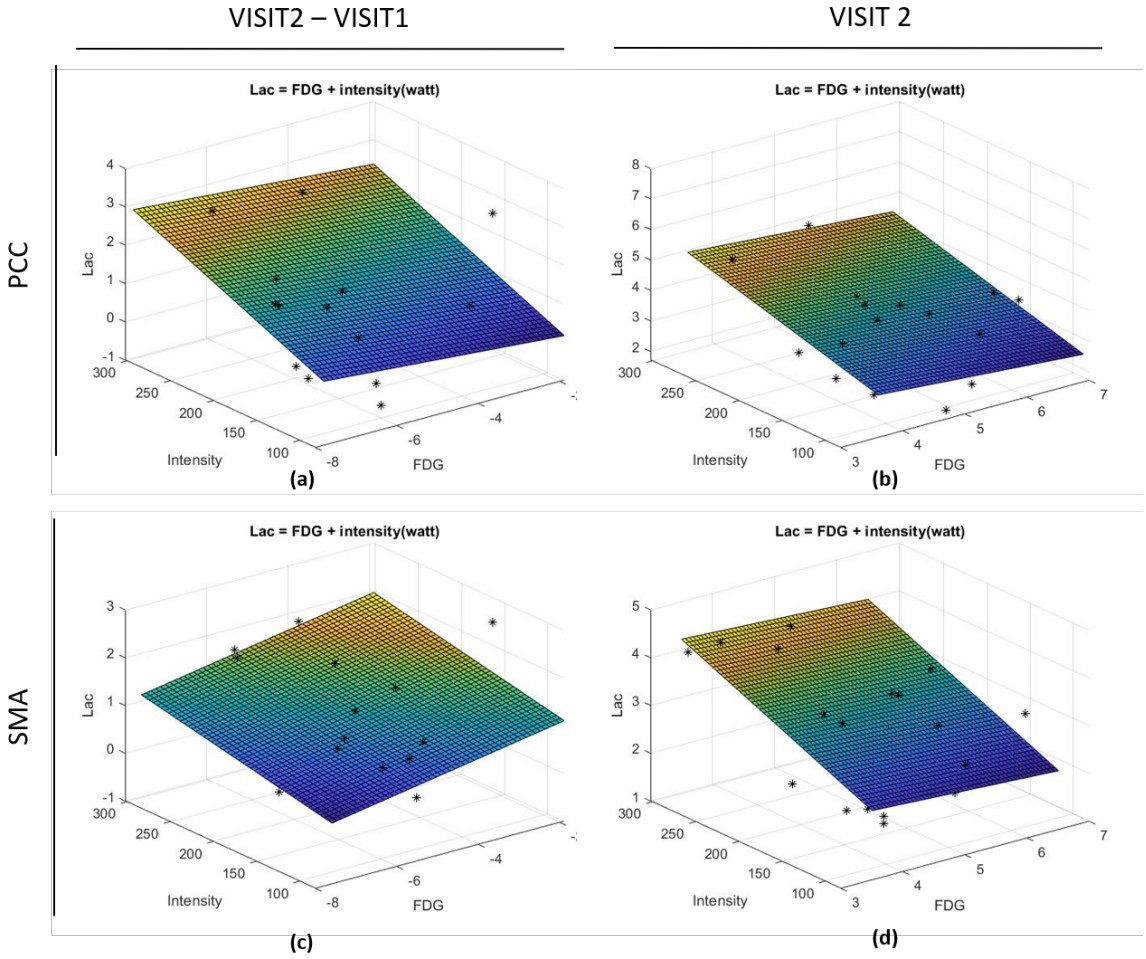


Figure 5.13: Linear regression model fitted to lactate, FDG, and intensity values: (a) Lac= FGD +Intensity in the PCC with normalization [V2-V1]. (b) Lac= FGD +Intensity in PCC without normalization [V2]. (c) Lac= FGD +Intensity in the SMA with normalization [V2-V1]. (d) Lac= FGD +Intensity in SMA without normalization [V2].

By performing the same analysis, the rest of the situations are summarized as follows:

- MRL on PCC without normalization:

  The regression model with two variables is significant (R=0.473, R^2=0.224, F (28) =3.749, p=0.037), the regression model is:

  $$Y = -0.191FDG + 0.008Intensity + 3.223$$

  There is a positive relationship between exercise intensity and lactate and a negative effect of FDG on lactate.

  P (FDG)= 0.417, P(Intensity)= 0.038. So, FDG variable in the model does not have a significant effect on Y.

- MRL on SMA with normalization:

  The regression model with two variables is not significant (R=0.187, R^2=0.035, F (28) =0.469, p=0.631), the regression model is:

  $$Y = 0.041FDG + 0.002Intensity + 0.782$$

  There is a positive relationship between exercise intensity and FDG with lactate. P (FDG)= 0.751, P(Intensity)= 0.389. So, FDG and Intensity variables in the model do not have a significant effect on Y.

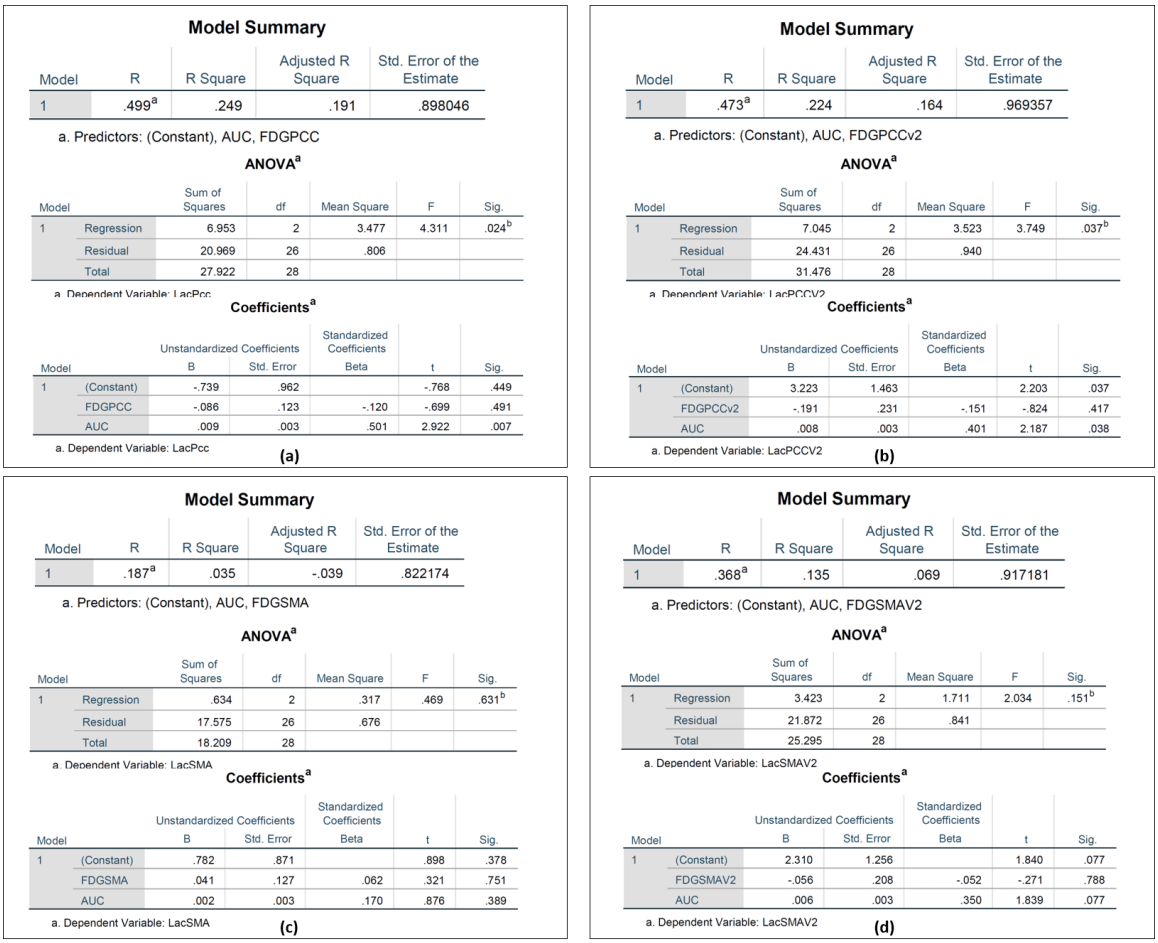
- MRL on SMA without normalization:

  The regression model with two variables is not significant (R=0.368, R^2=0.135, F (28) =2.034, p=0.151), the regression model is:

  $$Y = -0.056FDG + 0.006Intensity + 2.310$$

  There is a positive relationship between exercise intensity and lactate and a negative effect of FDG on lactate. P (FDG)= 0.788, P(Intensity)= 0.077. So, FDG and Intensity variables in the

model do not have a significant effect on Y.



Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.499[a]	.249	.191	.898046

a. Predictors: (Constant), AUC, FDGPCC

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	6.953	2	3.477	4.311	.024[b]
	Residual	20.969	26	.806		
	Total	27.922	28			

a. Dependent Variable: LacPcc

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	-.739	.962		-.768	.449
	FDGPCC	-.086	.123	-.120	-.699	.491
	AUC	.009	.003	.501	2.922	.007

a. Dependent Variable: LacPcc

(a)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.473[a]	.224	.164	.969357

a. Predictors: (Constant), AUC, FDGPCCv2

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7.045	2	3.523	3.749	.037[b]
	Residual	24.431	26	.940		
	Total	31.476	28			

a. Dependent Variable: LacPCCV2

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	3.223	1.463		2.203	.037
	FDGPCCv2	-.191	.231	-.151	-.824	.417
	AUC	.008	.003	.401	2.187	.038

a. Dependent Variable: LacPCCV2

(b)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.187[a]	.035	-.039	.822174

a. Predictors: (Constant), AUC, FDGSMA

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.634	2	.317	.469	.631[b]
	Residual	17.575	26	.676		
	Total	18.209	28			

a. Dependent Variable: LacSMA

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	.782	.871		.898	.378
	FDGSMA	.041	.127	.062	.321	.751
	AUC	.002	.003	.170	.876	.389

a. Dependent Variable: LacSMA

(c)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.368[a]	.135	.069	.917181

a. Predictors: (Constant), AUC, FDGSMAV2

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.423	2	1.711	2.034	.151[b]
	Residual	21.872	26	.841		
	Total	25.295	28			

a. Dependent Variable: LacSMAV2

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	2.310	1.256		1.840	.077
	FDGSMAV2	-.056	.208	-.052	-.271	.788
	AUC	.006	.003	.350	1.839	.077

a. Dependent Variable: LacSMAV2

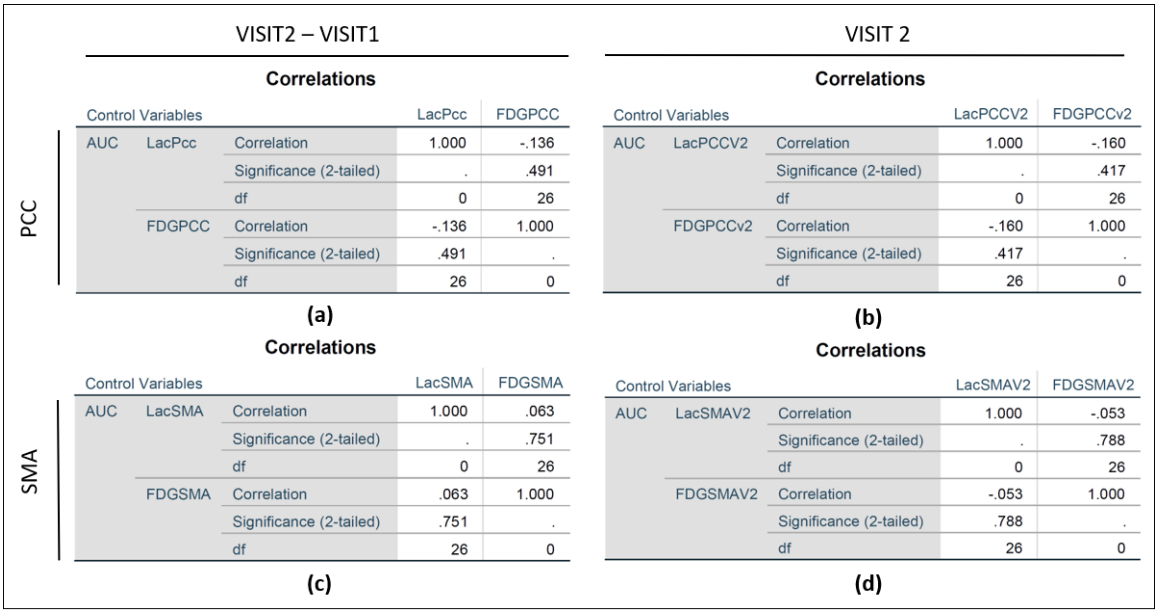
(d)

Figure 5.14: Multiple Linear Regression analysis in SPSS: (a) It presents the statistical results related to the present graph in Figure 5.13.a. (b) It presents the statistical results related to the present graph in Figure 5.13.b. (c) It presents the statistical results related to the present graph in Figure 5.13.c. (d) It presents the statistical results related to the present graph in Figure 5.13.d.

Although the MLR model is significant in the PCC region, as we have shown, the load of this significance was the responsibility of the intensity variable, and in this case, where we were looking for a relationship between lactate and FDG, it is not important. Therefore, with the presented results, it can be concluded that lactate and FDG were not related even considering the intensity variable. But it can be claimed that in all cases except MLR in SMA with normalization, there is an inverse relationship between those two brain metabolites.

5.5.3 Partial Correlation results

As we observed in sections 5.5.1 and 5.5.2, SLR and MLR were not able to prove the existence of a linear relationship between brain metabolites (lactate and FDG). Also, since we have almost found that lactate changes are related to exercise intensity (we have discussed this more in the next sections), there is a possibility that if we remove the effect of exercise intensity, we can see the desired relationship between the variables. For this reason, we decided to evaluate the relationship between these variables once again while controlling the effect of the third parameter (exercise intensity). Therefore, we have used partial correlation to analyze the linear relationship between brain lactate and FDG beyond the intensity variable.



PCC

VISIT2 – VISIT1

Correlations

Control Variables			LacPcc	FDGPCC
AUC	LacPcc	Correlation	1.000	-.136
		Significance (2-tailed)	.	.491
		df	0	26
	FDGPCC	Correlation	-.136	1.000
		Significance (2-tailed)	.491	.
		df	26	0

(a)

VISIT 2

Correlations

Control Variables			LacPCCV2	FDGPCCv2
AUC	LacPCCV2	Correlation	1.000	-.160
		Significance (2-tailed)	.	.417
		df	0	26
	FDGPCCv2	Correlation	-.160	1.000
		Significance (2-tailed)	.417	.
		df	26	0

(b)

SMA

Correlations

Control Variables			LacSMA	FDGSMA
AUC	LacSMA	Correlation	1.000	.063
		Significance (2-tailed)	.	.751
		df	0	26
	FDGSMA	Correlation	.063	1.000
		Significance (2-tailed)	.751	.
		df	26	0

(c)

Correlations

Control Variables			LacSMAV2	FDGSMAV2
AUC	LacSMAV2	Correlation	1.000	-.053
		Significance (2-tailed)	.	.788
		df	0	26
	FDGSMAV2	Correlation	-.053	1.000
		Significance (2-tailed)	.788	.
		df	26	0

(d)

Figure 5.15: Partial Correlation in SPSS: (a) It indicates the correlation between Lac and FDG, whilst controlling for ”AUC (intensity)” in the PCC with normalization [V2-V1]. (b) It indicates the correlation between Lac and FDG, whilst controlling for ”AUC (intensity)” in PCC without normalization [V2]. (c) It indicates the correlation between Lac and FDG, whilst controlling for ”AUC (intensity)” in the SMA with normalization [V2-V1]. (d) It indicates the correlation between Lac and FDG, whilst controlling for ”AUC (intensity)” in SMA without normalization [V2].

Figure 5.15 shows the report of the execution of partial correlation command of lactate and brain FDG variables with control of exercise intensity. The partial correlation of the two mentioned variables in PCC in normalized (Figure 5.15a) and non-normalized (Figure 5.15b) states is -0.136 and

-0.160, respectively, which means that by removing the effect of exercise intensity, the correlation of these two variables is equal to the values mentioned. These values show that there is a negative correlation between the dependent variable and the independent variable, which is not statistically significant (p=0.491, p=0.417). Also, similarly, for SMA in the normalization state (Figure 5.15c), there is a partial positive correlation (0.063) which is not statistically significant (p=0.751). In addition, no significant partial correlation (p=0.788) was observed in SMA without normalization (Figure 5.15d), although it represents a negative relationship between the two variables (R=-0.053).

Therefore, by performing this test, we realize that by controlling and removing the effects related to the intensity of exercise, we still do not see a significant relationship between brain metabolites. The results obtained from all three tests of SLR, MLR, and partial correlation lead us to conclude that there is at least no linear relationship between brain lactate and FDG, although a strong non-linear relationship between the two may exist. which requires modeling and estimation of optimal parameters. In addition, to model the non-linear relationship, it is necessary to have more information about the independent and dependent parameters, which are not included in this study.

5.5.4 Logit Regression results

Before drawing general conclusions about the relationship between the metabolites involved in brain energy metabolism (lactate and FDG), we are interested in evaluating whether we can predict the probability of resting or exercising by relying on lactate and FDG values as independent quantities.

In fact, by conducting this study, we intend to interpret the difference between the first and second visits by classifying the measured quantitative values. As the results showed, there may not be a linear relationship between lactate and FDG in the brain, but we have seen a significant increase and decrease in lactate concentration and FDG uptake in the brain, respectively, which we believe is due to events that happened during the first and second visits. Therefore, we have used logistic regression to find a relationship between independent quantities (lactate and FDG) and dependent quantity that has a binary form (first and second visits).

Table 5.1 shows the output of binary logistic regression analysis for PCC.

Based on the statistical information, the output shows that FDG reduction and lactate increase

are significantly (p=0.0000e^{-27}, p=0.0000e^{-27} respectively) related to the probability of being in the second visit (exercise mode). Therefore, the model is suitable and the probability that the changes of the independent variables can show the presence of the subject in the second visit (practice conditions) is calculated according to the following function:

$$p(x) = 1/(1 + e^{-(-174.3462+62.1914FDG-98.0835Lac)})$$

Table 5.1: The output of logistic regression analysis in PCC with two explanatory variables (FDG, Lac) and a binary categorical variable (V1, V2).

	Coefficient	**Std. Error**	**t-value**	**p-value**
Intercept ($\beta 0$)	-174.3462	15.728	11.085	0.1483 e^{-27}
FDG-PCC ($\beta 1$)	62.1914	2.252	27.615	0.0000 e^{-27}
Lac-PCC ($\beta 2$)	-98.0835	3.750	-26.149	0.0000 e^{-27}

Although metabolites are well able to classify data and predict the dependent variable in PCC, to better understand and visualize, the behavior of each metabolite (FDG and lactate) in predicting the dependent variable is shown as a single variable in Figure 5.16a and 5.16b for FDG and PCC lactate, respectively.

According to Figure 5.16a, we can see that FDG has been able to completely categorize the attendance of the subjects into two groups, rest and exercise. It also shows that the reduction of FDG increases the probability of being in the condition of exercise. But based on Figure 5.16b, we find that the lactate measured in PCC is not able to recognize and classify the subjects as well as the FDG value, although it has an effective and significant behavior in the model. As we can see, the measured values of lactate in the two groups of rest and exercise overlap and it is probably due to the difference in the order of data acquisition from the subjects.

Similar to the analysis we did about the binary logistic regression output in PCC, Table 5.2 also shows the regression output for SMA metabolites. According to the statistical values, both FDG and lactate metabolites have been able to be significantly (p=0.0000e^{-62}, p=0. 7927e^{-27} respectively) included in the model and predict the probability of the dependent variable. As the last column of this table shows, even though both metabolites are significant, FDG, with its decrease, has shown a

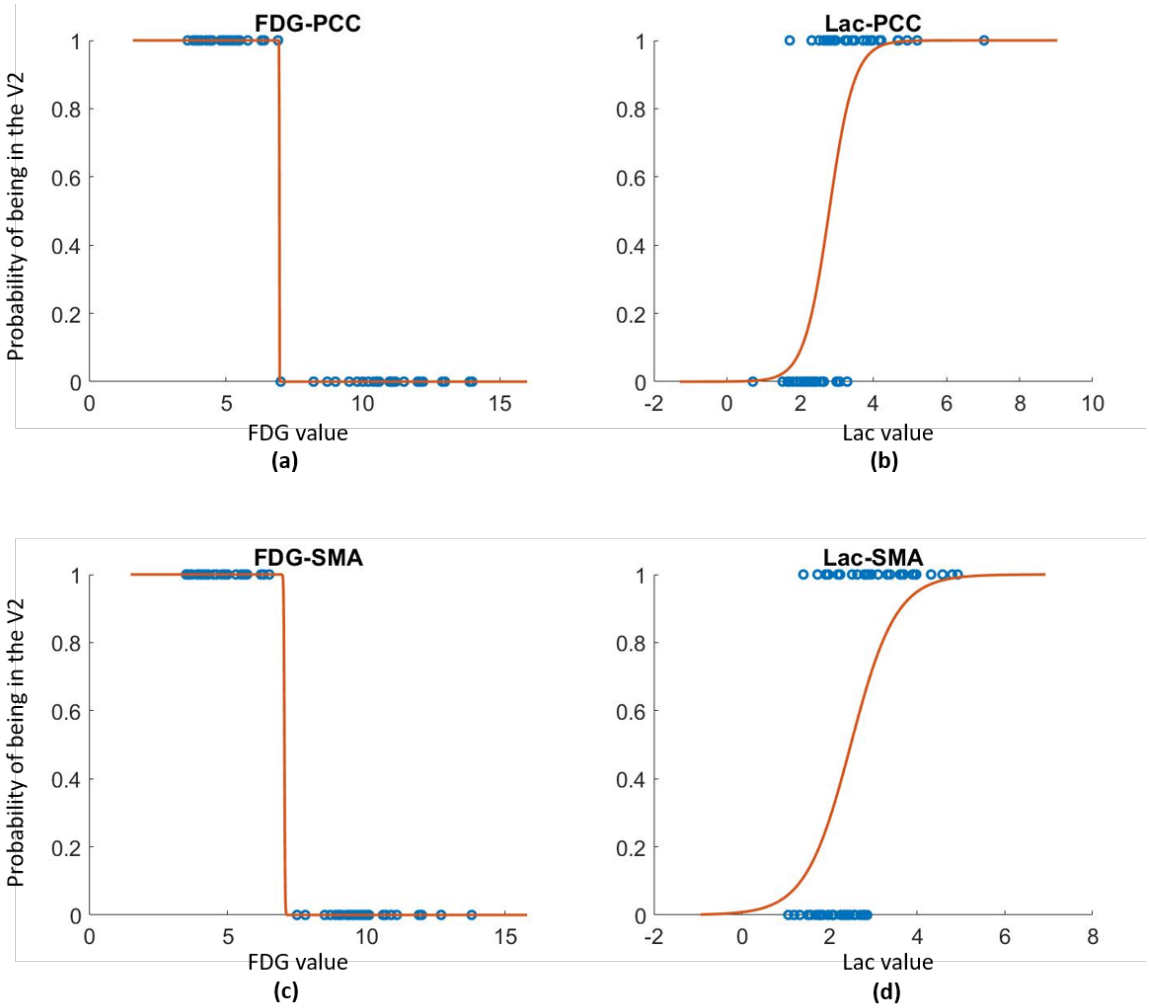


Figure 5.16: Graph of a logistic regression curve fitted to the brain data. (a) The curve shows the probability of being in the exercise condition versus the amount of the FDG-PCC. (b) The curve shows the probability of being in the exercise condition versus the amount of the Lac-PCC. (c) The curve shows the probability of being in the exercise condition versus the amount of the FDG-SMA. (d) The curve shows the probability of being in the exercise condition versus the amount of the Lac-SMA.

greater ability to classify subjects than lactate. The probability of predicting the dependent variable is calculated according to the following function:

$$p(x) = 1/(1 + e^{-(-432.0509+88.9647FDG-66.1667Lac)})$$

Figures 5.16c and 5.16d show the behavior of each variable of FDG and lactate, respectively, in

Table 5.2: The output of logistic regression analysis in SMA with two explanatory variables (FDG, Lac) and a binary categorical variable (V1, V2).

	Coefficient	**Std. Error**	**t-value**	**p-value**
Intercept ($\beta 0$)	-432.0509	20.528	-21.046	0.0000 e^{-62}
FDG-PCC ($\beta 1$)	88.9647	2.443	36.404	0.0000 e^{-62}
Lac-PCC ($\beta 2$)	-66.1667	3.954	-16.729	0.7927 e^{-62}

the prediction of being in the second visit (exercise mode). As we mentioned for PCC, in SMA, the lactate values overlapped in the first and second visits, which makes lactate unable to differentiate the groups as well as FDG.

5.6 Is there a relationship between plasmatic Lac and Glc?

In section 5.4, we obtained valuable information about the plasmatic behavior of lactate and glucose after exercise, which confirmed the initial hypothesis that plasma lactate and glucose levels increase after moderate to intense exercise. This increase was very significant for plasma lactate, which indicates an increase in lactate production in the muscle and release in the blood. Although the amount of plasma glucose has also faced a slight increase, it should be considered that during intense exercise, glucose is always being converted into lactate in the skeletal muscles. However, our initial hypothesis regarding the increase of both parameters was confirmed, and after that, similar to the hypothesis that was formed at the brain level, a secondary hypothesis was also proposed regarding these parameters, in which we seek to investigate the relationship between plasmatic lactate and glucose. The following analyzes are all similar to the operations in steps 5.5.1, 5.5.2, 5.5.3, and 5.5.4. Therefore, we refrain from additional explanations and present the results and their interpretation.

5.6.1 Simple Linear Regression results

We have used simple linear regression (SLR) to find the relationship between plasmatic lactate and glucose. As shown in Figure 5.9, the highest increase in the amount of lactate and glucose in the plasma was observed in BD3 and BD4, which was in 20 and 30 minutes after the start of exercise.

Therefore, if there is a relationship between plasma lactate and glucose, it is definitely in these two time zones. Therefore, in the following analysis, we have considered the average values in these two blood samples (Average BD3, BD4). Also, similar to the analysis done in the brain, we have used two modes of normalization with baseline (Average (BD3, BD4) - BD1) and without normalization with baseline.

The scatter diagrams of Figure 5.17 show the existence of a linear relationship between lactate and glucose in each case of normalizing with baseline (Figure 5.17a) and without normalizing with baseline (Figure 5.17b). In order to statistically check and evaluate the importance of this relationship, we refer to the results presented in Figure 5.18.

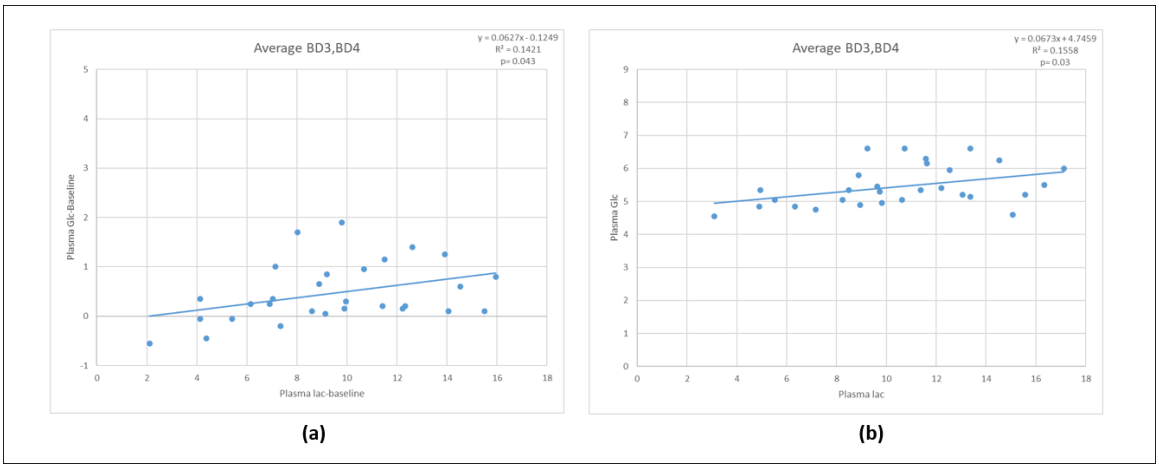
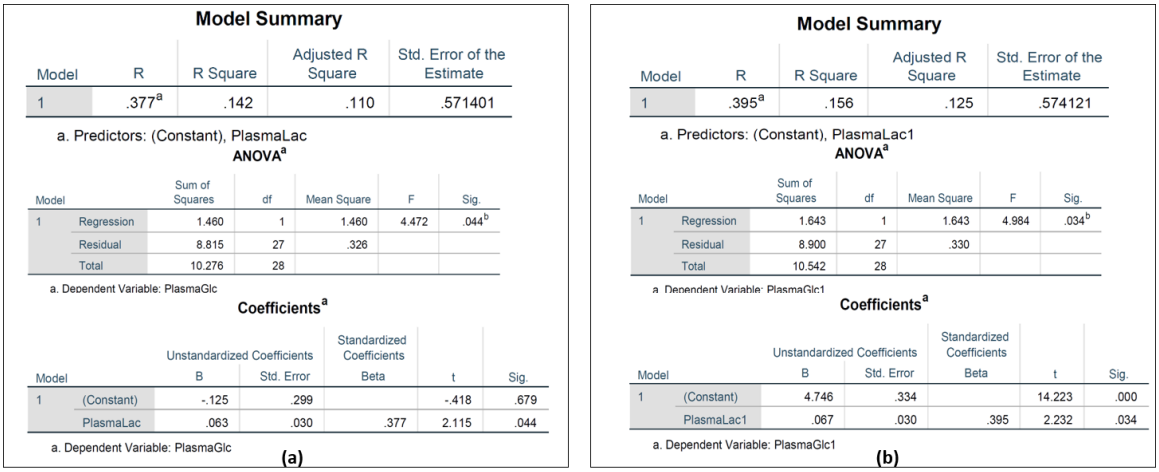


Figure 5.17: Linear regression model fitted to plasmatic lactate and glucose values: (a) Plasma-Glc vs. Plasma-Lac with normalization [Average (BD3, BD4)-BD1]. (b) Plasma-Glc vs. Plasma-Lac without normalization [Average (BD3, BD4)]. The horizontal axis represents lactate values and the vertical axis represents glucose values.

According to the information in the tables in Figure 5.18a and 5.18b, we find that there is a positive and significant average correlation between plasma lactate and glucose in both cases: normalization through BD1 (R= 0.377, R^2= 0.142, F (28) = 4.472, p= 0.04) and without normalization (R= 0.395, R^2= 0.156, F (28) = 4.984, p= 0.034).

Based on the results shown, as expected, there is a positive linear relationship between lactate and glucose at the plasma level, in the sense that increasing one increases the other and vice versa.



Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.377[a]	.142	.110	.571401

a. Predictors: (Constant), PlasmaLac

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.460	1	1.460	4.472	.044[b]
	Residual	8.815	27	.326		
	Total	10.276	28			

a. Dependent Variable: PlasmaGlc

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	-.125	.299		-.418	.679
	PlasmaLac	.063	.030	.377	2.115	.044

a. Dependent Variable: PlasmaGlc

(a)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.395[a]	.156	.125	.574121

a. Predictors: (Constant), PlasmaLac1

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.643	1	1.643	4.984	.034[b]
	Residual	8.900	27	.330		
	Total	10.542	28			

a. Dependent Variable: PlasmaGlc1

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	4.746	.334		14.223	.000
	PlasmaLac1	.067	.030	.395	2.232	.034

a. Dependent Variable: PlasmaGlc1

(b)

Figure 5.18: Simple Linear Regression analysis in SPSS: (a) It presents the statistical results related to the present graph in Figure 5.17.a. (b) It presents the statistical results related to the present graph in Figure 5.17.b.

5.6.2 Multiple Linear Regression results

Although in the previous part we observed a significant linear relationship between the metabolites in plasma (lactate and glucose), according to the difference in the two groups ”moderate-intensity” and ”high-intensity” in the graphs of Figure 5.10 Re-examining the linear regression by adding the exercise intensity variable can lead to a more accurate interpretation of the results and the statistical model.

For this purpose, we have implemented MLR with two independent variables (glucose and exercise intensity) and one dependent variable (lactate). We have considered the lactate variable to be dependent on the fact that based on the literature review, following intense exercise, glucose is converted into lactate in the muscles.

Figure 5.19 shows a representation of the MLR model in three-dimensional space in each of the two cases we consider with normalization and without normalization with the labels ”a” and ”b” respectively. For a more detailed analysis of these models, we refer to the statistical information presented in Figure 5.20.

The statistical outputs of the tables in Figure 5.20 are labeled with the same labels as Figure 5.19 and show their compatibility. Therefore, relying on the results of Figure 5.19a and 5.20a, we realize

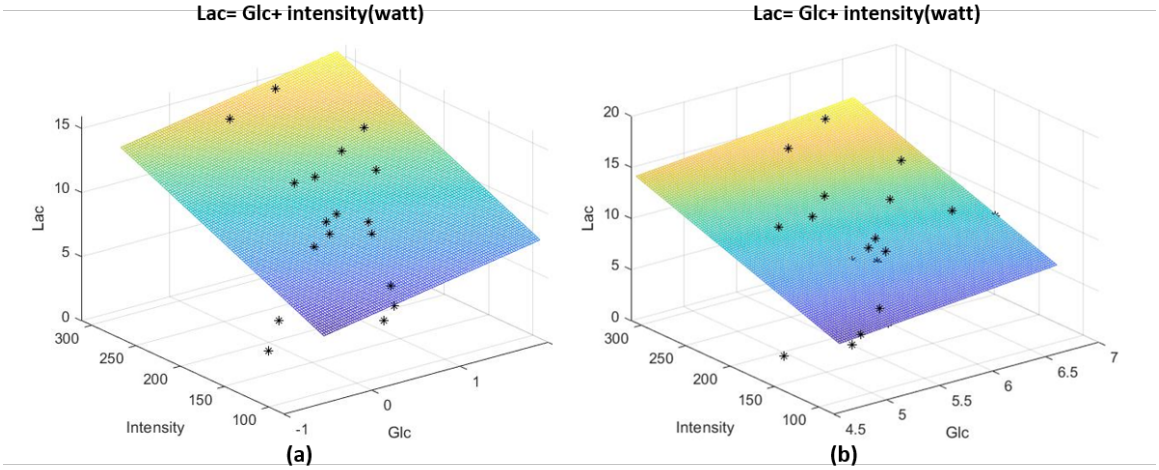


Figure 5.19: Linear regression model fitted to lactate, Glc, and intensity values: (a) Lac= Glc +Intensity in the plasma with normalization [Average (BD3, BD4)-BD1]. (b) Lac= Glc +Intensity in plasma without normalization [Average (BD3, BD4)].

that the regression model with two variables is significant (R=0.593, R^2=0.351, F (28) =7.040, p=0.004). By deepening the table of coefficients, the regression model is summarized as follows:

$$Y = 1.183Glc + 0.032Intensity + 3.3$$

This model indicates a positive relationship between exercise intensity versus lactate and glucose versus lactate. But more carefully, we notice that according to the t-test, the Glc variable in the model does not have a significant effect on Y, because the sig value for this variable is equal to 0.257, which is greater than 0.05. Therefore, the significance of the model depends on the intensity of the exercise (p=0.008).

By performing the same analysis, the results related to Figures 5.19b and 5.20b are summarized as follows:

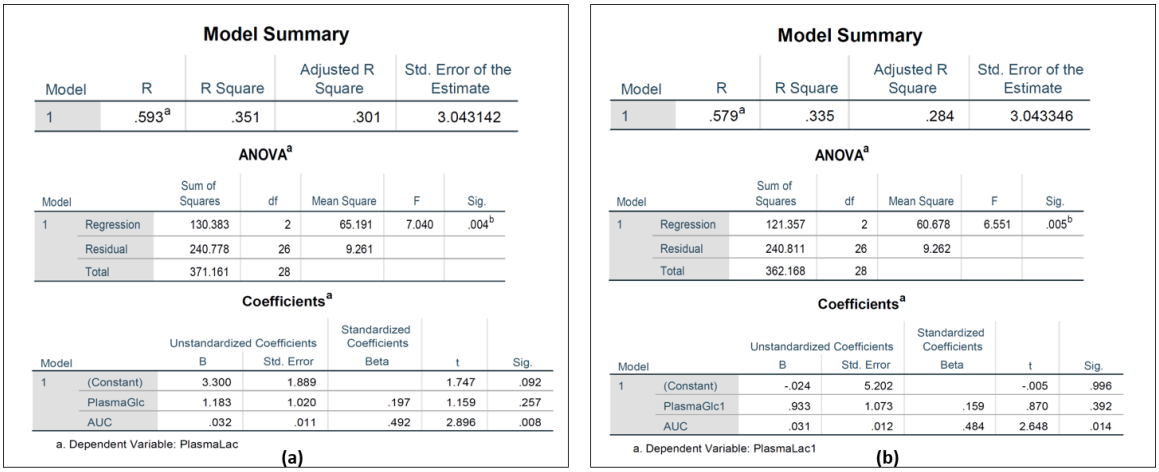
- MRL on plasmatic metabolites without normalization:

  The regression model with two variables is significant (R=0.579, R^2=0.335, F (28) =6.551, p=0.005), the regression model is:

$$Y = 0.933Glc + 0.031Intensity - 0.024$$

There is a positive relationship between exercise intensity versus lactate and glucose versus lactate.

P (Glc)= 0.392, P(Intensity)= 0.014. So, the Glc variable in the model does not have a significant effect on Y.



Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.593[a]	.351	.301	3.043142

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	130.383	2	65.191	7.040	.004[b]
	Residual	240.778	26	9.261		
	Total	371.161	28			

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	3.300	1.889		1.747	.092
	PlasmaGlc	1.183	1.020	.197	1.159	.257
	AUC	.032	.011	.492	2.896	.008

a. Dependent Variable: PlasmaLac

(a)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.579[a]	.335	.284	3.043346

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	121.357	2	60.678	6.551	.005[b]
	Residual	240.811	26	9.262		
	Total	362.168	28			

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	-.024	5.202		-.005	.996
	PlasmaGlc1	.933	1.073	.159	.870	.392
	AUC	.031	.012	.484	2.648	.014

a. Dependent Variable: PlasmaLac1

(b)

Figure 5.20: Multiple Linear Regression analysis in SPSS: (a) It presents the statistical results related to the present graph in Figure 5.19.a. (b) It presents the statistical results related to the present graph in Figure 5.19.b.

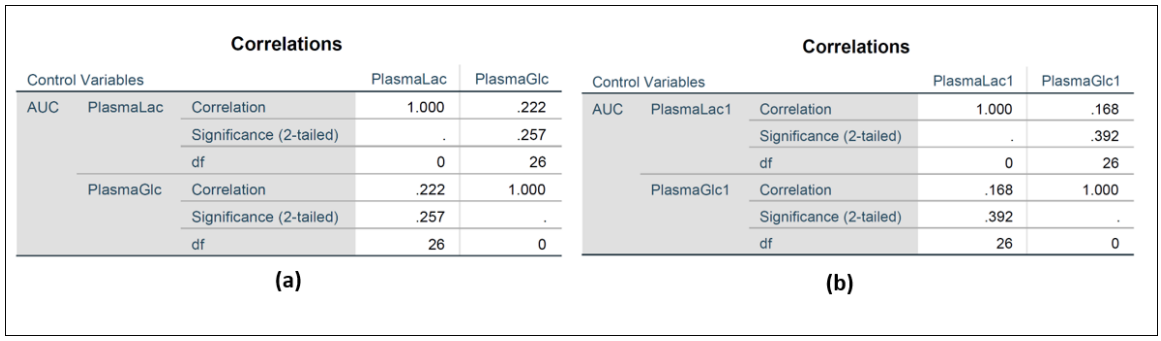
Although MLR shows us a significant model at the plasma level, as we have shown, the burden of this significance was on the intensity variable, and in this case, it did not matter where we looked for the relationship between lactate and glucose. Therefore, with the presented results, it can be concluded that by entering the third variable (exercise intensity) into the linear regression, and considering that the plasma lactate changes are significantly dependent on the exercise intensity, the significant linear relationship that was observed in the previous part has disappeared. But still, the results insist on the existence of a positive correlation (lactate increase as well as glucose increase).

5.6.3 Partial Correlation results

By using partial correlation, we intend to investigate the relationship between lactate and glucose at the plasma level without the influence of the difference in the intensity of the subjects' exercise. Figure 5.21 shows the partial correlation between lactate and glucose plasma variables by

controlling exercise intensity. The partial correlation of these two variables in normalization with BD1 (Figure 5.21a) and without normalization (Figure 5.21b) is equal to 0.222 and 0.168, respectively, which indicates a positive partial correlation. It is lactate and glucose in plasma. But this positive correlation is not statistically significant in any of the situations (p=0.257, p=0.392).

Therefore, by performing this test, we realize that by controlling and removing the effects related to exercise intensity, the significant linear relationship that we observed in part 5.6.1 disappears.



Correlations

Control Variables			PlasmaLac	PlasmaGlc
AUC	PlasmaLac	Correlation	1.000	.222
		Significance (2-tailed)	.	.257
		df	0	26
	PlasmaGlc	Correlation	.222	1.000
		Significance (2-tailed)	.257	.
		df	26	0

(a)

Correlations

Control Variables			PlasmaLac1	PlasmaGlc1
AUC	PlasmaLac1	Correlation	1.000	.168
		Significance (2-tailed)	.	.392
		df	0	26
	PlasmaGlc1	Correlation	.168	1.000
		Significance (2-tailed)	.392	.
		df	26	0

(b)

Figure 5.21: Partial Correlation in SPSS: (a) It indicates the correlation between Lac and Glc, whilst controlling for ”AUC (intensity: 10’-30’)” in the plasma with normalization [Average (BD3, BD4)-BD1]. (b) It indicates the correlation between Lac and Glc, whilst controlling for ”AUC (intensity: 10’-30’)” in plasma without normalization [Average (BD3, BD4)].

So far, the results obtained from all three parts SLR, MLR, and partial correlation can be interpreted as follows:

The results obtained from SLR, in which only two parameters lactate and glucose are involved, indicate the existence of a significant linear relationship. The partial correlation showed that this relationship disappears by removing the effect of intensity on the metabolites, which can prove that lactate and glucose are related to each other under intensity values. On the other hand, we know that there is a strong relationship between plasma lactate and the intensity of each subject, so entering the intensity values as the third variable in the linear regression causes the linear relationship observed in SLR to disappear because the intensity of exercise is sufficiently related to plasma lactate and this level of its significance makes the MLR model ignore the relationship between glucose and lactate.

5.6.4 Logit Regression results

Similar to the goal we had in the implementation of logistic regression in part 5.5.4, we are also interested in examining at the plasmatic level the ability of lactate and glucose values as independent values to predict the presence of the subject in the second visit.

Table 5.3 shows the output of binary logistic regression analysis at the plasmatic level.

Relying on the statistical information, the output shows that the increase in plasmatic lactate is significantly (p=0) related to the probability of being in the second visit (exercise mode), which proves the effect of exercise on plasma lactate production. While this is not true for plasmatic glucose (p=0.1860).

Table 5.3: The output of logistic regression analysis at the plasmatic level with two explanatory variables (Glc, Lac) and a binary categorical variable (V1, V2).

	Coefficient	**Std. Error**	**t-value**	**p-value**
Intercept ($\beta 0$)	94.4673	16.798	5.623	0.0000
FDG-PCC ($\beta 1$)	5.0732	3.836	1.322	0.1860
Lac-PCC ($\beta 2$)	-48.7474	0.972	-50.105	0

Based on the results, the plasmatic glucose variable is not able to predict the dependent variable. As shown in Figure 5.9b, although plasmatic glucose increased during exercise, its changes were minor and did not have significant changes in plasmatic lactate size. This is the reason why in figure 5.22a, lactate was completely able to distinguish the groups from each other, while glucose in figure 5.22b was not able to classify the groups due to the slight difference in the values of the first visit and the second visit and was not significantly added to the model.

Among the reasons for the slight increase in plasma glucose during exercise, we can mention the Cory cycle, in which glucose is converted into lactate during intense exercise in the skeletal muscles, and part of the lactate is converted back into glucose in the liver. Therefore, the production rate of lactate is higher than the production rate of glucose at the plasmatic level.

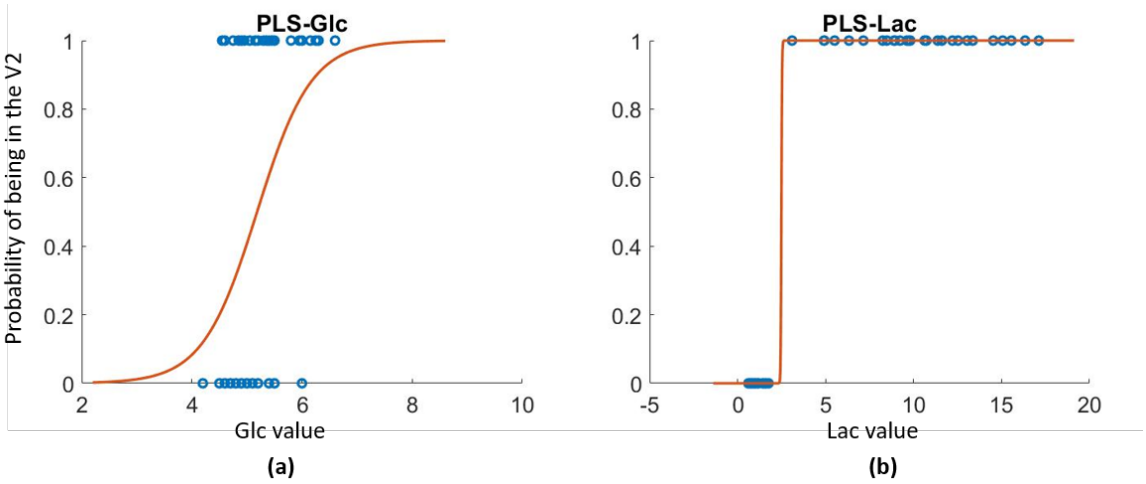


Figure 5.22: Binary Logistic Regression Analysis: (a) Plasmatic glucose during both visit1 and visit2. (b) Plasmatic lactate during both visit1 and visit2.

5.7 MRS-Lac and Plasma-Lac

After investigating the relationship between brain metabolites with each other and also evaluating the behavior of metabolites at the plasma level under the influence of exercise, our other goal is to investigate the relationship between brain metabolites and similar metabolites in plasma. Here, we investigated the behavior of brain lactate and plasma lactate after exercise.

As discussed in detail in the literature review, one of the important sources of lactate measured in the brain by MRS is blood lactate. According to the existing theories, lactate is produced in the muscles after intense exercise and through the bloodstream enters the brain as fuel. Therefore, it is expected that there is a strong correlation between brain lactate (MRA Lac) and plasma lactate.

The graphs presented in Figure 5.23 show the relationship between PCC-Lac and plasma-Lac (Figure 5.23a) and lactate measured in SMA versus plasma-Lac (Figure 5.23b).

Imaging of the PCC and SMA was done approximately 45 and 55 minutes after the start of the exercise (exact times were recorded for each subject). Also, as we mentioned in the framework of data collection, we have tried to observe the order of data acquisition from PCC and SMA regions between subjects. In addition, the collected values of plasma lactate are related before the subject enters the MR imaging suite (BD4) and after the completion of imaging (BD5). In other words, we have not been able to take blood samples during imaging from PCC and SMA. Therefore, in

order to investigate the relationship between MRS-Lac and plasm-Lac, it is necessary to report both values at the same time.

For this purpose, we have used linear interpolation to find accurate plasma-Lac values that correspond to the time of brain data acquisition.

As expected, Figure 5.23 shows a strong linear relationship between lactate measured in the brain and plasma. Also, the positive correlation between them indicates an increase in one after the other. More detailed statistical analyzes are reported in the tables in Figure 5.24.

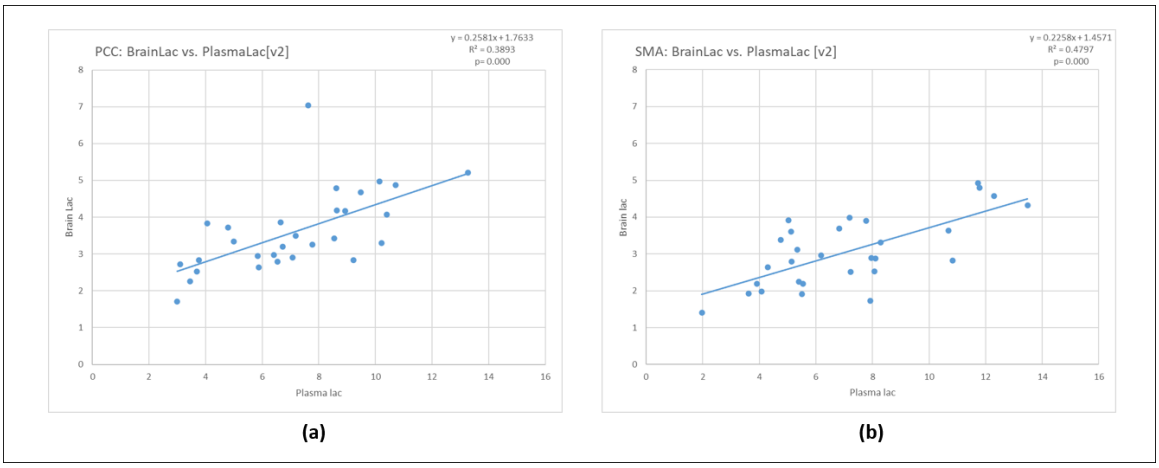


Figure 5.23: Linear regression model fitted to MRS-Lac and Plasma-Lac: (a) PCC-Lac vs. Plasma-Lac during the second visit. (b) SMA-Lac vs. Plasma-Lac during the second visit.

Based on the statistical information in Figure 5.24a, we find that the lactate measured in the PCC has a strong and significant linear correlation (R=0.624, R^2=0.389, F (28) =17.210, p=0.000) with the lactate measured in the plasma. According to the value of Sig in the ANOVA table, it can be said that the regression model has been able to describe the variance of the dependent variable well. Therefore, according to the regression coefficients or the estimation of the parameters in the Coefficients table, its linear equation is as follows:

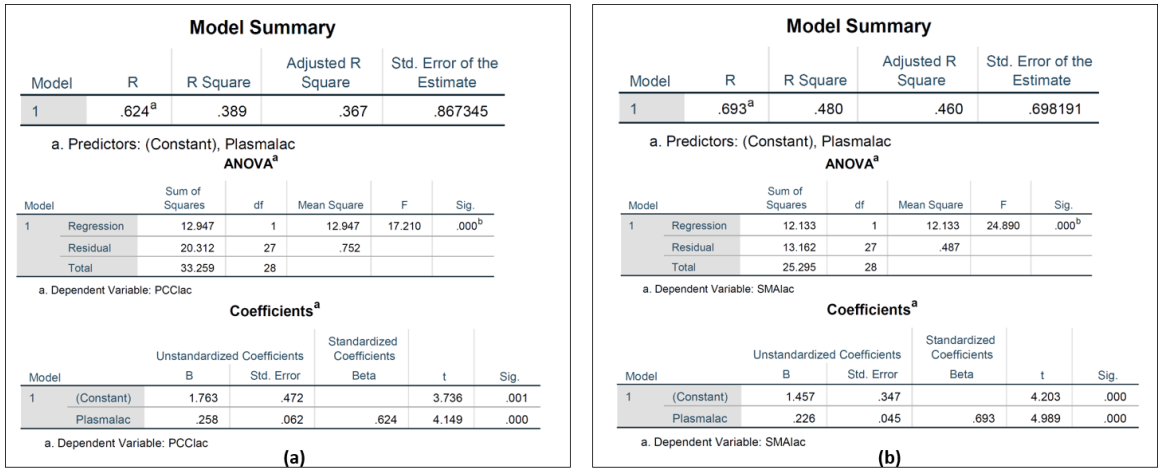
$$PCCLac = 0.258PlasmaLac + 1.763$$

SMA is no exception to this rule. Figure 5.24b shows that similar to the PCC, the SMA region has a significant positive correlation with plasma lactate values (R=0.693, R^2=0.480, F (28)

=24.890, p=0.000). The linear equation of this model is as follows:

$$SMALac = 0.226 PlasmaLac + 1.457$$

The results shown meet our expectations of the relationship between lactate measured in the brain and plasma. Also, these results confirm the review of the literature and prove that increased lactate in the blood following exercise is one of the primary sources of lactate measured in the brain and they are dependent on each other.



Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.624[a]	.389	.367	.867345

a. Predictors: (Constant), Plasmalac

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	12.947	1	12.947	17.210	.000[b]
	Residual	20.312	27	.752		
	Total	33.259	28			

a. Dependent Variable: PCClac

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	1.763	.472		3.736	.001
	Plasmalac	.258	.062	.624	4.149	.000

a. Dependent Variable: PCClac

(a)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.693[a]	.480	.460	.698191

a. Predictors: (Constant), Plasmalac

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	12.133	1	12.133	24.890	.000[b]
	Residual	13.162	27	.487		
	Total	25.295	28			

a. Dependent Variable: SMAlac

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	1.457	.347		4.203	.000
	Plasmalac	.226	.045	.693	4.989	.000

a. Dependent Variable: SMAlac

(b)

Figure 5.24: Simple Linear Regression analysis in SPSS: (a) It presents the statistical results related to the present graph in Figure 5.23.a. (b) It presents the statistical results related to the present graph in Figure 5.23.b.

5.8 Brain-FDG and Astrocyte-derived Lac

As we explained in the literature review, lactate observed by MRS has a secondary source in addition to plasmatic lactate. The secondary source is lactate derived from astrocytes under the ANLS theory.

After observing the strong correlation between MRS-Lac and plasmatic-Lac (in section 5.7), and considering that it is likely that a large part of the lactate observed by MRS is related to this source, a hypothesis formed in our minds. According to this hypothesis, it is possible that FDG is not related to the whole MRS-Lac, but only to a part of it that is produced in the brain!

In other words, there may be an inverse relationship between FDG and lactate derived from astrocytes.

For this purpose, first, in order to make sure that FDG has no relationship with that part of MRS-Lac that enters the blood, we evaluated the correlation between the two. Figure 5.25 shows FDG versus plasmatic-Lac.

As the scatterplots show, there is no significant linear relationship between PCC-FDG and plasmatic-Lac (Figure 5.25a) as well as between SMA-FDG and plasmatic-Lac (Figure 5.25b). The statistical information is presented in Figure 5.26a and 5.26b respectively for PCC-FDG vs. plasma-Lac (R=0.032, R^2=0.001, F (28) =0.028, p=0.866) and SMA-FDG vs. plasma-Lac (R=0.253, R^2=0.064, F (28) =1.855, p=0.184) also supports this claim.

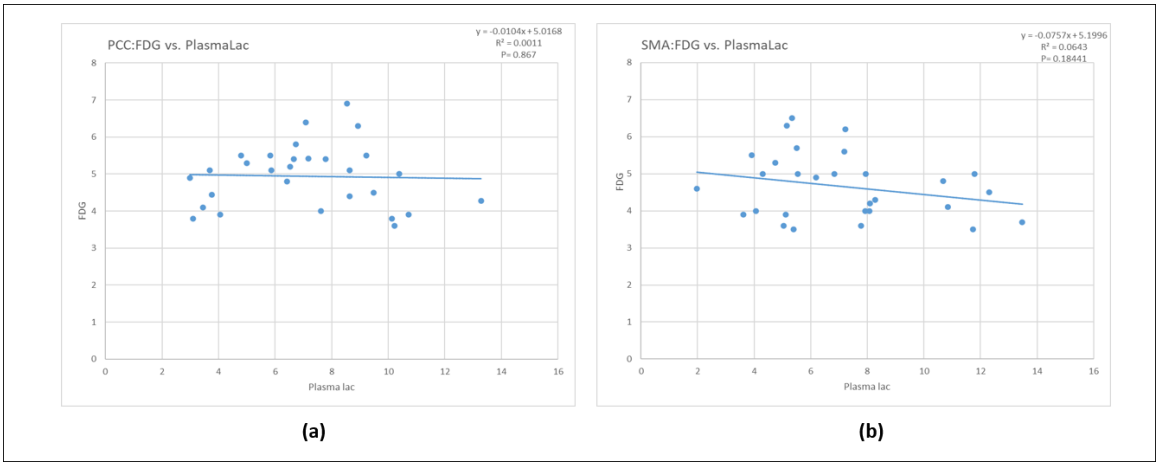


Figure 5.25: Linear regression model fitted to FDG and plasmatic lactate: (a) PCC-FDG vs. Plasma-Lac. (b) SMA-FDG vs. Plasma-Lac. The horizontal axis represents plasmatic lactate and the vertical axis represents FDG.

Therefore, in order to investigate the proposed hypothesis, we first calculated the remaining values of the line equations of part 5.7 and by removing the part related to plasmatic lactate, we considered it as lactate derived from astrocytes.

Figure 5.27 shows the correlation between FDG and Astrocyte-derived Lac, where the values related to PCC and SMA are labeled ”a” and ”b”, respectively.

As the statistical tables in Figure 5.28 confirm, contrary to our opinion, there is no significant relationship between FDG and Astrocyte-derived Lac either in PCC (R=0.375, R^2=0.128, F (28)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.033[a]	.001	-.036	.85532

a. Predictors: (Constant), PlasmaLac

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.021	1	.021	.029	.867[b]
	Residual	19.752	27	.732		
	Total	19.773	28			

a. Dependent Variable: FDG

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	5.017	.465		10.780	.000
	PlasmaLac	-.010	.061	-.033	-.169	.867

a. Dependent Variable: FDG

(a)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.254[a]	.064	.030	.85691

a. Predictors: (Constant), PlasmaLac

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.362	1	1.362	1.855	.184[b]
	Residual	19.826	27	.734		
	Total	21.188	28			

a. Dependent Variable: FDGSMA

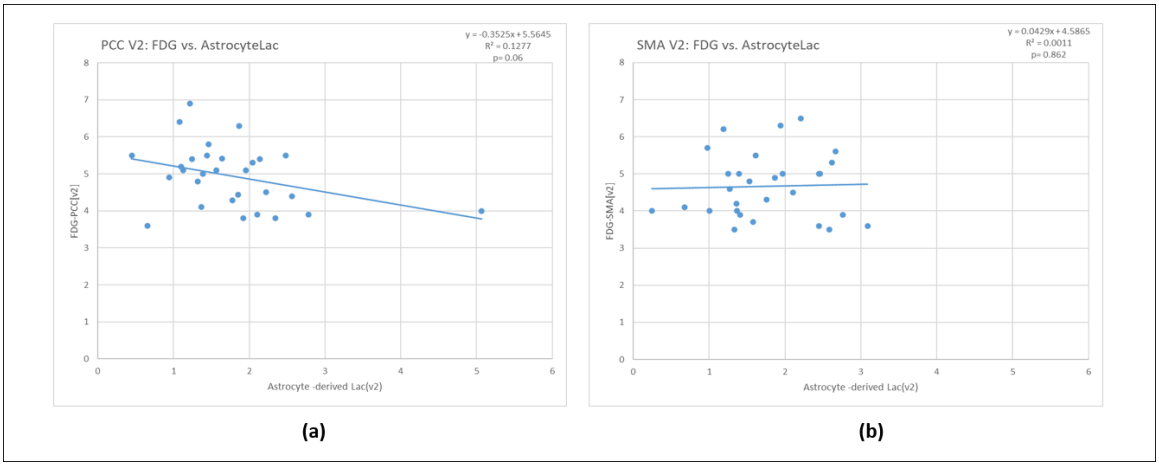
Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	5.200	.426		12.220	.000
	PlasmaLac	-.076	.056	-.254	-1.362	.184

a. Dependent Variable: FDGSMA

(b)

Figure 5.26: Simple Linear Regression analysis in SPSS: (a) It presents the statistical results related to the present graph in Figure 5.25.a. (b) It presents the statistical results related to the present graph in Figure 5.25.b.

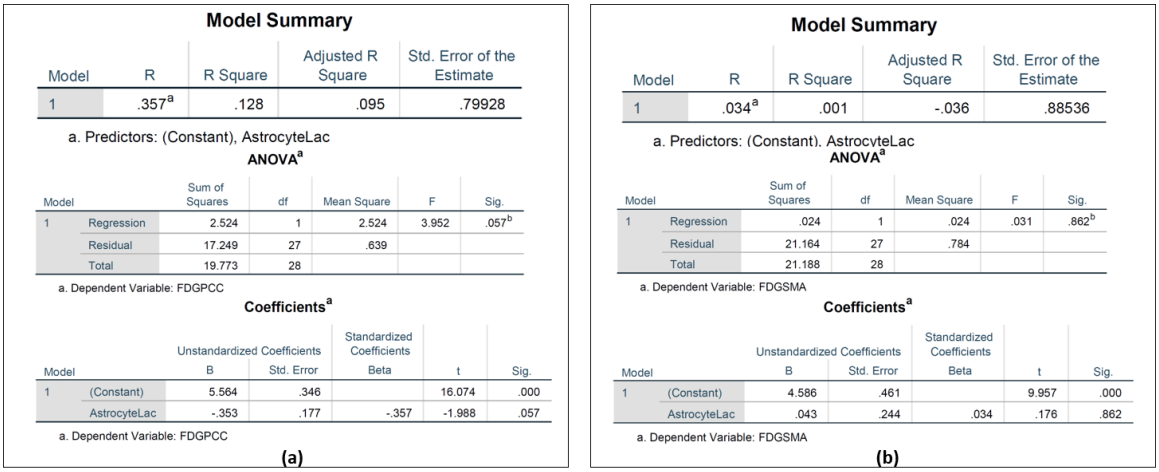


(a) (b)

Figure 5.27: Linear regression model fitted to FDG and Astrocyte-derived Lac: (a) PCC-FDG vs. Astrocyte-Lac. (b) SMA-FDG vs. Astrocyte-Lac. The horizontal axis represents Astrocyte lactate and the vertical axis represents FDG.

=3.952, p=0.057) or in SMA (R=0.034, R^2=0.001, F (28) =0.031, p=0.862). Although the value of Sig in the ANOVA table (Figure 5.28a) shows that the relationship between FDD and Astrocyte-derived Lac is close to significance for PCC, by being more precise in the scatter diagram presented in Figure 5.27a, we find the burden of this significance is due to the amount of Astrocyte-derived Lac of subject 29, which, by removing it, the p-value of the regression model increases to 0.12.

Therefore, based on the results shown, the FDG values measured in the brain in each region



Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.357[a]	.128	.095	.79928

a. Predictors: (Constant), AstrocyteLac

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.524	1	2.524	3.952	.057[b]
	Residual	17.249	27	.639		
	Total	19.773	28			

a. Dependent Variable: FDGPCC

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	5.564	.346		16.074	.000
	AstrocyteLac	-.353	.177	-.357	-1.988	.057

a. Dependent Variable: FDGPCC

(a)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.034[a]	.001	-.036	.88536

a. Predictors: (Constant), AstrocyteLac

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.024	1	.024	.031	.862[b]
	Residual	21.164	27	.784		
	Total	21.188	28			

a. Dependent Variable: FDGSMA

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	4.586	.461		9.957	.000
	AstrocyteLac	.043	.244	.034	.176	.862

a. Dependent Variable: FDGSMA

(b)

Figure 5.28: Simple Linear Regression analysis in SPSS: (a) It presents the statistical results related to the present graph in Figure 5.27.a. (b) It presents the statistical results related to the present graph in Figure 5.27.b.

of interest of PCC and SMA are not related not only to the total lactate (MRS-Lac) observed but also to the lactate produced in the brain (astrocyte-derived lactate). The obtained results reject our hypothesis on the existence of a significant linear relationship between brain glucose and brain lactate and it was contrary to our expectations.

5.9 Order of data acquisition in MRS

As we explained in the methodology section, in order to avoid the effects of systematic order, the order of data acquisition using MRS for the two ROIs, PCC and SMA, was balanced among the subjects.

The imaging of the first area was on average 45 minutes after the start of the exercise, and the imaging of the second area started with an interval of about 10 minutes. In other words, the imaging of the first area was between 45 and 55 minutes after the start of exercise and the imaging of the second area was between 55 and 65 minutes after the start of exercise.

Based on the presented results, we found that lactate increased after exercise in both ROIs (PCC and SMA). But we do not know about its stability. Therefore, it is possible that the increase in lactate will stop with the cessation of the exercise and the process of decrease will proceed.

On the other hand, in Figure 5.9, which shows the changes in plasmatic lactate and glucose during the second visit, we have shown that after exercise, the significant increase observed in plasma lactate quickly takes a downward trend and returns to its normal value. Therefore, it is highly likely that this process is also true for brain lactate because Figure 5.23 shows a strong correlation between MRS lactate and plasmatic lactate.

To investigate this issue more closely, we divided the subjects into two groups according to the order of data collection: PCC1st- SMA2nd (which includes 15 subjects) and SMA1st- PCC2nd (which includes 14 subjects)

According to the box plots in Figure 5.29, we find that the average lactate measured in PCC is almost the same in both groups (PCC1st and PCC2nd), although there is a decrease in group 2nd compared to group 1st. Unlike PCC, in SMA there is a significant difference between the two groups (SMA1st and SMA2nd) and it is well known that over time the average lactate measured in group 2nd decreased.

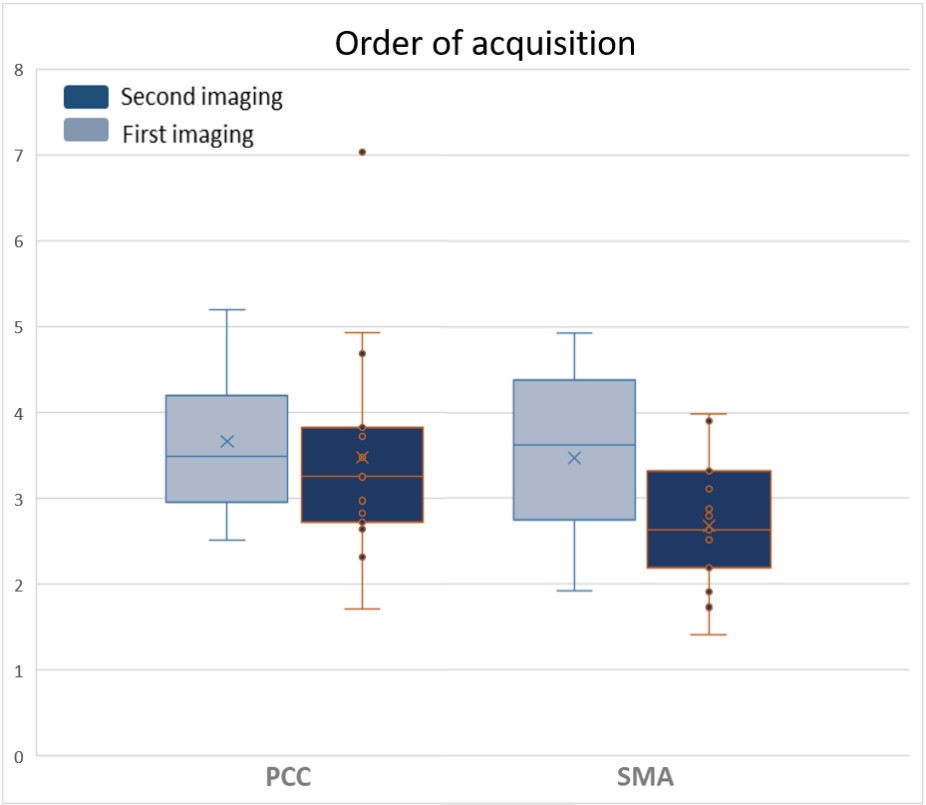


Figure 5.29: Order of acquisition for the two regions of interest PCC and SMA

Based on the presented results, it can be concluded that the increased lactate in the brain after exercise, at least in SMA, is not stable, and actually decreases after 20 minutes from the end of the exercise. Lactate not being stable in the brain, especially in SMA, can be one of the reasons for not observing a significant linear relationship between FDG and MRS lactate. In addition, the obtained information can be a document proving the existence of behavioral differences in PCC and SMA in the way of energy metabolism.

Although several analyzes were performed on the data, in this chapter we attempted to summarize the main findings of the data collection and analysis that were directly related to answering the research questions because it is not possible to present all the results due to the limitation of the length of the thesis.

Chapter 6

Conclusion and Future Work

6.1 Concluding Remark

By analyzing lactate and glucose changes in the brain and plasma after exercise, this thesis has shown how moderate to intense exercise can directly affect the energy metabolism of the brain and body.

Based on the quantitative analysis of the measured parameters, it can be concluded that the fueling of neurons under exercise conditions differs from the normal state. The results show that brain lactate concentration in the ROIs increases significantly after exercise while a significant decrease in FDG absorption occurs. These results, confirming the initial hypothesis, indicate the preference of neurons to use lactate as fuel over glucose during exercise.

In addition, with the measurements performed at the plasmatic level and the analysis of the relationship between the lactate produced in the plasma and the lactate recorded in the brain, we conclude that one of the primary sources of brain lactate is the lactate produced in the skeletal muscles, which is provided to neurons by crossing the blood-brain barrier.

Although this research clearly shows a significant decrease in FDG uptake following exercise and an increase in lactate, it rejects the hypothesis of a linear relationship between these two metabolites involved in brain energy metabolism. There may be a strong non-linear relationship between FDG and lactate. Still, this study was limited to recording these two quantities, and more variables are needed to estimate the non-linear model. Therefore, despite the limitations in recording

additional variables and based on the analyzes performed, it can be concluded that the observed changes in cerebral lactate and glucose due to exercise are two completely independent phenomena that occur in parallel in the brain; And it cannot be claimed that the increase of lactate follows the reduction of brain glucose.

This study also showed that the amount of changes in plasmatic lactate and brain lactate strongly correlates positively with the intensity of each subject's exercise. In comparison, glucose changes are an exception to this rule and do not show significant changes by manipulating exercise intensity. However, the logistic regression analysis showed that brain energy metabolism metabolites effectively predict the probability of the subject's presence during the visits (rest or exercise). These results support a strong relationship between the increase in lactate concentration and the decrease in FDG absorption with the possibility of the subject being present in two different positions (rest or exercise).

One of this study's most critical findings is that lactate's behavior in SMA is different from that of lactate in PCC. The analyzes carried out on the order of data acquisition by the MRS technique from the PCC and SMA confirmed that the behavior of lactate does not change much after 20 minutes after the end of the exercise in the PCC, but the trend in the SMA takes the reduction. Overall, these results reinforce the idea that the supplementary motor area needs to receive and metabolize energy faster than the posterior cingulate cortex. On the other hand, it has been well established that the rate of glucose metabolism is significantly faster than that of lactate. Therefore, it can be concluded that SMA relies more on glucose absorption due to its immediate need for energy, while PCC has a greater tendency to absorb lactate as fuel.

In general, the findings of this study make several contributions to the current literature and complement the findings of previous studies. Before this study, the prediction of how exercise affects brain energy metabolism had been limited to invasive and indirect measurement techniques (via arterial difference concentration measurements). Therefore, this is the first report examining brain lactate and glucose changes from a group of 29 healthy individuals following exercise, using a non-invasive imaging technique to record data. In addition, the findings reported here can be generalized to society due to the high sample size and fairness in the gender of the participants. Also, the insight gained from this study could lead to better-defined interventions with exercise or

other approaches in patients with various neurological disorders.

6.2 Future Work

Although this project has provided valuable information on brain function, further research using controlled experiments is needed to provide more definitive evidence of the exact mechanism of energy metabolism in the brain. In addition, if the discussion is to be pushed forward, it is necessary to overcome the limitations of the data collection protocol to lead to reliable findings. Below are some suggestions that can be relied on to expand the project:

- This study can be limited to recording lactate data from only one region of interest to eliminate the effect of the order of data acquisition. In this case, the data recorded from the subjects at the same time will be the same in time and lead to better conclusions about the behavior of lactate.

- By training the subjects through a training program for several weeks before the start of the test, it can be expected to a large extent that the participants will be able to withstand the conditions of the exercise test for 25 minutes with high exercise intensity (80% of VO2 max). In this case, the test can be implemented under better-controlled conditions, and the effect of exercise intensity on the results is reduced.

- Our information about the rate of return of increased lactate to its regular rate after exercise needs to be completed. Therefore, it is better to design the data collection protocol so that MRS records brain lactate data during the increase of lactate production in the body. Since it is impossible to use the MR imaging system when the subject exercises, I recommend that other methods, such as lactate injection, do lactate production in the body. In this case, the injection can be done only a few seconds before the data collection, and the data recording is done without time loss.

- Pyruvate is another essential metabolite involved in energy metabolism. As we explained in the literature review, in the process of glycolysis, glucose first turns into pyruvate and then into lactate. Therefore, measuring other involved variables, such as pyruvate, is suggested to

understand the relationship between lactate better and brain glucose and model it. It should be kept in mind that the measurement of pyruvate by MRS requires the injection of 13C-enriched substrates.

Appendix A

Recruiting participants procedure

We invited volunteers to participate in this research project using promotional email. The email included the topic, a brief description of the project steps, and some criteria necessary to participate in the study, which determined the target community. Some of these criteria are as follows:

- Participants must be between the ages of 25 and 45
- They must engage in regular, moderate-intensity physical activity (at least three times per week)
- They must not have any non-removable metallic piercings, metal fragments, or other non-removable metallic objects on their bodies.
- They must not be pregnant or breastfeeding.
- They must not have claustrophobia.
- They must not have a history of neurological disorders.
- They must not be smokers.

Further, the volunteers interested in participating were separately informed about the purpose of the study and the procedures governing it. They were invited to complete the online survey form to register and obtain more information.

Survey

One of the essential steps before collecting the desired data is determining the target community. The nature of our project is designed in such a way that subjects cannot be selected randomly. Therefore, the survey form has a significant role in providing more comprehensive information from the volunteers to evaluate their eligibility to participate in the study. This strategy causes the participants in this type of research project to be selected by sampling based on the established community, which leads to easy and targeted sampling.

The survey consists of two parts: an adult medical history form and a Get Active Questionnaire.

In the adult medical history part, we examine each volunteer's personal and family medical history, as well as the surgeries and medications they have taken. In the Get Active Questionnaire part, in addition to being informed about the hours of physical activity of the volunteer, several questions about the health and physical condition of the subjects in order to check the factors that may affect their ability to do physical activity (such as chest pressure or dizziness during physical activity, shortness of breath at rest, avoidance of specific physical activities due to medical reasons) are planned.

The primary purpose of designing the survey form is to evaluate the cardiovascular risk to obtain permission for the VO2max test and the exercise part of the protocol. In order to save the valuable time of the researcher as well as the participant, this form has been provided to the volunteers in the form of a web form.

Inclusion criteria

Subjects are selected based on three basic criteria to participate in the study:

I. Body Mass Index (BMI): It is a way to know the approximate level of body fat and fitness based on a person's weight-to-height ratio, which applies to the body of every adult man and woman. We use equation 28 to calculate body mass index. This scale is almost accurate and depends only on two variables: the weight and height of the candidate.

$$BMI = \frac{mass(kg)}{height(m)^2} \tag{28}$$

Eligible people to participate in the study should be in the normal range (18.5 – 24.9), according to Table A.1. But since BMI cannot distinguish between muscle and fat, an athlete's BMI may be in the range of an overweight person, while these people are perfectly fit and ideal. Therefore, by evaluating the sports history of the participants, we make an exception for athletes with high BMI (25.0 – 29.9). Otherwise, the candidate's request to cooperate in this research will be rejected at this stage because they do not have an excellent cardiopulmonary condition.

Table A.1: BMI, basic categories

Category	**BMI (kg/m2)**
Underweight (Severe thinness)	< 16.0
Underweight (Moderate thinness)	16.0 – 16.9
Underweight (Mild thinness)	17.0 – 18.4
Normal range	18.5 – 24.9
Overweight (Pre-obese)	25.0 – 29.9
Obese (Class I)	30.0 – 34.9
Obese (Class II)	35.0 – 39.9
Obese (Class III)	≥ 40.0

II. History of regular aerobic exercises: based on the protocol designed for this test, the participants should be able to handle the part related to the VO2max test and exercise. Therefore, according to *Canada's physical activity guidelines*, adults should have moderate-to-vigorous physical activity at least three times per week. Otherwise, the subject will not be able to complete the exercise part of the protocol, leading to recording inappropriate results. Therefore, the presence of people who do not have a history of regular aerobic exercises is prevented.

III. Medical background evaluation: If the candidate passes the first two stages, for a more accurate assessment of the possibility of cardiovascular risk and to ensure the physical ability of the participant in all stages of the test, the medical history of the candidate and immediate family members will be evaluated by the cardiopulmonary suite supervisor. Finally, if the candidate's information is confirmed, they will be selected to cooperate with this project.

Exclusion criteria

All people receiving medication for high blood pressure, blood cholesterol, diabetes, or neuroactive drugs, as well as people with a history of smoking, or cardiopulmonary diseases of any kind, are prohibited from participating in this project. Also, subjects with orthopedic restrictions or other restrictions preventing exercise testing on a stationary bike were excluded. According to the latest guidelines of the *American College of Sports Medicine*, people who are prohibited from any exercise test or exercise are also excluded from participating in the project. Furthermore, we ensured that any individual who had engaged in another study protocol involving radiation doses during the previous 12 months did not receive a total radiation dose of more than 20 mSv[1] by engaging in the current investigation. Otherwise, the volunteer is prohibited from participating in the experiment in order to comply with safety.

In addition to all the mentioned cases, the possibility of withdrawing from the cooperation has been available for any participant who wished to do so at any time without needing to explain. Also, the researchers could exclude any subject from the study if they noticed medical contraindications or other mentioned cases or if the subject did not follow the instructions. Then, the subjects were compensated according to the project participation percentage.

[1]The scientific unit for measuring whole-body radiation dose, called ”effective dose,” is the millisievert (mSv).

Appendix B

Sample Size

The sample size is determined based on the type of study. The larger the sample size, the lower the number of errors in the conclusion because it will better represent the community and provide more accurate results. However, in extensive samples, the increase in accuracy will be slight, and the additional time and expense of recruiting more participants are unnecessary. On the other hand, a smaller selection will need more statistical power to answer the primary research question and will lead to non-scientific results. In a clinical trial, a well-executed study may fail to determine significant effects and associations between variables simply because of insufficient sample size and will have no future benefit.

In this study, in a certain period, 61 people registered their request to participate in the experiment by filling out an online survey. But not all of them had the necessary criteria to enter the research project. Due to this, 34 qualified people were included in the analysis, and to respect the fairness and generalization of the results, it has been tried that women and men have an equal share in participating in the project. Meanwhile, 3 of the participants during the data collection, respectively, due to fear of the imaging environment, allergic reaction, and drop in blood pressure after sampling, refused to continue participating in the project or were excluded as determined by the researchers. As a result, data collection continued with 31 subjects. After completing the data collection, we found out that the brain glucose measurements of two of the subjects had an error, which led to the deletion of all the data recorded from them in the test process.

Finally, the sample size of this study is 29 healthy people, including 13 women and 16 men aged

25 to 45, and the population of each of the male and female groups is large enough to measure their differences. We believe that this sample size is good enough for this study because, as I mentioned, a smaller number leads to non-scientific and insignificant results. On the other hand, increasing accuracy in wide samples is not significant due to additional time and cost. Table B.1 provides complete information of each of the subjects.

Table B.1: Participant information

Participants	**Gender**	**Age**	**Height (m)**	**Weight (kg)**	**BMI (kg/m2**	**Training Hour (min/week)**
Subject 01	Female	33	1.6	53.8	21.02	540
Subject 02	Female	29	1.73	59.1	19.75	240
Subject 03	Male	31	1.78	77	24.30	337
Subject 04	Female	30	1.7	68	23.53	180
Subject 05	Male	43	1.83	80	23.89	120
Subject 06	Male	29	1.86	95	27.46	600
Subject 07	Male	29	1.66	71	25.77	105
Subject 08	Male	30	1.73	67.7	22.62	280
Subject 09	Female	45	1.68	54.5	19.31	160
Subject 10	Female	29	1.54	57.7	24.33	180
Subject 11	Female	30	1.62	54	20.58	60
Subject 12	Male	29	1.75	84	27.43	315
Subject 13	Female	28	1.63	57.7	21.72	180
Subject 14	Male	34	1.83	79	23.59	180
Subject 15	Male	29	1.83	84	25.08	360
Subject 16	Male	36	1.8	82	25.31	240
Subject 17	Female	45	1.7	63.6	22.01	180
Subject 18	Female	32	1.63	62	23.34	240
Subject 19	Male	35	1.87	88	25.17	180
Subject 20	Male	27	1.77	88	28.09	240
Subject 21	Male	34	1.85	84.1	24.57	150
Subject 22	Female	28	1.63	53.2	20.02	180
Subject 23	Female	26	1.68	66.8	23.67	120
Subject 24	Female	30	1.7	68.2	23.60	300
Subject 25	Male	33	1.72	54.6	18.46	120
Subject 26	Female	39	1.6	55.5	21.68	210
Subject 27	Male	44	1.78	76	23.99	360
Subject 28	Male	42	1.85	80.9	23.64	300
Subject 29	Male	30	1.83	75	22.40	180

Appendix C

Data acquisition protocol details

Interview

Before starting any data collection on the first day of the experiment, a short interview (15 to 20 minutes) will be conducted in person with the participant. During this interview, once again, the purpose of the project and all the steps are carefully explained to the subject, and the questions and concerns formed in the subject's mind about the project are answered. Then they are asked to fill out and sign the consent form. All the questions asked during the interview are only related to the research, and the participant has the right to remain completely anonymous or disclose personal information if necessary.

Pregnancy Test

Participating in this research project and undergoing a PET scan may have known or unknown risks to pregnant women, unborn children, or breastfed infants. Therefore, female participants cannot participate in this plan if they are pregnant or likely to be pregnant. In this regard, before any PET scan, female subjects are asked to perform a rapid pregnancy test strip (FaStep® hCG Rapid Test Strip (HCG-U11)), and only if the result of the pregnancy test is negative are they allowed to participate in the project.

Questionnaires

In the first visit of the experiment, four valid behavioral questionnaires designed to control interpersonal changes in the way of experiencing experimental sessions are implemented. This includes:

I. Sallis Self-Efficacy Questionnaire (SSE) (Figure C.1):

This questionnaire is a shorter version of the Sallis exercise effectiveness questionnaire. This questionnaire is used to control changes in regular physical activity. In fact, in this way, we check how confident the subject is to do things continuously (at least for six months) to take advantage of the opportunity to exercise. SSE consists of 12 questions, and the participants fill out the questionnaire based on the Likert scale. This scale includes five options from ”I know I can't” to ”I know I can.” SSE is filled in the interval between MRI and PET scan where the subject spends the waiting time.

II. Amsterdam Resting-State Questionnaire (ARSQ) (Figure C.2):

The ARSQ quickly provides information about the participants' state of thoughts and consciousness during the scanning session. This questionnaire is used to control the active state of mind or wakefulness. Participants fill out this 18-question questionnaire immediately after the MRA session. ARSQ is also composed on a Likert scale and includes five options from ”Completely Disagree” to ”Completely Agree.”

III. Positive and Negative Affect Questionnaire (PANAS) (Figure C.3):

The PANAS consists of several words describing different feelings and emotions that the subject may have felt during the data collection process. This questionnaire contains 20 words on a 5-point Likert scale, where the participant chooses the appropriate answer from ”very little or not at all” to ”extremely.” This questionnaire is also filled out while waiting for the PET scan.

IV. STAI Y-6 Questionnaire (Figure C.4):

This questionnaire is a 6-question version of the Spielberger State-Trait Anxiety Inventory (STAI). At the end of the exercise test to determine the ventilation threshold, the participants

rate these statements on a Likert scale of 1-4: I feel calm, upset, and content. I am relaxed, tense, and worried. We ask participants to spend only a little time on each comment and consider the answer that best describes their feelings.

Sallis Self-Efficacy Questionnaire (Exercise Confidence Survey)

ID:	
Date:	

Whether you exercise or not, please rate how confident you are that you could really motivate yourself to do things like these consistently, for at least six months.
Please circle one number for each question.How sure are you that you can do these things?

#		I know I can not		Maybe I can		I know I can	Does not Apply
1	Get up early, even on weekends, to exercise.	1	2	3	4	5	(8)
2	Stick to your exercise program after a long, tiring day at work.	1	2	3	4	5	(8)
3	Exercise even though you are feeling depressed.	1	2	3	4	5	(8)
4	Set aside time for a physical activity program; that is, walking, jogging. swimming, biking, or other continuous activities for at least 30 minutes, 3 times per week.	1	2	3	4	5	(8)
5	Continue to exercise with others even though they seem too fast or too slow for you.	1	2	3	4	5	(8)
6	Stick to your exercise program when undergoing a stressful life change (e.g., divorce, death in the family, moving).	1	2	3	4	5	(8)
7	Attend a party only after exercising.	1	2	3	4	5	(8)
8	Stick to your exercise program when your family is demanding more time from you.	1	2	3	4	5	(8)
9	Stick to your exercise program when you have household chores to attend to.	1	2	3	4	5	(8)
10	Stick to your exercise program even when you have excessive demands at work.	1	2	3	4	5	(8)
11	Stick to your exercise program when social obligations are very time consuming.	1	2	3	4	5	(8)
12	Read or study less in order to exercise more.	1	2	3	4	5	(8)

Score:	

Figure C.1: Sallis Self-Efficacy Questionnaire (SSE)

Amsterdam Resting-State Questionnaire (ARSQ)

ID:	
Date:	

Below is a list of the ways you might have felt during the scan. Please indicate which of these applied to you.

#		Completely Disagree	Disagree	Neither Agree nor Disagree	Agree	Completely Agree
1	I had rapidly switching thoughts.					
2	I thought about myself.					
3	I thought about things I need to do.					
4	I felt tired.					
5	I felt sleepy.					
6	I had difficulty staying awake.					
7	I felt comfortable.					
8	I felt relaxed.					
9	I was conscious of my body.					
10	I thought about my breathing.					
11	I thought about my heartbeat.					
12	I felt pain.					
13	I felt restless.					
14	I enjoyed the session.					
15	I had negative feelings.					
16	I felt bored.					
17	I felt nothing.					
18	I thought about the sounds around me.					

Score:	

Figure C.2: Amsterdam Resting-State Questionnaire (ARSQ)

Positive and Negative Affect Questionnaire (PANAS)

ID:	
Date:	

This scale consists of a number of words that describe different feelings and emotions. Read each item and then please circle the appropriate answer.

#	Indicate the extent you have felt this way **Today**	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
1	Interested	1	2	3	4	5
2	Distressed	1	2	3	4	5
3	Excited	1	2	3	4	5
4	Upset	1	2	3	4	5
5	Strong	1	2	3	4	5
6	Guilty	1	2	3	4	5
7	Scared	1	2	3	4	5
8	Hostile	1	2	3	4	5
9	Enthusiastic	1	2	3	4	5
10	Pround	1	2	3	4	5
11	Irritable	1	2	3	4	5
12	Alert	1	2	3	4	5
13	Ashamed	1	2	3	4	5
14	Inspired	1	2	3	4	5
15	Nervous	1	2	3	4	5
16	Determined	1	2	3	4	5
17	Attentive	1	2	3	4	5
18	Jittery	1	2	3	4	5
19	Active	1	2	3	4	5
20	Afraid	1	2	3	4	5

Score:	

Figure C.3: Positive and Negative Affect Questionnaire (PANAS)

STAI: Y-6

ID:	
Date:	

English version: Read each statement and then circle the most appropriate number to the right of the statement to indicate how you feel **right now, at this moment.**
There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

#		Not at all	Somewhat	Moderately	Very Much
1	I feel calm	1	2	3	4
2	I am tense	1	2	3	4
3	I feel upset	1	2	3	4
4	I am relaxed	1	2	3	4
5	I feel content	1	2	3	4
6	I am worried	1	2	3	4

French version : Lisez chacun des énoncés et cochez dans le case appropriée de droite ce qui convient le mieux à la façon dont vous vous sentez maintenant;
Il n'existe ni bonnes ni mauvaises réponses. Ne passez pas trop de temps sur chacun des points, mais donnez la réponse qui semble décrire le mieux. Répondez à toutes les questions et ne cochez qu'une case pour chacune d'entre elles.

#		Pas du tout	Un peu	Modérément	Beaucoup
1	Je me sens calme	1	2	3	4
2	Je suis tendu	1	2	3	4
3	Je me sens contraint	1	2	3	4
4	Je suis détendu	1	2	3	4
5	Je suis content	1	2	3	4
6	Je suis inquiet	1	2	3	4

Score:	

Figure C.4: STAI Y-6 Questionnaire

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