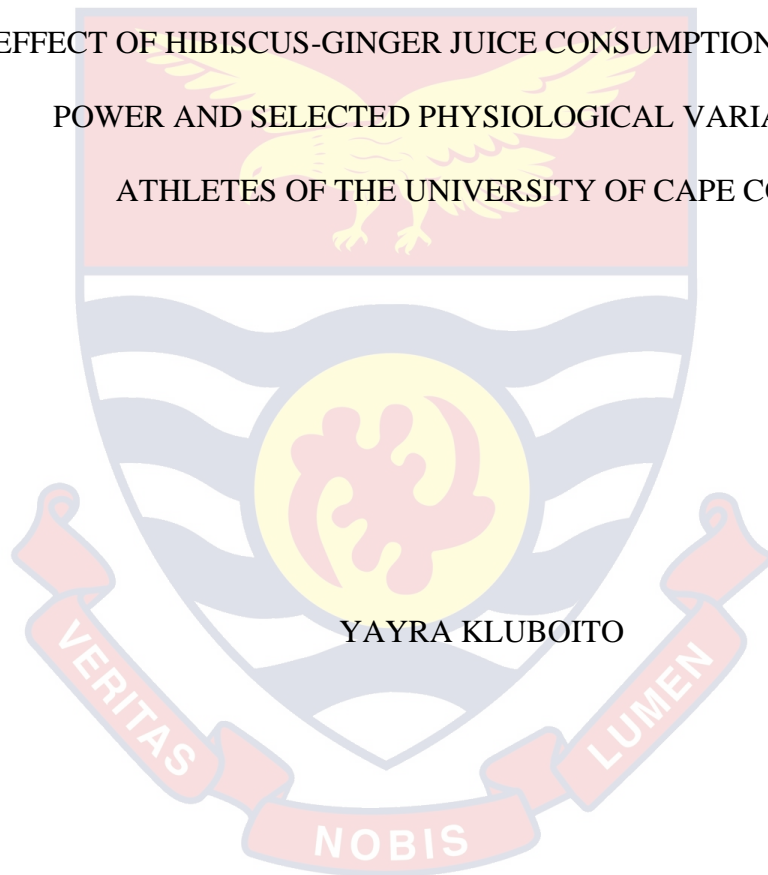


UNIVERSITY OF CAPE COAST

EFFECT OF HIBISCUS-GINGER JUICE CONSUMPTION ON AEROBIC
POWER AND SELECTED PHYSIOLOGICAL VARIABLES OF
ATHLETES OF THE UNIVERSITY OF CAPE COAST

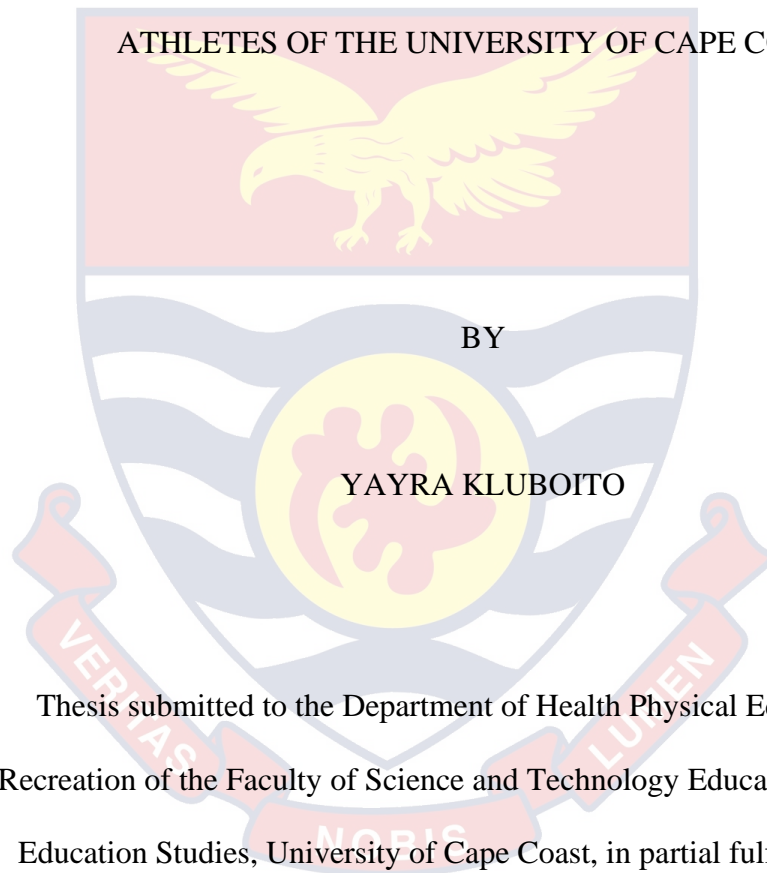


YAYRA KLUBOITO

2020

UNIVERSITY OF CAPE COAST

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BY

YAYRA KLUBOITO

Thesis submitted to the Department of Health Physical Education and
Recreation of the Faculty of Science and Technology Education, College of
Education Studies, University of Cape Coast, in partial fulfillment of the
requirements for the award of Doctor of Philosophy degree in
Physical Education.

OCTOBER 2020



DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature Date

Name: Yayra Kluboito

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature Date

Name: Dr Samuel Essien-Baidoo

Co-Supervisor's Signature Date

Name: Prof. Joseph Kwame Mintah

ABSTRACT

Sports places a demand on athletes which leads them to always look for an edge to improve their performance to remain competitive at all times. In this stead, the use of ergogenic aids has become common bu without side effects. Therefore in more recent times, natural dietary sources of ergogenic aids is becoming popular in sports. Hibiscus-ginger juice has high vitamin and mineral contents, making it important for the improvement of metabolism, recovery after exercise, reduction of inflammation, and enhancement of athletic performance. The study was therefore to explore the opportunity hibiscus ginger juice presents for performance improvement. This pretest-posttest experimental design included 28 university athletes aged between 20 and 37 years. The- athletes were randomly assigned to treatment and control groups with the treatment group allocated to daily dosages of 500ml hibiscus-ginger juice after training for 24 days. Physiological measures of heart rate, blood pressure, and blood lactate levels as well as aerobic power were measured on three different occasions within the period. Mixed model ANOVA results showed no significant results for all heart rate ($p = .645$), systolic blood pressure, ($p = .211$), diastolic blood pressure ($p = .904$), blood lactate levels ($p = .502$) and aerobic power ($p = .117$). 500ml of hibiscus-ginger juice taken daily for 24 days did not cause a significant change in the aerobic power of athletes in training. Therefore, athletes who consume hibiscus-ginger juice may not have an advantage over their colleagues who do not.

KEYWORDS

Nutrition

Performance

Hibiscus

Ginger

Aerobic power

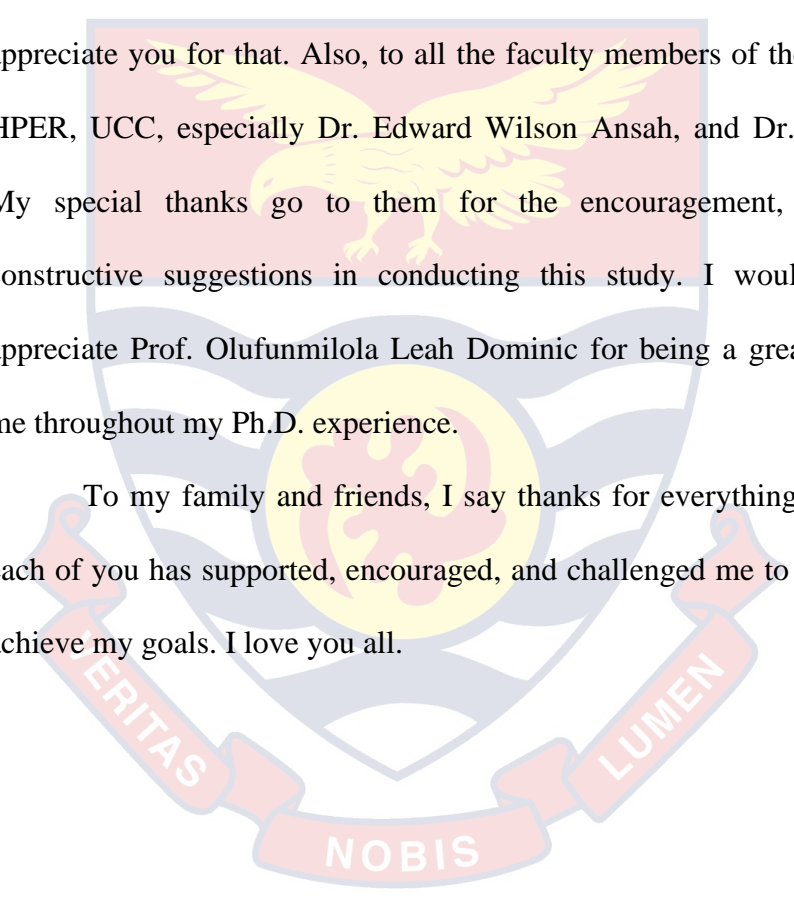


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To my family and friends, I say thanks for everything. In your ways, each of you has supported, encouraged, and challenged me to do my best and achieve my goals. I love you all.



DEDICATION

To my Family



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CHAPTER ONE

INTRODUCTION

Background to the Study

Sports require high levels of physical fitness coupled with adequate nutrition for good recovery to produce optimum performance (Marchetti *et al.*, 2015). In view of this, athletes often seek an advantage to boost their results to stay successful at all times (Beck, Thomson, Swift, & von Hurst, 2015). Good skill training, physical and physiological conditioning, nutrition as well as psychological profile make the difference between average and outstanding athletes (Wang *et al.*, 2013). Therefore, to become an outstanding athlete requires that a performer undergoes series of exercises that place stress on the metabolic pathways. This creates the need for increased energy expenditure and fluid replacement. Athletes, to keep up with the increase in energy needs and fluid intake, have to consume adequate food, fluids, and supplements. The challenge is, knowing the right type of food, fluids, and supplements, the amount taken in, and the timing of the intake to enhance athletic performance (Thomas, Erdman, & Burke, 2016).

Regular activity that raises energy costs and fluid needs also raises body turnover and loss of micronutrients (Tarazona-Diaz, Alacid, Carrasco, Martinez & Aguayo, 2013). As the body is subjected to exercise, its physical demand is increased, requiring the muscles to work continuously at more than normal rates over long periods in aerobic events and a faster rate in high-intensity anaerobic bouts (Bogdanis, 2012). For muscle groups to work constantly there need to be an optimum supply of micronutrients. For example, skeletal muscle contraction is maintained so far as there is a constant

supply of calcium ions in the sarcoplasm of the muscle cell (Babalola, 2011). This knowledge has made it a common practice for athletes to use ergogenic aids, protein supplements, vitamin supplements, and mineral supplements in their training, to achieve optimum muscle performance and facilitate their athletic performance (Sengpiel *et al.*, 2013). The concern, however, is about choosing the right kind of supplement as these ergogenic aids and supplements come in different forms including fluids, pills, bars, and powders (Alsunni, 2011).

Energy drinks have become one of the most common ergogenic aids consumed by athletes (An, Park, & Kim, 2014). As in other performance enhancing ingredients, such as taurine, herbal extracts, and B vitamins, energy drinks often contain caffeine and are known to be filled with calories (Heckman, Sherry, & Gonzalez de Mejia, 2010). The contents, availability, and potency of energy drinks have made them very attractive, and the likely choice of ergogenic aid amongst most athletes (Buxton, & Hagan, 2012). The potency of energy drink for both aerobic and anaerobic performances have been investigated (Carvajal-Sancho, & Moncada-Jimenez, 2005; Dawes *et al.*, 2014; Del Coso, Salinero, Gonzalez-Millan, Abian-Vicen, & Perez-Gonzalez, 2012; Goldstein, Jacobs, Whitehurst, Penhollow, & Antonio, 2010; Krammerer *et al.*, 2012; McCann *et al.*, 2012; Stojanovic *et al.*, 2011, Lufkin, 2011), however, with varying results.

Usually, caffeine improves endurance performance (Dawes *et al.*, 2014). Nevertheless, the usefulness of intake of caffeine for short-term high-intensity exercise is unclear. In order to enhance physical endurance, cognitive function, alertness and resilience, mood, and perception of exhaustion, for

example, moderate energy drink intake is required (Ruxton, 2008). Despite these improvements, excessive intake has also been associated with dehydration, anxiety, headache, and sleep disturbances (Alsunni, 2011). Caffeine intoxication, withdrawal, and dependence which are some of the side effects of energy drink consumption; have raised issues about the regulations governing their content labeling and health warnings (Reissig, Strain, & Griffiths, 2009).

Meanwhile, most energy drinks and supplements athletes consume contain micronutrients that work as catalysts for energy production. However, these micronutrients are better metabolized in their natural states and food than in their synthetic states (Babu, Church, & Lewander, 2008), leading to a new era in supplementation. There is therefore a surge in the use of naturally manufactured or occurring products in recent times for health benefits (Tarazona-Diaz *et al.*, 2013). The health benefits from these natural products make them more appealing sources of energy and essential nutrients among the general populace as well as sportsmen and women who seek to improve and maintain optimum performances in sports while maintaining optimum health (Mashhadi *et al.*, 2013; Tarazona-Diaz *et al.*, 2013). Evidence has emerged that coconut water (Kalman, Feldman, Kreiger, & Bloomer, 2012) and beetroot juice (Clifford *et al.*, 2016) could have effects on performance indices amongst athletes who consume them. These natural products contain carbohydrates, potassium, calcium, and antioxidants that are essential for cellular energy generation (Bentley, Ackerman, Clifford, & Slattery, 2015). The micronutrients in these natural products play the role of coenzymes and cofactors in the process of energy regeneration. The lack of these coenzymes

and cofactors therefore could lead to impairment in cellular energy production, causing an individual to have low energy levels (Huskisson, Maggini, & Ruf, 2007).

Micronutrients, classified as organic compounds (vitamins) and inorganic compounds (minerals), serve the purpose of enabling chemical reactions in the human body (Leicht, 2014). Micronutrients cannot boost energy stores but, they are crucial for converting food into energy through biochemical activities (Shenkin, 2006). Vitamins, an organic component of micronutrients, play the role of co-enzymes in many metabolic pathways in the body where they are directly involved in the synthesis of indispensable compounds during exercise (Lukaski, 2004). Exercise and training, especially endurance training increases oxidation due to the metabolic processes that take place for energy production, therefore, higher levels of antioxidants may be necessary to prevent free radicals from causing harm (Mason, Morrison, McConnel, & Wadley, 2016). Antioxidants, which are abundant in vitamins are the “defense players” against cellular deterioration induced by free radicals involved in muscle, joint, and tendon harm and swelling, as well as debilitating arthritis in the future (Reza, Rascol, Mansour, & Abdollah, 2013).

Micronutrients such as potassium, iron, zinc, and chromium also play various roles in the process of elevated physical activity. Potassium has been shown to help relieve muscle cramps and speed up recovery after exercise and training (Haakonssen *et al.*, 2015). Together with sodium, potassium also helps the muscles and nerves to work properly in exercise and training by balancing hydration in intracellular fluids. In this stead, they are essential for recovery after strenuous exercise or training. Inadequate consumption

combined with injury and menstruation may result in iron deficits (Leicht, 2014). Iron is important for carrying oxygen to working muscles and is important in sufficient amounts. As a result, resilience sportsmen with regular hemoglobin are well known to add iron to increase their red blood cells and blood hemoglobin levels.

Poor zinc status may result in decreased heart and lung function, as well as reduced strength and endurance (Huskisson *et al.*, 2007). Chromium may support the action of insulin which is responsible for glucose uptake by working muscles from the blood and are therefore important for exercise and training (Madden, Shearer, & Pamell, 2017). Consequently, an inadequate amount of chromium during exercise and training may produce an abnormal insulin response, resulting in reduced glucose uptake in muscle, and impaired glucose storage in the liver (contributing to elevated blood glucose levels). Given all these benefits from vitamins and minerals, there is a need for athletes to work towards keeping their micronutrients levels up.

Studies conducted to ascertain the role of micronutrients in sports performance have shown that the stresses that result from it cause micronutrient deficiencies which could even occur with supplementation (Madden *et al.*, 2017). This deficiency is a result of sweating and oxidative reactions that occur during training (Baker, 2005). Adequate intake and supplementation of micronutrients are therefore essential to help the body deal with the losses which could cause harmful inflammatory responses from the immune system (Elkington, Gleeson, Pyne, Callister, & Wood, 2015). This is possible because of the antioxidant nature of some micronutrients which causes a counter-reaction, to control the effect of the oxidative reactions.

Meanwhile, the extent to which plant products have been exploited in the recovery of micronutrients cannot be overemphasized. Plants such as the hibiscus and ginger which contain vitamins and minerals necessary for improving athletic performance have been studied. The calyces of *Hibiscus Sabdariffa* are rich in vitamins, natural carbohydrates, protein, iron, tannins, gums, and other antioxidants including minerals (Okereke, Iroka, & Chukwuma, 2015). Research has shown that the hibiscus flower contains protein (1.9g/100g), fat (0.1g/100g), carbohydrates (12.3g/100g), and fiber (2.3g/100g) (Ismail, Ikram, & Nazri, 2008). Also, it contains vitamin C (14mg/100g), calcium (1.72mg/100g), and iron (57mg/100g) (Anel, Thokchom, Subapriya, Thokchom, & Singh, 2016). Also, the extract from- the hibiscus calyces contains phenolic compounds and flavonoids responsible for its antioxidative properties (Afiune *et al.*, 2017). Early research which examined the effect of hibiscus extract on cholesterol level found that two capsules of *Hibiscus Sabdariffa* extract with a meal for one month significantly lowered the serum cholesterol level of participants (Lin *et al.*, 2007). It was also found to lower blood pressure in prehypertensive and mildly hypertensive adults (McCay, Chen, Salzman, & Blumberg, 2010), as well as increase blood hemoglobin levels (Anel *et al.*, 2016). Thus, hibiscus extracts have been found to improve metabolism, reduce blood pressure, and also have potent anti-inflammatory and antioxidant effects in humans (Joven *et al.*, 2014).

Ginger, a subtropical plant, is also rich in minerals and vitamins especially potassium, calcium, and magnesium (Pirculescu, Bordean, Popescu, Sirbulescu, & Draghici, 2015), which are essential in muscle contraction and

delays the onset of muscle fatigue (Haakonssen *et al.*, 2015). This herb has many therapeutic properties in combating respiratory and circulatory diseases, due to the rich content of potassium and sodium (Ozgoli & Goli, 2009). It reduces oxidative stress among athletes based on its nutritional value (Haakonssen *et al.*, 2015). Ginger assists in the absorption of iron which aids in the treatment of anemia (Kulkarni, Deshpande, Saxena, Varma, & Sinha, 2012). These micronutrient-rich plants (hibiscus flowers and ginger) are the main ingredients in preparing the hibiscus-ginger juice popularly known as *Sobolo* in the Ghanaian setting.

Statement of the Problem

University athletes consume at least one can of energy drink a week during or after training or in competition. The athletes do this intending to replenish lost energy, gain extra energy and fluids to the body, improve performance, reduce fatigue and promote recovery, as also observed among Ghanaian student-athletes (Buxton & Hagan, 2012). Even though many of the student athletes may be aware of and have experienced side effects including stomach pains, headaches, and increased heart rates, they still are likely to consume energy drinks, neglecting future side effects and associated chronic diseases (Seifert, Schaechter, Hershorin, & Lipshultz, 2013). Others mistakenly take in energy drinks to rehydrate and stimulate adequate recovery (Babu, Church & Lewander, 2008).

In the face of the side effects and misconceptions about energy drinks, there is growing interest to finding healthier sources of energy-boosting and recovery promotion drinks for sportsmen and women. Thus, natural supplements including hibiscus juice, ginger, watermelon, and pomegranate

have become very popular in the world of sports due to their widely published health and medicinal benefits (Barhe & Tchouya, 2014; Lin *et al.*, 2007; Roelofs, Smith-Ryan, Trexler, Hirsch, & Mock, 2016; Tarazona-Diaz *et al.*, 2013).

The hibiscus-ginger juice popularly called *Sobolo* or *Bissap* in Ghana and some West African countries is a popular herbal drink produced with hibiscus calyces and ginger roots as its main ingredients. It is consumed for its medicinal gains and used traditionally in the treatment of various disease conditions (Alshami, & Alharbi, 2014). Research has indicated that there are high vitamin and mineral contents of the two main constituents of *Sobolo* which are hibiscus calyces and ginger (Anel *et al.*, 2016; Ismail *et al.*, 2008). This makes it important for the improvement of metabolism, recovery after exercise, reduction of inflammation, and enhancement of performance among athletes with minimal or no side effects.

Even though research has shown that *Sobolo* has medicinal benefits (Joven *et al.*, 2014), its ability to promote recovery and sports performance has not been documented. If a naturally healthy drink is purported to have medicinal value and the potential to enhance athletic performance, then it is important to ground these assertions in literature. The focus of this research therefore was to investigate the performance benefits associated with the consumption of *Sobolo*.

Purpose of the Study

The purpose of this study is to explore the effect of hibiscus-ginger juice supplementation on selected physiological variables of university athletes.

Hypotheses

The following hypotheses were tested in the study

1. Consumption of hibiscus-ginger juice has significant effect on the heart rate of athletes of the University of Cape Coast.
2. Consumption of hibiscus-ginger juice has significant effect on the blood pressure of athletes of the University of Cape Coast.
3. Consumption of hibiscus-ginger juice has significant effect on blood lactate levels of athletes of the University of Cape Coast.
4. Consumption of hibiscus-ginger juice has significant effect on the aerobic power of athletes of the University of Cape Coast.

Delimitation of the Study

The study was delimited to athletes of the University of Cape Coast who have participated in Ghana University Sports Association games. Heart rate, blood glucose level, and blood lactate level were the only physiological variables measured in this study.

Limitations of the study

The relatively small sample size might have decreased the statistical power to detect group differences, and may have resulted in a type II error. The research was conducted on participants who were not in a controlled environment. Although they were all living together and eating the same food, there were no records of the other things they might have been eating or drinking, as well as the amount of rest they were getting. Again, the environment within which the study was conducted was not controlled for temperature, which might have affected the study outcome. There is the potential of confounding variables such as sleep patterns, rest periods and

testing periods affecting the outcomes of the current study. Therefore one cannot say for certain that the supplementation of 500ml of hibiscus-ginger juice is not effective for performance and physiological improvements among athletes or whether confounding variables interfered in the ability to detect group differences.

Significance of the Study

The findings of this study could be beneficial to society considering that hibiscus ginger juice, popularly known as *Sobolo* has become very popular amongst them for its documented and undocumented health benefits. Since the consumption of *Sobolo* is rising, it is important to find out more about the drinks content and its benefits to the human physiology. Also, for athletes, the findings of this study could provide adequate information to make the transition from synthetic and artificially produced supplements and ergogenic aids to naturally produced and prepared supplements if they decide to do so. Again, for the producers and marketers of *Sobolo* and its ingredients, this study could help boost their economic yields as there could be added current information on the importance of their produce, essentially creating a boost in its purchase and use. Lastly, for the researcher, this study will help uncover critical areas in the use of supplements made from natural products, especially *Sobolo*, and how it could affect athletic performance, which is an under-explored area of study.

Organization of the Rest of the Study

This research is organized in five chapters, one, two, three, four, and five. Chapter one deals with the background of the study, statement of the problem, the purpose of the study, hypotheses, delimitations of the study as

well as the significance of the study. In chapter two, relevant related literature was reviewed. Chapter three was organized under methodology; research design, population, sample and sampling procedures, instrument, data collection procedures, and data analysis. Chapter four deals with results and discussions, and chapter five includes a summary, conclusions, and recommendations.



CHAPTER TWO

REVIEW OF RELATED LITERATURE

The purpose of the study was to examine the effect of hibiscus-ginger juice as a recovery drink on selected physiological responses of university athletes. The following literature review is based on related research aimed at providing a basis for the need for this study.

Introduction

The human body needs carbohydrates, protein, fats, vitamins, and minerals to function properly. These nutrients are classified into macro-nutrients and micro-nutrients. Macro-nutrients are needed in larger quantities than micro-nutrients (Leicht, 2014). Macro-nutrients breakdown for energy production while micro-nutrients do not directly function for energy production but play the role of facilitating the processes necessary for energy production (Maughan, 1999). Adequate nutrition is important for all athletes, not only to provide the necessary energy for performance but also to ensure that the body is kept in good shape and health. Food serves as a source for these nutrients that athletes need. Therefore, athletes should consume nutrient-rich foods to get the essential nutrients needed to build and maintain a strong body as well as produce peak performances.

Athletes need balanced diet such as carbohydrates to boost glycogen stores before and after training and performance, protein to help facilitate the repair of worn-out muscles as well as building new muscle, fluids, and electrolytes to help enhance hydration and promote recovery. Fat is also included as a secondary source of energy, vital organ protection, and a vitamin carrier (Manore & Thompson, 2015). Along with these nutrients, the body

requires vitamins and minerals that play vital role during sustained exercise in promoting energy production, hemoglobin synthesis, bone health, muscle strength, immune function, oxidative regulation, as well as lean body mass repair and maintenance (Bentley *et al.*, 2015).

Vitamins and minerals, further classified as micro-nutrients are organic (vitamins) and inorganic compounds (minerals) and minerals (inorganic compounds), and they serve the purpose of enabling chemical reactions in the human body. While adequate vitamin and mineral status is necessary for normal health, states of marginal deficiency can only be evident when, in the case of athletes, the metabolic rate is high. Due to regular participation in medium to high-intensity physical activity, athletes have increased metabolism and so will need to pay special attention to the intake of micronutrients. Increased food consumption would typically increase the intake of dietary micro-nutrients to satisfy these requirements.

Exercise and training activate a lot of physiological processes in the body (Maughan *et al.*, 1997). This involves the interplay of the physiological systems of the body to produce at the right time, the desired skeletal contractions to produce the right kinds of movements required for performance (Babalola, 2011). In the face of these disruptions, the body needs to make some adjustments. In doing so, to avoid dehydration and replace critical salts lost in sweat, as well as macro-nutrients, the body would need water and electrolytes to provide the sufficient total energy necessary to fuel the exercise and maintain body weight (Thomas *et al.*, 2016). Prolonged daily exercise can result in increased body loss of micro-nutrients or an increased turnover rate, resulting in the need for increased dietary intake (Burke & Cox,

2010). In particular, sports performance require additional hydration and energy before and during physical activity, as well as a sufficient intake of the necessary nutrients to support subsequent recovery. Many micro-nutrients play a key role in the metabolism of energy and the rate of energy turnover in skeletal muscle will increase to up to 20-100 times the resting rate during strenuous physical activity (Maughan, 1999).

In general, endurance training increase oxidation due to the metabolic processes involved in energy production and may require higher levels of antioxidants to prevent harm from free radicals (Mason *et al.*, 2016). Antioxidants are the actors of protection against cell harm induced by free radicals that are involved in muscle, joint, and tendon harm and inflammation, degenerative arthritis, and in the aging phase in the future (Reza *et al.*, 2013). Antioxidants also help restore damage to the affected tissues in the face of injury and, in doing so, promote healing. Taking vitamin A, C, and E supplements (the most potent antioxidants) can also improve the antioxidant defense mechanism by reducing the metabolism of reactive oxygen. Antioxidants can reduce unproductive cell damage, help the healing process, help prevent injury, reduce short-term as well as long-term fatigue. In optimizing the training impact, they also play an important role, contributing to changes in performance and body composition. However, some research has suggested that by stimulating the development of more hormones and enzymes that aid in the recovery process and protect against potential damage, free radicals can be good for the body (Pingitore *et al.*, 2015).

The diets of many athletes, especially women and vegetarians, are likely to have low levels of minerals and trace elements, mainly calcium,

magnesium, iron, zinc, and chromium (Howat, Brooks & Cavalier, 1999). Inadequate consumption of calcium has also been shown to increase the risk of low bone mineral density (osteoporosis) and stress fractures, making it especially important in weight-controlled sports for athletes' bone health. Therefore, experts suggest taking calcium supplements combined with vitamin D to boost bone mineral density, which will play an important role in sports related to weight (IOC, 2016). Consequences for the future include the development of insufficient mass and strength during skeleton growth, excessive bone depletion, and inadequate formation of new bone during remodeling, leading to fragile bone tissue (Haakonssen, *et. al.*, 2015). For weight-conscious athletes such as wrestlers, dancers, and gymnasts, magnesium intake has been documented as it forms the component of more than 300 enzymes involved in energy metabolism and also plays a functional role in bone formation. Magnesium is especially important since it is involved in many processes of metabolic nucleic acids, and a deficiency can lead to muscle cramps and reduced performance of the muscle.

Iron is necessary for carrying oxygen to the working muscles, and iron deficiency can be caused by insufficient intake, combined with injuries and menstruation (Liecht, 2014). Iron deficiency anemia may improve performance through supplementation. Iron supplementation is thought to theoretically help endurance athletes with average hemoglobin status (Howat *et al.*, 1999). Poor zinc status can lead to, as well as decreased strength and endurance decreased heart and lung function. Chromium can stimulate the action of the insulin hormone, which is responsible for the absorption of glucose by working blood muscles and is therefore essential for exercise and

training. An abnormal insulin response can result in decreased muscle glucose uptake and impaired liver glucose storage during exercise and training (contributing to elevated blood glucose levels).

Potassium has been shown to help relieve muscle cramps and speeding up recovery after exercise and training (Haakonssen *et al.*, 2015). Again, together with sodium, potassium also helps the muscles and nerves to work properly in exercise and training. In intracellular fluids, these minerals are the primary electrolytes playing a role in balancing hydration and so are considered for recovery after strenuous exercise or training. This could account for the reason marathon runners and other athletes who engage in strenuous activities usually eat a banana during and after their events (Nieman *et al.*, 2012).

Exercise and training increase the physiological requirements of the body, raising the need for the body to generate energy. An increase in food intake to meet the energy requirement may increase the intake of dietary micronutrients, but athletes engaged in strenuous exercise or hard training will need to pay careful attention to their intake of micronutrients, especially calcium, iron, and antioxidants, to help improve performance while keeping the body safe, preventing deficiencies caused by calcium, iron, and antioxidants, to help with performance improvements while keeping the body healthy, avoiding deficiencies.

The calyces of the hibiscus plant are rich in natural carbohydrates, protein, and most especially, vitamins and minerals including vitamin C, calcium, and iron (Okereke *et al.*, 2015). Also, the extract of the calyces has been found to contain compounds that have antioxidant properties. Similarly,

ginger, also rich in vitamins and minerals, especially potassium, calcium, and magnesium, is a subtropical plant (Pirculescu *et al.*, 2015). These two are the main ingredients of a hibiscus-ginger juice popularly known as *Sobolo* in Ghana. The following review looks at the role of nutrition in sports recovery and performance, hibiscus, and ginger which are the two main ingredients of *Sobolo*, then the nutritional value of the *Sobolo* and how it could contribute to athletic performance.

Nutrition and Athletic Performance

To the majority of athletes, nutrition in terms of sports is about carbohydrate-loading to help prepare for competitions. To others, it's about having the latest food supplement. Nonetheless, one primary component with the greatest potential to impact sports performance training is diet (Thomas *et al.*, 2016). Training diet is the component of overall nutrition most likely to have an impact on the body of an athlete based on time alone. It also sets the basis for the long-term success of athletic performance. In sport, daily eating keeps one healthy, uninjured, and in top condition (IOC, 2016). For an athlete, daily training creates unique nutritional needs, particularly for a professional athlete, whose training dedication is almost a full-time job, not ignoring the fact that even recreational sport can create nutritional challenges (IAAF, 2007). Whatever the degree of participation in sport, athletes are to gain the optimum return from their preparation, one must face these challenges. Much of the intent of an athlete's training could be lost without sound feeding. Dietary problems and deficiencies can directly impair training and performance under the worst-case scenario (Howat *et al.*, 1999). There may be changes in some cases, but at a pace that is below the target potential or slower

than other competitors. However, there is a bright side to it, which entails having a strong and sufficient proper eating schedule and training dedication.

There is no ideal mix of foods or a single eating plan to meet each athlete's nutritional challenges, but eating a diverse diet is one of the ways one can be sure that they consume as much as they need, especially young athletes (Davis, Esslinger, Munene, & St Pierre, 2019). For all-athlete categories, a full-value optimum diet provides conditions for optimum athletic efficiency, enhances the susceptibility of a body to fatigue, and the impacts of any unfavorable influences. To ensure timely identification of health and performance dynamics, command over the adequacy of nutrition, as well as its optimization, is integrated into the design of the required athletes' check-ups (IOC, 2016). Nutritional disorders dramatically decrease the efficacy of training exercises, especially in cases of traumatic stress, and raise the risk of having diseases. They can adversely affect the efficacy and length of the professional activity of athletes, along with other factors (Valenta & Dorofeeva, 2018)

Macro-nutrients and Athletic Performance

Macro-nutrients, that is protein, fat, and carbohydrates, are important energy-supplying nutrients and are required by the body in large amounts. Fats and proteins, with several uses in the body, are functional building blocks. Some of these uses include growth, repair, as well as hormone precursors, and immune system components. It is important to note that all these macro-nutrients have the same components: carbon (C), oxygen (O), nitrogen (N), and hydrogen (H). The following are the importance of macro-nutrients to

athletic performance as well as the required amount for every athlete to boost performance.

Macro-nutrients Energy Pathways

A fundamental understanding of how training-nutrient interactions influence energy systems, substrate availability, and training adaptations is underpinned by recommendations for the timing and quantity of intake of macro-nutrients in an athlete's diet (Burke, 2010). Exercise is fueled by an interconnected set of energy systems that use substrates that are both endogenous and exogenous in origin, including non-oxidative (phosphagen and glycolytic) and aerobic (fat and carbohydrate oxidation) pathways. Adenosine triphosphate and phosphocreatine (phosphagen system) provide a rapidly available source of muscle contraction energy, but not at sufficient levels to guarantee a continuous energy supply for more than 10 seconds or so. The anaerobic glycolytic pathway metabolizes glucose and muscle glycogen rapidly via the glycolytic cascade and is the primary pathway that supports 10-180 seconds of high-intensity exercise. Because neither the phosphagen nor the glycolytic pathway is capable of sustaining energy demands to enable muscles to contract for long-lasting events at a very high pace, oxidative pathways provide the primary fuels for events longer than about 2 minutes. Muscle and liver glycogen, intramuscular lipid, adipose tissue triglycerides, and amino acids from muscle, liver, and gut are the main substrates. When the working muscle becomes more exposed to oxygen, more aerobic (oxidative) pathways, and less anaerobic (phosphagen and glycolytic) pathways are used by the body. The greater reliance on aerobic processes does not occur suddenly, nor is one direction ever exclusively relied on. The

individual's strength, length, frequency, type of training, sex, and level of training, as well as previous nutrient intake and availability of substrates, determine the relative contribution of energy pathways and when there is overlap between pathways (Maughan & Gleeson, 2010).

The skeletal muscle of an athlete has outstanding plasticity to react quickly to mechanical loading and the availability of nutrients, resulting in metabolic and functional adaptations unique to the condition (Hawley *et al.*, 2011). These adaptations influence performance nutrition guidelines with the overall objective of training energy systems to provide the most economical support for an event's fuel demands, while other techniques should achieve sufficient availability of substrates during the event itself. Adaptations that increase metabolic versatility include improvements in transport molecules that carry nutrients across membranes, improvements in enzymes that activate or influence metabolic pathways, improvement in the ability to withstand metabolism side products, and an increase in the size of muscle fuel stores (Spriet, 2014). Although relatively large amounts of certain muscle substrates (e. g. body fat) are present, others will need to be manipulated according to particular requirements (e. g. carbohydrate supplementation to replace muscle glycogen stores).

Carbohydrate

Carbohydrates are abundant in diets; they are present in bread, grains, rice, pasta, fruits, cereals, and vegetables. They provide the main source of muscle and body fuel, delivering 17 kJ /g. The special source of energy for red blood cells is glucose, a monosaccharide (simple sugar), which provides a large portion of the energy needed for the brain. Excess glucose is processed

in the body as glycogen. Around 5000 kilo-joules of glucose in the form of glycogen are processed by the average individual, which is then quickly converted to glucose again to be used by the body as blood glucose levels decrease. In the body, carbohydrates have multiple biological functions. They also have a systemic function, apart from their important role in supplying electricity. The five-C atom monosaccharide is ribose, which is a component of coenzymes and the backbone of RNA, and the closely related deoxyribose is a component of DNA. In the immune system and blood clotting, carbohydrates often play important roles.

Due to a variety of special features of its role in the success of, and adaptation to training, Carbohydrate has rightly gained a great deal of interest in sports nutrition. Second, the size of the stores of body carbohydrates is relatively small and can be acutely manipulated by dietary consumption or even a single exercise session regularly (Spriet, 2014). Third, because of its use of both anaerobic and oxidative pathways, carbohydrate provides a crucial fuel for the brain and central nervous system and a flexible substrate for muscular work where it can help exercise over a wide range of intensities (Koopman *et al.*, 2007). Carbohydrate also offers advantages over fat as a substrate even when operating at the maximum intensities that can be sustained by oxidative phosphorylation, since it provides a greater yield of adenosine triphosphate per oxygen amount that can be provided to the mitochondria, thereby enhancing gross exercise efficiency (Cole, Coleman, Hopker, & Wiles, 2014). Fourth, there is substantial evidence that strategies that maintain high carbohydrate availability (i.e., match glycogen stores and blood glucose to the fuel demands of exercise) enhance the efficiency of

sustained continuous or intermittent high-intensity exercise, whereas depletion of these stores is correlated with exhaustion in the form of reduced work rates, impaired skills and focus.

Research has established that glycogen plays important direct and indirect roles in controlling muscle adaptation to training in addition to its function as a muscle substratum (Philp, Hargreaves, & Baar, 2012). The amount and position of glycogen inside the muscle cell affect the physical, metabolic, and hormonal atmosphere under which exercise signaling responses are exercised. A coordinated up-regulation of the transcriptional and post-translational responses to exercise is generated in detail by beginning a session of endurance exercise with low muscle glycogen content (e.g. by conducting a second training session in the hours after the previous session has exhausted glycogen stores).

The training schedule of an athlete should be considered before making recommendations for regular carbohydrate intakes and should be made with the priority of encouraging high-quality exercise efficiency. Unfortunately, for many of the training sessions performed by athletes, there is not enough detail on the basic substratum specifications. Therefore, researchers have needed to rely on guesswork, backed by data from technologies such as consumer-based activity and heart rate monitors (Lee, Kim, & Welk, 2014), power meters, and global positioning systems on work requirements for exercise.

To encourage or decrease the availability of carbohydrates, the timing of carbohydrate intake over the day concerning training may also be manipulated (Burke, Hawley, Wong, & Jekendrup, 2011). Fueling activities, however, are important within the periodized training program to facilitate

high-quality workouts. Besides, they add value spontaneously in fine-tuning intended event eating strategies and in promoting adaptations such as gastrointestinal tolerance and enhanced intestinal absorption that enable fully effective competition strategies (Cox *et al.*, 2010).

It may be less necessary to achieve high carbohydrate availability during other training program sessions, or there may be some benefit in actively exercising with low carbohydrate availability to improve training stimulation or adaptive response. Various techniques can be used to encourage or facilitate low availability of carbohydrates, including reducing total intake of carbohydrates or manipulating the timing of training concerning carbohydrate intake, such as training in a fast state, performing two workout sessions closely without the ability to refuel between sessions (Burke, 2010).

Carbohydrates, the principal nutrients for muscle contraction during physical activities of varying intensity, are the primary energy factor for both aerobic and anaerobic metabolism pathways. For different sports, the degree of use and depletion of carbohydrates stored in muscles varies and largely depends on the length and strength of the training phase, as well as the degree of hydration of the body and the level of training of the athletes (Khanferyan *et al.*, 2018). In addition to this, the scarcity of carbohydrates is a limiting factor in athletes' cognitive functions (Welsh, Davis, Burke & Williams, 2002; Winnick *et al.*, 2005).

Carbohydrates that are ingested during prolonged exercise can provide the brain and muscles with an additional source of fuel. Running and cycling studies have shown that fuel intake can boost stamina and efficiency during exercise (Manore *et al.*, 2015). In the second half of the team and racquet play,

other studies indicate enhanced abilities and activity patterns (Coyle, 2004). In these trials, not all sports have been covered, and not all studies indicate consistent benefits after the ingestion of carbohydrates. This says as much about the challenge of researching sports-science as it does about the strength of the evidence. Information on the benefits of carbohydrate intake during prolonged exercise is not fresh (Burke & Cox, 2010), as it was recorded during the Boston Marathon in the 1920s that runners performed better when they ate candy during the race which was a smart thing to do. Also during the early years of this race, Tour de France cyclists undoubtedly ingested some sort of carbohydrate en route, long before sports scientists demonstrated that it was a smart thing to do.

Carbohydrate Intake by Athletes

Some guidelines for the consumption of carbohydrates by athletes were provided by Burke *et al.* (2011). The guidelines are intended to provide high carbohydrate availability for different exercise loads in circumstances where high quality and/or high-intensity exercise is required. It is important to fine-tune the general recommendations made below, taking into account the total energy requirements, the specific training requirements, and the training performance inputs individually. For other cases, meeting these carbohydrate targets or arranging carbohydrate consumption over the day may be less necessary to maximize availability for specific sessions where the quality or duration of exercise is less necessary. In such cases, the intake of carbohydrates may be chosen to fit the energy targets, dietary preferences, or food availability.

In certain cases, where the emphasis is on enhancing the training stimulus or adaptive response, low availability of carbohydrates can be intentionally accomplished by minimizing the overall intake of carbohydrates or by manipulating the intake of carbohydrates associated with training sessions (e.g. fasted training, second training session without sufficient opportunity for refueling after the first session)

Table 1- Situational target carbohydrate consumption for athletes

Training type	Situation	Carbohydrate Targets
Light	Low intensity or skill-based activities	3–5 g/kg of the athlete’s body weight /day
Moderate	Moderate exercise program (eg, ~1 hr per day)	5–7 g/kg of the athlete’s body weight /day
High	Endurance program (eg, 1–3 hrs/day moderate-high-intensity exercise)	6-10g/kg of the athlete’s body weight /day
Very High	Extreme commitment (eg, 4–5 hrs/day moderate-high intensity exercise)	8-12g/kg of the athlete’s body weight /day

Source: Burke (2010).

Acute carbohydrate fueling strategies

The following guidelines have been recommended by the American Gatorade Sports Science Institute (2017), IAAF (2007), International Olympic Committee (2016), to promote high carbohydrate availability to promote optimal performance in competition or key training sessions.

1. Athletes may choose carbohydrate-rich sources that are low in fiber/residue and easily consumed to ensure that fuel targets are met, and to meet goals for gut comfort or lighter “racing weight”
2. There may be benefits in consuming small regular snacks. Carbohydrate-rich foods and drinks may help to ensure that fuel targets are met
3. Timing, amount, and type of carbohydrate foods and drinks should be chosen to suit the practical needs of the event and individual preferences/experiences.
4. Choices high in fat/protein/fiber may need to be avoided to reduce the risk of gastrointestinal issues during the event.
5. Low glycemic index choices may provide a more sustained source of fuel for situations where carbohydrates cannot be consumed during exercise.
6. A range of drinks and sports products can provide easily consumed carbohydrates. The frequent contact of carbohydrates with the mouth and oral cavity can stimulate parts of the brain and central nervous system to enhance perceptions of well-being and increase self-chosen work outputs.
7. Carbohydrate intake provides a source of fuel for the muscle to supplement endogenous stores.
8. Opportunities to consume foods and drinks vary according to the rules and nature of each sport.
9. A range of everyday dietary choices and specialized sports products ranging in form from liquid to solid may be useful

10. The athlete should practice finding a refueling plan that suits their individual goals including hydration needs and gut comfort.
11. Higher intakes of carbohydrates are associated with better performance.
12. Products providing multiple transportable carbohydrates (Glucose: fructose mixtures) achieve high rates of oxidation of carbohydrate consumed during exercise.

Table 2- Acute carbohydrate fueling strategies

Fueling type	Situation	Carbohydrate targets
General fueling up	Preparation for events <90 min exercise	7-12g/kg per 24hrs as for daily fuel needs
Carbohydrate loading	Preparation for events >90 min of sustained/intermittent exercise	36-48h of 10-12g/kg body weight per 24h
Speedy refueling	<8 hrs recovery between 2 fuel demanding sessions	1-1.2g/kg/hr for the first 4hrs then resume daily fuel needs
Pre-event fueling	Before exercise >60 min	1-4 g/kg consumed 1-4 hrs before exercise
During brief exercise	<45 min	Not needed
During sustained high-intensity exercise	45-75min	Small amounts including mouth rinse
During endurance exercise including 'stop and start' sports	1-2.5 hrs	30-60 g/hr
During ultra-endurance exercise	92.5 min-3 hrs	Up to 90 g/hr

Sources: Gatorade Sports Science Institute (2017), IAAF (2007), National Institute of Nutrition & Sports Authority of India (2016), International Olympic Committee (2016).

Proteins

Proteins are a group of essential nutrients that provide about 17kJ/g of energy. Single units known as amino acids are made up of proteins, a group of basic nutrients that provide about 17kJ / g of energy. These amino acids are the human body's building blocks and are used to synthesize cells, muscles, organs, hormones, and immune factors, as well as to control the body's acidity or basicity by serving as buffers. They are involved in cell development, repair, and replacement, including blood, muscles, skeletal system, tissues and organs, and in the regulation of the body's homeostatic management and optimization (NHMRC, 2006).

Enzymes, biological catalysts, are synthesized from amino acids as well as other elements such as zinc and selenium that accelerate chemical reactions in the body. To aid in the repair and development of the body, they are used by every organ and cell. Enzymes are also involved in the synthesis of proteins that are involved in the body's homeostatic control, immune function, management of fluid balance, transport of nutrients and other molecules, and body detoxification. In addition to the essential function of the protein in the body's development and control, protein may also be used as an energy source when the intake of carbohydrates and fats is limited, as in the case of starvation. When required, muscle is broken down to provide additional energy if protein dietary intake is also reduced. When energy demands are maintained over a long period, such as in ultra-endurance events lasting 3 to 4 hours or more, protein can also be metabolized for energy.

Protein and Athletic Performance

The dietary protein interacts with exercise, offering both a catalyst and a substrate for contractile and metabolic protein synthesis (Philips & van Loon, 2011; Philip, 2012) and improving structural changes in non-muscle tissues such as tendons and bones (Babraj *et al.*, 2002). Resistance training response studies have demonstrated consistency in the regulation of muscle protein synthesis (MPS) for at least 24 hours in response to a single exercise session, with enhanced sensitivity over the whole time to dietary protein intake (Burd *et al.*, 2011). As such, in future studies that involve several protein feedings after exercise and during the day, it could lead to changes in skeletal muscle protein mass observed. Likewise, during aerobic exercise or other forms of exercise, responses can also be observed, examples of which are intermittent sprinting exercises and concurrent exercise, but with possible variations in the form of proteins synthesized. While classical nitrogen balance work has helped determine protein requirements to avoid energy balance deficiency in sedentary humans (WHO, 2007), athletes do not fulfill this profile, and achieving nitrogen balance is secondary to an athlete with the primary objective of adjusting to training and improving performance (Philips, 2012).

Existing data indicate that the consumption of dietary protein required to facilitate metabolic adaptation, repair, remodeling, generally varies from 1.2 to 2.0 g / kg / d for protein turnover (Mettler, Mitchell, & Tipton, 2010). During intensified training or when decreasing energy intake, higher intakes can also be suggested for short periods (Philips & van Loon, 2011). Therefore, with a meal plan offering a routine distribution of sufficient quantities of high-

quality protein during the day and after intensive physical activity, daily protein consumption targets can be met. Most training regimens are protected by these guidelines and make flexible changes with regular training and experience (Rosenbloom, & Coleman, 2012; Moore et al., 2015). The intake of sufficient energy to match energy expenditure, especially from carbohydrates, is necessary so that amino acids are spared and not oxidized for protein synthesis (Rodriguez, Vislocky, & Gaine, 2007). Elevated protein intakes as high as 2.0 g / kg/day or higher in cases of energy restriction or sudden inactivity as a result of injury can be advantageous in avoiding fat-free mass loss when distributed over the day (Mettler *et al.*, 2010; Wall, Morten, & van Loon, 2015).

It is not yet conclusively proven higher doses raise MPS further and may only be prudent for the biggest athletes or during weight loss (Moore *et al.*, 2009). MPS exercise-enhancement, defined by protein intake timing and pattern, responds to further protein intake within the 24 hours after exercise, and can eventually translate into chronic accretion and functional shift of muscle protein (Areta *et al.*, 2013). Longitudinal training research, however, currently indicates that with immediate post-exercise protein supply, gains in strength and muscle mass are greatest (Joose, Tang, Tarnopolsky, & Phillips, 2010). Although conventional protein intake guidelines focused on total protein intake over the day (g / kg), newer recommendations now highlight that by ingesting these goals as 0.3 g / kg body weight after main exercise sessions and every 3-5 hours over several meals, muscle tolerance to training can be maximized (Moore *et al.*, 2009; Moore, & Philips, 2014; Phillips, & Slater, 2015).

Research has shown that consumption of milk-based protein helps enhance muscle strength after resistance exercise (Hartman *et al.*, 2007; Joose *et al.*, 2010; Joose, Atkinson, Tarnopolsky, & Phillips, 2011). Elevated muscle protein synthesis (MPS) and protein accretion have also been documented with whole milk, lean meat, and dietary supplements, some of which include isolated whey, casein, soy, and egg proteins. Dairy proteins today tend to exceed other proteins studied, primarily due to the quality of leucine and the digestion and absorptive kinetics of branched-chain amino acids in dairy foods dependent on fluid (Smock, Yadid, Dym, Clarke, & Tawfik, 2016). Other research, however, is now testing other sources of protein, such as eggs, beef, pork, and concentrated vegetable protein. Then compact, third-party tested dietary supplements with high-quality ingredients can serve as a realistic option to help athletes meet their protein needs when whole food protein sources are not convenient or available.

Fats

Fat is a critical part of a healthy diet, providing energy, important cell membrane components, and promoting the absorption of fat-soluble vitamins. Years ago, based on the finding that the standard Australian diet contains more fat than is essential or safe, it was simply said 'eat fewer fats and oils' (NHRMC, 2013). Promoting good weight or weight loss and reducing the risk of certain lifestyle disorders are the direct benefits of reducing the consumption of fats (Liu *et al.*, 2017). Indirect advantages include allowing room for certain more valuable foods and nutrients in the energy budget. After all, fats and oils are commonly distributed in foods and have many benefits.

Some of these involve the provision of essential fatty acids and vitamins that are fat-soluble and are vital for health.

The types of fats and oils consumed and not just the amount of fat in our diets are targeted by recent dietary recommendations (Amend, 2019). This is because different fats have varying effects on weight, how well the body of a person responds to insulin, and the levels of blood fat and cholesterol. Most individuals, however, over-eat the 'unhealthy' fats over and under eat the 'healthy' ones (Davis *et al.*, 2019). The saturated forms and trans fatty acids are the most overeaten fats. Saturated fats come primarily from animal sources such as meat and dairy foods, as well as coconut and palm oils (Boateng, Ansong, Owusu, & Steiner-Asiedu, 2016). The unhealthy form of blood cholesterol known as LDL (low-density lipoprotein) cholesterol is increased by both saturated and trans fats, while trans fats also decrease the safe cholesterol known as HDL. Therefore, attempts to decrease the consumption of fats and oils should concentrate on unsaturated forms. Fat consumption by athletes should be in line with recommendations for public health and should be individualized based on training level and expectations for body composition (Rosenbloom & Coleman, 2012).

Fats and Athletic Performance

Fat, in the form of plasma-free fatty acids, intramuscular triglycerides, and adipose tissue, provides a fuel substrate that, as a result of endurance training, is both relatively abundant and increased in muscle availability (Spriet, 2014). Exercise-induced adaptations, however, do not tend to increase oxidation rates, as dietary interventions such as fasting, acute fat intake pre-exercise, and chronic exposure to high-fat, low-carbohydrate diets can further

improve them. Although interest in chronic adaptation to high-fat low-carbohydrate diets has been historical and recently revived (Volek, Noakes, & Phinney, 2015), current evidence suggests that enhanced fat oxidation rates can only match exercise capacity or performance achieved by diets or strategies promoting high availability of carbohydrates at moderate intensities (Purdom, Kravitz, Dokladny, & Mermier, 2018). Even when glycogen is available, this appears to occur as a result of the down-regulation of carbohydrate metabolism (Stellingwerff *et al.*, 2006). While there may be particular situations where high-fat diets may provide some advantages or at least the absence of performance drawbacks, they usually tend to decrease metabolic versatility rather than increase it by decreasing the availability and capacity to use carbohydrates effectively as an exercise substrate (Burke, 2015). Therefore, it would be unwise for professional athletes to surrender their capacity to undergo high-quality preparation or high-intensity competition efforts that could decide the outcome. Dietary fats and oils are the most concentrated source of food energy, however, they do not provide the main source of fuel for exercising muscles (Spriet, 2014). Instead, body carbohydrate stores provide a vital energy supply for strenuous exercise. These, however, are restricted in scale. On the other hand, body fat reserves, also in the leanest of athletes, are in ample supply and could provide fatty acids for hours and days of exercise.

Micro-nutrients

Consuming the right amounts of micro-nutrients is very important since both deficiency and excess in intake can be detrimental to health. Two major groups of micro-nutrients exist, vitamins and minerals. Vitamins are

organic compounds that are graded as fat-soluble (A, D, E that K) or water-soluble (B-group vitamins and vitamin C). Inorganic chemical elements (such as magnesium) or element compounds (such as sodium chloride) are the minerals (Heffernan, Horner, De Vito, & Conway, 2019). Based on the quantities required by the body, they are often grouped. For macro-minerals (calcium, chloride, magnesium, phosphorus, potassium, sodium, sulfur), the recommended intake reaches 100 micro-grams a day. In smaller quantities, micro-minerals (copper, iron, zinc, molybdenum, manganese, selenium, fluoride) are needed (Maggini, Pierre, & Calder, 2018). Exercise stresses many of the metabolic pathways that involve micro-nutrients (Thomas *et al.*, 2016) and lead to biochemical modifications of the muscles that increase the need for those micro-nutrients.

Athletes who frequently restrict energy intake, rely on extreme weight-loss practices, eliminate one or more food groups from their diet, or consume poorly chosen diets, may be required to consume sub-optimal amounts of micro-nutrients and benefit from micro-nutrient supplementation (Farajian, Kavouras, Yannakoulia, & Sidossis, 2004). This occurs most frequently in the case of calcium, vitamin D, iron, and some antioxidants (Lukaski, 2004; Woolf, & Manore, 2006).

Vitamin B

A lot of the B Vitamins (thiamin, riboflavin, niacin, pantothenic acid) is needed for energy production, protein and fatty acid synthesis, and carbohydrate metabolism. Many of these nutrients are co-factors in the Krebs cycle which is a series of chemical reactions that result in the release of chemical energy and carbon dioxide. During exercise, skeletal muscle's use of

energy increases by up to one hundred folds (Rodriguez, DiMarco & Langly, 2009). Thiamin and riboflavin intake are sometimes reported as ‘micro-grams per 100-kilo calories’, because of their importance in energy production (Maggini *et al.*, 2019). Provided athletes eat a balanced diet, they can achieve adequate intakes of B vitamins to meet energy needs since they are widely distributed throughout food types (IOC, 2016).

Supplementation of vitamin B is less harmful because it is water-soluble and excreted through urine. Conversely, athletes must be aware that there is an upper level of intake (UL) set for B6, niacin, and folate (Lukaski, 2004). Excess supplementation of vitamin B6 can lead to sensory neuropathy, which often comes as pain or numbness in the hands and feet. Other symptoms of niacin toxicity include itchy, red, or warm skin, dizziness, leg cramps, muscle pain, and insomnia.

Vitamin B12 and folate are important for tissue repair, protein synthesis, and the proper functioning of the nervous system. In addition to that, they are needed for the formation of red blood cells. In the diets of vegetarians athletes and female athletes, these nutrients are usually low. Insufficient intake of folate and vitamin B12 will lead to folate deficiency anemia and vitamin B12 deficiency anemia. These deficiencies cause low performance in athletes (Lukaski, 2004). Folate can be found in green leafy vegetables and whole grains while vitamin B12 is found in animal foods including meat, chicken, fish, eggs, and dairy products. The upper limit for folate is set because excess in folic acid can mask vitamin B12 deficiencies.

Calcium

Calcium is a structural component of bone. It combines with phosphorus to form hydroxyapatite, a hard, crystalline structure that gives their strength. Calcium is very important for growth, maintenance, and repair of bone tissue; regulation of muscle contraction; nerve conduction; and normal blood clotting. The risk of low bone-mineral density and stress fractures is increased by low bone mineral density and stress fractures is increased by low energy availability, and in the case of female athletes, menstrual dysfunction, with low dietary calcium intake contributing further to the risk (Nattiv *et al.* 2007; Nickols-Richardson, Beiseigel, & Gwazdauskas, 2006). Restricted energy intake, disordered eating, and specific avoidance of dairy products or calcium-rich foods are all causes of low calcium levels in the body. Calcium intakes of 1,500 mg/d and 1,500–2,000 IU/day of vitamin D are needed to enhance bone health in athletes with low energy availability or menstrual dysfunctions (Mountjoy *et al.*, 2014). Some of the dairy sources of calcium include milk, cheese, and yogurt.

Vitamin D and Phosphorus

The absorption of calcium and phosphorus from the gut is improved by vitamin D. Fluoride and magnesium play apart in the mineralization of bones. On the other hand, phosphorus is present in most high-protein foods. There are also small quantities of vitamin D in dairy eggs, mushrooms and fortified margarine. In other situations, sufficient exposure to sunlight is required to obtain sufficient levels of vitamin D. Sun exposure is suggested to be approximately six minutes per day to 40 minutes per day (Nowson *et al.*, 2012). Over-training, however, is known to decrease the development of the

sex hormone estrogen, which, especially in women, plays a vital role in maintaining bone mass (Maughan, 1999). The probability of stress fractures can be increased by low bone mass and density and may also have a negative impact on the capacity of an athlete to compete.

There is a particular risk of stress fractures for female athletes with eating disorders because their calcium intake is likely to be smaller, and they are likely to have menstrual dysfunction associated with reduced development of estrogen (Rodriguez et al. 2009). Among athletes, as well as non-athletes, vitamin D deficiency is very common. Dark-skinned athletes, indoor participants, and athletes living at high altitudes are at higher risk of vitamin D deficiency (Powers, Nelson, & Larson-Meyer, 2011). Athletes with disordered eating and amenorrhea are advised to use, in addition to their diet, 1500 micrograms of calcium and 400 to 800 International Units (IU) of vitamin D a day as supplements.

Iron

One of the main components of hemoglobin is iron. Hemoglobin is responsible for the transport of oxygen. Iron is also a co-factor for enzymes that participate in the electron transport chain, a series of reactions that are needed for the synthesis of ATP, the body's energy carrier, making it an essential nutrient for athletes, especially endurance athletes. The amount of iron needed by athletes can be more than up to 70% more than the recommendations for non-athletes (Rodriguez *et al.*, 2009). Just like vitamin D, iron deficiency anemia (IDA) is the most common nutrient deficiency among athletes and the general population. However, athletes who are at risk of iron deficiency include:

1. Athletes that follow energy-restricted diets
2. Adolescent athletes
3. Vegetarian athletes
4. Female athletes who are menstruating
5. Athletes who undertake altitude training
6. Runners
7. Injured athletes
8. Athletes who donate blood

Iron is carried around the blood by a protein called transferrin; when blood iron stores are low, total iron-binding capacity (TIBC) increase so that transferrin can bind to more of the available iron and, at the same time, serum ferritin (SF) levels drop. When transferrin saturation (serum iron/ TIBC) is below 16%, the body is said to be experiencing an early functional iron deficiency. If iron deficiency progresses further, the body is unable to make hemoglobin and the mean cell volume (MCV) of red blood cells decreases, leading to iron deficiency anemia (IDA). Iron deficiency anemia has deleterious effects on athletic performance and also impacts concentration and hence the ability to make tactical decisions during play (Deakin, 2009). Using supplements to correct iron deficiency anemia could increase work capacity, reduce heart rate as well as blood lactate concentration (Rodriguez *et al.* 2009). At-risk athletes should be regularly screen (through blood tests) and also focus on preventing the development of IDA by obtaining sufficient sources of iron. Whole grains, leafy greens, nuts, and seeds also provide some iron.

Zinc and Magnesium

For most enzymes involved in energy production, zinc and magnesium usually serve as co-factors. Zinc is involved in growth, muscle building, and repair in athletes. Magnesium, on the other hand, is needed for immune function, protein synthesis, and muscle contraction. In athletes, zinc and magnesium are lost in sweat, urine, and feces. The deficiency of zinc can impair athletic performance by reducing cardio-respiratory function, muscle strength, and endurance. Deficiency of magnesium could also lead to increased oxygen requirements for performing submaximal activities (Heffernan *et al.*, 2019).

Potassium and Sodium

Sodium is the main cation in extra-cellular fluid, potassium is the main cation in intracellular fluid, and chloride is the main anion in intracellular fluid. These electrolytes help the body to maintain fluid balance. Sodium acts to ensure the acid-base balance of body fluids and both sodium and potassium have additional roles in nerve-impulse transmission and muscle contraction. Athletes have higher sodium and chloride need than non-athletes (Davis *et al.*, 2019). Sodium and chloride are found in foods as sodium chloride (salt). Table salt, soy sauce, processed foods, meat, milk, and bread are all sources of sodium chloride. Sports drinks containing electrolytes are frequently recommended for athletes involved in endurance exercises.

Nutrition and Recovery

The reproduction of muscle cells and their repair as well as the remodeling of muscle protein are influenced by nutrition and are also important for post-exercise recovery after intense exercises. It is therefore

important that athletes maximize their recovery potential through optimal nutrition. Athletes need to be advised about the number of meals and snacks, timing and composition of these meals based upon their training load. As such, a multidimensional nutritional strategy targeting the combined ingestion of dietary carbohydrates and protein (rather than either one alone) will be most effective in achieving these recovery goals. In early topics, proteins and carbohydrates were discussed extensively. However, this part of the project will now talk about their importance to recovery. After thorough literature, it has been found that protein and carbohydrates are the most important nutrients when recovery of muscles is in question.

Carbohydrate and Recovery

Intake of carbohydrate (CHO) is very critical to enhance muscle and glycogen stores after a very intensive exercise. The rate of muscle glycogen is greatest during the initial ~1hr after exercise and is enabled by the contraction-induced recruitment of GLUT-4 transporters to the muscle membrane and enhanced activity of the rate-controlling enzyme glycogen synthase (Jentjens, & Jeukendrup, 2003). The translocation of GLUT4 transporters to the membrane accelerates the transport of glucose into the muscle fibers where it goes through the anabolic process of glycogen re-synthesis. The activation of GLUT4 transporters is extended when carbohydrate is consumed after exercise because of the rise in plasma insulin concentration. The consequence is that re-synthesis continues to deplete glycogen stores. This is why eating carbohydrates immediately after exercise is so strongly recommended (Jensen, & Richter, 2011).

The rate of glycogen re-synthesis depends on the amount of carbohydrate consumed after and most importantly the intensity and duration of the exercise (Price, Laurent, Peterson, Rothman, & Shulman, 2000). The lower the post-exercise glycogen stores the greater the need to consume the larger amounts of carbohydrate. However, when exercise is neither prolonged nor intense then there is no need to consume large amounts of carbohydrate during the recovery period. The addition of protein to carbohydrate increases the concentrations of insulin values than when the only carbohydrate is consumed (van Loon, Kraris, & Wagenmakers, 2000; Zawadzki, Yaspelkis, & Ivy, 1992). It was reported that consuming a mixture of carbohydrates and protein immediately after exercise increased the rate of post-exercise muscle glycogen re-synthesis beyond that which occurs with carbohydrates alone (Fogt & Ivy 2000; Ivy *et al.*, 2002).

Protein and Recovery

A most important way to improve muscle protein synthesis (MPS) is the consumption of the dietary protein (Levenhagen *et al.*, 2001) which provides the necessary amino acid building blocks for repairing, remodeling, and building new muscles. In other words, protein consumption after exercise enhances muscle protein synthesis and the net protein balance (Philips, & van Loon, 2011), predominantly by increasing mitochondrial protein fraction with endurance training, and myofibrillar protein fraction with resistance training (Wilkinson *et al.*, 2008). Carbohydrate ingestion alone does not affect MPS (Levenhagen *et al.*, 2001) and does not augment the dietary amino acid-induced stimulation of MPS after exercise (Koopman *et al.*, 2007; Staples *et al.*, 2011)

Dose-response studies suggest that approximately 20 g of high-quality protein is sufficient to maximize MPS at rest (Witard *et al.*, 2014), following resistance (Moore *et al.*, 2009), and after high-intensity aerobic exercise (Rowlands *et al.*, 2015). The rate of MPS has been found to approximately triple 45–90 minutes after protein consumption at rest, and then return to baseline levels, even with continued availability of circulating essential amino acids (termed the “muscle full” effect) (Atherton *et al.*, 2010). Previous studies have demonstrated that a single 20-g bolus of high-quality protein including egg or whey, is sufficient to maximize MPS after resistance exercise with greater protein amounts, resulting in increased amino acid oxidation (Moore *et al.*, 2009; Witard *et al.*, 2014). It has also been shown that 16 g of milk protein (Lunn *et al.*, 2012) and 20 g of whey protein (Breen *et al.*, 2011) augment post-exercise rates of MPS after aerobic-based exercise. The timing of the protein ingestion, also, may be an important factor to initiate the recovery process after a bout of endurance exercise. Consuming a source of protein immediately after endurance exercise is critical to enhancing MPS, as delaying this ingestion by as little as 3 hrs has been shown to markedly attenuate the anabolic effects of the dietary amino acids (Levenhagen *et al.*, 2001). For long-term recovery, the repeated ingestion of 20 g of protein every 3 hrs (60 g in total) have been shown to support greater rates of myofibrillar protein synthesis over 12 hrs after resistance exercise as an identical amount ingested as either eight feedings of 10 g every 1.5 h or two feedings of 40 g every 6 h (Areta *et al.*, 2013).

Hibiscus Plant

Hibiscus sabdariffa L. (HS), also known as Roselle is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems, and can also be used as food and fiber (Da-Costa-Rocha, Bonnlaender, Pischel, & Heinrich, 2014). The Roselle is a profusely flowering, perennial, woody ornamental shrub distributed widely in the tropical region (Mak, Chuah, Ahmed, & Bhat, 2012). Roselle is an annual crop used in food, animal feed, nutraceuticals, cosmeceuticals, and pharmaceuticals and takes about five months from planting to harvesting.

The crop is native to India but was introduced to other parts of the world such as Central America, West Indies, and Africa. It is best grown in tropical and sub-tropical regions. The plant is resistant to short periods of drought, and it can be cultivated throughout the tropics and subtropics during hot and rainy seasons (Sharara, 2017). Roselle is a robust branched shrub-like annual or biennial plant that gets 4 to 7 feet tall and almost as broad. Leaves are dark green leaves, about 6 inches across, deeply dissected into 5 narrow lobes. The stems, branches, leaf veins, and leaf stalks are reddish-purple. The hibiscus-like flowers are yellow and about 3 inches across. The sepals of the flowers are prominent, fleshy, bright red. The sepal cup is about 1 inch in diameter. Sepals of Roselle are used in making juices, squashes, jellies, wines, and pies. Roselle is native to tropical Africa, cultivated in many places, but has escaped cultivation and become naturalized in tropical America and Asia.

In Africa, Roselle is widely accepted to have originated from West Africa where diverse wild types are found particularly in Ghana, Niger, Nigeria, Senegal, and Mali (McClintock, & El Tahir, 2004). The species

Hibiscus sabdariffa L. is believed to have originated from the hybridization between *Hibiscus cannabinus* (AA) and an unknown Y genome species (YY) followed by doubling of the chromosomes of the resulting diploid species (Singha, & Kumar, 2008). Roselle was first domesticated in Western Sudan before it was disseminated throughout the tropical regions of the world such as Asia where it was developed as a fiber crop in Central and Northern America (Wilson, 2006). It is known by different names across the world; karkade in the Middle East and Sudan, Sorrel in the Caribbean, and Bissap in some West African countries

The juice from the calyces is said to be a health-enhancing drink due to its high content of vitamin C, anthocyanins, and other antioxidants (Barhe, & Tchouya, 2014). Nutritionists have also reported that Roselle calyces are high in calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), niacin, riboflavin, and iron (Islam, Jamini, Islam, & Yeasmin, 2016). The calyces of the plant have been used for many purposes, including as a hot or cold beverage, food flavoring and color, and as herbal medicine (Salman *et al.*, 2017). They have also been used as jams, jellies, ice-cream, as well as salads, which is an indication that it is highly consumed among many people across several cultures.

In most West African countries including Ghana, the Roselle is commonly cultivated as a leafy vegetable and to supply raw material for making the very popularly beverage known as ‘Bissap’ or ‘Sobolo’ across the Sub-region respectively (McClintock, & El Tahir, 2004).

Table 3- Names of Hibiscus in different countries

Countries	Names
Indonesia	Rosella, Rosella fruit
Ghana	Bissap, Sobolo
Senegal	Bissap
France	Bissap
Mali	Dah, Dah Blendi, Datou,
The Gambia	Furundu
Nigeria	Wongo
Iran	Zobo
Egypt	Chaye-Torosh
Saudi Arabia	Karkade
Sudan	Karkade
Namibia	Karkade
Caribbean	Omutete
Latin America	Sorrel
Mexico	Sorrel
Panama	Flor de Jamaica
Burkina Faso	Saril
Niger	Bikalga Dawadawa-botso

Source: Darkwa (2016).

Uses of Hibiscus Sabdariffa

Hibiscus sabdariffa calyces are used in the preparation of herbal beverages (Gibbon, & Pain, 1985), hot and cold beverages, fermented drinks, wine, jelly, jellied confectionery, ice cream, chocolates, flavoring agents, puddings as well as cakes (Bako, Mabrouk, & Abubakar, 2009). The fleshy calyces are used in Egypt to manufacture "*cacodyl tea*" and fermented beverages (Ismail, Ikram, & Nazri, 2008), while the calyces are boiled with sugar in Sudan and Nigeria to manufacture a drink known as "*Karkade*" or "*Zoborodo*" (Bolade, Oluwalana, & Ojo, 2009; Okoro, 2007). This drink is called Jamaica in Mexico, or "*agua de Jamaica*" or "*tea de Jamaica*" (Plotto, 2004). Calyces are also used as coloring and flavoring ingredients in rum in the West Indies (Ismail *et al.*, 2008), while the seeds are eaten roasted or

ground in meals, and the leaves and shoots are eaten raw or fried, or as a vegetable or condiment with a sour flavor (Wilson, & Menzel, 1964). The leaves are eaten green or dried in nations such as Sudan, cooked with onions and groundnuts, while the cooked leaves are eaten as vegetables in Malaysia (Tsai, McIntosh, Pearce, Camden, & Jordan, 2002). The seeds are roasted or ground into a powder in Africa and used in meals, such as oily soups and sauces (Bolade *et al.*, 2009). In China and West Africa, the seeds are also used for their oil (Atta, & Imaizumi, 2002).

Leaves or calyx infusions are historically used in India, Africa, and Mexico for their diuretic, choleric, febrifugal, and hypotensive effects, reducing blood viscosity, and stimulating intestinal peristalsis (Wilson, & Menzel, 1964). It is also prescribed in Senegal as a hypotensive medication (Morton, 1987). In Egypt, calyx preparations are used to treat heart and nerve disorders as well as to improve urine production (diuresis). An injection of "Karkade" calyces is used to help lower elevated body temperatures from fevers in Egypt and Sudan (Leung, & Foster, 1996). It is used to treat drunkenness in Guatemala (Morton, 1987). Calyx preparations are used in North Africa to treat painful throats and coughs, as well as genital problems, while emollient leaf pulp is used to treat external wounds and abscesses (Neuwinger, 2000). A decoction from the seeds is used in India to ease urination pain and indigestion. The roots are thought to have stomach and emollient properties in Brazil. It is used for treating liver diseases and high blood pressure in Chinese folk medicine (Morton, 1987). Sour hibiscus tea is reportedly a conventional hypertension remedy in Iran (Burnham, Wickersham, & Novak, 2002), while seed decoction is historically used in

Nigeria to increase or induce lactation in cases of low milk production, low letdown, and maternal mortality (Gaya, Mohammad, Suleiman, Maje, & Adekunle, 2009).

In India, other Hibiscus species are used in the manufacture of clothing, linen, fishing nets, ropes, and similar products as a jute substitute (Clydesdale, Main, & Francis, 1979). Although this species is slow-growing, as it requires about 180 days to produce a satisfactory fiber yield, the plant still has interest because some varieties of Hs (not edible but type of fiber) have a high degree of genetic resistance to root-knot nematodes. In comparison with other Hibiscus plants, the key drawback of growing Hibiscus sabdariffa is the slow growth rate, which increases the cost of weed control and land occupation of the crop. Compared to, Hibiscus cannabinus, it is often difficult to distinguish the ribboning stalks from the bark (Wilson, & Menzel, 1964). By 2008, world production of Kenaf fibers (*H. cannabinus*) had reached 272,000 tons, while Hibiscus *sabdariffa* fibers had not acquired the same economic significance. However, when used as a replacement for synthetic or mineral fibers in composite materials and as a source material for high-quality paper production, Hibiscus *sabdariffa* fibers are subject to ongoing research showing promising technical properties (Dutt, Upadhyaya, & Tyagi, 2010; Kumar, Dutt, & Bharti, 2013; Singha, & Kumar, 2008). The leaves are used for fiber and animal feed (Plotto, 2004). The seeds can be used both for feeding poultry and sheep, and the residue from the extraction of seed oil can also be used to feed cattle and chicks (Al-Wandawi, Al-Shaikhly, & Abdul-Rahman, 1984).

Effects of *Hibiscus sabdariffa* on Blood Pressure and Heart Rate

High blood pressure is a global health issue with a large level of morbidity and mortality, with around 1 billion people suffering from hypertension globally, causing up to 7.1 million deaths each year, which is around 13 percent of the world's overall deaths (Brown, 1997). Angiotensin-converting enzyme (ACE) inhibitors and diuretics are among the drugs that are used to treat hypertension (Neal, Macmahon, & Chapman, 2000; Gallagher, Perkovic, & Chalmers, 2006). There are, however, still criteria for other resistant hypertension agents, as well as for non-pharmacological approaches that could be promoted at the population level. One possible non-pharmacological remedy is *Hibiscus sabdariffa* (Onyenekwe, Ajani, Ameh, & Gamaniel, 1999). The calyx infusion is used in folk medicine for the treatment of many disorders, including high blood pressure (Gautam, 2004). Compounds of anthocyanins and proanthocyanidins detected in abundance in the aqueous infusion of *Hibiscus* calyces, maybe the bioactive compounds responsible for lowering blood pressure based on earlier studies that demonstrated the antihypertensive effects of anthocyanins by inhibiting the transforming enzyme of angiotensin II and thus a vasodilating activity (Jonadet *et al.*, 1990) in additives.

Studies based on the effectiveness of aqueous extract in hypertensive humans showed a substantial decrease in both systolic and diastolic blood pressure relative to the control group, although the diastolic pressure remained unchanged relative to the significant decrease in systolic pressure (McCay, Chen, Salzman, & Blimberg, 2010; Mckay *et al.*, 2010). A study in rats have also been carried out and results have been shown to support the common

belief that roselle extract contains antihypertensive components (Onyenekwe *et al.*, 1999). Anthocyanin extract, which has been tested in humans for its therapeutic effectiveness, safety, and tolerability along with the antihypertensive drug captopril, lisinopril, found comparable results and indicates that the synergistic mechanism of inhibition of diuretic and angiotensin-converting enzyme (ACE) results in hypotensive effects (Herrera-Arellano *et al.*, 2004).

There is a chance of coronary heart disease (CHD) and cardiovascular disease (CVD) at elevated resting heart rates. Through other risk factors such as blood pressure, cigarette smoking, exercise, or cholesterol level, elevated heart rates can also be secondarily linked to coronary heart disease (Astrand, & Rodahl, 1977; Dyer *et al.*, 1980; Goldbourt, & Medalie, 1977; Keys *et al.*, 1971; Wood *et al.*, 1983). A great deal of evidence suggests that high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL) and their sub-fractions are correlated with various risks of CHD. High serum HDL cholesterol, apolipoprotein (apo) A-I (Maciejko *et al.*, 1983), and total HDL lipoprotein mass appear to protect against coronary heart disease, while lipoproteins of low density tend to raise the heart rate and increase the risk of coronary heart disease. However, the inhibitory effects of plant extract on low-density lipoprotein oxidation and fructose and cholesterol-fed anti-hyperlipidemia have been shown (Chau-Jong *et al.*, 2004). Consequently, it was found that the Hibiscus Sabdariffa extract decreases LDL levels and the ratio of LDL-cholesterol to HDL-cholesterol. Dried calyx ethanol extract intake decreases the lipid profile in rats (Onyenekwe *et al.*, 1999).

Aqueous extracts are also reported to have hypocholesterolemic and antioxidant effects in hypercholesterolemic rats (Hirunpanich *et al.*, 2006), where the protective effect of Roselle on LDL oxidation was demonstrated by the antioxidant effects of aqueous dried calyx extracts using rat low density. The plant's biochemical dynamics and hypocholesterolemic action have shown that its administration causes a substantial decrease in serum GOT, GPT, alkaline, and acid phosphatase activity as well as in total serum protein activity. However, these values almost returned to initial amounts after administration for 9 weeks (El Saadany, Sitothy, Labib, & El-massry, 1991). Red and green plant petal aqueous extracts have also been found to decrease overall plasma concentrations in rats (Olatunji *et al.*, 2005).

Effect of Hibiscus on Exercise and Athletic Performance

Nutrient deficiencies among athletes are not widely documented (Hefferman *et al.*, 2019). However, it is conceivable that a nutrient deficiency may lead to an increased susceptibility of either malnutrition or reactive oxygen species to exercise-induced damage and thus lead to impaired performance of exercise among athletes (Davis *et al.*, 2019). To enhance their success, therefore, the nutritional needs of athletes must be met. Most nutrients that athletes need for success are found in Roselle extracts. Depending on the variety and geographical location, its calyces, leaves, and seeds are rich in organic acids, minerals, amino acids, carotene, vitamin C, and total sugar at varying amounts (Yakubu, Hannatu, & Garba, 2018). Some compounds have also been isolated and characterized in Roselle including flavonoids, anthocyanidins, triterpenoids, steroids, and alkaloids (Cisse *et al.*, 2009).

Table 4- Hibiscus sabdariffa plant parts and their nutrient contents.

Nutrients	Calyxes	Seeds	Leaves
Protein [g]	2.0	28.9	3.5
Carbohydrates[g]	10.2	25.5	8.7
Fat [g]	0.1	21.4	0.3
Vitamin A [I.E.]	-	-	1000
Thiamine [mg]	0.05	0.1	0.2
Riboflavin [mg]	0.07	0.15	0.4
Niacin [mg]	0.06	1.5	1.4
Vitamin C [mg]	17	9	2.3
Calcium [mg]	150	350	240
Iron [mg]	3	9	5

Source: Naturland, 2002; Singh, Khan, & Hailemariam, 2017.

Antioxidant Effect of *Hibiscus Sabdariffa* on exercise and athletic performance

Regular exercise is considered to be good for health by raising the body's antioxidant defenses (Baker, 2005). Exhaustive exercise, -however, can create excessive reactive oxygen species (ROS), leading to damage to tissue related to oxidative stress and reduced muscle contractility (Bogdanis, 2012). In both aerobic and anaerobic exercises, ROS is developed. As possible contributors to ROS development, mitochondria, NADPH oxidases, and xanthine oxidases have all been identified. It has been shown that muscle activity among athletes is correlated with ROS development (Steinbacher, & Eckl, 2015). In light of this, there was consensus that during physical activity, especially among athletes, ROS is produced primarily by contracting skeletal muscles (Finsterer, 2012). For the development of normal muscle force, moderate levels of ROS are truly required, but excess ROS can lead to muscle fatigue and contractile dysfunction (Powers, Ji, Kavazis, & Jackson, 2011). Some of the main endogenous origins of ROS in skeletal muscles are

mitochondria, NADPH oxidase (NOX), and xanthine oxidase (XO) (Steinbacher, & Eckl, 2015). ROS is produced by mitochondria under biological conditions as a by-product of cellular respiration. Oxygen extracted from mitochondria can therefore be found in both muscle rest and exercise (Sakellariou *et al.*, 2013; Zuo, Zhou, Pannell, Ziegler, & Best, 2015). Also, clear evidence suggests that the primary cause of exercise-induced disruptions in muscle oxidation-reduction status is reactive oxygen species (Powers, & Jackson, 2008). Significant cellular redox equilibrium disruptions have been shown to lead to oxidative injury and muscle fatigue (Powers *et al.*, 2011). According to Hadi *et al.* (2017), both the whole aqueous and anthocyanin-rich extracts of Roselle are effective antioxidants. Studies have also highlighted the polyphenolic acid, flavonoids, and anthocyanins which are found in Roselle are potent antioxidants (Formagio *et al.*, 2015; Riaz, & Chopra, 2018). Existing studies generally report decreased oxidative damage after antioxidant supplementation (Hadi *et al.*, 2017; Jordan, Lukaszuk, Misic, & Umoren, 2010; Sadeghi, & Husseini, 2017). However, only limited data demonstrate the ability of antioxidant supplementation to prevent the exercise-associated rise in markers of oxidative stress.

The next significant antioxidant is vitamin C, also known as ascorbic acid, in addition to the antioxidant properties of some of the roselle extracts discussed. Vitamin C in aqueous conditions is hydrophilic and works better (Traber, & Stevens, 2012). Since the ascorbic acid pKa is 4.25, the predominant shape that occurs at physiological pH is the ascorbate anion (Mogaddam, Kamyabnia, Gholamian, & Mosaddegi, 2012). Ascorbate is widely distributed in mammalian tissues but is present in the adrenal and

pituitary glands in relatively large concentrations (Yu, 1994). Vitamin C's function as an antioxidant is two-fold and can scavenge the radicals of superoxide, hydroxyl, and lipid hydroperoxide directly. Higher cellular concentrations of vitamin C from Roselle extracts should protect against muscle-related injuries caused by radical-mediated injury, provided the role of vitamin C in the recycling of vitamin E (Meščić Macan, Gazivoda Kraljević, & Raić-Malić, 2019). The use of Roselle extracts as supplements may be a breakthrough for athletes to overcome oxidative stress (Hadi *et al.*, 2017), as Roselle's antioxidant nature can help avoid muscle fatigue and harm throughout exercises.

Ginger

Ginger, also known as *Zingiber officinale* rhizome, belongs to the Zingiberaceae family. It is widely used as a culinary spice and for more than thousands of years, it has been used as treatment for various ailments as a traditional medicine (Sharifi-Rad *et al.*, 2017). Ginger was first discovered in southern China and has in time, spread to other parts of Asia and all over the world (Agrahari, Panda, Verma, Khan, & Darbari, 2015). Ginger belongs to the Zingiberaceae family, just like other spicy plants, including turmeric, cardamom and galangal (Leung *et al.*, 1996). Ginger is spicy in nature making it appealing to its users. The spicy nature of the ginger is due to its chemical structure, including the presence of ketones making it possess a spicy aroma (Dhifi, Bellili, Jazi, Bahloul, & Mnif, 2016). Ginger is used as a dietary supplement as well as a spice in alternative medicine and as a food and beverage flavoring agent. It has also been used in the treatment of various illnesses and discomforts including nausea in pregnant women, arthritis,

muscle aches, sore throats, cramps, fever, and a few infectious diseases (Fiorucci, Meli, Bucci, & Cirino, 2001; Nigam, Bhui, Prasad, George, & Skukla, 2009; Shukla, & Singh, 2007)

Ginger is a slender perennial plant that was first grown in China and then spread to India, West Africa, Southeast Asia, and the Caribbean. It is a tropical plant and its underground stem is used for medicinal and culinary spices. The rhizome of the ginger is from underground stems surrounded by the sheathing bases of two graded leaves (Pratap, Gangadharappa, & Mruthunjaya, 2017). It is typically an erect annual plant that grows in the soil and grows to a height of about 41.44 cm. Rhizomes are approximately 7-15 cm long, 1-1.5 cm wide, and compressed laterally. The branches emerge from the rhizome obliquely and are about 1-3 cm long and end in depressed scars or undeveloped buds. The outer surface is buff-colored and fibrous or longitudinally striated. A thin cortex, a well-marked endodermis, and a wide stele are seen on the broken surface of a ginger rhizome. The ginger plant has yellow, greenish flowers with a pungent, herbal flavor.

The *Zingiber officinale* rhizome is one of the most commonly used species in the family of Zingiberaceae and is a popular condiment for different foods and beverages (Pratap *et al.*, 2017). Ginger is common used as a spice in cooking throughout the world and specially used in kitchen as a hot aroma flavor spice that is said to also have healing effects (Sachan, Kumar, Kumari, & Singh, 2018). This is because ginger it has been shown to have anti-inflammatory, anti-apoptotic, anti-tumour activities, anti-pyretic, anti-platelet, anti-tumourigenic, anti-hyperglycaemic, antioxidant anti-diabetic, anti-clotting and analgesic properties (Shahrajabian, Sun, & Cheng, 2019)

Nutritional Profile of Ginger

Fresh ginger contains numerous phytochemicals that -have antioxidant, antimicrobial, gastroprotective, and anti-inflammatory properties (Cafino, Lirazan, & Marfori, 2016). The rhizome of ginger is an excellent source of dietary fibers that contain certain health benefits, essential oils, moisture, protein, fat, minerals, vitamins, and carbohydrates (Shahrajabian *et al.*, 2019). The composition and nutritional profile of ginger are varying with the type, variety, extraction & curing methods, drying, and storage conditions (Govindarajan, 1982). As per USFDA in Shirin Adel, & Prakash (2010), the nutritional profile of ginger is given in the following table.

Table 5- Nutritional composition of ginger (100g)

Constituent	Value
Moisture	15.02 ± 0.04
Protein (g)	5.087 ± 0.09(5.98)
Fat (g)	3.72 ± 0.03 (4.37)
Insoluble fibre (%)	23.5 ± 0.06 (27.65)
Soluble fibre (%)	25.5 ± 0.04 (30.0)
Carbohydrate (g)	38.35 ± 0.1
Vitamin C (mg)	9.33 ± 0.08 (10.97)
Total carotenoids (mg)	79 ± 0.2 (9296)
Ash (g)	3.85 ± 0.61 (4.53)
Calcium (mg)	88.4 ± 0.97 (104.02)
Phosphorus (mg)	174±1.2 (204.75)
Iron (mg)	8.0 ± 0.2 (9.41)
Zinc (mg)	0.92 ± 0 (1.08)
Copper (mg)	0.545±0.002 (0.641)
Manganese (mg)	9.13 ± 001 (10.74)
Chromium (µg)	70 ± 0 (83.37)

Source: Shirin Adel, & Prakash (2010).

Uses of Ginger

To flavor foods and also as a seasoning, ginger is used in cooking. It is also used for lowering blood sugar, lowering seizures, strengthening bones, and treating eyes, cough, colic, palpitations, swelling, dyspepsia, loss of appetite, and rheumatism (Tapsell *et al.*, 2006; Wang, & Wang, 2005). It is also frequently used to treat dyspepsia, gastro-paresis, symptoms of slow motility, constipation, or colic. It is most commonly used to mask the taste of narcotics. Pharmacological activities such as antipyretic, anti-nausea, anti-inflammatory, anthelmintic, fungicidal, antibacterial, antitussive, cardiogenic, sedative, hypoglycemic, and positive inotropic cardiac activities were demonstrated by Ginger and its constituents. Ginger increases appetite and digestion, boost bile and decreases gastric secretions, and promotes vasomotor and respiratory centers.

The primary promoter of antioxidant activity is phenolic compounds in plants (Maizura, Aminah, & Wan Aida, 2011). It is a good source of antioxidants because of the polyphenolic content of ginger (Yassin, ElRokh, El-Shenawy, & Ibrahim, 2012). Ginger is a natural antioxidant (El-Ghorab, Nauman, Anjum, Hussein, & Nadeem, 2010; Yeh *et al.*, 2014). In the prevention of certain diseases, antioxidants are important and slow down the process of aging (Fusco, Collaca, Monaco, & Cesari, 2007). Research has been carried out on more than 120 plant foods, in which ginger ranked first in the anti-oxidants' five affluent food wellsprings (Yahin, Yashin, Xia, & Nemzer, 2017). Ginger also improves the production of antioxidants in the body by avoiding free radicals while showing antioxidant effects (Fuhrman, Rosenblat, Hayek, Coleman, & Aviram, 2000; Raizur *et al.*, 2015). The

antioxidant constituent present in ginger appears to be 6-gingerol, as it has been demonstrated to protect HL-60 cells from oxidative stress (Wang, Chen, Lee, & Yang, 2003). Therefore, ginger oil has dominative protective effects on H₂O₂-induced DNA damage, acts as an oxygen radical scavenger, and could be used as an antioxidant (Peng *et al.*, 2012).

The discovery of the inhibitory effects of ginger on prostaglandin biosynthesis was frequently confirmed in the early 1970s (Grzanna, Lindmark, & Frondoza, 2005), which shows that ginger is a therapeutic herbal product that shares pharmacological properties with anti-inflammatory nonsteroidal drugs. By inhibiting cyclooxygenase-1 and cyclooxygenase (Vanden, & Letsky, 2000), Ginger suppresses prostaglandin synthesis. The discovery that ginger also suppresses leukotriene biosynthesis by inhibiting 5-lipoxygenase was a significant extension of this early work. It is distinguished from nonsteroidal anti-inflammatory drugs by this pharmacological feature of ginger. This result goes before the observation that cyclooxygenase and 5-lipoxygenase dual inhibitors could have a stronger therapeutic profile and have fewer side effects than anti-inflammatory nonsteroidal drugs (Fiorucci *et al.*, 2001).

The activation of several genes involved in the inflammatory response is inhibited by ginger extract (EV.EXT.77) derived from *Zingiber officinale* (family Zingiberaceae) and *Alpina galanga* (family Zingiberaceae). These include cytokine encoding genes, chemokines, and the inducible cyclooxygenase-2 enzyme. The first proof that ginger regulates biochemical pathways activated in chronic inflammation was provided by this discovery. The previous study indicated that in patients with rheumatoid arthritis (RA)

and osteoarthritis (OA), the use of powdered ginger for a period of 3 months to 2.5 years decreases pain and inflammation in 75 % of patients without any adverse effect and indicates that ginger is an anti-inflammatory agent (Srivastava, & Mustafa, 1992). 6-gingerol serves as an anti-inflammatory agent that can be effective in treating inflammation without interfering with macrophage antigen function (Tripathi, Maier, Bruch, & Kittur, 2007).

With several active components, ginger is rich. In vitro and in vivo, 6-gingerol, a major pungent ginger ingredient, has a potent antiangiogenic effect. Also, through its anti-angiogenic action, 6-gingerol can inhibit tumor growth and metastasis, establishing that it may have anticancer properties (Kim *et al.*, 2005). Ginger's natural antioxidant and anticarcinogenic dietary components modulate in vitro secretion of angiogenic factors in ovarian cancer cells and function as potent dietary preventive agents for chemotherapy (Rhode- *et al.*, 2007). An extract from the ginger plant is a novel anticancer drug β -element and activates apoptosis mediated by the mitochondrial release of cytochrome c in non-small - cell lung cancer cells. The β -element induces the activities of caspase-3, -7, and -9, reduces the expression of Bcl-2, triggers the release of cytochrome c, and raises the cleaved caspase-9 and poly (ADP-ribose) polymerase levels in cells (Wang *et al.*, 2005). Enhanced glutathione reductase (GR) enzyme activity, glutathione peroxidase (GPX), glutathione-S-transferase (GST) suppresses ginger supplement colon carcinogenesis, effectively minimizing colon cancer (Manju, & Nalini, 2005). In-vivo, inhibiting necrosis factor-kappa B (NF-kB) and also interleukin-8 (IL-8) (Rhode *et al.*, 2007), ginger and its component 6- gingerol are effective against ovarian cancers.

Six-gingerol is also effective in suppressing colon tumor growth in mice (Yusof, Ahmad, Das, Sulaiman, & Murad, 2009), works against skin cancer (Nigam *et al.*, 2009), breast cancer, ovarian cancer (Rhode *et al.*, 2007), and inhibits gastric cancer by 6-gingerol and [6] shogaols (Shukla *et al.*, 2007). Ginger has some severe effects on vomiting and nausea and has some advantages (Ernst, & Pittler, 2000; Viljeon, Visser, Loen, & Musekiwa, 2014). In 2006, a meta-analysis and five randomized trials were performed to test the use of ginger for vomiting and nausea in a study conducted on 363 patients, showing that ginger was more successful than a placebo (Chaiyakunapruk, Kitikannakorn, Nathisuwan, & Leorakoboon, 2006).

During pregnancy, for vomiting and nausea, ginger is quite useful (Jewell & Young, 2010). In 2005, 33 studies examining the productivity of ginger in relieving vomiting and nausea caused at the time of pregnancy were reviewed in a study (Borrelli, Capasso, Aviello, Pittler, & Izzo, 2005). The included parameters are double-blind, randomized controlled trials (RTC's), with a total of 675 participants in just six studies. Four out of 33 of these experiments found that ginger was imperious to placebo. Two of these studies have indicated that it is similar to vitamin B6, which is effective in the treatment of pregnancy-induced nausea. The study found that there were no negative effects of ginger on pregnancy outcomes.

Antiviral activity was demonstrated by Ingenol and 6-shogaol, isolated from a ginger rhizome (Bordia, Verma, & Srivastava, 1997). The active inhibitor of mycobacterium avium and mycobacterium tuberculosis in vitro (Rahmani, Al shabrmi, & Aly, 2014) was identified as 10-gingerol. For antimicrobial activities, gingerol and related compounds have been studied.

Antibacterial activity against periodontal bacteria was demonstrated by 6-gingerol and 12-gingerol, isolated from a ginger rhizome (Levita *et al.*, 2018). Other literature has also shown that Ginger's ginger constituents prevent bacteria and fungi from developing and have both cidal and static behavior. Antimicrobial activity against E Coli, Salmonella typhi, and Bacillus subtilis was demonstrated by Ginger (Azu & Onyeagba, 2006). Antibacterial agent against periodontal bacteria (Park, Bae, & Lee, 2008), and Candida albicans (Atai, Attapour, & Mohseni, 2009), are recognized as Ginger and its significant constituent of gingerol and shogaol.

Other Medicinal Benefits of Ginger

Some studies have shown that components of ginger, namely compound classes of gingerol and shogaol, can have many therapeutic effects, including anti-inflammatory, antioxidant, and hypocholesterolemic effects (Ernst, & Pittler, 2000; OZgoli, & Goli, 2009; Chaiyakunapruk, Kitikannakorn, Nathisuwan, Leepakoboon, & Leelasettagool, 2006). Through stimulation of the heart muscle and by diluting circulating blood, ginger also improves blood circulation throughout the body. This improves the metabolism of cells and helps to relieve cramps and stress (Al-Awwadi, 2017). When administered at 0.3-3 mg/kg, ginger was shown to have a hypotensive effect (Ghayur & Gilani, 2005). In this study, by blocking the calcium channel or acting on the muscarinic receptor, ginger helped reduce atrial blood pressure. Because of high blood pressure, the narrowing of blood vessels - caused by the accumulation of cholesterol and/or fats (lipids) in the blood vessels is most often the result (Fuhrman *et al.*, 2000). Therefore, narrowing appears to place more pressure on the heart and raise the heart rate in most

cases. Ginger extract, however, is said to interfere with cholesterol biosynthesis, resulting in decreased levels of cholesterol (White, 2007). Blood coagulation leads to the formation of plaque or thrombosis that can lead to various heart diseases (Nagareddy & Smyth, 2014). Studies have shown that by reducing blood clotting, ginger prevents heart diseases and also helps open blood vessel blockage that helps reduce blood pressure and peripheral vascular resistance (Vasanthi & Parameswari, 2010). Studies have shown that ginger prevents heart disease. Increased cholesterol levels that make the heart unhealthy are significantly reduced (Ghayur *et al.*, 2005). By reducing thermogenesis and high lipid levels, ginger extracts also have antilipidemic effects, which help to increase serum HDL-cholesterol (Al-Awwadi, 2017; Ernst & Pittler, 2004; Ozgoli & Goli, 2009; Vutyavanich, Kraissarin, & Ruangri, 2000). Portoni *et al.*, 2003; In vitro research shows that at low doses and cardiogenic properties, gingerols and related shogaols exhibit cardio depressant activity at higher doses (Ashraf, Shah, Azra, Niaz, & Hakro, 2019). Powerful enzymatic inhibitors of prostaglandin, thromboxane, and leukotriene biosynthesis are reportedly both 6-shogaol and 6-gingerol and gingerdiones respectively (Wang *et al.*, 2003). Also, by getting rid of or scavenging free radicals, the antioxidant property of *Zingiber officinale* helps it to reduce blood lactate. Ginger is a free-radical scavenger. Ginger oil has scavenging effects and various studies have proven this (Avato, Tursil, Vitali, Miccolis, & Cadido, 2000; Duke & Ayensu, 1985; Kamtchouing, Mbongue, Dimo, & Jasta, 2000; Kumar *et al.*, 2015).

Effect of Ginger on athletic performance

Some of the most important parameters that make up a complete athlete are speed, strength, and endurance. Speed, strength, and endurance are highly dependent on the athlete's muscles. How quickly the muscle can expand and contract, how long the muscles are functioning, and how quickly the muscle can recover from injury are also important for athletic performance (Wall *et al.*, 2015). By compensating their muscles with supplements and other medications, most athletes tend to optimize their performance. Two ginger types is considered in this review and these are Zingiber officinale and Black ginger (*Kaemferia parviflora*). Physical exhaustion reduces the effectiveness of physical fitness and muscular endurance. Due to the accumulation of lactic acid, fatigue was previously thought to reduce intracellular pH (acidosis) (Favero, 1999). Recent results, however, indicate that lactic acid accumulation is not a direct cause of fatigue, but rather a fatigue-recovering factor (Pedersen, Nielson, Lamb, & Stephenson, 2004; Nielsen, de Paoli, & Overgaard, 2001; Stary, & Hogan, 2005).

Also, various factors have been suggested to play a role in the complex fatigue processes, including ATP metabolism, acidosis, and oxidative stress (Allen, Kabbara, & Westerblad, 2002; Finsterer, 2012; Gosker, & Schols, 2008; Lui *et al.*, 2015; Nielsen, & Clausen, 2000; Powers *et al.*, 2011; Vandenoorn, 2004). A variety of stimulators and suppressors within inflammatory pathways, including the COX-prostaglandin cascade (Davis *et al.*, 2007), regulate exercise-induced increases in cytokines, such as TNF-alpha, leading to further increased inflammatory cytokine production, pain, and performance deficits among athletes in muscle function. Elevated blood

levels of creatine phosphokinase, also referred to as creatine kinase (CK), and lactate dehydrogenase (LDH) are indirectly quantified by muscle damage in athletes (Overgaard, Lindstorm, Ingemann-Hanson, & Clausen, 2002; Totsuka, Nakaji, Suzuki, Sugawara, & Sato, 2002). Therefore, as an indicator of muscle fiber damage, CK, and LDH measurements can be used (Ohtani, Sugita, & Maruyama, 2006). Delayed onset muscle soreness (DOMS) (Hilbert, Sforzo, & Swensen, 2003) is more commonly recognized as skeletal muscle pain, which may be attributed to muscle fiber alteration, followed by ion imbalances and inflammation. Individuals more commonly recognize DOMS as muscle pain and tenderness, which develops over many hours and is 1 to 2 days after exercise at most (Prasartwuth, Taylor, & Gandevia, 2005). Given that ginger has anti-inflammatory and analgesic properties, it follows that it can be used after high-intensity exercise to decrease the damage and consequent DOMS.

Effect of *Zingiber officinale* on muscle recovery, and performance

A study by Matsumura, Zavorsky, and Smoliga (2015) aimed at examining the effects of ginger supplementation pre-exercise on muscle damage and the onset of delayed muscle soreness. In their study, non-athlete male and female subjects were recruited and assigned randomly to the ginger and placebo groups. Ginger supplements were given to subjects in the ginger group and asked to consume their supplements for 5 days, whereas placebo (dextrose) was given for 5 days to subjects in the control group. There was no specified precise period for consumption, but subjects were asked to be consistent in their time of administration. During the early stages of delayed

onset of muscle soreness (DOMS), the ginger group showed a substantial rise, but this did not happen in the placebo group.

Interestingly, after other eccentric exercises, the placebo group continued to show improvements in strength, yet the ginger group showed no other significant improvements, but ginger subjects experienced an accelerated recovery compared to the placebo group. As shown by a more incremental rise in creatine kinase, this was due to ginger delaying real muscle damage itself. It was also said that it was possible for ginger loading to have increased muscle function, which allowed people to perform more physical work during the eccentric muscle damage protocol, which later during the study may have led to more muscle damage. Another possibility was linked to the fact that the constituents of ginger have a limited window of anti-inflammatory function, thus eliminating the protective effect against muscle damage by terminating ginger supplementation. It was therefore concluded that continued supplementation of ginger was necessary for the analgesic effect for ginger to be used as a supplement to increase athletic performance. It also seemed that this regimen for ginger supplementation postponed muscle damage but did not stop it. Although many unknowns remain, the results of this study, combined with several others, make it clear that ginger does indeed have an impact on basic exercise-related measures of muscle function, and further studies in this area are needed. Another study has suggested that exercise-associated inflammatory markers in male distance runners may be attenuated by 6 weeks of 1.5 g of ginger per day (Zehsaz, Farhangi, & Mirheidari, 2014).

An experiment was designed to determine whether 11 consecutive dietary supplementations with 2 grams of raw or heat-treated ginger would

affect delayed muscle pain caused by eccentric exercise in a different study that sought to demonstrate that dietary ginger (*Zingiber officinale*) decreases muscle pain caused by eccentric exercise (Black, Herring, Hurley, & O'Conner, 2010). However, the new finding was that 24 hours after eccentric exercise, supplementation with both raw and heat-treated ginger attenuated the intensity of muscle pain. During the experiment, it was found that raw ginger consumption resulted in a 25 percent decrease, while heat-treated ginger resulted in a 23 percent decrease. The biological plausibility of ginger having hypoalgesia effects is supported by considerable evidence (Perna *et al.*, 2020). It has been shown that Ginger and its components, specifically 6-gingerol and 6-shogaol, inhibit COX 1 and 2 enzymes (Nurtjahja-Tjendraputra *et al.*, 2003; Tjendraputra *et al.*, 2001), leukotriene synthesis (Kiuchi, Iwakami, Shibuya, Hanaoka, & Sankawa, 1992) and proinflammatory cytokine release (Tripathi *et al.*, 2007) *in vitro*. These established biological actions indicate that ingested ginger may reduce the increase in muscle tissue mechanical hypersensitivity by reducing the direct activation of type III and type IV afferent nerve fibers by substances such as bradykinin and the sensitivity of prostaglandins and cytokines such as IL-1 and IL-6 to afferent fibers. Ginger may also act centrally, in addition to its ability to act at peripheral sites. Transient receptor potential vanilloid 1 (TRPV1) receptor agonists are known to be gingerol, shogaol, and zingerone (Dedov *et al.*, 2002).

In peripheral (dorsal root ganglion) and central neural tissue, TRPV1 receptors are expressed and are thought to play a role in the treatment of nociception and pain (Cho & Valtschanoff, 2008; Mezey *et al.*, 2000). The role TRPV1 receptors play in mechanical hyperalgesia after eccentric exercise

in rodents 16 was shown by a recent study (Ota *et al.*, 2013). Initially, TRPV1 receptor activation by agonists such as capsaicin can be painful (Jaraoseguera, Simon, & Rosenbaum, 2008). However, it has been demonstrated that large doses of long-term administration desensitize nociceptive afferents to mechanical and chemical stimuli. Therefore, in the study by Dedov *et al.* (2002), ginger consumption reduced muscle-pain intensity in part by desensitizing peripheral and/or central TRPV1 receptors. This could also be a demonstration that 11 consecutive days of 2 grams of raw and heat-treated ginger dietary supplementation reduces muscle pain caused by eccentric exercise, raw ginger is as effective in achieving this effect as heat-treated ginger. The biological activities of black ginger extract (*Kaempferia parviflora* extract: KPE) and poly methoxy flavones (PMFs), including antioxidant activity, have been demonstrated in some recent studies (Akase *et al.*, 2011; Shimada, Fujita, Yamamoto, & Ishihama, 2011; Kusirisin *et al.*, 2009; Rujjanawate, Kanjanapothi, Amornlerdpison, & Pojanagaroon, 2005).

In *Kaempferia parviflora* extract (KPE), poly methoxy flavones (PMFs) are found. In clinical studies, KPE has been shown to improve physical fitness performance (Toda, Hitoe, Takeda, & Shimoda, 2016), thus implicating KPE's anti-oxidative activity in its beneficial effects. PMFs in KPE has been reported to increase energy production through activation of adenosine monophosphate kinase (AMPK) induced myocyte metabolism enhancements (Toda *et al.*, 2016). In the regulation of energy homeostasis, AMP-activated protein kinase (AMPK) is known to be critically involved (Khan, Alquier, Carling, & Hardie, 2005) and its activation has been shown to improve glucose and lipid metabolism (Hardie, Schaffer, & Brunet, 2016).

AMPK has therefore been an attractive target for the discovery of anti-diabetic and anti-obesity therapies.

Physical activity and muscular endurance are related to AMPK. Previously, 5-Aminoimidazole4-carboxamide ribonucleotide (AICAR), an AMPK agonist, has been reported to increase running stamina by up to 44% and decrease body fat in mice when given orally for 4 weeks (Narkar *et al.*, 2008). The study assessed the effects of KPE on the performance of physical fitness and muscular endurance in mice. Male mice received oral KPE for 4 weeks, and subsequently underwent a forced swimming test (ST), open-field test, inclined plane test (PT), and wire hanging test. The swimming time, motility after swimming and grip strength were substantially increased by KPE. Therefore, it was concluded that KPE and PMFs suppressed in vitro and in vivo muscular inflammation, increased mitochondrial KPE, and decreased in vivo blood lactic acid (LA) levels, thereby reducing blood lactate levels. PMFs have also promoted in vitro glycogen accumulation. Black ginger has also been reported to improve the performance of physical fitness in the elderly, and one of the mechanisms for the physical improvement effect of KPE has been suggested to be improved antioxidant enzyme activities (increased superoxide dismutase, glutathione peroxidase, and serum catalase activities) and decreased MDA levels (Tende, Ayo, Mohammed & Zezi, 2015). Based on the above studies, black ginger may be an appropriate ingredient for improving physical fitness, muscular endurance, fatigue, and metabolism.

CHAPTER THREE

RESEARCH METHODS

The purpose of the study was to explore the effect of hibiscus-ginger juice supplementation on selected physiological variables of university athletes. This chapter discusses the research design, the population, the processes through which participants were recruited and grouped for the study, preparation of treatment, and instrument for data collection. Details of the data collection procedures including the experimental protocol for this study as well as details of data processing and analyses are discussed in this chapter.

Research Design

The research, being quantitative, sought to establish a cause and effect. Therefore, an experimental approach was used since it offers the strongest tests of causal relationships compared to other social techniques altogether, providing evidence about the influence of the independent variable on the dependent variable (Neuman, 2014). A pretest-posttest experimental design was used. Using this design enabled the researcher the opportunity to explore the effect of treatment while comparing the outcomes of both the experimental and control groups. The design further helped to measure the degree of change occurring as a result of the treatment (Creswell, 2013). Random assignment of participants was done to ensure the groups are equal on variables of interest, which makes the internal validity of the design strong.

Population

Athletes of the University of Cape Coast made up the population of the study. These athletes according to the Sports Section of the university number about two hundred and ten (210). They include track and field athletes (40),

racquet game players (24), footballers (40), basketball players (24), handball players (28), netball players (14), and hockey players (40). To the best of the researcher's knowledge, these athletes possessed the physical fitness and discipline required for the study. Specifically, the researcher targeted footballers, basketball players, handball players, and hockey players. This group of athletes provided the researcher with a group with very similar characteristics, both physically and physiologically as the games they play have similar movement patterns, as well as the similar duration of play. The athletes of the University of Cape Coast, train for at least two months in every semester of every academic year, in preparation for inter-halls. The inter-hall games serve as a selection process for the University teams for inter-university games. If an athlete is selected, they continue to train after the inter-halls towards Ghana University Sports Association (GUSA) games. This implies that they train for most of the year and so were better suited for this study.

Sampling Procedure

To get the sample for the study, athletes from the University of Cape Coast sports teams who were selected to be part of the GUSA team were gathered and briefed on the purpose of the study and what is expected of participants. The athletes were then allowed to volunteer or not, to be participants of the study. At the end of the briefing, 28 athletes volunteered to take part in the study.

So that the requirements for a true experiment which is randomization, randomized grouping was done to ensure that the group a participant belonged to was left to chance. The procedure for the randomized groupings is fully described under phase one (1) of the data collection procedure.

Data Collection Instruments

Data was collected on aerobic power (VO_2 max) and selected physiological responses including heart rate, blood pressure, blood lactate level, as well as physical measurements of height and weight. The following are the details of the instrument used to measure the dependent measures

1. Aerobic power: High-intensity intermittent recovery running test was used to measure aerobic power (Bangsbo, Iaia, & Krustup, 2008).

This test is also called the Yo-Yo intermittent recovery test (YYIR test). Required equipment for conducting the Yo-Yo test included

- I. Facility: a consistent, flat, and non-slip floor with a minimum length of 30m,
- II. Marking cones,
- III. Measuring tape,
- IV. YYIR1 test audio,
- V. Loudspeaker for audio,
- VI. A performance recording sheet and
- VII. An official in charge of recording the number of shuttles completed.

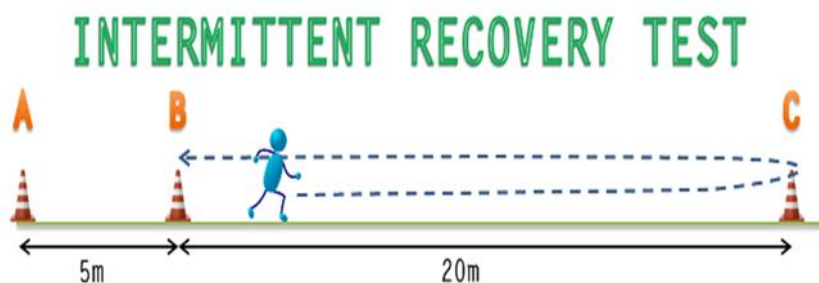


Figure 1: YoYo Test Setup

Test procedure

The performance procedure (regarding the above setup) is as follows

- A. Starting stance is standing with feet behind the line at cone B and wait for a signal from an audio recording to start.
- B. When the audio gives the signal to start, the participant begins running towards cone C.
- C. Once the participant gets to cone C, they turn at the sound of a first beep from the audio recording and run back towards cone B. Participants will have to get to cone B before the second beep from the audio recording.
- D. Once back at cone B, the participant takes 10 seconds of active rest by walking or jogging from cone B to cone A and back. They then resume the ready position for the next shuttle run.
- E. If the participant is unable to return to cone B before the second beep, they receive a warning. The next time they miss this target, the test ends for them and a final score is recorded.

Scoring

Distance covered in meters is recorded and VO_2 max is calculated using the formula by Bangsbo, Iaia, and Krustrup (2008).

$$VO_2 \text{ max} = \text{distance in meters} \times 0.0084 + 36.4$$

2. Heart rate and blood pressure: Heart rate and blood pressure were measured using the Omron BP 742N 5 series upper arm blood pressure monitor. This device is an automated sphygmomanometer used to check both blood pressure and heart rate.

3. Blood lactate: A venous blood sample of 4ml was collected from each study participant into a gel separator tube and allowed to clot. The gel separator tubes' blood sample was centrifuged for 15 minutes at 1000g using a centrifuge to plasma, and these were stored at -20°C. Serum samples were used for Lactate dehydrogenase (LDH) estimation.
4. Height: The height of the participants was measured using a portable researcher-made stadiometer. The stadiometer was constructed using standardized procedures and its reliability was determined during the pilot test.
5. Weight: Weight was also measured with a bathroom weighing scale. The weighing scale is an already standardized device however its consistency in measurements was tested during the pilot testing.

Preparation of Hibiscus-Ginger juice and participant recruitment

The hibiscus-ginger juice used as treatment for the study was prepared using a previously recommended protocol by Darkwa (2016). The following recipe was used to prepare of a portion of three (3) liters of the hibiscus-ginger juice. Ingredients used include;

1. 100 grams of dried hibiscus calyces
2. 100 grams of ginger rhizome
3. 200 grams of pineapple peels
4. 10 grams of peppercorn
5. 10 grams of cloves
6. 2.5 liters of drinking water
7. 0.5 liter of pineapple juice

Procedure

1. Pour dried hibiscus calyces into a pot preferably with a cover big enough to contain 1.5 liters of water,
2. Add crushed ginger rhizomes, washed pineapple peels, peppercorn, and cloves.
3. Add 2 liters of drinking water to the combination and bring to boil. Take the mixture off fire 5 minutes after it starts to boil and leave to cool till room temperature.
4. When the mixture has cooled, strain into a bowl and add sugar. Stir the mixture till all the sugar is dissolved.
5. Add pineapple juice.
6. Bottle and store in the refrigerator.

Pilot study

A pilot study was conducted before the main study using ten (10) students from the Department of Health, Physical Education and Recreation who did not form part of the target group. These participants were taken through the experimental protocol, but only for two weeks. Five research assistants were recruited and trained to assist with data collection in the pilot study as well as the main study.

The pilot study was conducted to address any practical challenges that could arise in the main data collection of the definitive study to be carried out. The pilot study also guided decisions about designing approaches to the recruitment of participants as well as how to go about measurements for the various dependent measures. The pilot study also gave the researcher an idea of what the true recruitment rate was (not just the number of subjects

available, but also their willingness to participate). It was even more helpful in identifying any methodological issues related to applying the intervention or measuring outcome variables, which were appropriately addressed.

Data Collection Procedures

After the necessary ethical clearance, data were collected in three phases. Phase one involved the recruitment of participants, familiarization, and randomized grouping of the participants for the study. Phase two comprised of the pretest data collection and phase three involves post-test data collection. Treatment was administered to the participants who belonged to the treatment group from between the period of the pretest and the final post-test data collection.

Ethical considerations

The research protocol was submitted to the Institutional Review Board of the University of Cape Coast for approval before data collection (UCCIRB/CES/2019/38). Introductory letters were obtained from the Department of Health Physical Education and Recreation to the University Sports Section and for all other permissions needed to proceed with data collection.

Participants were adequately informed about the purpose of the study as well as their rights. Participation was solely voluntary and participants were given the option to withdraw from the study at any stage if they wished to. The researcher, however, reserved the right to withdraw any participant who was found to be going contrary to the requirements for the study. Participation was also based on the participant readiness and signing informed consent which provided sufficient information and assurances about taking part to allow the

participants understand the implications of participation, be fully informed of the processes involved in the study to consider and decide freely about whether to participate or not without any pressure or coercion.

Participants were assured of the confidentiality of the research data and anonymity. To ensure that participants could take part in the study without any harm to their health, they were screened for health risks and given pre-participation health questionnaire to complete, which was used to decide whether a participant was at a health risk by participating in the study or not. They were also given physical activity readiness questionnaire to complete. Participants were taken through nutritional screening to ascertain their nutritional levels before the study. Participants with health problems, injuries, allergies to any of the ingredients of the treatment, and questionable health screening results were excluded from the study.

Phase one: Recruitment of participants, familiarization and group assignments

Members of the study population were first contacted at a team meeting and the purpose of the study was explained to them. At this briefing, these athletes were fully informed of the risks, benefits, issues about confidentiality as well as their responsibilities for the study if they chose to participate. After this exercise, they were given the option to participate or opt-out if they had any conditions that could hinder their participation, or for any other reasons which were not probed into.

Athletes who opted to participate in the study were then taken through the necessary protocol to establish their eligibility and fitness to become participants. After a week, familiarization test run for the YYIR -test, demographic data (age, gender, weight, and height) collection as well as

resting physiological data (heart rate, blood pressure, and blood lactate level) data were taken from participants. Data from the familiarization test run was then used as a basis for which randomized grouping was done. Participants at this point were still given the option of opting out of the study if they wished to. These were done after the test procedure had been explained to the participants, consent obtained and physical activity readiness ascertained.

Before participants were taken through the YYIR test, to ascertain entry performance levels and familiarization exercise, they were given ten (10) minutes to warm up and stretch to get ready for the test. Results from the familiarization test were then used to systematically group participants according to their levels of performance to ensure even distribution of performance abilities across all groups. In doing this, the researcher established two groups for the study; the treatment group, (TG) and the control group (CG) using the fishbowl method. Participants in TG consumed hibiscus-ginger juice. They took the hibiscus-ginger juice together with water as part of their after-training fluid intake for the period of the intervention. The control group proceeded with training as the treatment group but did not receive any treatment but took only water after training.

After the test, the participants were refreshed and the researcher established a means of communication with the participants by which the participants could contact the researcher with any questions or concerns. This was also done to enable the researcher to get in touch with the participants in the participants. A date for the pretest was also set in consultation with the participants to ensure that the date would be appropriate for them.

Phase two: Pretest data collection and treatment administration

On the day of the pretest, the participants arrived ready to go through the test. Resting blood pressure and heart rate were measured before the YYIR test. The participants were then given 10 minutes to warm up for the test, after which they went through the test in groups as determined during the pilot test. The physiological measures of blood pressure and heart rate were taken immediately after completion of the test as well as blood lactate levels. This was to serve as baseline measures for these parameters. The intervention involved the participants in the treatment group receiving 500ml of hibiscus-ginger juice after every afternoon training for the 24 day period for which the study was conducted. They took the treatment before eating evening meals together with water.

Phase three: Posttest data collection

Two post-tests were conducted for the study. The first YYIR posttest was conducted 2 weeks after the pretest and treatment and the second posttest happened ten days after the first post-test. This was done to keep a progressive report of the athletes' performance in the test. Physiological measures of blood pressure and heart rate were recorded after each posttest. Blood lactate measures were however taken only during the pretest and the final posttest. The same protocol used for the pretest data collection was used for both post-test data collections. Participants were refreshed, briefed, and then dispatched after the final post-test data collection.

Data Processing and Analysis

All data was processed using SPSS version 20. Data collected were screened for missing data and data entry errors using frequencies and

percentages. To ensure that the data meet the assumptions of statistical tests used, data were checked for outliers using the box plot, and normality was checked using the histogram. Demographic data were analyzed using descriptive statistics of means and standard deviations. Data collected on the hypotheses being tested were done at three different times and in two groups, treatment, and control. Data collected in the pretest and posttests were on a continuous scale, therefore, quantitative data analyses were adopted for analyses of the data. Therefore, having met the assumptions for conducting ANOVA, hypotheses one to four were analyzed using mixed-design ANOVA, with a within-subjects factor (pretest, posttest 1, and posttest 2) and a between-subject factor (experimental and control), to find mean differences at a significance level of $p < 0.05$. This was done using the repeated measure in the General Linear Model in. In order to verify the association of repeated measures, the Mauchly sphericity test was used and analysis of inside-subject and between-subject effects were carried out using repeated measure ANOVA in a general linear model.

CHAPTER FOUR

RESULTS AND DISCUSSION

The purpose of the study was to explore the effect of hibiscus-ginger juice supplementation on selected physiological variables of university athletes. This chapter focuses on the presentation of results and result interpretation. The findings of the results were discussed with existing literature.

The current study is an experimental type following a pretest-posttest design. Data were collected in three phases which included one pretest and two posttests. The participants for the study were volunteers from the University of Cape Coast sports team for the GUSA games, 2020. These participants were part of the basketball, football, handball, and hockey teams. Out of the initial 46 volunteers, 28 completed the experiment while 16 did not make the final team after final selections for their various teams. The participants were taken through a familiarization where their VO_{2max} test was used to randomly assign them into treatment and control groups. Treatment was administered over a 24 days period when the athletes were training in preparation for GUSA games, 2020. During the duration of the study, two members of the treatment group opted out of the study due to injuries incurred from training. Therefore, the result presented in this study is on 28 athletes, 13 in the treatment group, and 15 in the control group. Demographic data of age, weight, and height were collected. The demographic characteristics of the participants are presented in Table 6.

Table 6- Demographic data of participants

Demographic Characteristic	Number	Minimum	Maximum	Mean	Standard Deviation
Age (yrs)	28	20.00	37.00	24.43	3.65
Weight (kg)	28	48.10	170.00	70.46	23.24
Height (cm)	28	155.00	196.00	171.16	10.02

Source: Kluboito (2020)

Hypothesis One: Consumption of Hibiscus-Ginger Juice has a Significant Effect on the Heart Rate of Athletes of the University of Cape Coast

Heart rate was measured at rest and after YoYo on three different occasions. These measures were recorded and classified as resting heart rate (RHR) and exercise heart rate (EHR) respectively. Separate analyses were run for either of these measures, and the details are shown in tables 7 and 8.

Resting heart rate

The initial resting heart rate (RHR1) of the participants which was taken on the first meet ranged from 54bpm to 88bpm ($M = 70.43$, $SD = 10.82$). Resting heart rate taken before the first posttest (RHR2) ranged from 53bpm to 94bpm ($M = 73.17$, $SD = 10.07$) and resting heart before the second posttest (RHR3) also ranged from 54bpm to 88bpm ($M = 72.79$, $SD = 10.10$).

Table 7- ANOVA results for the effect of consumption of hibiscus-ginger on resting heart rate

	<i>Df</i>	F-value (<i>F</i>)	Significance (<i>p</i>)	Partial eta squared (η_p^2)
RHR	2	1.214	.305	.045
RHR * GRP	2	1.760	.182	.063
GRP	1	.874	.323	.033
Intercept	1	2190.92	.000	.988

Source: Kluboito (2020)

N = 28 (treatment = 13, control = 15).

Error df (within-subject = 52, between-subject = 26)

A 3(Time) x 2(Group) mixed model ANOVA revealed that the main effect for Group was not significant $F(1, 26) = .874, p = .323, \eta_p^2 = .033$. Thus, there was no overall significant difference in the RHR of treatment ($M = 70.59$) compared to control ($M = 74.47$). Main effects for time (RHR) were also not statistically significant $F(2, 52) = 1.214, p = .305, \eta_p^2 = .045$. However, RHR increased over from RHR1 ($M = 70.19$) to RHR2 ($M = 73.21$) then dropped to RHR3 ($M = 72.69$). Time x Group was also not significant, $F(2, 52) = 1.760, p = .182, \eta_p^2 = .063$. Examination of the cell means indicate that for the treatment group, there was an increase from RHR1 ($M = 66.76$) to RHR2 ($M = 73.69$) which later dropped in RHR3 ($M = 71.31$). In the control group however, with time, resting heart rate dropped from RHR1 ($M = 73.60$) to RHR2 ($M = 72.73$) and rose again in RHR3 ($M = 74.07$).

Exercise heart rate

Heart rate after pretest (EHR1) ranged from 83bpm to 138bpm ($M = 109.57, SD = 13.62$). Heart rate after first posttest (EHR2) ranged from 81bpm to 151bpm ($M = 113.23, SD = 14.40$) and heart rate after second posttest (EHR3) ranged from 68bpm to 153bpm ($M = 110.28, SD = 20.45$).

Table 8- ANOVA results for the effect of consumption of hibiscus-ginger on exercise heart rate

	<i>df</i>	F-value (<i>F</i>)	Significance (<i>p</i>)	Partial eta squared (η_p^2)
EHR	1.595	.770	.442	.029
EHR * GRP	1.595	1.203	.302	.044
GRP	1	.217	.645	.008
Intercept	1	1961.45	.000	.987

Source: Kluboito (2020)

N = 28 (treatment = 13, control = 15).

Error df (within-subject = 41.469, between-subject = 26)

A 3(Time) x 2(Group) mixed model ANOVA revealed that the main effect for Group was not significant $F(1, 26) = .217, p = .645, \eta_p^2 = .008$. Thus, there was no overall significant difference in the EHR of treatment ($M = 112.28$) compared to control ($M = 109.94$). Main effects for time (EHR) were also not statistically significant $F(1.595, 41.469) = .770, p = .442, \eta_p^2 = .029$. However, EHR increased over time from EHR1 ($M = 109.45$) to EHR2 ($M = 113.38$) then dropped to EHR3 ($M = 110.51$). Time x Group effect was also not significant, $F(1.595, 41.469) = 1.203, p = .302, \eta_p^2 = .044$. Examination of the cell means indicated that for the treatment group, there was an increase from EHR1 ($M = 107.77$) to EHR2 ($M = 115.39$) which later dropped in EHR3 ($M = 113.69$). In the control group, with time, exercise heart rate increased from EHR1 ($M = 111.13$) to EHR2 ($M = 111.37$) and dropped in EHR3 ($M = 107.33$).

Hypothesis Two: Consumption of Hibiscus-Ginger Juice has a Significant Effect on the Blood Pressure of Athletes of the University of Cape Coast

Systolic blood pressure was measured at rest and after YoYo test on three different occasions. These measures were recorded and classified as resting systolic blood pressure (RSBP) and exercise systolic blood pressure (ESBP) respectively. Separate analyses were run for either of these measures, and the details are shown in tables 9 and 10.

Resting systolic blood pressure

Initial resting systolic blood pressure (RSBP1) ranged from 104mmHg to 162mmHg ($M = 129.75, SD = 14.09$). Resting systolic blood pressure before first posttest (RSBP2) ranged from 107mmHg to 158mmHg ($M = 130.57, SD = 14.21$) and resting systolic blood pressure before second posttest (RSBP3) ranged from 104mmHg to 168mmHg ($M = 132.18, SD = 16.58$).

Table 9- ANOVA results for the effect of consumption of hibiscus-ginger on resting systolic blood pressure

	<i>df</i>	F-value (<i>F</i>)	Significance (<i>p</i>)	Partial eta squared (η_p^2)
RSBP	2	.492	.614	.019
RSBP * GRP	2	.748	.478	.028
GRP	1	.010	.922	.000
Intercept	1	2648.70	.000	.990

Source: Kluboito (2020)

N = 28 (treatment = 13, control = 15).

Error *df* (within-subject = 52, between-subject = 26)

A 3(Time) x 2(Group) mixed model ANOVA revealed that the main effect for Group was not significant $F(1, 26) = .010, p = .922, \eta_p^2 = .000$. Thus, there was no overall significant difference in the RSBP of treatment ($M = 130.56$) compared to control ($M = 131.07$). Main effects for time (RSBP) were also not statistically significant $F(2, 52) = .492, p = .614, \eta_p^2 = .019$. However, RSBP increased over time from RSBP1 ($M = 129.85$) to RSBP2 ($M = 130.47$) then dropped to RSBP3 ($M = 132.13$). Time x Group was also not significant, $F(2, 52) = .748, p = .478, \eta_p^2 = .028$. Examination of the cell means indicate that for the treatment group, there was a decrease from RSBP1 ($M = 131.23$) to RSBP2 ($M = 129.08$) which later increased in RSBP3 ($M = 131.39$). In the control group however, with time, resting systolic blood pressure increased from RSBP1 ($M = 128.47$) to RSBP2 ($M = 131.87$) and rose again in RSBP3 ($M = 132.87$).

Exercise systolic blood pressure

Systolic blood pressure after pretest (ESBP1) ranged from 129mmHg to 205mmHg ($M = 164.82, SD = 20.80$). Systolic blood pressure after first posttest (ESBP 2) ranged from 128mmHg to 211mmHg ($M = 165.40, SD =$

23.19) and systolic blood pressure after second posttest ranged from 125mmHg to 260mmHg ($M = 182.90$, $SD = 34.21$).

Table 10- ANOVA results for the effect of consumption of hibiscus-ginger on exercise systolic blood pressure

	<i>df</i>	F-value (<i>F</i>)	Significance (<i>p</i>)	Partial eta squared (η_p^2)
ESBP	1.451	5.339	.016 ^x	.170
ESBP * GRP	1.451	.503	.550	.019
GRP	1	1.648	.211	.060
Intercept	1	2265.225	.000	.989

Source: Kluboito (2020)

N = 28 (treatment = 13, control = 15).

Error df (within-subject = 37.716, between-subjects = 26)

A 3(Time) x 2(Group) mixed model ANOVA revealed that the main effect for Group was not significant $F(1, 26) = 1.648$, $p = .211$, $\eta_p^2 = .060$. Thus, there was no overall significant difference in the ESBP of treatment ($M = 166.10$) compared to control ($M = 175.31$). Main effects for time (ESBP) were however statistically significant, $F(1.451, 37.716) = 5.339$, $p = .016$, $\eta_p^2 = .170$. This is further indicated with an increase in ESBP over time from ESBP1 ($M = 164.71$) to ESBP2 ($M = 165.10$) and ESBP3 ($M = 182.37$), implying that ESBP was significantly affected overtime. Time x Group was not significant, $F(1.451, 37.716) = .503$, $p = .550$, $\eta_p^2 = .019$. Examination of the cell means indicate that for the treatment group, there was a decrease from ESBP1 ($M = 163.10$) to ESBP2 ($M = 160.69$) but increased in ESBP3 ($M = 174.54$). In the control group also, with time, exercise systolic blood pressure increased from ESBP1 ($M = 166.33$) to ESBP2 ($M = 169.48$) and rose again in ESBP3 ($M = 190.13$).

Diastolic blood pressure

Diastolic blood pressure was measured at rest and after YoYo test on three different occasions. These measures were recorded and classified as resting diastolic blood pressure (RDBP) and exercise diastolic blood pressure (EDBP). Separate analyses were run for either of these measures, and the details are shown in tables 11 and 12.

Resting diastolic blood pressure

Initial resting diastolic blood pressure (RDBP1) ranged from 57mmHg to 95mmHg ($M = 75.96, SD = 9.45$). Resting diastolic blood pressure before first posttest (RDBP2) ranged from 65mmHg to 102mmHg ($M = 80.11, SD = 10.69$) and resting diastolic blood pressure after second posttest (RDBP3) ranged from 55.00mmHg to 94.00mmHg ($M = 75.21, SD = 9.46$).

Table 11- ANOVA results for the effect of consumption of hibiscus-ginger on resting diastolic blood pressure

	<i>df</i>	F-value (<i>F</i>)	Significance (<i>p</i>)	Partial eta squared (η_p^2)
RDBP	1.639	2.686	.089	.094
RDBP * GRP	1.491	.871	.406	.032
GRP	1	.307	.848	.001
Intercept	1	3053.838	.000	.992

Source: Kluboito (2020)

N = 28 (treatment = 13, control = 15).

Error *df* (within-subject = 42.606, between-subjects = 26)

A 3(Time) x 2(Group) mixed model ANOVA revealed that the main effect for Group was not significant $F(1, 26) = .307, p = .848, \eta_p^2 = .001$. Thus, there was no overall significant difference in the RDBP of treatment ($M = 77.39$) compared to control ($M = 76.84$). Main effects for time (RDBP) were also not statistically significant $F(1.639, 42.606) = 2.686, p = .089, \eta_p^2 = .094$.

However, RDBP increased over time from RDBP1 ($M = 75.93$) to RDBP2 ($M = 80.06$) then dropped to RDBP3 ($M = 75.35$). Time x Group was also not significant, $F(1.491, 42.606) = .871, p = .406, \eta_p^2 = .032$. Examination of the cell means indicate that for the treatment group, there was an increase from RDBP1 ($M = 75.46$) to RDBP2 ($M = 79.39$) which later dropped in RDBP3 ($M = 77.31$). In the control group however, with time, resting diastolic blood pressure increased from RDBP1 ($M = 76.40$) to RDBP2 ($M = 80.73$) and then dropped in RDBP3 ($M = 73.40$).

Exercise diastolic blood pressure

Diastolic pressure after pretest (EDBP1) ranged from 67mmHg to 112mmHg ($M = 86.39, SD = 12.22$). Diastolic blood pressure after first posttest (EDBP2) ranged from 54mmHg to 110mmHg ($M = 95.18, SD = 21.02$) and diastolic blood pressure after second posttest (EDBP3) ranged from 67.00mmHg to 110.00mmHg ($M = 93.82, SD = 13.50$).

Table 12- ANOVA results for the effect of consumption of hibiscus-ginger on exercise diastolic blood pressure

	<i>df</i>	F-value (<i>F</i>)	Significance (<i>p</i>)	Partial eta squared (η_p^2)
EDBP	2	3.124	.052	.107
EDBP *GRP	2	.995	.377	.037
GRP	1	.015	.904	.001
Intercept	1	1782.903	.000	.986

Source: Kluboito (2020)

N = 28 (treatment = 13, control = 15).

Error df (within-subject = 52, between-subject = 26)

A 3(Time) x 2(Group) mixed model ANOVA revealed that the main effect for Group was not significant $F(1, 26) = .015, p = .904, \eta_p^2 = .001$. Thus, there was no overall significant difference in the EDBP of treatment (M

= 91.51) compared to control ($M = 92.04$). Main effects for time (EDBP) were also not statistically significant $F(2, 52) = 3.124, p = .052, \eta_p^2 = .107$. However, EDBP increased over from ESBP1 ($M = 86.45$) to EDBP2 ($M = 95.30$) then dropped to EDBP3 ($M = 93.59$). Time x Group was also not significant, $F(2, 52) = .995, p = .377, \eta_p^2 = .037$. Examination of the cell means indicated that for the treatment group, there was an increase from EDBP1 ($M = 87.23$) to EDBP2 ($M = 97.00$) which later dropped in EDBP3 ($M = 90.31$). In the control group also, with time, exercise diastolic blood pressure increased from EDBP1 ($M = 85.67$) to EDBP2 ($M = 93.60$) and rose again in EDBP3 ($M = 96.87$).

Hypothesis Three: Consumption of Hibiscus-Ginger Juice has a Significant Effect on the Blood Lactate Levels of Athletes of the University of Cape Coast

Blood lactate levels of the participants classified as blood lactate 1 (BL1) and blood lactate 2 (BL2). Blood lactate levels after pretest ranged from 8.74mmol/L to 11.61mmol/L ($M = 10.18, SD = 3.27$) for the treatment group and 6.32mmol/L to 8.99mmol/L ($M = 7.66, SD = 1.61$) for the control group. After the second posttest, blood lactate levels ranged from 6.51mmol/L to 11.72mmol/L ($M = 9.11, SD = 4.04$) for the treatment group and 7.56mmol/L to 12.41mmol/L ($M = 9.98, SD = 4.99$) for the control group.

Table 13- ANOVA result for the effect of consumption of hibiscus-ginger on blood lactate levels

	<i>df</i>	F-value (<i>F</i>)	Significance (<i>p</i>)	Partial eta squared (η_p^2)
Blood lactate	1	.811	.376	.030
Blood lactate * GRP	1	5.832	.023 ^x	.183
GRP	1	.463	.502	.018
Intercept	1	232.621	.000	.899
Error	26			

Source: Kluboito (2020)

N = 23 (treatment = 13, control = 15).

A 2(Time) x 2(Group) mixed model ANOVA revealed that the main effect for Group was not significant $F(1, 26) = .463, p = .502, \eta_p^2 = .018$. Thus, there was no significant overall difference in the blood lactate of treatment ($M = 9.65$) compared to control ($M = 8.82$). Main effects for time (Blood lactate) were also not statistically significant $F(1, 26) = .811, p = .376, \eta_p^2 = .030$. However, blood lactate increased over time from BL1 ($M = 8.92$) to BL2 ($M = 9.55$). Time x Group was significant, $F(1, 26) = 5.832, p = .023, \eta_p^2 = 1.83$, implying that for each of the groups, blood lactate levels changed significantly with time. Examination of the cell means indicated that for the treatment group, there was a decrease from BL1 ($M = 10.18$) to BL2 ($M = 9.11$). In the control group however, with time, blood lactate increased from BL1 ($M = 7.66$) to BL2 ($M = 9.98$).

Hypothesis Four: Consumption of Hibiscus-Ginger Juice has a Significant Effect on the Aerobic Power of Athletes of the University of Cape Coast

Aerobic power was measured in the pretest and two posttests with a Yo-Yo test on three different occasions. These measures were recorded and classified as aerobic power 1 (VO₂ max1), aerobic power 2 (VO₂ max2), and aerobic power 3 (VO₂max3). Pretest measures of VO₂max 1 ranged from

38.75_{ml/kg/min} to 53_{ml/kg/min} ($M = 44.61, SD = 3.55$). First posttest measures of VO₂max 2, ranged from 38.58_{ml/kg/min} to 54.54_{ml/kg/min} ($M = 45.19, SD = 3.87$) and second posttest measures of VO₂max 3 ranged from 39.10_{ml/kg/min} to 54.88_{ml/kg/min} ($M = 44.74, SD = 4.06$).

Table 14- ANOVA results for the effect of consumption of hibiscus-ginger on aerobic power

	<i>df</i>	F-value (<i>F</i>)	Significance (<i>p</i>)	Partial eta squared (η_p^2)
VO ₂ max	2	1.117	.335	.041
VO ₂ max * GRP	2	.691	.506	.026
GRP	1	2.631	.117	.002-
Intercept	1	4562.64	.000	.994

Source: Kluboito (2020)

N = 28 (treatment = 13, control = 15).

Error *df* (within-subject = 52, between-subject = 26)

A 3(Time) x 2(Group) mixed model ANOVA revealed that the main effect for Group was not significant $F(1, 26) = 2.631, p = .117, \eta_p^2 = .002$. Thus, there was no overall significant difference in the VO₂max of treatment ($M = 46.00$) compared to control ($M = 43.84$). Main effects for time (VO₂max) were also not statistically significant $F(2, 52) = 1.117, p = .335, \eta_p^2 = .041$. However, VO₂max increased over time from VO₂max1 ($M = 44.67$) to VO₂max2 ($M = 45.27$) then dropped to VO₂max3 ($M = 44.83$). Time x Group was also not significant, $F(2, 52) = .691, p = .507, \eta_p^2 = .026$. Examination of the cell means indicated that for the treatment group, there was an increase from VO₂max1 ($M = 45.47$) to VO₂max2 ($M = 46.42$) which later dropped in VO₂max3 ($M = 46.12$). In the same way, the control group, with time, had VO₂max increase from VO₂max1 ($M = 43.86$) to VO₂max2 ($M = 44.13$), but dropped in VO₂max3 ($M = 43.55$).

Discussion

The present experimental study investigated the effect of hibiscus-ginger juice on selected physiological variables of university athletes including aerobic power, heart rate, blood pressure, and blood lactate levels. These variables were examined in the study as parameters likely to be influenced by the consumption and utilization of the nutrients in the hibiscus-ginger juice. The novelty of the idea implies not much research, however, the individual main ingredients, hibiscus, and ginger have been used to find out how they affect sports performance (Hadi *et al.*, 2015; Mardanpour-Shahrekordi, Banitalebi, Faramazi, Ghafari, & Mardanpour-Shahrekordi, 2017; Matsumura *et al.*, 2015; Perez, Dobson, Ryan, & Riggs, 2019; Vahdhatpoor, Shakerian, Alizadeh, & Tabatabaei, 2019). Also, other extracts containing similar nutrients and properties with hibiscus and ginger have been investigated for their effects on physiological variables and physical performance (Mogaddam *et al.*, 2012; Nyakayiru *et al.*, 2017).

Hibiscus is known to have an effect on cardiovascular parameters due to the abundant presence of anthocyanins and proanthocyanin compounds, which have inhibiting effects on angiotensin II converting enzyme, hence causing a vasodilation effect as well as increasing the concentration of urinary sodium while maintaining normal potassium levels, leading to antihypertensive effects (Jonadaet *et al.*, 1990; Onyenekwe *et al.*, 1999). Ginger is also known for its hypotensive tendencies, exhibiting cardio-depressant activities at low doses and cardiostimulant properties at high doses, which have been shown to help with the lowering of resting blood pressure, as well as resting heart rate (Ernst, & Pittler, 2000; Wang *et al.*, 2003). The first hypothesis “consumption of

hibiscus-ginger juice has a significant effect on the heart rate of athletes” was rejected after hypothesis testing, as the results produced were not statistically significant. This result is contrary to those of Nyakayiru *et al.* (2017) and Salman *et al.*, (2017). Salman *et al.*, (2017) reported that acute drinking of cold hibiscus beverage significantly reduced the heart rate of adult females. Unlike in the current study where the heart rate seemed to have increased, even though this was not statistically significant. However, this could have occurred because the athletes in the current study were under the stress of training which is known to fluctuate heart rate readings in individuals (Forjaz, Matsudaira, Rodrigues, Nunes & Negrao, 1998). In a more similar group of participants who consumed beetroot juice and performed high-intensity intermittent exercise (Nyakayiru *et al.*, 2017), after six days of beetroot ingestion, mean heart rate was significantly lowered among the treatment group. Like the current study, these participants were trained, however, they were not in active training and so the stress would have been minimal. More so, as much as hibiscus-ginger juice contains similar nutrients as beetroot juice, there is the presence of nitrate in beetroot which might have played a further role in the result obtained. However, in a study by Jordan *et al.*, (2010), heart rate amongst the participants who consumed beta-alanine as supplementation over 28 days recorded a significant increase in heart rate, which is similar to the current study, even though the current study’s result was not statistically significant. Better inferences could have been made with recurrent measures of heart rate during and after exercise (Forjaz *et al.*, 1998). Therefore future studies could consider that form of measurement for a better picture of how hibiscus-ginger juice affects heart rate among training athletes.

The second hypothesis was to find out if the consumption of hibiscus-ginger juice would affect the blood pressure of athletes. Results showed no statistically significant changes in blood pressure except for exercise systolic blood pressure, which was significantly affected by time interactions ($p = 0.16$). This change was an increase in the systolic blood pressure reading taken after each Yo-Yo test. The insignificant nature of the results is similar to previous studies which also looked into the effect of hibiscus or ginger on blood pressure (Abubakar, Ukeyima, Spencer, & Lovegrove, 2019; Faraji, & Tarkhani, 1999; Herrera-Arellano, Flores-Romero, Chaves-Soto, & Conway, 2004; Khosravi-Mozaffari, Ahadi, & Barzegar, 2013; Jalalyazdi *et al.*, 2019). Herrera-Arellano *et al.* (2004) found no significant difference in blood pressure after 4 weeks of taking in an infusion of *Hibiscus sabdariffa* and water in mildly hypertensive patients. Abubakari *et al.* (2019) also found a non-significant decrease in blood pressure after acute consumption of *hibiscus sabdariffa* extracts in normotensive individuals, similar to the participants in the current study. For studies in which ginger was used as a treatment, 12-week supplementation with ginger powder in the form of capsules one hour after meals did not significantly affect blood pressure in individuals with non-alcoholic fatty liver disease (Rafie, Hosseini, Hajiani, Malehi, & Mard, 2020).

Contrary to these outcomes, Khosravi *et al.* (2013) found that mildly hypertensive patients with type two diabetes experienced significant decreases in their blood pressure after consuming *hibiscus sabdariffa* tea three times daily for 4 weeks. This is an indication of high doses, unlike the current study where participants consumed their treatment once a day. Again, the participants in the current study were normotensive individuals, while those in

the study by Khosravi *et al.* (2010) were mildly hypertensive as well as diabetic implying more potency of hibiscus among hypertensive patients. This is supported by another study (Faraji *et al.*, 1999) where hypertensive individuals experienced significant decreases in their blood pressure after consuming hibiscus for 12 days. Again hibiscus supplementation was potent in reducing blood pressure in patients with stage one hypertension (Jalayazdi *et al.*, 2019). In the same way, systolic blood pressure was significantly affected by the treatment of 2 cups of hibiscus tea for one month in diabetic and mildly hypertensive patients (Mozaffari-Khosravi, Jalali-Khanabadi, Afkhami-Ardekani, & Noori-Shadkam, 2009).

In the current study, blood pressure and heart rate were not significantly affected by the three weeks consumption of 500ml of hibiscus-ginger juice. In this case, both hibiscus and ginger were present in the extract and so the biochemistry could be different from if these two ingredients were taken separately (Rafie *et al.*, 2020). Blood pressure not being significantly affected by the consumption of the hibiscus-ginger juice might have been as a result of the fact that these athletes were not hypertensive anyway, unlike existing studies (Faraji *et al.*, 1999; Khosrav-Mozafarri *et al.*, 2013; Jalalyazdi *et al.*, 2019), most of which were conducted among persons with compromising health status. Therefore, the potency of hibiscus and ginger in decreasing blood pressure could be more profound in people with underlining ailments (Ngamjarus, Pattanittum, & Sombonporn, 2010). Even though measures of systolic blood pressure was not different between the treatment and control groups for the current study, exercise systolic statistically significantly increased by time interactions. This could be due to fatigue over

the period (Babalola, 2011) since they were training twice daily every day for 24 days. Going back to the data, however, there seemed to have been a drop in almost all the parameters between the first and second posttests which could be an indication that a longer period of treatment could have yielded different results. Future studies could explore longer study periods as well as control for the other activities aside from training and treatment which could have affected the results in the current study.

The Antioxidant property of hibiscus and ginger has been shown to help in the reduction of blood lactate by getting rid of or scavenging free radicals (Al-Awwadi, 2017). The fourth hypothesis of this study “consumption of hibiscus-ginger juice will have a significant effect on blood lactate levels of athletes” was however refuted by the results. This result is similar to another study that also found non-significant changes in blood lactate levels after supplementation with antioxidants (Klarod, Gatterer, Frontul, Philipe & Burtscher, 2015). In Klorad *et al.*, (2015), it was found that reactive oxygen metabolites and maximal exercise performance remained unchanged in the cold as well in the heat with and without short-term antioxidant supplementation. Even though their tests were done under different temperatures, comparing it to the current study, they also used a test which was done till exhaustion and blood samples taken after the test. Also, the treatment used has antioxidant properties as the current study.

Contrary to these outcomes, studies have found significant changes in blood lactate levels after supplementation with natural antioxidants (Hadi *et al.*, 2017; Jordan *et al.*, 2010; Sadeghi & Hussein, 2017). Jordan *et al.* (2010) found that supplementation with beta-alanine caused a delay in onset of blood

lactate accumulation, leading to performance improvements. In another study (Hadi *et al.*, 2017), hibiscus tea extracts were found to have a beneficial effect on oxidative stress status in athletes. Jordan *et al.* (2010) and Hadi *et al.* (2017), did not directly measure the blood lactate levels of participants, unlike the current study. However, in a study where actual blood lactate levels were measured, short-term supplementation with glutamine reduced blood lactate levels in elite swimmers (Sadeghi & Husseini, 2017). Matsumura *et al.* (2015) also reported that supplementation with 4g of ginger may be used to accelerate muscle strength recovery following intense exercise and reducing muscle damage due to oxidative stress. This also could help to improve overall athletic performance since muscle strength is important for athletic performance.

In the current study, athletes in the treatment group consumed both hibiscus and ginger extracts in one juice which from a layman's perspective should have caused improvements in the oxidative capacity of the athletes which was measured in the form of blood lactate. Blood lactate was however not significantly affected by this treatment, contrary to the expectation. This could be a result of the intensity at which the participants worked during the testing period, which may have not produced significant oxidative stress for the athletes hence the insignificant changes in blood lactate readings (Klarod *et al.*, 2015). Going back to the data in this study, there seemed to have been a decline in the blood lactate levels of the treatment group as opposed to the control group, even though this was not statistically significant to reflect between-subject difference for the hypothesis test on the effect of hibiscus-ginger juice on blood lactate levels. There is therefore the need for further

research on this with different approaches of blood lactate measurements which might produce better results.

Hypothesis five was on the effect of hibiscus-ginger juice on the aerobic power of athletes. Aerobic power here was measured as $VO_2\text{max}$ using the YoYo test and was found not to be significantly different among treatment and control groups after 24 days of hibiscus-ginger juice supplementation. This result confirms findings of similar studies (Mogaddam *et al.*, 2012; Perez *et al.*, 2019; Vahdhatpoor *et al.*, 2019), in which no improvements were found in $VO_2\text{max}$ after supplementation with similar treatments. Mogaddam *et al.* (2012) reported no significant difference in aerobic and anaerobic power after 3 weeks of vitamin C supplementation among male college students. In another study, Vahdhatpoor *et al.* (2019) also found that ginger supplementation did not have a significant effect on the improvement of aerobic capacity in overweight girls. Again, Perez *et al.* (2019) reported non-significant results on the effect of beetroot juice on $VO_2\text{max}$ in healthy recreationally trained individuals. In contrast, others have shown increases in $VO_2\text{max}$ in obese middle-aged women with type two diabetes with ginger supplementation over a ten-week training period (Mardanpour-Shahrekordi *et al.*, 2017), and also trained soccer players with supplementation with beetroot for six days (Nyakayiru *et al.*, 2017). Lane *et al.* (2014), also found that caffeinated beetroot juice improves cycling time trial of 50 to 60 minutes in competitive cyclists and triathletes. These studies are similar to the current study in terms of the antioxidant nature of the treatments used. However, the time and duration within which the treatment was administered and training times are slightly different. In the above

mentioned studies related to $VO_2\text{max}$, participants in the study by Reinks, Vanderwoude, Mass, Blea and Subudhi (2015), were not in training and so were not engaged in daily exercise as is the case in the current study. Athletes in the current study were engaged in a twice-daily training session and so the possibility of fatigue setting in and affecting the results of the Yo-Yo is high, which could be a reason for the inconsistent results. Again, significant results recorded by Nyakayiru *et al.* (2017) happened within six days of supplementation, which is not long enough period for fatigue to have set in, affecting the results, unlike the current study, where athletes were constantly training twice daily for 24 days.

The participants of the current study had been training before the start of this study which could have had an influence on their training gains during the duration of the study, unlike in Mardanpour-Shahrekordi *et al.* (2017) where the participants were obese persons who during the 10 weeks of the study lost weight which could have contributed to improvements in their aerobic power. In the current study, aerobic power seems to have increased from the pretest to the second posttest for the treatment group, but this was not significant statistically, which could be an implication that given more time, the dynamics of the hibiscus-ginger juice in aerobic power could have been more understood. Dosage of the supplementation is an issue in determining its potency for performance enhancements as stipulated in similar studies (Mogaddam *et al.*, 2012; Perez *et al.*, 2019; Vahdhatpoor *et al.*, 2019). This could have been the case in the current study as well since athletes consumed 500ml of the hibiscus-ginger juice daily as their treatment. It could also be

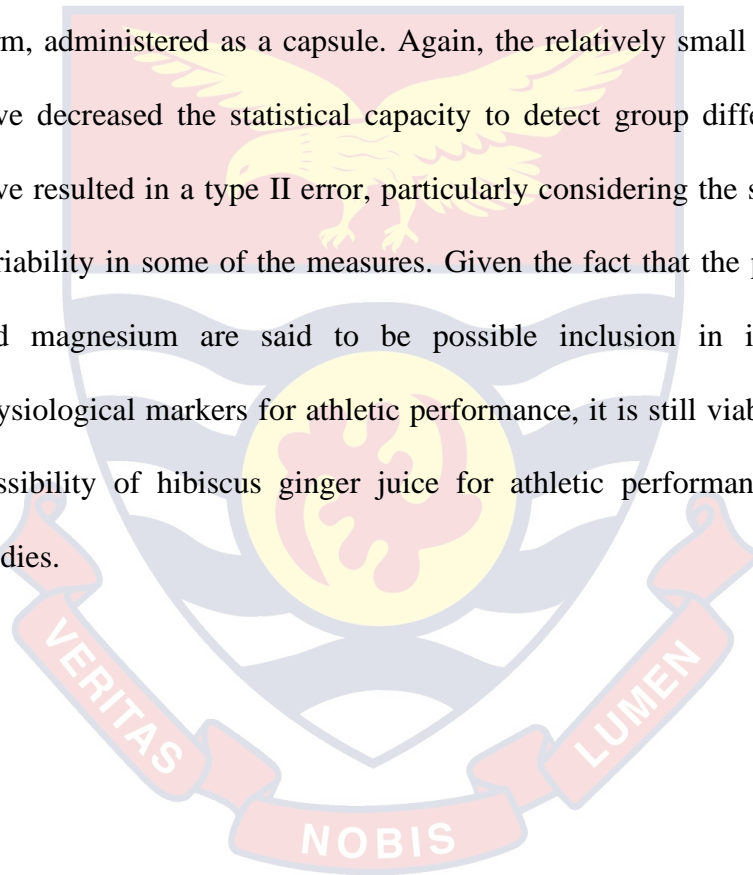
speculated that the concentration of the supplement was not enough to cause a change (Perez *et al.*, 2019).

Explanations for the conflicting findings are not clear, but all factors that may explain these differences are variations in the strength and duration of the exercise tests (Zamzow, 2017), the dosage and duration of supplement administered (Wylie *et al.*, 2016), and the duration between the administration and the test. Also unlike the previously mentioned studies, in the current study, the treatment was administered after training every day as the athletes were in training since supplementation after training. This is important for muscle recovery (Matsumura *et al.*, 2015) which leads to improved performance, however, it turned out not to be the case in the current study. Despite the results of this study on aerobic power, there might still be a potential for aerobic power improvements with further studies with modified treatment and method, since the data showed a bit of an increase in the aerobic power of the treatment group, showing that even though the statistical test for between-subjects and within-subjects effects were not significant. Also, future prospective studies could consider consuming the hibiscus-ginger juice before a workout, as is the case in Reinks *et al.* (2015).

Summary

The findings of the study partially refute the initial working hypotheses. There were no significant effects observed for neither aerobic power nor physiological variables due to the supplementation of hibiscus-ginger juice between treatment and control groups. Based on this outcome, it appears that hibiscus-ginger juice might not be a potent enough supplement to induce performance changes in athletes with daily dosages of 500ml over 24

days. Due to the novelty of the supplement chosen for this study, it was initially believed that its potency among other groups of people could be the same or more, for athletes, which has turned out not to be so with the current study. Some of the possible explanation for the results could be the dosage of hibiscus ginger juice given the athletes, which might not have been enough, especially in the form in which it was administered, as most studies have used either hibiscus, ginger, or any other natural supplement in their powdered form, administered as a capsule. Again, the relatively small sample size may have decreased the statistical capacity to detect group differences and may have resulted in a type II error, particularly considering the subject to subject variability in some of the measures. Given the fact that the potentials of iron and magnesium are said to be possible inclusion in improvements in physiological markers for athletic performance, it is still viable to explore the possibility of hibiscus ginger juice for athletic performance in the future studies.



CHAPTER FIVE

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The purpose of the study was to explore the effect of hibiscus-ginger juice supplementation on selected physiological variables of university athletes. This chapter focuses on the summary, key findings, conclusions, and recommendations of the study.

Summary

High levels of physical fitness coupled with good nutrition for good recovery are required to produce optimum performance. This places the demand for athletes to always look for an edge to improve their performance to remain competitive at all times. These athletes use several ergogenic aids, some of which may be harmful to their health, either short term or long term. Ergogenic aids with more natural ingredients, most of which are consumed for their health benefits are encouraged and being researched into to establish the point. Hibiscus-ginger juice is a drink consumed widely for its healing benefits and research has indicated that there are high vitamin and mineral contents of the two main constituents of hibiscus and ginger, making it important for the improvement of metabolism, recovery after exercise, reduction of inflammation and enhancement of athletic performance. For this reason, this research was conducted to explore the effect of hibiscus-ginger juice supplementation on the aerobic power of athletes while assessing the effect of the supplementation on physiological measures of heart rate, blood pressure, and blood lactate levels. To do this, athletes were randomly assigned to two groups; treatment, and control with the treatment group, taking hibiscus-ginger juice as part of their after training fluid intake for 24 days. This was the period

for which these athletes were training in preparation for GUSA Games. Aerobic power was measured using the Yo-Yo test, heart rate, and blood pressure with an automated sphygmomanometer, and blood lactate levels were measured from blood samples drawn after the pretest and the last posttest. The 28 participants in the study were taken through 1 pretest and two posttests on different occasions. On each of these occasions, they went through the Yo-Yo test to ascertain if there had been any improvements in their aerobic power. Hypotheses were analyzed using mixed-model ANOVA.

Key findings

1. Heart rate was not significantly affected by the supplementation with 500ml hibiscus-ginger juice for 24 days. Both the resting heart rate and exercise heart rate were not affected by this supplementation.
2. Consumption of 500ml of hibiscus-ginger juice for 24 days did not affect the blood pressure of athletes of the University of Cape Coast. Systolic blood pressure after Yo-Yo tests was however affected over time.
3. Only time interaction effects for blood lactate levels for within factor effects on groups and not between-subject effect for treatment and control groups was significant following the 24 days consumption of 500ml daily of hibiscus-ginger juice.
4. Consumption of 500ml of hibiscus-ginger juice for 24 days did not affect the aerobic power of athletes.

Conclusions

1. Healthy athletes do not experience improvements in heart rate and blood pressure after 24 days of consuming hibiscus ginger-juice as part of their after training a fluid replacement.

2. Supplementation with hibiscus-ginger juice does not cause a significant change in blood lactate levels of athletes in training.
3. 500ml of hibiscus-ginger juice daily for 24 days did not cause a significant change in the aerobic power of athletes in training. Therefore, athletes who consume hibiscus-ginger juice may not have an advantage over their colleagues who do not.

Recommendation

The results of this study are inconclusive on how much an athlete in training's physiology and performance can be affected by supplementation with hibiscus-ginger juice. However, other natural sources of micronutrients have been found to be potent, therefore, athletes should consider using those products when looking to improve on their performances.

Suggestions for Further Studies

Due to the novelty of the study of hibiscus-ginger juice supplementation in athletes, further studies are needed in this area before firm conclusions and recommendations about the supplementation with hibiscus-ginger juice can be made. Therefore, further research in hibiscus-ginger juice supplementation in athletes could focus on:

1. Using a larger sample size is needed to confirm or deny these findings since the sample size was a limitation for this study.
2. Administering hibiscus and ginger together in a different form or extract to athletes to find possible performance changes.
3. Comparing the dose-effect of the hibiscus-ginger juice on performance variables of athletes.

4. Investigating the nature and mechanisms surrounding the benefits of hibiscus-ginger juice to the training athletes.



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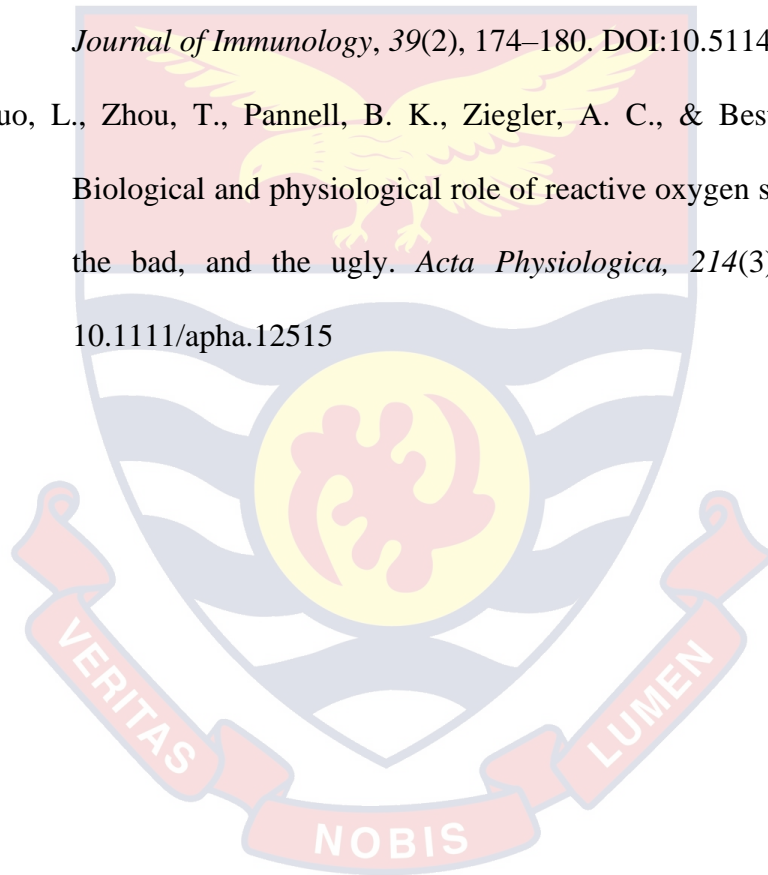
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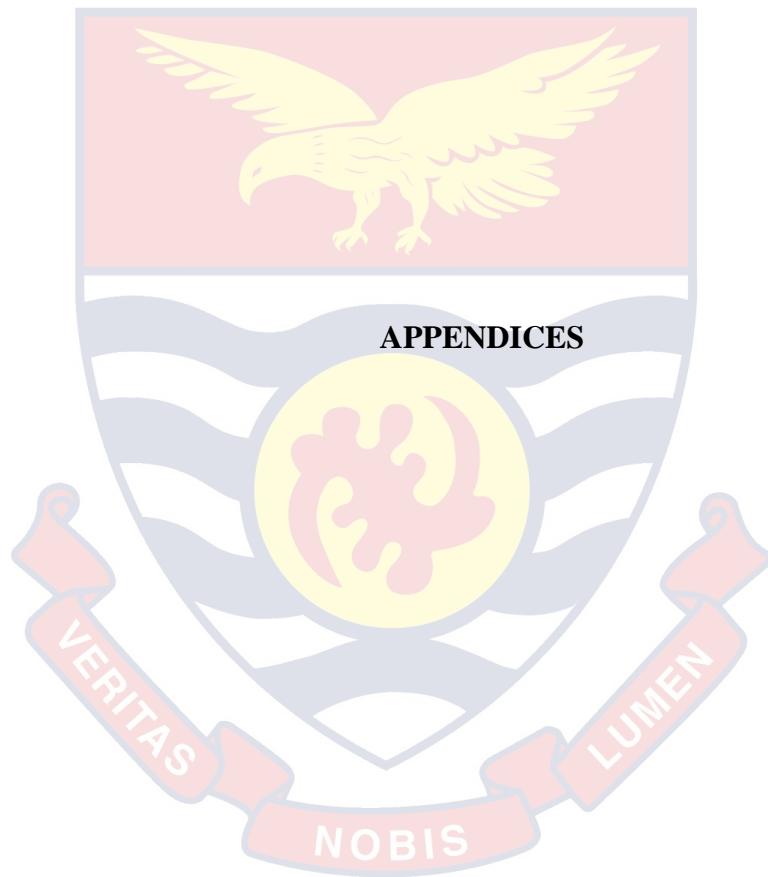
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APPENDIX A
DATA RECORDING SHEET

Participant ID:

Date:

Age:

Height:

Body weight:

Gender:

Resting heart rate:

Resting blood pressure:

Resting blood lactate level:

Test conditions:

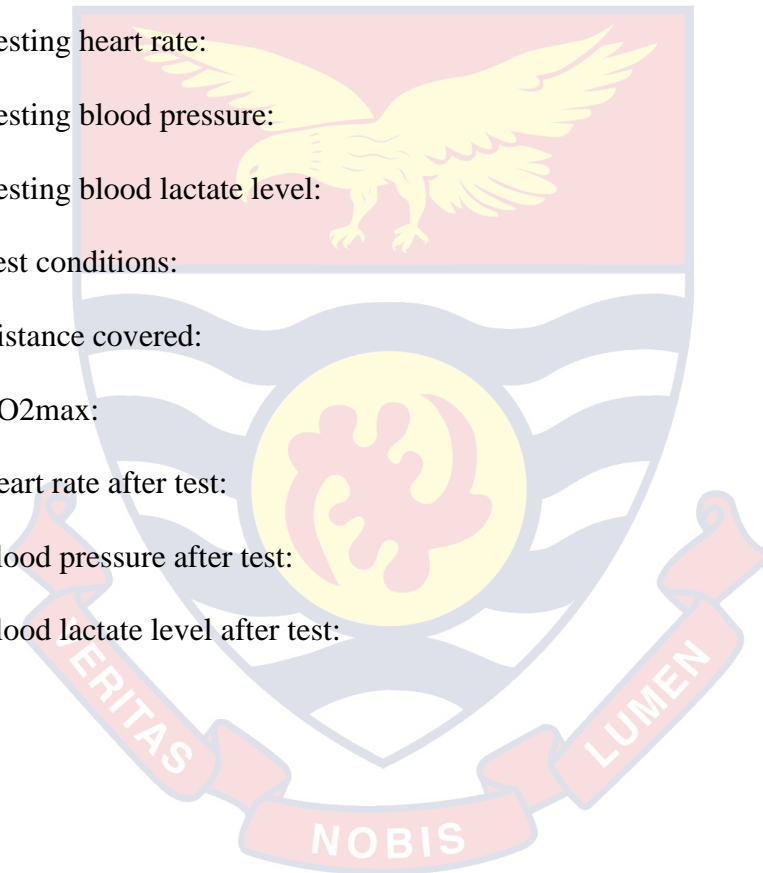
Distance covered:

VO₂max:

Heart rate after test:

Blood pressure after test:

Blood lactate level after test:



APPENDIX C

ETHICAL CLEARANCE

UNIVERSITY OF CAPE COAST

INSTITUTIONAL REVIEW BOARD SECRETARIAT

TEL: 0558093143 / 0508878309 / 0244207814

C/O Directorate of Research, Innovation and Consultancy

E-MAIL: irba@ucc.edu.gh

OUR REF: UCC/IRB/A/2016/539

YOUR REF:

OMB NO: 0990-0279

IORG #: IORG0009096



8TH OCTOBER, 2019

Ms. Yayra Kluboito

Department of Health Physical Education and Recreation
University of Cape Coast

Dear Ms Yayra,

ETHICAL CLEARANCE – ID (UCCIRB/CES/2019/38)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted **Provisional Approval** for the implementation of your research protocol titled **Effect of hibiscus-ginger juice on aerobic power of athletes**. This approval is valid from 8th October, 2019 to 7th September, 2020. You may apply for a renewal subject to submission of all the required documents that will be prescribed by the UCCIRB.

Please note that any modification to the project must be submitted to the UCCIRB for review and approval before its implementation. You are required to submit periodic review of the protocol to the Board and a final full review to the UCCIRB on completion of the research. The UCCIRB may observe or cause to be observed procedures and records of the research during and after implementation.

You are also required to report all serious adverse events related to this study to the UCCIRB within seven days verbally and fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithfully,

A handwritten signature in black ink, appearing to read 'Samuel Asiedu Owusu'.

Samuel Asiedu Owusu, PhD

UCCIRB Administrator

ADMINISTRATOR
INSTITUTIONAL REVIEW BOARD
UNIVERSITY OF CAPE COAST

APPENDX D

METHODOLOGY FOR PROXIMATE ANALYSIS OF HIBISCUS- GINGER JUICE

Protein Determination

Protein was determined by pipetting 5ml of the juice into a numbered kjedahl digestion flask. About 4.5ml of digestion mixture was added and the sample was digested at 360°C for two hours (AOAC, 2005). The digest was allowed to cool and diluted to 100ml with distilled water. Twenty milliliters (20ml) of the digested was immediately distilled after adding 10ml of alkali mixture using 5ml of boric acid as indicator. 50ml of the distillate was collected and titrated against 0.00712M HCl until it turns to a pink colour which determined the endpoint. The remaining diluted digest was reserved for the mineral determination as ascribed by Food and Agriculture Organisation [FAO], (2008).

Percentage protein was calculated using the formula;

$$N \text{ (mg/L)} = T \text{ (ml)} \times 100 / \text{aliquot} \times \text{Dilution factor}$$

$$\% N = N \text{ (mg/L)} / 10000$$

$$\% \text{Protein} = \% N \times 6.25$$

Carbohydrate Determination

One millilitres of the Bissap juice was pipetted into a conical flask diluted and kept for colour development. Two millilitres of glucose standard solution and the extract were pipetted into a set of boiling tubes, 10ml of anthrone solution was rapidly added to the boiling tubes mixed thoroughly and cooled under running tap water or ice bath. The tubes were placed in a beaker containing boiling water in a dark fume cupboard for 10minutes. The tubes

were allowed to cool in cooled water in the dark (FAO, 2008; Page, Miller & Keeney, 1982). The optical density of the standards and the sample solution was measured at 625nm using the spectrophotometer. A calibration graph was prepared from the standards and used to obtain mg glucose in the sample aliquot.

$$\text{Soluble Carbohydrate (mg/L)} = \frac{C(\text{mg}) \times 100}{\text{aliquot}} \times \text{Dilution factor}$$

Where C (mg) = carbohydrate concentration from the graph.

Calcium Determination

An aliquot of 10ml of the reserved digest was pipetted into a 250ml conical flask and 150ml of distilled water was added. 1 ml each of potassium cyanide, hydroxylamine hydrochloride, potassium ferrocyanide and triethanolamine were added. 20ml of 10% sodium hydroxide was added to raise the pH and then 10 drops of calcon indicator was added to the solution and titrated against 0.005M EDTA solution (AOAC, 2005).

Magnesium Determination

An aliquot of 10ml of the reserved digest solution was pipette with a 250ml conical flask. One hundred and fifty millilitres (150ml) of distilled water was added. Fifteen milliliters (15ml) of buffer solution was added and allowed to stand for few minutes. One millilitre (1 ml) of each of potassium cyanide, hydroxylamine hydrochloride, potassium ferrocyanide and triethanolamine were added. Ten (10) drops of erichrome Black T indicator was added and titrated against 0.005m EDTA solution (Page, Miller & Keeney, 1982).

Phosphorus Determination

One millilitres of the digested sample solutions was pipette into a 25ml volumetric flask. 1ml of the blank digest was also added to the 2ml of standard phosphorus solution to give it the same background as the digest. Ten milliliters of distilled water was added to the standards as well as the sample solutions. Four milliliters of reagent B made up of ascorbic acid and reagent (Page, Miller& Keeney, 1982). A reagent was added to the standard and sample solutions. Distilled water was added to the volumetric flask to make up to the volume of 25ml and allowed to stand for about 15 minutes for the colour to develop. After colour development, the absorbances of the standard and sample solutions were determined using a spectrophotometer at a wavelength of 882mm. A standard calibration curve was plotted using their concentration against absorbance.

Calculations

If C= P ug/ml obtained from the graph then

$$P \text{ (mg/L)} = \frac{C \text{ mg} \times \text{diluted factor}}{\text{aliquot}}$$

Sodium and Potassium Determination

Potassium and sodium concentrations in the digested samples were determined using the flame photometer. The following standard concentrations of both potassium and sodium were prepared 0, 2,4,6,8 and 10 ug/ml (Page, Miller, Keeney, 1982). Both the working standards and the sample solutions were aspirated individually into the flame photometer and their emissions recorded. A calibration curve was plotted using the concentration and missions of the working standards. The concentration of

potassium and sodium in the sample solution were extrapolated from the curve using their emissions

$$K \text{ or } Na \text{ (mg/L)} = \frac{C(ppm) \times \text{solution volume}}{\text{aliquot}}$$

Iron, Copper and Zinc Determination

Standard solutions of 1, 2 and 5ug/ml solutions of Fe, Cu and Zn were prepared(FAO, 2008). The standard solutions of Fe, Cu and Zn were aspirated into the atomic absorption spectrophotometer (AAS) and their respective curve plotted on the AAS. The sample solutions were also aspirated on the AAS with their respective concentrations provided by the AAS.

$$Fe/Cu/Zn(mg/L) = \frac{C(ppm) \times \text{solution volume}}{\text{aliquot}}$$

Aliquot = Volume of Juice taken for the digestion

Result

Sample	P mg/L	Ca	Mg	K mg/L	Na mg/L	CHO mg/L
		mg/L	mg/L			
1	65.323	383.165	64.178	225.352	103.093	142471.910
1	66.713	382.363	66.123	234.742	103.093	142404.494
1	66.018	381.962	64.908	234.742	101.031	142471.910

Sample	Fe mg/L	Cu mg/L	Zn mg/L	% Protein
1	44.860	2.940	7.720	1.425
1	44.960	2.980	7.820	1.438
1	44.920	2.960	7.680	1.463

APPENDIX E

BLOOD LACTATE LEVELS DETERMINATION

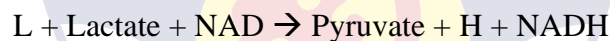
Collection of blood samples

A venous blood sample of 4ml were collected from each study participant into a gel separator tube and allowed to clot. The gel separator tubes blood sample was centrifuged for 15 minutes at 1000g using centrifuge to plasma, and these were stored at -20°C. Serum samples were used for Lactate dehydrogenase (LDH) estimation.

Method

UV-assay according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)

Reaction principle



Lactate Dehydrogenase catalyzes the conversion of L-lactate to pyruvate in the presence of lactate dehydrogenase (LDH). In the process, B-nicotinamide adenine dinucleotide (NAD) is deoxidized to NADH. This change in absorbance is directly proportional to the activity of LDH in the sample.

Calculation

The analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process

Conversion factor of traditional units (U/L) into SI -units (ukat/L)

$$1\text{U/L} + 16.67 \times 10^{-3} \text{ukat/L}$$

1ukat/L= 60U/L

Calibration

Human multi-calibrator from Mindray was used. Calibration was stable for approximately 30 days (720 hours) and was required with each change in reagent lot number. Calibration was verified with at least two levels of controls according to the established quality control requirements for the laboratory. If control results fall outside acceptable ranges, recalibration was done.

Quality Control

Two levels of controls (normal and abnormal) were run every 24 hours. If quality control results did not meet the acceptance criteria defined by the laboratory. The established quality control procedures for the laboratory were followed. Recalibration was done.

