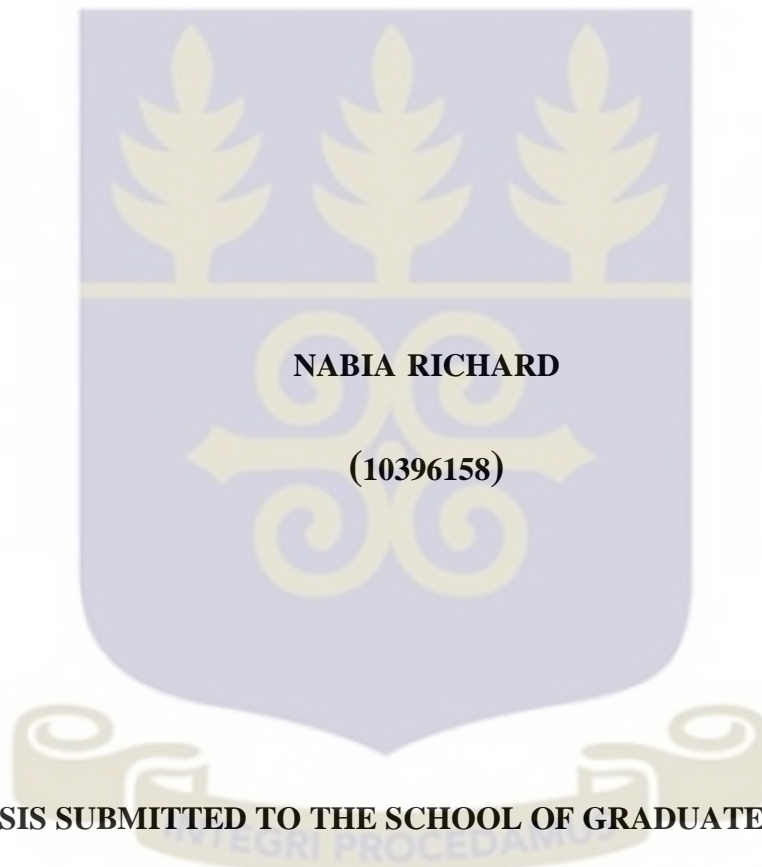


**UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES**

**ENERGY EXPENDITURE AND IRON STATUS OF
PREMIER LEAGUE FOOTBALLERS IN GHANA**



**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN
PARTIAL FULFILMENT OF THE AWARD OF DEGREE OF MASTER OF
SCIENCE IN DIETETICS**

DEPARTMENT OF NUTRITION AND DIETETICS

JULY, 2015

DECLARATION

I, Richard Nabia, declare that this work is the report of the research I undertook at the Department of Nutrition and Dietetics, School of Biomedical and Allied Health Sciences, University of Ghana towards the award of a Master Science Degree and that to the best of my knowledge, it contains no material which has been accepted for the award of any other degree of this University, except where due acknowledgements has been made in the text.

Signature----- Date-----

Richard Nabia
(Candidate)

Signature----- Date-----

Dr Charles Brown
(Supervisor)



ABSTRACT

Introduction: Football is a popular sport. Footballers will perform better if their nutritional status is optimum. Iron is a nutrient required for performance. Energy expenditure must be met to maintain performance. Creatinine levels indicate kidney function and electrolyte balance to some extent.

Aim: This study assessed energy expenditure and iron status of premier league footballers in Ghana

Methods: Registered footballers in three teams in the First Capital Plus Premier League, Bechem United Football Club (BUFC) in Bechem (Brong Ahafo Region), Brong Ahafo United Football Club (BAFC) in Sunyani (Brong Ahafo Region) and Liberty Professionals Football Club (LPFC) in Dansoman (Greater Accra Region) were recruited. Full blood counts, serum ferritin levels and serum creatinine levels were determined from blood samples collected from the footballers. Their heights and weights were measured and BMI and body composition determined. Energy expenditure was estimated using the Schofield equation.

Results: Sixty-three footballers from the 3 teams were recruited but only 52 were included in the data analyses. Mean weight was 69.89 ± 7.41 kg, mean height was 1.74 ± 0.07 M, mean muscle mass was 29.94 kg and mean body fat was 15.56%. Low haemoglobin levels were reported in 9% (n=4). Nineteen percent of footballers (n=9) had creatinine levels above the reference range. Ferritin levels were normal for all footballers (44.90- 115ng/dL). Mean daily energy expenditure was 4329.36 ± 269.69 kcal/day. Correlations between creatinine and ferritin with anthropometric indices were not significant (all $p > 0.05$).

Conclusion: Mean height and weight of premier league footballers appeared lower when compared with that in other countries. Haemoglobin levels of most of the footballers were normal. Creatinine levels though high in some footballers was possibly physiological.



DEDICATION

To my father whom I wish were here. To the Premier League Footballers of Ghana who continue to thrill and entertain us.



ACKNOWLEDGEMENTS

The pages of this thesis cannot contain my gratitude to my family for immense support. I also extend my immense appreciation to Charles Brown, PhD and Nana Owusu Kofi, R.D for their supervision and guidance throughout this research. Finally, thank you Adam Yussif for everything. Sincerely, I treasure you all pricelessly. You are blessed by God Almighty for His name sake. Amen.



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LIST OF ABBREVIATIONS

ACSM	American College of Sports Medicine
ADA	American Dietetic Association
AT	Activity Thermogenesis
BAFC	Brong Ahafo United Football Club
BEE	Basal Energy Expenditure
BMR	Basal Metabolic Rate
BUFC	Bechem United Football Club
DAC	Dietitians Association of Canada
DIT	Diet Induced Thermogenesis
DLW	Doubly Labelled Water
FAO	Food and Agriculture Organisation
FFA	Free Fatty Acid
FIFA	Federation of International Football Association
GFA	Ghana Football Association
Hb	Haemoglobin
Hct	Haematocrit
LPFC	Liberty Professionals Football Club
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MPV	Mean Platelet Volume
MTN	Mobile Telecommunication Network
MUFA	Mono Unsaturated Fatty Acid

PA	Physical Activity
PDW	Platelet Distribution Width
PLB	Premier League Board
PLT	Platelet Distribution Width
PUFA	Poly Unsaturated Fatty Acid
RBC	Red Blood Cell
RDW	Red cell Distribution Width
RMR	Resting Metabolic Rate
SFA	Saturated Fatty Acid
TEF	Thermic Effect of Food
WBC	White Blood Cells
WHO	World Health Organisation



CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Football, more formerly called association football is unarguably the world's most popular sport (Reilly *et al.*, 2000). As at 2007 there were 265 million footballers in the world, representing about 4% of the world's population, and 46 million in Africa (Kunz, 2007). With 207 countries being members of the Federation of International Football Associations (FIFA), the game is played in every country in the world.

Football is also about money. It is a big source of business; FIFA ended the year 2014 with an income well above 5.7 billion dollars in cash alone (FIFA, 2015a). At club and country levels, sponsorship packages given to teams have made the game an attractive profession (Reilly *et al.*, 2000). The huge sums of money paid to professional footballers make the game overwhelmingly attractive to many young people.

Ghanaians enjoy football and have produced some world class footballers over the years. Even the senior national team is considered a formidable side at least at the continental level. Ghana was ranked 3rd best team in Africa and 25th best in the world by FIFA in July, 2015 (FIFA 2015b). In Ghana the game is played in all communities irrespective of their economic or social status. It is a social, cultural, tribal and political affair in this country. Teams bear names that suggest tribal, ethnic, corporate and geographical affiliations or belonging. Local teams have support bases that are very passionate about the results of matches and want their teams to win. The premier league (the First Capital Plus Premier League) is the highest domestic football competition in Ghana and involves 16 teams. These teams compete during the football season for the league championship.

Just as any other sport, there are many factors that contribute to success in football. These include talent, training, motivation and resistance to injury (Ron and Louise 2012; Daneshvar *et al.*, 2013). In football competitions, whenever well trained and motivated players meet, the margin between victory and defeat is usually small thus making the need for attention to every detail including health and nutrition very important (Maughan *et al.*, 2010).

Athletes are thought as being normal and healthy. The high training workloads however modify their homeostasis and induces some physiological changes (Bangsbo, 2014). These changes can be haematological or biochemical. The results of tests may show apparently pathological values. Thus diagnostic tests using normal population reference may indicate health problems when in fact the changes observed are not pathological (Milic *et al.*, 2011). In sports medicine, creatinine is a common parameter used in the assessment of an athlete's health status, particularly in sports such as football where hydroelectrolytic balance is crucial (Banfi and del Fabbro, 2006). Creatinine levels are elevated in elite sportsmen in the absences of kidney damage (Banfi *et al.*, 2006). Nonetheless, creatinine tests give an idea about health status and have been claimed to be suggestive of muscle breakdown in athletes without kidney diseases (Silva *et al.*, 2008).

It is common knowledge that nutrition status affects training performance and completion outcomes in the long run (Montfort-Steiger and Williams, 2007). This is because the choice, composition and timing of food intake does affect training intensity and duration (ADA, DC and ACSM, 2009). Training in turn affects physical and physiological adaptations such muscle build up, haemodilution and increased cardiac volume. Going

into competitions, nutrition and nutritional status affect quality of performance because food provides the energy needed for the game at hand whilst nutritional status affects susceptibility to injury and cardiovascular endurance (Medina *et al.*, 2014).

Elite players can cover distances up to 13 km in a competitive match (Mohr *et al.*, 2003), with an overall energy cost of approximately 2,000 kcal during a match depending on individual body composition (Bangsbo, 2014). To do this, both anaerobic and aerobic energy systems are heavily utilized. Losing only 2% of lean body weight can significantly impair performance in sports (Reilly *et al.*, 2000). For this reason, regular training and competition in sports require that the individual eats more food than usual if his/her health is to be maintained, and peak performance attained and maintained. Even regular non-significant calorie deficit may lead to decrease in body mass including loss of muscle mass (Bell *et al.*, 2005). This calorie deficit will also impact on recovery rate, training adaptation, immune system and cognitive function (Bell *et al.*, 2005).

Maintaining body fat within an optimal range can preserve power to mass ratio, allowing for more efficient movement during training and matches (James & Ian, 2014). This is achieved through a combination of sound nutrition and training. Again, achieving an appropriate body composition is considered a very important aspect of preparing for performance in professional football. The reason is that excess fat acts as a dead weight in activities in which the body is lifted against gravity (Sutton *et al.*, 2010). This added dead weight can effect general soccer specific skills such as jumping to head the ball, sprinting and tackling (Reilly *et al.*, 2000). Therefore, body mass and composition affect performance in soccer.

In football, players are mostly in some form of movement. It has periods of intermittent high intensity exercises (Reilly *et al.*, 2000). Coupling this with the big dimensions of the field (110 m long and 90m wide), football can be termed an endurance sport. Cardio-respiratory endurance should be a basic ability of elite footballers as they have to cover a lot of distances (Ostojic and Ahmetovic, 2009). Since much energy is spent and cardio-respiratory endurance is required, the issue of adequacies in micronutrient intake comes into perspective. Micronutrient deficiencies are of concern because their adequacies make available sufficient enzymes, co-enzyme and cofactors that are needed for energy production and enhanced aerobic endurance. As a fact, nutrient sufficient footballers cover more distance in a game and have higher work rates (Helgerud *et al.*, 2001).

Iron for instance is one such micronutrient required in sports. It plays roles in oxygen transfer to tissues and electron transfer in energy production. Footballers with low iron level will have a reduced aerobic endurance owing to reduced oxygen supply to tissues (Hale, 2003). Footballers have a risk of anaemia due to haemolysis caused by repeated foot strikes and physical contact, iron loss through the gastrointestinal tract and through sweating (Robinson *et al.*, 2006) and thus an increased iron requirement (Reinke *et al.* 2012).

Stronger, faster, higher and smarter athletes are likely to succeed. While talent and genetics cannot be overlooked in all sports, it is a reasonable assumption that a well-nourished footballer is likely to perform better than a poorly nourished player of equal genetic predisposition and talent.

1.2 PROBLEM STATEMENT

The technical and tactical abilities of team are dependent on physiological and physical characteristics of its players (Helgerud *et al.*, 2001). Physiological and physical characteristics are components of health and nutritional status.

Elite athletes do represent a specific population in many regards. Specific reference values of biochemical variables for sportsmen are still challenging to define. Those used for the general population, including serum creatinine concentration, are routinely applied to athletes (Banfi and del Fabbro, 2006) with some misinterpretations. This study will add to data being gathered to define reference intervals for creatinine in elite sportsmen.

Knowledge of the nutritional status of sportsmen in Ghana is insufficient for which reason there is a gap between sports and dietetic practice. Previous work on nutritional status of Ghanaian footballers did not undertake any haematological or biochemical studies. However, these factors are crucial predictors of optimal performance in sports and must be studied. Therefore a study such as this will report whether footballers in the premier league are nourished to perform well or not. Without these assessments, nutritional problems affecting players may not be detected and the need to allocate resources at the team and national level to properly feed footballers may not have any justification.

In other continents namely Europe and South America, there has been a remarkable development and an increase in the scope of research on physiology, nutrition and medicine of footballers. The response to these findings has been an increased investment in those aspect of the game with the outcome being more patronised (in terms of fans/viewership) leagues. This study follows that lead.

Again, the outcome of an unresolved diminishing nutritional status is that footballers lack speed, flair and thrills that beautify the game. This may lead to a reduced interest and investments made by teams and corporate bodies may not also yield the expected benefits. Losses may discourage future investments. Malnutrition in local footballers probably explains why foreign based players are seen to perform better. Thus it is necessary that footballers are regularly nutritionally assessed and managed accordingly.

1.3 SIGNIFICANCE OF STUDY

The entertainment and business that follows the game of football depends largely on the footballers. If malnutrition reduces their performances, then there will be an accompanying reduction in patronage and business. A clear understanding of the energy expenditure and iron status of premier league footballers will give insight into efforts that aim at optimizing their nutrition. When it is observed that there are deficiencies, then appropriate nutritional attention will be given. Without an understanding of the usual nutritional status of footballers, the provision of services by dietician may be suboptimal because it will be based on little scientific evidence.

1.4 AIM

To assess the energy expenditure, iron status and serum creatinine levels of premier league footballers in Ghana.

1.5 SPECIFIC OBJECTIVES

The specific objectives were:

1. To determine the anthropometric indices of premier league footballers.

2. To assess some haematological parameters and ferritin levels of premier league footballers.
3. To estimate energy expenditure of premier league footballers.
4. To determine serum creatinine levels of premier league footballers.
5. To determine the association between anthropometric indices and serum creatinine levels in premier league footballers



CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF FOOTBALL

Football or association football is also called soccer. At the competitive level, the governing body of football in the world is FIFA. At the continental level, the Confederation of Africa Football governs the game in Africa and at the national level, the Ghana Football Association over sees the game in Ghana. A competitive match is one that is organised under the auspices of FIFA. All premier league matches are competitive matches (FIFA, 2009).

In a competitive FIFA match, two opposing teams made up of 10 outfield players and a goal keeper start the match. A match may not start if a team has less than 7 players. A competitive match lasts 90 minutes which is divided into two 45 minute halves separated by a 15 minute break. Additional time of up to 10 minutes may be added at the end of the 90 minutes. Sometimes an extra 30 minutes may be added plus the duration of a penalty shoot-out depending on the type of competition (FIFA, 2009).

Fig. 1 shows the dimension of a football field. There are roles or positions assigned to each footballer in the field. Whether a role or a position is given to footballer depends on team strategy and tactics. Often players are assigned both a position and role. Generally, the positions in a field of play are defense, midfield and offence (George, 2014).

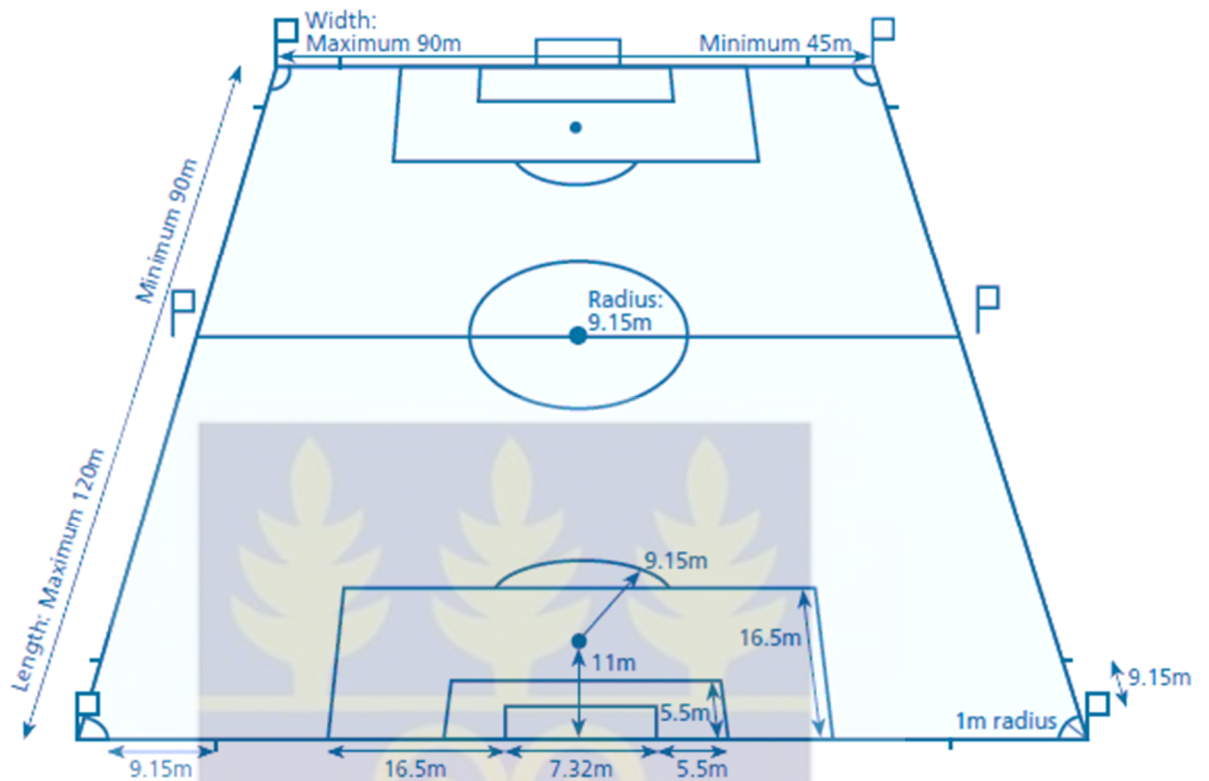


Figure 1 Dimensions of a standard football pitch. Source FIFA, (2009)

The names of these position vary but are about the same location. The defense position is manned by the centre-backs and full-backs. The aim is to stop the opponents from scoring. The midfielders supply the ball to the forwards. They operate in the middle of the field. Forward play very closely to opponent goal area with the main aim being to score. Scoring means to legitimately put the ball into goal post.

2.2 THE PREMIER LEAGUE

The premier league in Ghana, the First Capital Plus Premier League, is the highest level of domestic soccer competition in Ghana. Its activities are controlled by the Premier

League Board (PLB) which is under the Ghana Football Association (GFA). Premier league football started in Ghana in 1958(George, 2014).

There are 16 teams in the league. Each team will compete with all the other teams on home and away basis. Teams are awarded points depending on whether they draw, lose or win their matches (FIFA, 2009). The team with the highest number of points at the end of the completion wins. Each team will play a total of 30 matches in the premier league on home and away basis. These teams may also be involved in other national level competitive matches such as the Mobile Telecommunication Network (MTN) Football Association (FA) cup which is a knock-out championship involving up to 70 teams. Unlike the League, any team that loses in the FA cup leaves that competition (knocked out) (George, 2014).

2.3 THE ENERGY NUTRIENTS

Energy for metabolic and physiologic functions in humans are derived from the chemical energy bound in food and its macronutrient constituents .These macronutrients are carbohydrates, fats and oils, alcohol and proteins (FAO, 2001). Although proteins also provide important amounts of energy, fats and carbohydrates are the main sources of dietary energy, especially when total dietary intake of proteins is limited (FAO, 2001).

The products of the digestion of these macronutrients are mainly glucose, amino acids, fatty acids and glycerol, (Tortora andDerrickson, 2012). To release energy, all the products of digestion are metabolized to a common product, acetyl-CoA, which is then oxidized by the citric acid cycle as shown in figure 2 (Murray *et al.*, 2003) .

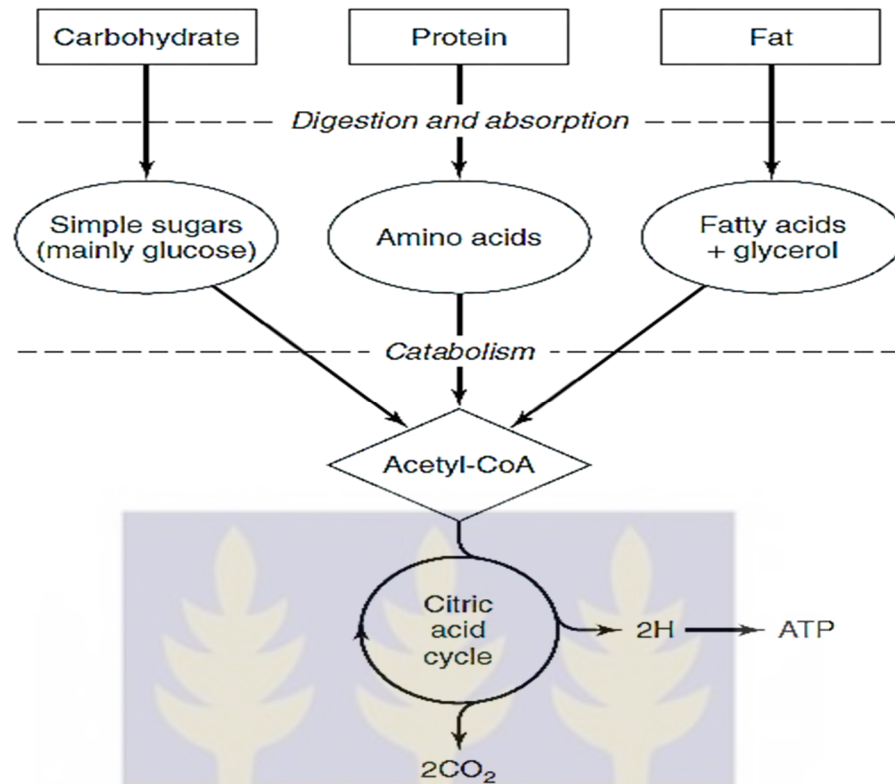


Figure 2 Outline of the pathways for the catabolism of dietary carbohydrate, protein, and fat. All the pathways lead to the production of acetyl-CoA, which is oxidized in the citric acid cycle, ultimately yielding ATP in the process of oxidative phosphorylation. (Murray *et al.*, 2003)

The position statement of The American Dietetic Association (ADA), Dietitians Association of Canada (DAC), and the American College of Sports Science (ACSM) (2000) is that, for high intensity exercises, energy and macronutrient needs of carbohydrate and protein must be met in order to maintain body weight, replenish glycogen stores and provide adequate protein for building and repair of tissues. Fat intake should also be adequate to provide essential fatty acids and fat soluble vitamins and as well help provide adequate energy for weight maintenance. For athletes, not only intake but timing of macronutrient intake is very important if performance is to be optimized (Phillips, 2006; Campbell *et al.*, 2013).

2.3.1 Carbohydrates

Carbohydrates are manufactured by plants and are a major energy source to the body (Mahan and Escott-Stump, 2008). Carbohydrates in food are present in the form of sugars and starch (polymers of sugar) and cellulose which is a non-starch polysaccharide (Murray *et al.*, 2003). The simplest component of carbohydrate is glucose. Glucose is the most important carbohydrate in humans (Sareen *et al.*, 2009).

2.3.1.1 Classification of carbohydrates

Carbohydrates are classified into:

- i. Monosaccharide such as glucose, fructose and galactose; the simple carbohydrates are crystalline solids and water soluble (Mahan & Escott-Stump, 2008)
- ii. Disaccharides made of two monosaccharide joined together. Examples are lactose in milk, maltose in malt and sucrose in table sugar;
- iii. Oligosaccharides such as verbascose, stachyoses and raffinose in legumes; and polysaccharides such as glycogen in muscles and the liver, starch in cassava and cellulose in vegetables (Murray *et al.*, 2003).

2.3.1.2 Metabolism and functions of carbohydrates

The digestion of carbohydrates starts in the mouth by the action of saliva and is finally broken down to a monosaccharide units (glucose, galactose or fructose) which are absorbed in the small intestines. Carbohydrate digestion involves hydrolysis of disaccharides and polysaccharides to monosaccharides (Sareen *et al.*, 2009). The monosaccharides are transferred across epithelial cells of the gastrointestinal tract and enter the portal vein. There is faster absorption of glucose and galactose than fructose (Tortora and Derrickson, 2012).

Glucose is the most important form of carbohydrate in humans. The liver converts all fructose and nearly all galactose into glucose (Arthur and John, 2006). Carbohydrates yield 16.74kJ/g or 4kcal/g when metabolised for energy in the human body (FAO, 2001).

Because glucose is the main carbohydrate in tissues and the primary source of energy, its roles are;

- i. Used in Adenosine triphosphate (ATP) production (energy production)
- ii. Amino acid synthesis, glycogen synthesis and triglyceride synthesis (Tortora & Derrickson, 2012).

Glucose is metabolized to pyruvate by the pathway of glycolysis. When this occurs anaerobically such as during high intensity exercises, the product is lactate. It has been suggested that lactate concentration in exercising muscle may be the cause of muscle cramps or pain (Hale, 2003). Aerobic tissues metabolize pyruvate to acetyl-CoA, which can enter the citric acid cycle for complete oxidation to carbon dioxide, water and ATP (Murray *et al.*, 2003). Figure 2 summarises the metabolism of glucose.

2.3.1.3 Recommended Dietary Allowance of carbohydrates

The recommended daily allowance of carbohydrate for the general population is based on their contribution to total energy intake. It is recommended that 45-65 % of energy intake comes from carbohydrates. (Judith, 2011). Refined sugar should not contribute more than 25% (Mahan & Escott-Stump, 2008). In the optimum diet for most sportsmen, carbohydrates contribute 60-70% of total energy intake (Burke *et al.*, 2004).

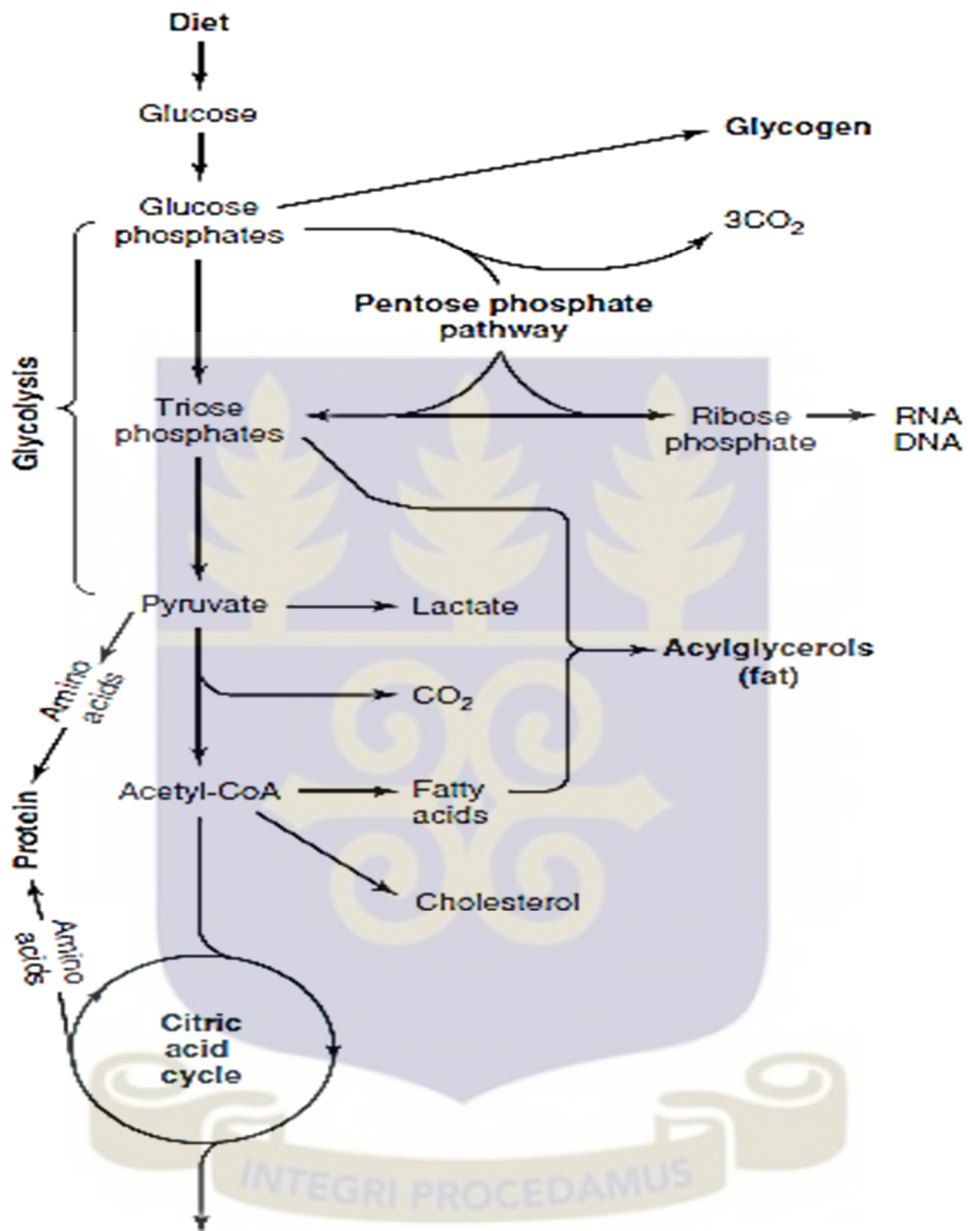


Figure 3 : Overview of dietary glucose metabolism showing the major pathways and end products. (Murray *et al.*, 2003)

The American Dietetic Association (ADA), Dietetians Association of Canada(DAC), and the American College of Sports Science (ACSM) in their position statement on nutrition and athletic performance (2000), recommended that sportsmen take 6-10g of carbohydrates per kilogram body weight. Intakes at this amount maintain body glucose levels during exercise and restore glycogen stores post exercise. It continues that these guidelines only provide a crude approximation of requirements. According to Kerksick *et al.* (2008), maximal endogenous glycogen stores for athletes are best promoted by following a high-glycaemic, high-carbohydrate diet at 8 – 10g per kilogram body weight daily. Also, during exercise, fluids containing carbohydrates may be given to replace losses. Approximately 30-60g is recommended per hour to maintain blood glucose levels. This is particularly important for endurance sports events lasting more than an hour and when the athlete has not consumed adequate food prior to the event or in extreme environments such as cold, heat or altitude.(ADA, DAC &ACSM. ,2000).

2.3.1.4 Dietary Sources of carbohydrates

The sources of major sources carbohydrates include cereals and grains, roots and tubers, legumes and table sugar (FAO, 2012). Recent inclusions in the market such as sports drinks, energy drinks and energy bars may be a significant source of carbohydrates for sports men who consume them.

2.3.2 Proteins

Protein is also a macronutrient. It contains carbon, hydrogen, oxygen and nitrogen. Some proteins also contain sulphur and phosphorus (Murray *et al.*, 2003). The basic structure or building unit of protein is amino acid.

The amino acids are joined by peptide linkages to form protein. Amino acids are water soluble, crystalline and insoluble in organic solvents (Sareen *et al.*, 2009).

2.3.2.1 Classification of proteins

There are about 20 amino acids present in the body. Nine amino acids are essential for humans, as humans cannot synthesize them (Murray *et al.*, 2003; Arthur & John, 2006). These are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine. The nonessential amino acids are glycine, alanine, serine, cysteine, cystine, aspartic acid, glutamic acid, arginine, hydroxylysine, tyrosine, proline, and hydroxyproline (Sareen *et al.*, 2009). Since humans cannot synthesize essential amino acids, they have to be acquired from the diet (Mahan *et al.* 2012)

2.3.2.2 Metabolism and functions of proteins

Digestion of protein starts from stomach and finally completes in the small intestine. Proteins on hydrolysis break down to polypeptides and finally into amino acids which are absorbed in the small intestines (Arthur and John, 2006). The amount and the type of protein taken in diet is important because digestibility and absorbability vary between different proteins (Tortora and Derrickson, 2012). Once absorbed, amino acids in the circulation are converted to proteins as a storage form. The main types of protein in plasma are albumin, globulin, and fibrinogen. A major function of albumin is to provide colloid osmotic pressure in the plasma, which prevents plasma loss from the capillaries. The globulins perform a number of enzymatic functions in the plasma and are also responsible for the body's both natural and acquired immunity. Fibrinogen is involved in blood clot formation. Proteins are required for general growth, maintenance and repair of body tissues and the synthesis of protoplasm, enzymes and hormones (Arthur and John,

2006). Since excess amino acids are not stored, those not immediately incorporated into new protein are rapidly degraded (Murray *et al.*, 2003). They are not excreted in the urine or faeces but converted into glucose or triglycerides (Tortora and Derrickson, 2012). As an energy source, it yields 16.74kJ/g or 4kcal/g (Mahan *et al.*, 2012).

2.3.2.3 Recommended Dietary Allowance for proteins

Protein requirements are increased in highly active people (Campbell, *et al.*, 2007). Recommendations for endurance athletes is 1.2-1.4g/kg body weight per day. (ADA, DAC and ACSM., 2000). Similarly, Ron and Louise (2012) recommended a daily intake of 1.2-1.6 g/kg body weight for these same group of people. Also, Campbell and his colleagues (2007) recommended 1.4-2.0g/kg per day for active individuals. They all agree that daily requirements could be met by diet alone without the use of protein or amino acid supplements once energy intake is adequate. That notwithstanding, protein needs of athletes have received considerable investments in the form of supplements (ADA, DAC & ACSM, 2000). There are contradictory reports about whether supplemental protein is really needed in sports (ADA, DAC and ACSM, 2000).

Ron and Louise (2012) recommended that eating about 20-25g of protein from a high quality protein immediately after exercise promotes muscle synthesis. Also, appropriately timed protein intake is essential for recovery, immune function growth and maintenance of lean mass. High protein diets of 30-35% of energy intake have been shown to lead to weight reduction and fat loss and the preservation of lean mass (Phillips, 2006).

2.3.2.4 Dietary sources of proteins

Protein containing foods can be divided into two categories

- i. Complete proteins or high quality proteins
- ii. Incomplete proteins or low quality proteins (Sareen *et al.*, 2009).

A complete protein contains all the essential amino acids in the approximate amounts needed by humans. Sources of complete proteins are mostly foods of animal origin such as milk, eggs, meat, fish, and poultry (Mahan *et al.*, 2012). One exception is soy protein from soya beans, which is of plant origin but is a complete protein (FAO, 2012). Incomplete proteins or low-quality proteins are lacking in one or more essential amino acids. They are derived from plant foods such as legumes, nuts, cereals, and grains (FAO, 2012).

2.3.3 Lipids

Lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are grouped more by their physical than by their chemical properties (Murray *et al.*, 2003).

2.3.3.1 Classification of lipids

Murray *et al.* (2003) classified lipids in terms of their molecular structure as follows:

1. Simple Lipids - these are esters of fatty acids with various alcohols
 - i. Fats: Esters of fatty acids with glycerol. Oils are fats in the liquid state.
 - ii. Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols.
2. Complex Lipids - Esters of fatty acids containing groups in addition to an alcohol and a fatty acid

- i. Phospholipids: Lipids containing a phosphoric acid residue in addition to fatty acids and an alcohol examples are glycerophospholipids and sphingophospholipids.
 - ii. Glycolipids: Lipids containing a fatty acid, sphingosine, and carbohydrate
 - iii. Other complex lipids: Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.
3. Precursor and derived lipids - These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones

Fatty acids (FA) are the simplest of lipids. They are of vital importance as an energy nutrient (Sareen *et al.*, 2009). The length of the carbon chain of fatty acids in food and human tissues vary from 4 - 24. Fatty acids may be in the form of saturated fatty acids (SFA) if they contain no double bonds, Monounsaturated fatty acids (MUFA) if they contain one double bond and polyunsaturated fatty acids (PUFA) if they contain two or more double bonds (Mahan *et al.*, 2012). Unsaturated fatty acids exist in two forms. The naturally occurring *cis* form and the *trans* form which often results from processing. Concerns have been raised about the possible adverse nutritional effects of dietary *trans* fatty acids, particularly their role in the etiology of cardiovascular diseases (Nikolaos *et al.*, 2010). Polyunsaturated fatty acids of note are omega 6 fatty acids and omega 3 fatty acids. These two FA are essential to humans and other animals because they can only be synthesised by plants (Sareen *et al.*, 2009).

2.3.3.2 Metabolism and Functions of lipids

Most of the lipid component of human diet is in the form of triglycerides with smaller quantities of cholesterol and phospholipids (Arthur and John, 2006). The dietary lipids are masticated and mixed with lingual lipase, followed by hydrolysis by gastric lipase in the stomach and then by pancreatic lipase in the small intestine (FAO, 2010). Lipids are emulsified by bile and then hydrolysed to fatty acids, glycerol and monoglycerides in the small intestines (Tortora and Derrickson, 2012). Once in the intestinal cells, they are converted back into triglycerides and chylomicrons and transported via the lymphatic vessels which empties into the blood vessels (Arthur and John, 2006). Chylomicrons are broken by enzymes on hepatocytes and adipocytes by hydrolysing triglycerides into fatty acid and glycerol (Tom, 1999). These diffuse through the cell membranes into the plasma (Sareen *et al.*, 2009). The liver cells and intestinal cells to a lesser extent convert free FAs and lipids into lipoproteins.

The primary function of the lipoproteins is to transport their lipid components in the blood. Lipoproteins are much smaller than chylomicrons, but qualitatively similar in composition containing triglycerides, cholesterol, phospholipids, and protein. Arthur and John (2006) classified lipoproteins into 4 types as follows:

- i. Very low density lipoproteins (VLDL), which contain high concentrations of triglycerides and moderate concentrations of both cholesterol and phospholipids.
- ii. Intermediate-density lipoproteins, which are very low density lipoproteins from which a share of the triglycerides has been removed, so that the concentrations of cholesterol and phospholipids are increased.

- iii. Low-density lipoproteins (LDL), which are derived from intermediate-density lipoproteins by the removal of almost all the triglycerides, leaving an especially high concentration of cholesterol and a moderately high concentration of phospholipids.
- iv. High-density lipoproteins (HDL), which contain a high concentration of protein (about 50%) but much smaller concentrations of cholesterol.

High density lipoproteins are usually termed good cholesterol because of their role in the prevention of cardiovascular diseases. Because low density cholesterol is associated with the incidence of cardiovascular diseases, they are termed bad cholesterol (Tom, 1999).

Dietary fat is essential for the digestion, absorption, and transport of the fat-soluble vitamins and phytochemicals such as carotenoids and lycopenes (Mahan *et al.*, 2012). They are building blocks for synthesis of biologically important lipids such as phospholipids, sphingolipids and cholesterol esters which have many metabolic and regulatory roles (Sareen *et al.*, 2009). Essential fatty acids (EFA) are important for the function and structure of body cells membranes, for hormones like prostaglandin. Monounsaturated fatty acids (MUFA) lower the risk of coronary heart disease, cancer, cataract, and other inflammatory disorders (Elmadfa and Kornsteiner, 2009). These are α -linolenic acid (ALA) and linoleic acid. Fats as adipose tissue act as an insulator and padding for vital organs (Tortora and Derrickson, 2012). It is well documented that high-fat diets, which are more energy-dense than low-fat diets, increase the risk of over-consumption of energy and play an important role in the development of obesity, cardiovascular diseases, cancer and diabetes.

Lipids are a concentrated source of energy providing 37.656 kJ or 9 kcal per gram (FAO, 2010). In the post-absorptive state, muscle, heart, liver and renal cortex use fatty acids as their primary fuel (Jequier, 1999; Jensen, 2003). Fatty acids are a major fuel source for humans both at rest and during exercise. Fatty acids may be oxidized to acetyl-CoA or esterified with glycerol, forming triglycerides (fat) as the body's main fuel reserve. Acetyl-CoA formed by β -oxidation may undergo several fates (Figure 3). Plasma free fatty acids (FFA), although present only in micromolar concentrations, are the major circulating lipid fuel. FFA availability can increase two- to four-fold with moderate intensity exercise (Jensen, 2003).

2.3.3.3 Recommended Dietary Allowance for lipids

The acceptable macronutrient distribution range (AMDR) for total fat intake can vary between 20 and 35% of daily total energy intake. Maximum intake is 35% and minimum intake is 15 % (Elmadfa and Kornsteiner, 2009). Jequier (1999) reported the lower limit of fat intake to meet energy needs of adults as ranging between 10- 15% provided that enough carbohydrates are available. While for most individuals with moderate physical activity 30% is recommended, for those with high physical activity levels this can reach 35% of total energy intake per day (Elmadfa and Kornsteiner, 2009; FAO, 2010) . The minimum intake levels of essential fatty acids to prevent deficiency symptoms are 2.5% of energy intake for linoleic acid plus 0.5% energy intake α -linoleic acid.

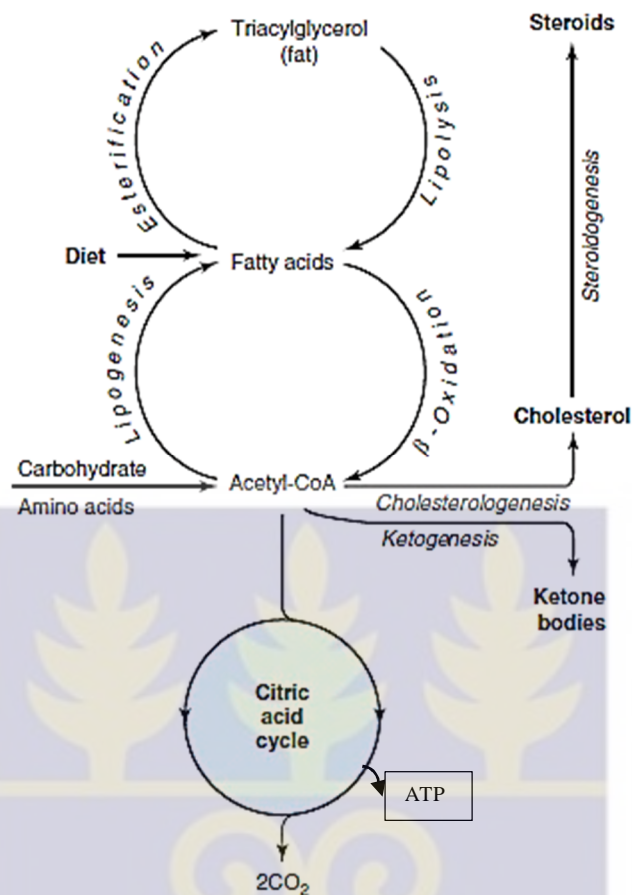


Figure 4 Overview of fatty acid metabolism showing the major pathways and end products. Adopted from Murray *et al.*, (2003)

It is well known that excess carbohydrates are converted to lipid and stored. Jequier (1999) concluded that when high carbohydrate and low fat diets are ingested, de novo lipogenesis is stimulated in adults, but the rate of conversion of glucose to fatty acids is low, which means that carbohydrate intake does not have much influence on fat requirements. Again, he added that energy from fats should not be below 10% of total energy intake in order to ensure an unrestricted absorption of fat-soluble vitamins, particularly vitamins A and E.

2.3.3.4 Dietary Sources of lipids

These include cooking oils, legumes and pulses, meat, dairies, poultry and oily fish (FOA, 2001)

2.4 ENERGY EXPENDITURE

Energy expenditure is a measure of the total amount of energy used by an individual in a day or a given time. In humans, energy is expended in the form of basal energy expenditure (BEE) or basal metabolic rate (BMR), thermic effect of food (TEF) or diet induced thermogenesis (DIT) and physical activity (PA) or activity thermogenesis (AT) (Mahan *et al.*, 2012).

BEE or BMR is the energy expended when an individual is lying at complete rest, in the morning after sleep, in the post-absorptive state which is usually about 10-12 hours after ingestion of food, drink or nicotine (Levine, 2007; Mahan *et al.*, 2012). In individuals with sedentary occupations, basal metabolic rate accounts for approximately 60-70% of the total daily energy expenditure (Mahan *et al.*, 2012). For practical reasons, the BEE is now rarely measured. Rather, it is resting energy expenditure (REE) or resting metabolic rate RMR that is measured. In most cases REE are higher than the BEE by 10% to 20% (Mahan *et al.*, 2012). Resting energy expenditure is the energy expended in the activities necessary to sustain normal body functions and homeostasis (Arthur and John, 2006). These activities include respiration and circulation, the synthesis of organic compounds and the pumping of ions across membranes. It also includes the energy required by the central nervous system and for the maintenance of body temperature (Mahan *et al.*, 2012). The REE is affected by age, body composition, body size, climate, gender, hormonal status and body temperature.

Thermic effect of food (TEF) is the increase in energy expenditure resulting from the digestion, absorption and storage of food ((Mahan *et al.*, 2012); Arthur & John, 2006; Levine, 2007). It ranges from about 10% of TEE (Mahan *et al.*, 2012) to 8 % of TEE (Arthur & John, 2006). The type of meal eaten may affect TEF. For example, a high protein meal is known to increase metabolic rate to maximum of 30% above normal, and this lasts for 3 to 12 hours. This effect of protein on the metabolic rate is called the specific dynamic action of protein (Arthur and John, 2006).

Activity thermogenesis is the energy cost of doing physical activities. These are exercise and non-exercise activity thermogenesis (Levine,2007). Most individuals do not do any purposeful sporting exercise and so their exercise-related activity thermogenesis is zero. For those who do exercise regularly, exercise related energy expenditure is generally, 10% of the total daily energy expenditure (Mahan *et al.*, 2012).

Owing to the large differences in the amount of physical activity among individuals, activity thermogenesis is the most important reason for the differences in caloric requirements of individuals.

2.4.1 Methods of Energy Expenditure Assessment

Measurement of energy expenditure in humans is used to assess metabolic needs and fuel utilization (Levine, 2007). Assessing daily energy expenditure in free living individuals such as footballers usually presents some form of challenges (Noda *et al.*, 2009). For any given context such as in footballers, these methods are either accurate but inapplicable , or applicable and accurate but expensive or applicable, affordable but inaccurate (Levine, 2007). Usually, when requirements cannot be measured by the more accurate calorimetric

methods and the doubly labelled water method, predictive equations have been used. Therefore, though precise, the doubly labelled water method and the calorimetric methods are impractical in this research. On the other hand, methods such as the predictive equations which have some degree of inaccuracy but are cheap to employ.

On the whole methods are selected after considering any set of the following factors that border but are not limited to accuracy, precision, objectivity, simplicity, time efficiency, minimal disruption of usual activity, social/cultural religious acceptability and applicability to large populations (Levine, 2007; Volp *et al.*, 2011; Mahan *et al.*, 2012).

2.4.1.1 Indirect Calorimetric Methods

In indirect calorimetry, oxygen consumption and/or carbon dioxide production is measured and converted into energy expenditure using formulae (Levine, 2007). Because 95% of energy expended in the body is derived from reactions with oxygen, energy expenditure can be calculated from the rate at which oxygen is being used with a high degree of accuracy; for the average diet, a litre of oxygen consumed yields 4.825 calories (Arthur and John 2006). The formulae used are detailed by Weir (1949) and Bursztein *et al.*, (1989).

A number of systems are employed to measure TEE by this method. Their operations and components have been elaborated by Levine (2007). These are;

1. Total Collection Systems; expired air is collected into container and analysed.
2. Open-circuit Indirect Calorimeter Systems; Subjects inspire air and the expired gases are analyse.

3. Closed Circuit System; Consists of a sealed respiratory gas circuit in which gaseous concentrations are measured over time (Benedict, 1909).
4. Confinement system (respiratory chambers); the subject is placed inside an air -tight sealed container of known volume. Oxygen consumption and carbon dioxide production are estimated from changes in the concentrations of these gases in chamber air over time(Aulick *et al.*, 1983).

Setting up an indirect calorimeter system may cost at least 10,000-20,000 US dollars (Levine, 2007). This high cost limits its application in research. Also the equipment do not make it possible to apply this method in studying free living persons. However, it has a high degree of accuracy (Levine, 2007; Roberts *et al.*, 1986; Livesey & Elia, 1988; Rosado *et al.*, 2013).

2.4.1.2 Direct Calorimetry

In the direct calorimetry method, heat lost from the body is measured and used in equations that derive energy expenditure (Volp *et al.*, 2011). The apparatus consist of a chamber lined with insulation material. Heat changes in the chamber is measured and attributed to the subject (Conway *et al.*, 2002). The apparatus is very expensive to build. It Costs more than one million US dollars. Once installed, it less expensive to use in measuring energy expenditure. It is applied in laboratories where heat lost by the body is of value (Levine, 2007).

2.4.1.3 Doubly Labelled Water (DLW)

This method is accurate and precise for measuring energy expenditure in free living individuals. it is about 97-99% accurate when compared with the indirect circuit method

(Volp *et al.*, 2011). It is based on the principle of isotope dilution. The doubly labelled water is made of O^{18} and H^2 .

The doubly labelled water method considers that the O_2 turnover is determined by the body water flow and the inspired O_2 and expired CO_2 , while the H_2 turnover is determined exclusively by the water flow through the body (Levine, 2007). To measure the total body water, a pre-established volume and concentration of the H^2 and O^{18} isotope is orally administered, which diffuses throughout the body over 2 to 6 hours (Volp *et al.*, 2011). As the energy is spent by the body, CO_2 and water are produced. The CO_2 is eliminated by the lungs, and the water, by lungs, skin and urine (Arthur & John, 2006). The H^2 and O^{18} disappearance rate is determined by measuring repeatedly their concentrations in the body fluids (saliva, urine or blood). The difference between the disappearance rates of the two isotopes is used to estimate the CO_2 production and with this, determine the energy expenditure based on the equation of Weir (Weir, 1949). One major setback of the DLW method is that it is very expensive and requires skilled personnel to run.

2.4.1.4 Physical Activity Records

In this method the study subject is made to complete a sheet by filling all the activities carried out over a 24 hour period for a specified number of days (Mahan *et al.*, 2012). The subject will specify the type of activity and the time spent doing the activity. The researcher or clinician then compares the activity to a coded list such as the compendium of physical activity published in 1993. The compendium consist of physical activity and their metabolic equivalents (MET). Once the MET of a given physical activity is gotten from the list, the following equation is one of many used to derive the amount of energy spent in that activity;

Equation: $0.0175 \times \text{weight (kg)} \times \text{METs} = \text{kcal/min}$ (Ridley *et al.*, 2008)

This method is simple to use and not expensive. The challenges lie in the information being collected by the subject. That is, there is a burden on the subject which could lead to misreporting (Levine, 2007). Also the subject must be literate and willing. Again, the list is not exhaustive when applied to the context in Ghana.

2.4.1.5 Predictive Equations

These are formulae used to estimate the energy requirements of individuals. They were developed from studying groups of individuals by using regression analysis based on independent variables such as weight, height, age and gender. A summary of some predictive equations follows

1. Harris- Benedict equation was formulated after analyzing the data base of normal weight individuals (Flancbaum *et al.*, 1999). It has been found to underestimate energy expenditure in obese individuals when compared to the indirect calorimetric method (Volp *et al.*, 2011).
2. The Food and Agriculture Organisation (FAO)/ United Nation University/World Health Organisation (WHO) adopted the Schofield's equation based on 114 healthy individuals in 1985 after some modification and considered it appropriate for international use (FAO, 2001).
3. Ireton- Jones equation is used to estimate energy requirements of obese patients (Mahan *et al.*, 2012).
4. The Mifflin-St Jeor equation was derived from studies of normal weight, overweight and obese individuals (Mifflin *et al.*, 1989)

2.4.2 Energy Intake and Expenditure in Footballers

The energy expenditure of a footballer is an estimate of how much energy he/she spends per day. When the energy expenditure is compared to the amount of energy consumed, then the difference is the energy balance (FAO, 2001). The issue of energy intake and/or expenditure among footballers has to be approached with some caution because;

- i. Dietary intake tools used and the researcher in person do have significant margins of error when collecting or analysing data. For example Caccialanza and his colleagues (2007) whilst studying self-reported energy intakes, energy expenditure and weight changes in Italian footballers observed that under reporting of dietary intake was rampant. Again when different dietitians were given the same dietary records of the same athletes to analyse using the same food data base and the same software, the results they gave varied considerably (Andrea *et al.*, 2003)
- ii. Energy expenditure measurement in free living footballers is only accurate when the doubly labelled water (DLW) method is used. Here, though equally accurate, the calorimetric methods are not applicable (Conway *et al.*, 2002). The doubly labelled water method is very expensive (Levine, 2007). For this reason probably, most studies use physical activity recall, the predictive equations or physical activity records to determine the energy expenditure of footballers. Results from these methods have been said to be inaccurate by Levine (2007) and Volp *et al.*, (2011). Conway and his colleagues had earlier in 2002 proven that the methods do not only just vary significantly but largely when compared with gold standards (calorimetric methods or the doubly labelled water method) in 24 individuals.

Studies have reported the mean energy expenditure and intakes of footballers in European, Asian and South American countries. Table 1 summarizes the findings of some of these studies;

Table 1 Summary of mean energy intakes and expenditure of footballers

Study	Population	Mean Energy Intakes (kcal/day)	Method of Assessment	Mean Energy Expenditure (kcal/day)	Method of Assessment
Maughan (1997)	Professional footballers, Scotland. n=51	2,857.14	7 day weighed intake	-	-
Ebine et al., (2002)	Professional footballers, Japan. n= 7	3113.00	4 Diet Record	3523.56	Doubly Labelled Water
Leblanc et al., (2002)	National youth team, France. n=180	2,873.5	7 Diet Record	-	-
Luiz et al.,(2006)	Professional Footballers, Brazil. n=118	3,361.43	4 Diet History	-	-
Costas et al., (2009)	Professional footballers, Greece. n=16	2935.50	7 day diet record	-	-

Given that matches are more competitive than training sessions, it is likely that more calories are spent on match days for the footballer who plays the entire length of the game and lower for the one that does not play. Travelling to play away matches may also vary

nutrient intake if not calorie intakes because a new environment may present different food choices.

Players usually under report their food intakes. For what the reason or cause of that may be, under reporting should always be taken into account when reporting calorie intakes (Caccialanza *et al.*, 2007).

2.5 ENERGY INTAKE ASSESSMENT

Dietary intake data is collected retrospectively or prospectively. Retrospective methods include the 24-hour dietary recalls, the food frequency questionnaire and the diet history. Prospective methods are the daily food record and the food diary. Each method has specific purposes, strengths, and weaknesses (Volp *et al.*, 2011).

The purpose of dietary assessment may be to measure nutrients intake, foods or eating habits, eating pattern and assess risk for certain diet associated diseases. The reality is that, there is widespread uncertainty about the reliability and validity of these methods. For this reason, many studies have tried to validate and/or improve the reliability of these tools before applying them (Thompson and Byers, 1994; NOO, 2010; Barrie and Pirjo, 1997; Block, 1982; Bingham *et al.*, 1994). That notwithstanding, they are used. What is advised is that the appropriate tool to employ for dietary assessment be dependent on the purpose for which it is needed. (Wrienden *et al.*, 2003). Their strengths and weaknesses need to be considered when selected one method or the other (Wrienden *et al.*, 2003). Another way to improve the accuracy of dietary assessment tools is to combine them where possible. For example, FAO / WHO (1998) reported that, the United State

Department of Agriculture combined the 24 hour recall and the food frequency questionnaire in collecting dietary data with view to improve accuracy and facilitate the interpretation of dietary data.

2.5.1 Estimated Dietary Record

In the dietary record method, the respondent records the foods and beverages and the amounts of each consumed over one or more days (Thompson and Subar, 2013). Ideally, the recording is done at the time when eating in order to avoid reliance on memory (Mahan *et al.*, 2012). The amounts consumed may be measured, using a scale or household measures such as cups or tablespoons (Thompson and Subar, 2013; Thompson and Byers, 1994). Amounts may also be estimated using models or pictures.

If multiple days are recorded, they are usually consecutive, and no more than 7 days are included. Recording periods of more than 4 consecutive days are usually unsatisfactory. The individual's nutrient intake is then calculated and averaged at the end of the desired period. (Mahan *et al.*, 2012). To complete a dietary record, each respondent must be trained in the level of detail required to adequately describe the foods and amounts consumed, including the name of the food (brand name, if possible), preparation methods, recipes for food mixtures, and portion sizes (Thompson and Subar, 2013). Therefore, it requires a high degree of cooperation from study participants.

A potential disadvantage of the dietary record method is that it is subject to bias both in the selection of the sample and in the sample's completion of the number of days recorded. Dietary record keeping requires that respondents or respondent proxies be both motivated and literate (if done on paper), which can potentially limit the method's use in

some population groups. In fact, Block (1982) suggests that it is impractical for large studies.

2.5.2 24 Hour Dietary Recall

Requires individuals to remember the specific foods and amounts of foods they consumed in the past 24 hours. The information is then analysed by the person or professional gathering the information (Mahan *et al.*, 2012). Reliability and validity of dietary recall methods are important. When people are questioned about their diet, they may consciously or unconsciously alter their intake either to simplify recording or impress the interviewer, thus decreasing the information's validity (Mahan *et al.*, 2012). The primary limitation of this method is that recording consumption for a single day is seldom representative of a person's usual intake due to day-to-day variation. Any self-reported method of obtaining data can be challenging because it is difficult for people to remember what they ate, the content, or even an accurate statement of portion size (Mahan *et al.*, 2012).

2.5.3 Food Frequency Questionnaires (FFQs)

The food frequency questionnaire consists of a list of foods and a selection of options relating to the frequency of consumption of each of the foods listed such as times a week, day or month. Food frequency questionnaires are designed to collect dietary information from large numbers of individuals such as 100 or more (Mahan *et al.*, 2012; Thompson and Subar, 2013). They can easily be self-administered or interviewer administered. Not much detail is collected on other characteristics of the foods eaten such as the methods of cooking and the combinations of foods in meals. Some FFQs also attempt to collect information about portion size in addition to frequency of consumption. They are referred

to as semi-quantitative FFQs. FFQs are useful for gathering information on groups of individuals as well as for looking at habitual intake of a range of foods

2.6 IRON

Iron is a d-block transition element that can exist in oxidation states ranging from -2 to +6. In biological systems, these oxidation states are limited primarily to the ferrous (+2) also called haem iron, ferric (+3) also called non haem iron and ferryl (+4) states (Beard, 2001). The inter conversion of iron oxidation states is not only a mechanism whereby iron participates in electron transfer but also a mechanism whereby iron can reversibly bind ligands (Sareen *et al.*, 2009) Iron can bind to many ligands by virtue of its unoccupied d orbitals (Beard, 2001). The preferred biological ligands for iron are oxygen, nitrogen and sulphur atoms (Beard 2001). In terms of daily requirements, it is a micro mineral (Sareen *et al.*, 2009).

According to Arthur and John (2006), the total quantity of iron in the body averages 4 to 5 grams. Haemoglobin constitutes 65%, 4 % is in the form of myoglobin, 1 % is in the form of the various haem compounds that promote intracellular oxidation, 0.1 per cent is combined with the protein transferrin in the blood plasma, and 15 to 30 per cent is stored for later use, mainly in the reticuloendothelial system and liver parenchymal cells, principally in the form of ferritin. Sareen *et al.*, (2009) gave a significantly different report; the human body contains approximately 2 to 4 g iron, or about 38 mg iron per kg body weight for women and approximately 50 mg iron per kg body weight for men. Over 65% of body iron is found in hemoglobin, up to about 10% is found as myoglobin, about 1% to 5% is found as part of enzymes, and the remaining body iron is found in the blood

or in storage. Commonly, these two groups of researchers agree that most of the iron in the body exist in haemoglobin.

Haemoglobin is a molecule composed of four units, each containing one haem group and one protein chain. The structure of haemoglobin allows it to be fully loaded with oxygen in the lungs and partially unloaded in the tissues (FAO and WHO, 2001). The iron-containing oxygen storage protein in the muscles, myoglobin, is similar in structure to haemoglobin but has only one haem unit and one globin chain. Several iron-containing enzymes, the cytochromes, also have one haem group and one globin protein chain (Harvey and Denise, 2011). These enzymes act as electron carriers within the cell and their structures do not permit reversible loading and unloading of oxygen (Arthur & John, 2006). The other types of heme proteins and classification are summarised elsewhere (Beard, 2001).

Athletes, particularly females and adolescents, are at increased risk of depleting their iron stores to the stage of functional or absolute iron deficiency (Auersperger *et al.*, 2013). This is because exercises induces iron loses through haemolysis, haematuria, sweating and gastrointestinal bleeding.

2.6.1 Metabolism and functions of Iron

Iron is both an essential nutrient and a potential toxicant to cells (Nikolaos *et al.*, 2010). Therefore, it requires a highly sophisticated and complex set of regulatory approaches to meet the demands of cells as well as prevent excess accumulation (Beard, 2001).

Haem iron (Fe^{+2}) in food must be hydrolysed from the globin portion of haemoglobin or myoglobin before absorption. This digestion is accomplished by proteases in both the

stomach and the small intestine and results in the release of haem iron from the degradation of globin. It is readily absorbed intact across the brush border of the mucosal cells of the small intestines by the haem carrier protein 1 (Sareen *et al.*, 2009). Iron absorption occurs throughout the small intestine. Non haem iron bound to components of foods must be enzymatically hydrolysed in the gastrointestinal tract to be absorbed. Gastric secretions, including hydrochloric acid and proteases in the stomach and small intestine, aid in the release of non-haem iron from food components. Once released from food components, most non haem iron is present as ferric (Fe^{3+}) iron in the stomach. Non haem iron forms insoluble compounds in the small intestines which renders it not absorbable (Mahan *et al.*, 2012). Ascorbic acid (vitamin C), along with citric, lactic, and tartaric acids, for example, acts as a reducing agent and forms a chelate with non-haem ferric iron at an acid pH. The chelate remains soluble and so improves iron absorption (Sareen *et al.*, 2009). Factors that reduce the absorption in the intestines are phosphates, phytates, oxalate and tannins. These form insoluble chelates with iron and render it unabsorbable (Kraemer & Zimmermann, 2007).

Arthur & John (2006) explained transport, storage, and metabolism of iron in the body as follows: When iron is absorbed it immediately combines in the blood plasma with a beta globulin, apotransferrin, to form transferrin, which is then transported in the plasma. The iron is loosely bound in the transferrin and, consequently, can be released to any tissue cell at any point in the body. Excess iron in the blood is deposited especially in the liver hepatocytes and to a less extent in the reticuloendothelial cells of the bone marrow. In the cell cytoplasm, iron combines mainly with a protein, apoferritin, to form ferritin. This iron stored as ferritin is called storage iron. Smaller quantities of the iron in the storage pool are in an extremely insoluble form called hemosiderin. This is especially true

when the total quantity of iron in the body is more than the apoferritin storage pool can accommodate. When the quantity of iron in the plasma falls low, some of the iron in the ferritin storage pool is removed and transported in the form of transferrin in the plasma to the areas of the body where it is needed. When red blood cells have lived their life span and are destroyed, the haemoglobin released from the cells is ingested by monocyte-macrophage cells. There, iron is liberated and is stored mainly in the ferritin pool to be used as needed for the formation of new haemoglobin. (Figure 5).

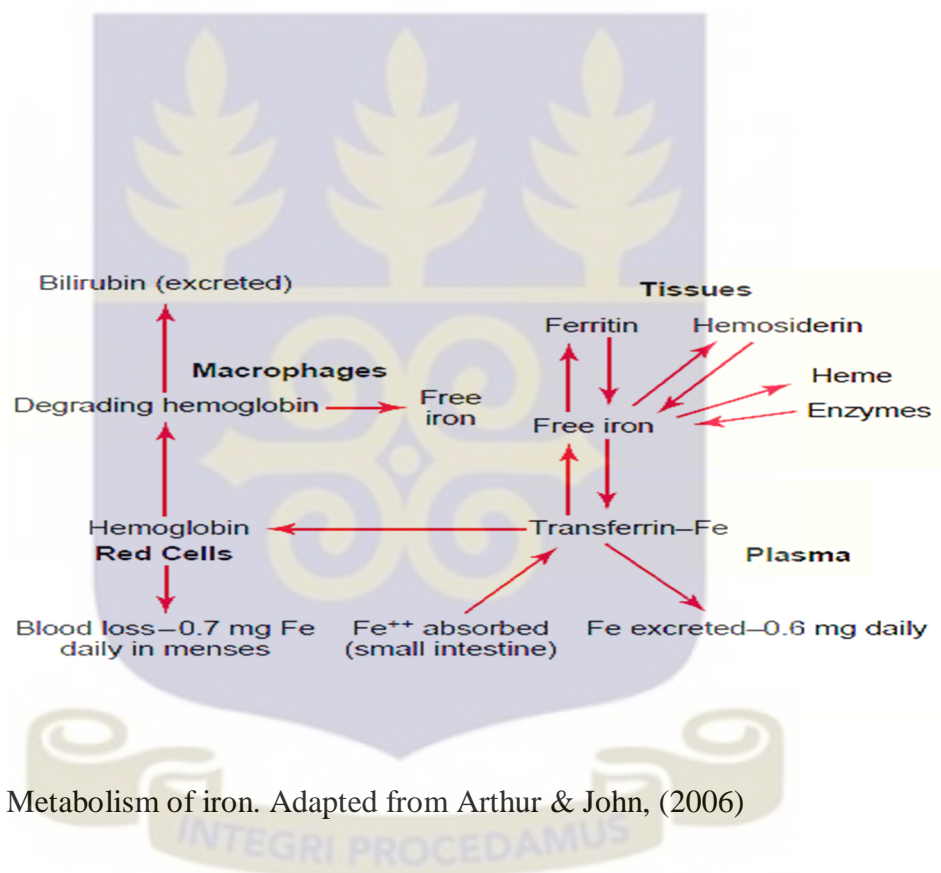


Figure 5: Metabolism of iron. Adapted from Arthur & John, (2006)

Iron is not actively excreted from the body in urine or in the intestines. Iron is only lost with cells from the skin and the interior surfaces of the body such as the intestines, urinary tract, and airways. The total amount lost has been estimated in several reports; 14 mcg/kg body weight/day (Green, 1968), non-menstruating 55-kg women lose about 0.8 mg /day and a 70-kg man loses about 1 mg. (FAO and WHO, 2001). Studies once suggested that iron losses from sweat are substantial in hot and humid climates especially. When

later studies took extensive precautions to avoid the contamination of iron from external sources during the collection of total body sweat, iron losses through sweat were found to be negligible (Brune, 1986). Beard (2001) reported that total iron losses in faeces, urine and sweat in endurance-trained athletes are approximately 1.75 mg/d in males and approximately 2.3 mg/d in females because of the additional iron losses with menstruation. Losses in normal population is set at 0.6 mg per day (Arthur and John, 2006).

Iron has several well documented functions. These include:

- i. The heme proteins myoglobin and haemoglobin maintain a supply of oxygen which is vital for oxidative metabolic processes. Myoglobin is found in muscle. Its role is to store oxygen as a reserve against oxygen deprivation. Haemoglobin, transports oxygen from lungs to the tissues and returns carbon dioxide and protons to the lungs (Murray *et al.*, 2003)
- ii. It is a vital component of the electron transfer chain that is involved in the control of energy release in cells (Burke and Deakin, 2000)

Iron is very important for sports because it is needed for oxygen supply and energy release. In fact as iron status declines, athletic performance is reduced (Weaver, 1992).

2.6.2 Iron Deficiency Anaemia

Iron deficiency is a state in which there is insufficient iron to maintain the normal physiological function of tissues such as the blood, brain, and muscles (Ellie and Sharon, 2008; WHO and CDC, 2005). In clinical terms anaemia is an insufficient mass of RBCs circulating in the blood; in public health terms anaemia is defined as a haemoglobin concentration below the 5th percentile of the haemoglobin concentration of a normal

population of the same sex and age group (WHO and CDC, 2005). Iron deficiency is probably the most common cause of anaemia. However, there are other causes. These include folate deficiency, cobalamin deficiency, vitamin A deficiency and genetically inherited conditions such as thalassemia (Provan, 2003; Mahan *et al.*, 2012). These were not investigated in this research and therefore will not be discussed.

Two billion people, more than over 30% of the world's population are anaemic with about 1 billion suffering from iron deficiency anaemia. In many developing countries one out of two pregnant women and more than one out of every three preschool children are estimated to be anaemic (Kraemer and Zimmermann 2007; WHO 2011a). The symptoms are summarized in Table 2.

Table 2 : Symptoms of Iron deficiency (Beard, 2001)

i. Increased insulin sensitivity	ii. Increased absorption of lead and cadmium
iii. Anaemia	iv. Impaired physical performance
v. Impaired thermoregulation	vi. Glossitis
vii. Impaired immune function	viii. Angular stomatitis
ix. Impaired mental function	x. Koilonychia
xi. Pica	xii. Complications of pregnancy
xiii. Fatigue	xiv. Blue sclera
xv. Altered drug metabolism	

Iron- deficiency anaemia is a cause of maternal and child mortality, poor cognitive development and increased morbidity in children and reduced productivity in adults

(FAO and WHO, 2001; Kraemer and Zimmermann, 2007; WHO and UNICEF, 2004) .

Initially, adaptation to IDA may be an increased cardiac output. This may offset some of the symptoms but a further demand for increased cardiac output in sports will not be met and performance declines (Weaver, 1992).

Iron deficiency occurs in 3 stages. The presence of anaemia is evidence of an advanced stage of the deficiency. Table 3 summarizes the stages of iron deficiency and their clinical indicators.

Stage	Clinical Indicator
1. Iron stores in bone marrow, liver and spleen are depleted	Plasma Ferritin decreases
2. Erythropoiesis diminishes as the supply of iron to erythroid marrow is reduced	Transferrin Saturation (TS) increases Total iron binding capacity (TIBC) increases Serum iron Red cell distribution width (RDW) decreases
3. Haemoglobin production falls resulting in anaemia	Haemoglobin concentration is low Haematocrit is low

Table 3 : Stages of iron deficiency

For the clinical diagnosis of iron deficiency anaemia using haemoglobin concentration at sea level, the following cut-offs (Table 4) have been set by WHO in 1968 and still applies till date (WHO, 2011a).

Clinical observation of blood smears of anaemic persons may reveal the characteristic findings of microcytic and hypochromic red blood cells. Automated haematology analysers record mean cell haemoglobin (MCH) as indicator of red cell colour (WHO and CDC, 2005). These changes in the red cell result from decreased rates of globin synthesis when haem is not available.

Iron deficiency can and does exist without anaemia. Even in the absence of anaemia, iron deficiency has metabolic consequences (Weaver, 1992). These consequence extend into sports performance. Reduced levels of iron stores increase fatigue and affect sport performance.

Table 4: Haemoglobin levels to diagnose anaemia at sea level (g/dl). Adapted from WHO, 2011a

Population	Non-Anaemia (g/dl)	Anaemia (g/dl)		
		Mild	Moderate	Severe
Children 6-59 months of Age	≥ 11.0	10.0-9.0	7.0-9.9	< 7.0
Children 5 - 11 years of age	≥ 11.5	11.0-11.4	8.0-10.9	< 8.0
Children 12 - 14 years of age	≥ 12.0	10.0-11.9	8.0-10.9	< 8.0
Non-pregnant women (15 years of age and above)	≥ 12.0	10.0-11.9	8.0-10.9	< 8.0
Pregnant women	≥ 11.0	10.0-10.9	7.0-9.9	< 7.0
Men (15 years of age and above)	≥ 13.0	11.0-12.9	8.0-10.9	< 8.0

Another important indicator for iron status is the measurement of ferritin (WHO 2011b). The plasma content of ferritin correlates well with the iron stores (Table 5), and in the first stage of iron deficiency the concentration of ferritin already decreases. This makes it the most sensitive parameter. Low ferritin always indicates storage iron depletion. To use ferritin as a diagnostic indicator, the following cut-offs have been set as indicators of iron depletion (WHO, 2011b).

Table 5: Serum iron concentration reflective of iron stores.

	Serum ferritin ($\mu\text{g/l}$)			
	Less than 5 years of age		Five years of age or older	
	Male	Female	Male	Female
Depleted iron stores	<12	<12	<15	<15
Depleted iron stores in the presence of infection	<30	<30	-	-

Adapted from WHO, 2011b

In the absence of genetic or metabolic disorders, causes of iron deficiency anaemia include insufficient dietary intake of iron, parasitic infections (malaria, hook worm and schistosomiasis are most common) and haemorrhage (Reinke *et al.*, 2012; Robinson *et al.*, 2006; WHO and CDC, 2005; Beard, 2001)

2.6.3 Haematological Indices for the diagnosis of Anaemia

The average adult has approximately 5.5 L of blood. Blood consists of plasma and cells. Plasma makes up 55% of the blood components and consists of proteins, water, and some

waste products. The cellular component is 45%. These are white blood cells (WBC) which has subtypes, RBCs (erythrocytes); and platelets (thrombocytes). All blood cells are produced in the bone marrow from a mother cell called the pluripotent stem cell. It undergoes stages of differentiation until it becomes committed to either the erythrocyte, thrombocyte, or one of the leukocyte subtypes (Tortora and Derrickson, 2012; Mader, 2004; Arthur and John, 2006).

The diagnosis of anaemia using blood relies on a number of parameters. These parameters may be divided into cellular components of blood and certain chemicals in the plasma. The cellular indices are red blood cell (RBC) count, haemoglobin concentration (Hb), haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV) and platelet distribution width (PDW). The biochemical indices are ferritin, transferrin, total iron binding capacity (TIBC), hepcidin, transferrin saturation and zinc protoporphyrin (WHO & CDC 2005). These indices and their importance are presented in Table 6

Table 6 Indices for the diagnosis of anaemia

Parameter	Indication
Haemoglobin	Anaemia
Haematocrit	Proportional volume of RBCs
Mean Cell Volume	Average size of RBCs. Small cells are microcytic, large cells are macrocytic
Mean Cell Haemoglobin	Haemoglobin in an average RBC. When low hypochromic.

Mean Cell Haemoglobin Concentration	Average haemoglobin in RBCs
Red Blood Cell Count	Number of RBCs in blood.
Red Cell Distribution Width	Abnormal sizes of RBCs. Value less than 11.5 or greater than 14.5 is termed anicytosis
Ferritin	Amount of iron stores
Serum Iron	Measure of iron supply to the bone marrow
Total Iron Binding Capacity (TIBC)	Total capacity of circulating transferrin bound to iron
Zinc Protoporphyrin	lack of iron to developing RBCs
Hepcidin	Regulation of iron absorption from the gut

Adapted from WHO, 2011b

2.6.4 Sports Anaemia

Sports anaemia describes an adaptive condition following strenuous exercise in which and expansion of plasma volume results in a drop in the concentration of haemoglobin and some other blood indices such as ferritin (Ottomano and Franchini, 2012; Wilkinson *et al.*, 2002; Eichner, 2001). The term sports anaemia is a misnomer because the haemodilution and resulting decrease in some blood indices is a positive adaptation to prevent haemoconcentration that occurs following sweating during exercise. It also results in an increased in cardiac stroke volume. In fact, it has no negative effect on performance (Mahan *et al.*, 2012). Eichner (2001) refers to it as pseudoanaemia, and Eric,

(1989) calls it athletes anaemia. It is not a pathological conditions and treatments such as use of iron supplements are not advised (Mahan *et al.*, 2012) Excessive sweating and an increase in lactic acid in tissues during exercise reduce plasma volume (Eichner, 2001). In response, the aldosterone, renin and vasopressin mechanism is activated to increase plasma volume. Also more albumin is added to blood (Nagashima *et al.*, 2000). The combined effect is an increase in plasma volume with the accompanying decrease in haemoglobin and ferritin concentration.

2.6.5 Iron status of Footballers

There is much research in the area of iron status of footballers in Europe and Latin America. Literature on iron status of sub-Saharan footballers was not found. Table 7 summarizes the findings of some research on selected mean iron indices.

2.6.6 Anthropometric Status of Footballers

Anthropometry of adults vary substantially due to many factors including genetics, race and diet. In soccer playing positions may be assigned based on the stature and size of a footballer alone before considering his talent. Also anthropometric characteristics of a team has been known to pre suggest their tactical approach to an opposing team.

Anthropometric status of most top footballers may have been modified by training to allow them achieve certain purposes in the game (Reilly *et al.*, 2000). Finding of various studies that investigated the anthropometric status of elite footballers are summarized in Table 8.

Table 7 : Iron indices of footballers in some countries.

Study	Country	Population of footballers	Haemoglobin (g/dL)	Hematocrit (%)	Mean Cell Volume(fL)	Ferritin ($\mu\text{g/l}$)
(Valtuna <i>et al.</i> , 2006)	Spain	Real Madrid junior team n=46	15.48 \pm 1.18	45.5 \pm 3.24	89.47 \pm 4.23	75.5 \pm 38.96
(Reinke <i>et al.</i> , 2012)	Germany	Bundesliga n=9	14.5 \pm 1.0	-	86.8	36.7 \pm 10.9
(Alper, 2013)	Turkey	2 nd Division league n=24	15.7 \pm 0.9	-	-	-
(Maria <i>et al.</i> , 2013)	Brazil	Club Athletico Ponte Preta(a 1 st Division Team) n=38	14.05 \pm 0.6	44.5 \pm 2.4	-	-
(Michailidis, 2013)	Greece	1 st Division league n=16	15.49	46.06	-	118.17
(Andelkovic <i>et al.</i> , 2015)	Serbia	Partisan Belgrade (1 st Division Team) n=19	15.2 \pm 2.1	46.2 \pm 0.05	86.81	69.0



Table 8 Anthropometric indices of footballers

Study	Footballers	Anthropometry (Mean \pm SD)			
		Height (cm)	Weight (kg)	Body Fat %	Muscle mass(kg)
Luiz et al. 2006	Sao Paulo, Brazil. n= 119	180.05 ¹	75.88 ¹	11.60 ¹	-
Ostojic 2003	1 st National League, Serbia. n=42	-	76.80 \pm 6.10	10.9 \pm 1.30	-
Marco et al. 2015	1 st Professional Division, Peru. n=44	180.00 \pm 0.1	74.90 \pm 5.60	13.30 \pm 2.90	-
Michailidis, 2013	Greek League, Greece. n=16	180.00 \pm 0.08	77.91 \pm 6.72	10.14 \pm 1.92	-
Maria et al. 2013	1 st Division league. Brazil. n=38	179.40 \pm 6.10	78.90 \pm 6.90	10.80 \pm 2.50	-

¹ Standard deviations (SD) were not given

2.7 EFFECT OF SPORTS ON IMMUNE CELL INDICES

The immune cells are the white blood cells (WBC) or leucocytes and the platelets. The leukocytes, also called white blood cells (WBC), are the mobile units of the body's protective system. They are formed partially in the bone marrow and partially in the lymph tissue. After formation, they are transported in the blood to different parts of the body where they are needed to protect the body against infection and other disease causing conditions. Platelets are formed from fragments of the cytoplasm of megakaryocytes in the bone marrow. (Arthur & John, 2006).

The types of WBC are neutrophils, eosinophils, basophils, lymphocytes and monocytes. Each type plays a role in body defense these together with the mechanism of haemostasis involving platelets are detailed elsewhere (Tortora and Derrickson, 2012; Mader, 2004; Sherwood, 2010).

It appears from literature that the effect of intense training on reductions in WBC counts is well reported and accepted as a physiological adaptation. However the mechanism and physiological advantage or disadvantage this phenomenon confers is unclear. It is yet to be explained how or why in the absence of underlying inflammation, exercise alone affects WBC populations (Pyne and Barnes, 2010; Nieman, 1998).

However, there are speculations from other studies that the reference ranges used in studies were not appropriate for athletes; or probably neutrophils were recruited to areas of inflammation following exercise thereby reducing circulating numbers or that high training affects haematopoiesis (Watson and Meiklejohn, 2001). Neutropenia has been reported in a mixed group of athletes of both sexes during a cross sectional study (Orysiak

et al., 2012), reduced WBC counts have been reported in exercising women (Johannsen *et al.*, 2012), neutropenia was reported in English Premier League footballers (Watson & Meiklejohn, 2001). There were no cases of neutropenia or low counts of WBC in *Serie A* footballers of Italy (Dolci *et al.*, 2003).

Anecdotes claim that reduced WBC count or neutropenia increased incidence of upper respiratory tract infections. However studies that reported neutropenia did not support the claim (Gannon *et al.*, 1997; Nieman, 1998).

2.8 CREATININE

Creatinine is an amino acid derivative with a molecular mass of 113 Da. It is a waste product of creatine and phosphocreatine (Murray *et al.*, 2003). Creatinine is formed from the irreversible non enzymatic dehydration of creatine with the loss of a phosphate. Almost all creatinine in the body is from creatine. The amount of creatine in the body depends on the amount of skeletal muscle in the individual (Arthur and John, 2006).

About 98% of all creatine is found in the muscles (Mahan *et al.*, 2012). Since creatinine levels depend on creatine levels, 24 hour creatinine excretion depends on the muscle mass. It has no known biologic function and must be cleared. It is almost entirely absorbed by the glomerulus. Therefore high levels may be indicative of impaired renal function or protein energy malnutrition (Mahan *et al.*, 2012). A low sodium diet increases creatinine clearance because of the angiotensin II secretion (Murray *et al.*, 2003).

Clinically, creatinine levels are used to estimate glomerular filtration rate. About 1.8g of creatinine is secreted daily which is cleared by the kidney. The normal muscle

concentration of total creatine is about 125 mmol/kg dry mass. About 2% of the body's creatine is converted to creatinine every day, resulting in the daily generation of creatinine at a fairly constant rate (Milic *et al.*, 2011).

In studying over 200 male and female athletes across various sports disciplines, it was observed that creatine levels in those athletes did correlate with their body mass index (BMI). That is as BMI levels increased, creatinine levels also increase (Banfi & del Fabbro, 2006).



CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was cross sectional

3.2 STUDY SITES

The study was carried out in 3 different locations in Ghana (Figure 3.1) which corresponded to the training ground of the three First Capital Plus Premier League (FCPPL) teams involved. The teams were Bechem United Football Club (BUFC) in Bechem (South Tano District, Brong Ahafo Region), Brong Ahafo United Football Club (BAFC) in Sunyani and Liberty Professionals Football Club (LPFC) in Dansoman, a suburb of Accra Metropolis in Greater Accra Region.

According to the Ministry of Local Government and Rural Development (MoLGRD) Sunyani is the regional capital of Brong Ahafo Region. It lies between Latitudes 7° 55'N and 7° 35'N and Longitudes 20° W and 20° 30'W (MoLGRD, 2014). Footballers of BAFC meet at the Sunyani Polytechnic soccer field for their training sessions. The institution is located along the Sunyani- Bechem road. Official matches of BAFC are played at the Sunyani Coronation Park located near the central market in Sunyani.

Bechem is the district capital of Tano South District in the Brong Ahafo Region. The district lies between latitudes 7°00'N and 7°25' N and between longitudes 1°45' W and 2°15' W (MoLGRD, 2014) . The Footballers of Bechem United Football Club stay in the Bechem Guest House which serves as their Club house. It is located along the Bechem-Kumasi road.

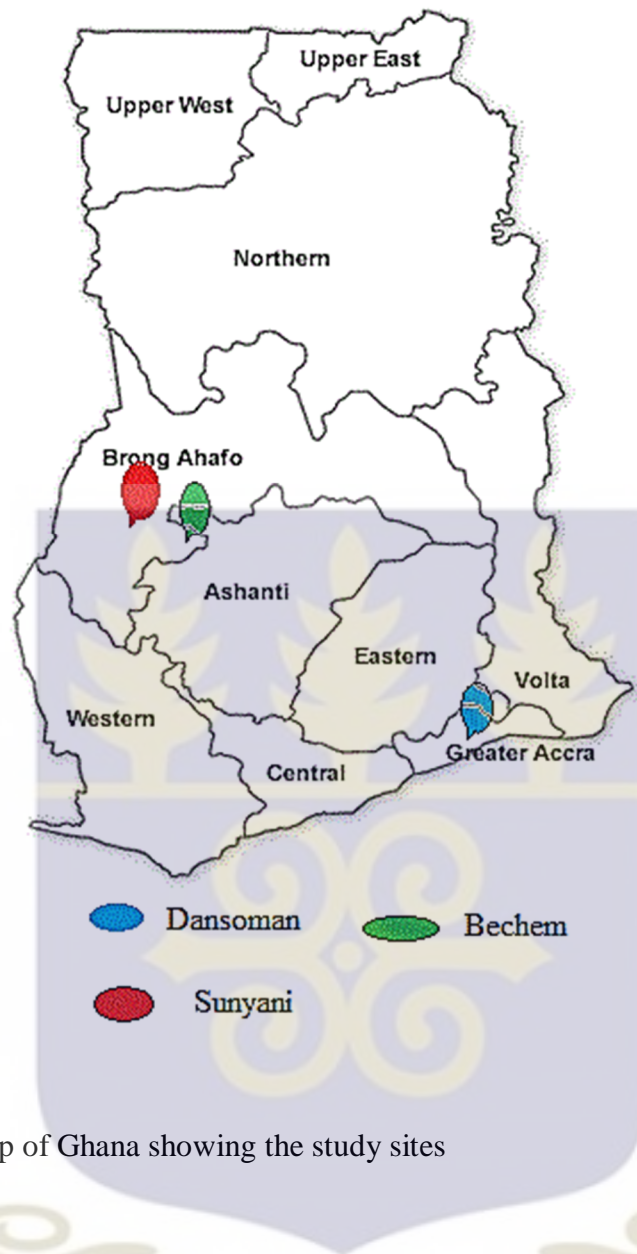


Figure 5 Map of Ghana showing the study sites

They also have a training park which doubles as an official match centre in the Bechem Township.

Dansoman is a suburb of Accra Metropolis. It is a residential area. The training pitch of LP FC is called the Karl Reindorf Park. It doubles as an official match centre. Karl Reindorf Park is near the Dansoman roundabout located around the market. |

3.3 DESCRIPTION OF RESEARCH SETTINGS

Data was collected from footballers (all males) in the three teams competing in the FCPPL in the 2014-2015 league season. Data collection for LPFC was done at the Karl Reindorf Park. At the park, data collection was done by the entrance to the dressing rooms. Tables and chairs were provided by the team management for data collection. Footballers were seated as they waited their turn. After data collection, they were given a snack.

In Sunyani, data collection for BAFC was done at their training pitch in Sunyani Polytechnic. Footballers were seated on benches provided by the institution. Because furniture was minimal, blood collection had to be done while footballers were seated on the benches. Anthropometric data was collected by a road very close to the training pitch. This was because the road provided the nearest source of a hard levelled surface from which accurate measurements of height and weight could be done. At the end of data collection, footballers were given a snack.

In Bechem, data collection for BUFC was done at the club house called Bechem Guest House. Data was collected in the one of the sheds at the club house. Footballers were called out from their room in turns for the data collection. To make adjustments for a meeting the team scheduled earlier, only blood collection was done at the team house. Anthropometry was done at the training pitch in Bechem town while the scheduled meeting went on. Anthropometric measurements were done in the dressing rooms.

3.4 TEAM RECRUITMENT AND SAMPLE SIZE

Teams were selected based on their willingness or interest to participate in the study.

The sample size was determined using the following formula for a population less than 10,000 (Araoye, 2003)

$$S = \frac{z^2 pq / d^2}{1 + \frac{(z^2 pq / d^2)}{N}}$$

n- Minimum sample size

S – sample size when population is >10,000

z- Standard normal deviate of 1.96 for a set confidence interval of 95%

p- Proportion of success. Arbitrary set at 0.50

q- Proportion of failure which is 0.50

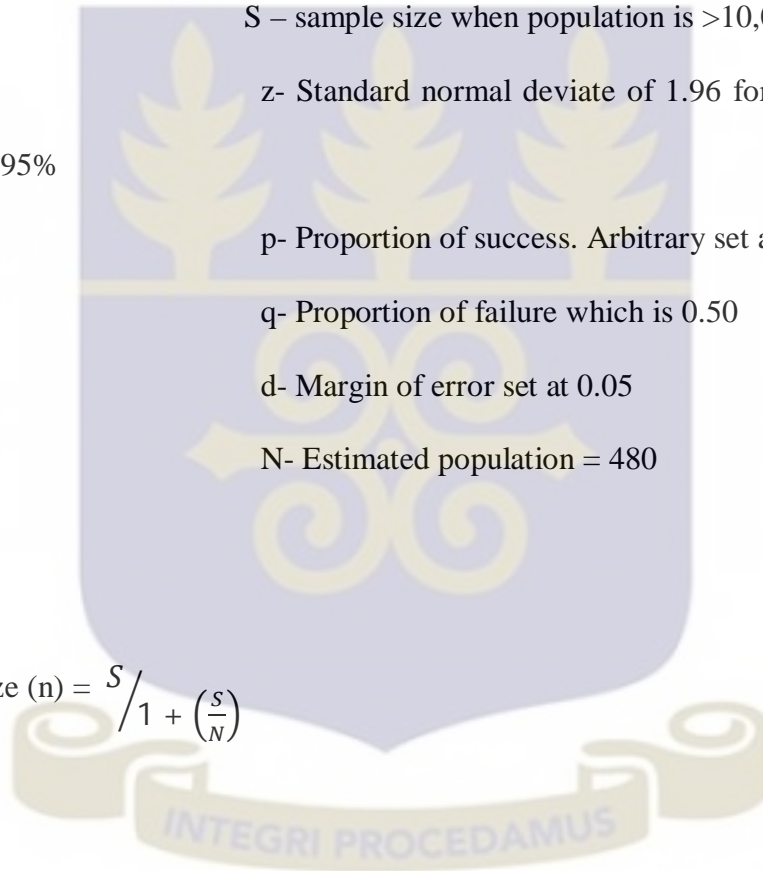
d- Margin of error set at 0.05

N- Estimated population = 480

S=213.

$$\text{Sample Size (n)} = \frac{S}{1 + \left(\frac{S}{N}\right)}$$

n =147



3.4.1 Inclusion Criteria

Only footballers in the selected teams that were registered to play in the premier league were included.

3.4.2 Exclusion Criteria

Footballers in a selected teams who were not registered to play in the premier league and any footballer though registered to play, reported sick or was injured.

3.5 ETHICAL APPROVAL

Ethical approval for the study was given by the School of Biomedical and Allied Health Sciences Ethical and Protocol Review Committee (Ethic identification number: SAHS-ET./10396158/AA/12A/2013-2014). Approval for the research was also given by the football regulatory body in Ghana, the Ghana Football Association (GFA) through the office of Premier League Board (PLB) of the GFA. The PBL provided letters which informed the teams about the research. On the field informed consent was obtained from team management and the footballers before data was collected.

3.6 PRE-TESTING OF TOOLS AND PROCEDURES

The tools to be used for data collection in the research except for blood collection were first tested on a 2nd division football team called Juventus in Korle-Bu, Accra. The team trains on the Nurses' and Midwives Training College (NMTC) soccer pitch on the Korle-Bu campus. The lesson learnt was that was that footballers were unwilling to spend time at data collection. Either they could not manage the burden of an extensive questionnaire or possibly, they found the questions too sensitive. They often referred most questions about themselves to their managers who were not available at the data collection sites. From this experience, only age, educational level and playing position on the field was demanded from them. All other questions were as much as possible directed to team management.

3.7 PROCEDURE FOR DATA COLLECTION

Data was collected at one meeting which preceded at least 2 days without strenuous exercise by the footballers. The footballers went through anthropometric measurements and blood collection. For all the 3 teams, data collection was done in the morning between 6-9am.

At each data collection site, anthropometry was carried out, blood samples were taken and team management filled a questionnaire. Once seated, a data collection sheet with a unique identification number for the footballer was given him. Data collection was done in this sequence;

- i. Blood samples were collected.
- ii. Height was measured.
- iii. Weight was measured
- iv. Body mass index (BMI) was calculated
- v. Body composition was determined
- vi. Calculations to estimate energy daily expenditure of the footballers was done later away from the training grounds.
- vii. A questionnaire was given to the team management to fill.

3.8 HAEMATOLOGICAL AND BIOCHEMICAL MEASUREMENTS

For the purpose of the research, full blood count, ferritin and creatinine levels were assessed.

3.8.1 Blood Collection Methods

Fasting blood samples were taken from study subjects after at least 48 hours since the last vigorous training or competitive activity. Footballers came from their homes to data collection sites were asked to sit and rest for at least 30 minutes. Blood was sampled (5ml) from the antecubital vein by a trained phlebotomist. Of the 5 ml of blood sample collected, 3 ml were aspirated into tri-potassium ethylene diamine tetra-acetic acid (K₃EDTA) or EDTA anticoagulant blood tubes for full blood count analysis and 2 ml were aspirated into fluoride/ heparin fluoride tubes. Blood samples were stored on ice packs until they were transported in an ice chest to the laboratory analysis.

3.8.2 Laboratory Methods

Blood samples in the EDTA tubes were analysed for full blood count (FBC) using an automated haematology analyser in a commercial laboratory. Blood samples in the heparin fluoride were centrifuged at 3500 rpm for 5 minutes to separate the serum. Sera were then pipetted into Eppendorf tubes and stored at -20°C until time of analysis. Ferritin and creatinine analysis were done on the sera using their respective automated analysers.

3.8.2.1 Full Blood Count

The SYSMEX[®] KX-21N automated haematology analyser (Sysmex Corporation, Japan) was used to do a full blood count (FBC) of all blood samples collected. The SYSMEX[®] KX-21N automated haematology analyser is a multi-parameter blood cell counter for *in vitro* clinical diagnostic use. FBC gives a clearer picture of the iron status than only haemoglobin measurements (Raik, 2004). The automated haematology analyser used processed and gave result in 19 parameters. These are;

1. WBC count in 1 mL of whole blood
2. RBC (red blood cell) RBC count in 1 mL of whole blood
3. HGB (Haemoglobin) Volume (gram) of haemoglobin in 1 dL of whole blood
4. HCT (Haematocrit value) Ratio (%) of whole RBC volume in whole blood
5. MCV (Mean RBC volume) Mean RBC volume (fL) in whole blood, which is calculated by Hct/RBC
6. MCH (Mean RBC haemoglobin) Mean haemoglobin volume (pg) per RBC, which is calculated by Hgb/RBC .
7. MCHC (Mean RBC haemoglobin concentration) Mean haemoglobin concentration (g/dL), which is calculated by Hgb/Hct .
8. LYM% [W-SCR] (WBC-Small Cell Ratio) Ratio (%) of lymphocytes (small cells) to whole WBC
9. MXD% [W-MCR] (WBC-Middle Cell Ratio) Ratio (%) of the summation of basophils, eosinophils and monocytes (middle cells) to whole WBC
10. NEUT% [W-LCR] (WBC-Large Cell Ratio) Ratio (%) of neutrophils (large cells) to whole WBC
11. LYM# [W-SCC] (WBC-Small Cell Count) Absolute count of lymphocytes (small cells) in 1 mL of whole blood
12. MXD# [W-MCC] (WBC-Middle Cell Count) Absolute count of the basophils, eosinophils and monocytes (middle cells) in 1 mL of whole blood
13. NEUT# [W-LCC] (WBC-Large Cell Count) Absolute count of neutrophils (large cells) in 1 mL of whole blood

14. RDW-CV (RBC distribution width - CV) RBC distribution width (%)
calculated from the points defining 68.26% of the entire area spreading from the peak of the RBC particle distribution curve.
15. RDW-SD (RBC distribution width - SD). The distribution width (fL) at the height of 20% from the bottom when the peak RBC particle distribution curve is taken as 100%.
16. PLT (Platelet) (Analysis principle: DC detection method) Platelet count in 1 mL of whole blood
17. PDW (Platelet distribution width). The distribution width (fL) at the height of 20% from the bottom with the peak of platelet particle distribution curve taken as 100%.
18. MPV (Mean platelet volume). Mean volume of platelet (fL)
19. P-LCR (Large platelet ratio) Ratio (%) of large platelet volume exceeding 12fL to the platelet volume.

3.8.2.2 Ferritin Assessment

Measurement of ferritin was based on the sandwich principle with a total duration time of 18 minutes. The instrument used was the Roche Elecsys-E170 modular analyser. The first incubation used 10 μL of each sample, a ferritin-specific antibody and a labelled ferritin-specific antibody to form a sandwich complex. The reagents were supplied by Roche Diagnostics in a ready-for use set. The second incubation occurred after the addition of micro particles that caused the complex to bind to the solid phase. The reaction mixture was aspirated into the measuring cell where the micro particles were magnetically captured onto the surface of the electrode. Unbound substances were then removed. Application of a voltage to the electrode induced a chemiluminescent emission which was

measured by a photomultiplier. All calculations were performed by the Hitachi Mod PE® Software system using a machine-stored calibration curve. Results were determined by observing a calibration curve. The concentration of ferritin was displayed on the screen and printed. The reference range of 17.9-464ng/L (males) was given by the laboratory.

3.8.2.3 Creatinine

The serum creatinine concentration was determined by using the Roche Modular P unit (Roche Diagnostics, UK). It is based on the principle of the Jaffe rate method (kinetic alkaline picrate). Specifically, the O'leiry modified Jaffe method was used to determine the concentration of creatinine. The creatinine calibration was done by using lyophilized human-serum-based Cfas calibrator (Roche Diagnostics) against an isotope dilution mass spectrometry (ID-MS) reference method. A precise volume of each sample (40 µl) was added to 1.27 ml of buffer reagent, and 40 µl added to 3.23 ml of reference reagent which was then introduced into a reaction cup containing an alkaline picrate solution. Absorbance readings were taken at 520 nm between 19 and 25 seconds after sample injection. Creatinine from the sample combined with the reagent to produce a red colour complex. The absorbance rate was the direct measure of the concentration of the creatinine in the sample. The Roche Modular P Unit performed all calculations internally to produce the final reported result. When all ordered tests were completed for each sample, the results were printed out by an external desktop computer. The reference range of 60-120µmol/L for males was given by the laboratory..

3.9 ANTHROPOMETRIC MEASUREMENTS

Anthropometric measurements were done on all study participants after blood sample collection.

3.9.1 Height

The Seca 213 portable Stadiometer (Seca Corp., Hamburg, Germany) was used to measure the height of the participants. It has a measuring range of 20-205 cm at 0.1 cm interval. Though the device was calibrated in centimetres, measurements were converted into metres at an interval of 0.01 m.

To measure the height, the stadiometer was set up according to the manufacturer's instructions. The footballer took out his footwear including socks if it were worn. He then mounted the stadiometer backwards so that his back was against the measuring rod of the stadiometer. The arms were held by the sides. The occiput, buttocks and heels came into contact with the measuring rod of the stadiometer. The feet were brought close together so that the ankles touched each other. The footballer was guided to stand erect looking straight forward with the head in the Frankfort position. The height was measured as the footballer inhaled deeply and held his breath. The height was measured by moving the scale of the rod to level with the top most part of the head and then reading the height from the rod. Approximations had to be made for those players with hair styles that hindered access to the top most part of the head.

3.9.2 Weight and Body Composition Measurement and Calculation of Body Mass Index (BMI)

The measurement of weight, calculation of BMI and the assessment of body composition were done with use of the Omron HBF-516B Body Composition Analyser and Electronic Scale (Omron Corp., USA). It measures weight to 0.01kg. Body fat and muscle mass were given as a percentage of the total body weight at intervals of 0.1%.

The Omron HBF-516B Body Composition Analyser and Electronic Scale is a tetra-polar bioelectrical impedance analyser (BIA). It has electrodes on the surface of the scale and on a hand held device with electrodes that is attached to the scale by a retractable cord. It works by passing a painless, imperceptible electrical current (500 μ A) at a fixed frequency of 50 kHz through the body while determining resistance and reactance. There was a display screen in which all measurements were read. To use the instrument to collect data, the footballer wore minimal clothes. His height (cm), age (years) and gender were manually entered into the instrument. BMI was calculated by dividing the weight in kilograms by the square of the height in meters or $BMI = \text{weight (kg)} / \text{height (m)}^2$.

All the footballers arrived in soccer shorts and sports shirts that were considered minimal. They willingly took off their jewellery, caps and arm bands. Each footballer mounted the scale bare footed with the sole of the feet in full contact with the electrodes. He then held the hand held device with bare hands and stretched his arms straight so that it was perpendicular to the body. The position was maintained for about 10 seconds allowing the device to measure the weight, calculate the BMI, measure percentage muscle and fat mass. These were then recorded. Muscle mass in kilograms was manually calculated as $(\% \text{ muscle mass} / 100) \times \text{weight of footballer in kilograms}$.

3.10 ENERGY EXPENDITURE ESTIMATION

Energy expenditure was estimated using the Schofield equation as adapted by the Food and Agricultural Organisation (FAO), World Health Organisation (WHO) and United Nations University (UNU) for the age group between 19 and 30 years for males (FAO, 2001). The factors involved in the calculation are age, weight, basal metabolic rate (BMR)

and physical activity level (PAL). PAL factor that match PAL of footballers during the league Season was 2.5 (FAO, 2001).

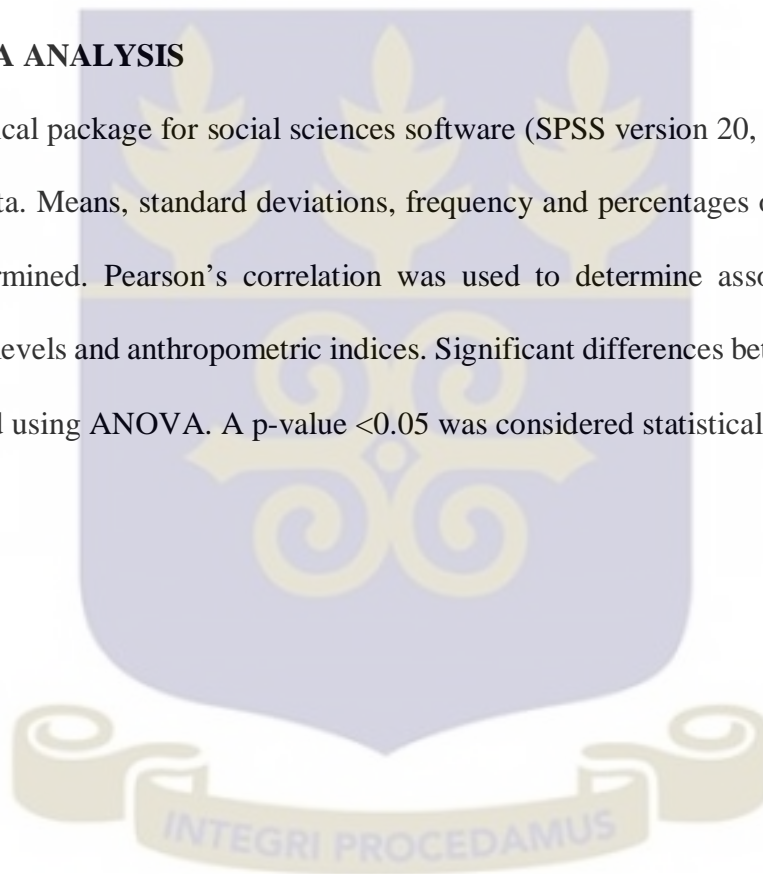
The is equation is give as:

$$\text{BMR} = 15 \times \text{Weight (kg) of Footballer} + 690$$

$$\text{Energy expenditure} = \text{BMR} \times 2.5$$

3.11 DATA ANALYSIS

The statistical package for social sciences software (SPSS version 20, IBM) was used to analyse data. Means, standard deviations, frequency and percentages of parametric data were determined. Pearson's correlation was used to determine associations between creatinine levels and anthropometric indices. Significant differences between means were determined using ANOVA. A p-value <0.05 was considered statistically significant.



CHAPTER FOUR

RESULTS

4.1 DEMOGRAPHIC DATA

The estimated sample size was 147 footballers. However 63 consented and were initially recruited for the study. Only 52 were able to complete all assessments and were used in the data analyses.

The demographic data of the footballers are presented in Table 9. Bechem United Football Club (BUFC) provided the highest number (44.2%) of footballers in this study. In terms of playing positions, mid-fielders (17) and full-backs (15) were in the majority. The mean age (years) of the footballers was 21.4 ± 2.7 SD. The youngest player was 15 years and the oldest was 27 years. Brong Ahafo United Football Club (BAFC) footballers were the youngest (19.6 ± 1.8 years) while oldest team was Liberty Professionals Football Club (LPFC) footballers (22.3 ± 2.7 years). All the footballers have had some amount of education and the majority (84.6%) of them had completed Senior High School (SHS).

4.2 ANTHROPOMETRY

There were no significant differences (all p s > 0.05) between any of the teams when height (m), weight (kg), BMI (kg/m^2), muscle mass (kg) and percentage body fat were compared (Table 10). Mean weight was highest in BAFC players (70.93 ± 5.03 kg). They also had the highest mean BMI of 23.59 ± 1.53 kg. Liberty Professionals Football Club players recorded the lowest mean BMI (22.44 ± 1.54 kg).

Mean muscle mass (kg), a measure of the absolute weight of muscles was $29.96 \pm 2.4\text{kg}$ in all footballers. The muscle mass of footballers in the 3 teams did not differ much. BUFC footballers recorded the highest mean percentage body fat ($15.75 \pm 2.93\text{kg}$).



Table 9 : Demographic characteristics of footballers.

Variable	Category	Team ¹ (n)			TOTAL
		BUFC (23)	LPFC (19)	BAFC (10)	
Age (Years), Mean ± SD		21.4 ±3.4	22.3 ±1.6	19.6 ± 1.8	21.4 ±2.7
Education Level	JHS	4	0	0	4
	SHS	17	18	9	44
	Tertiary	2	1	1	4
Usual Position	CB	2	4	0	6
Played*	FB	4	7	4	15
	GK	3	1	2	6
	MF	10	5	2	17
	F	4	2	2	8

¹ Bechem United Football Club (BUFC); Brong Ahafo United Football Club (BAFC); Liberty Professionals Football Club (LPFC)

*centre-back (CB), full-back (FB), goalkeeper (GK), midfielder (MF), forward (F)

Table 10: Anthropometric indices of footballers according to their teams

Team ¹	Anthropometric index (Mean ±SD)				
	Height (m)	Weight (Kg)	BMI (Kg/m ²)	Muscle mass (Kg)	% Body Fat
LPFC	1.75 ± 0.07	68.63 ± 7.12	22.44 ± 1.54	29.68 ± 2.38	15.02 ± 2.72
BUFC	1.75 ± 0.07	69.57 ± 8.09	22.73 ± 1.82	29.71 ± 2.62	15.75 ± 2.93
BAFC	1.73 ± 0.04	70.93 ± 5.10	23.59 ± 1.53	30.49 ± 2.18	15.56 ± 2.61
Total Mean	1.74 ± 0.70	69.89 ± 7.41	22.85 ± 1.74	29.94 ± 2.4SD	15.56 ± 3.03

¹ Bechem United Football Club (BUFC); Brong Ahafo United Football Club (BAFC); Liberty Professionals Football Club (LPFC)

4.3 HAEMATOLOGICAL ASSESSMENT

Values for the red blood cell indices for the 3 teams are shown in Table 11. There were no significant differences between the teams (all $ps > 0.05$). The overall mean for haemoglobin was 14.3 ± 0.8 g/dL. Low levels of haemoglobin were recorded in 8% of footballers. The mean MCV was for all footballers was low (79.98 ± 4.60 fL) and that for MCHC was high (36.15 ± 1.22 g/dL).

Table 11: Haemoglobin indices of footballer according to their teams

Red Blood Cell Index (Mean \pm SD)	Team ¹			Total Mean	Reference Range ²
	LPFC	BUFC	BAFC		
Hb (g/dL)	13.9 \pm 0.7	14.5 \pm 0.9	14.4 \pm 0.9	14.3 \pm 0.88	13-18
Hct (%)	38.7 \pm 2.0	39.8 \pm 2.1	39.9 \pm 1.0	39.4 \pm 2.0	39-52
MCV (fL)	80.96 \pm 5.09	79.13 \pm 5.28	79.30 \pm 1.70	79.98 \pm 4.60	80-95
MCH (pg)	29.12 \pm 2.3	29.19 \pm 3.12	28.65 \pm 1.30	29.13 \pm 2.40	27-32
MCHC (g/dL)	35.92 \pm 1.14	36.36 \pm 1.33	36.11 \pm 1.17	36.15 \pm 1.22	32-36
RBC $\times 10^6/\mu\text{L}$	4.70 \pm 0.69	5.09 \pm 0.48	5.03 \pm 0.26	4.93 \pm 5.6	3.50-5.50
RDW_SD/fL	42.35 \pm 2.54	41.47 \pm 3.33	36.66 \pm 13.70	40.86 \pm 6.68	39.0-46.0

¹ Bechem United Football Club (BUFC); Brong Ahafo United Football Club (BAFC); Liberty Professionals Football Club (LPFC)

² Reference ranges were set by the local laboratory used for the study

4.3.1 Leucocyte Indices

Absolute counts for white blood cells (WBC) and its deferential are presented in Table 12. There were no significant differences between the teams (all $ps > 0.05$). However low WBC counts and neutropenia were recorded in 19.2% and 23% of footballers, respectively.

Table 12 Leucocyte indices of footballers according to the teams

Leucocyte Index	² Team (Mean \pm SD)			Mean Total	¹ Reference range
	LPFC	BUFC	BAFC		
WBC $\times 10^3/\mu\text{L}$	4.55 \pm 1.14	5.06 \pm 1.03	5.39 \pm 1.02	4.94 \pm 1.09	4.0-10.0
LYM%	45.16 \pm 10.69	56.33 \pm 9.85	41.76 \pm 7.5	49.17 \pm 10.9	20-40
MXD%	11.93 \pm 5.6	12.91 \pm 7.11	14.33 \pm 5.47	11.75 \pm 6.06	1-15
NEUT%	43.92 \pm 12	40.23 \pm 16	42.01 \pm 7	41.15 \pm 12.96	50-70
LYM# $\times 10^3/\mu\text{L}$	2.03 \pm 0.63	4.78 \pm 9.36	2.22 \pm 0.46	2.42 \pm 0.72	0.6-4.1
MXD# $\times 10^3/\mu\text{L}$	1 \pm 0.54	1.07 \pm 0.54	1 \pm 0.78	0.74 \pm 0.39	0.1-1.8
NEUT# $\times 10^3/\mu\text{L}$	2.06 \pm 1	2.39 \pm 1	2.06 \pm 1	2.18 \pm 0.81	0.1-1.8

¹ Reference ranges were set by the local laboratory used for the study

² Bechem United Football Club (BUFC); Brong Ahafo United Football Club (BAFC); Liberty Professionals Football Club (LPFC)

4.3.2 Thrombocyte Indices

There were no significant differences between thrombocyte indices of the 3 teams (all $ps > 0.05$) (Table 13). However the mean PDW of all three teams (15.76 \pm 3.17 fL) were above the reference range. This was also observed in MPV (11.58 \pm 3.17 fL).

Table 13 Thrombocyte Indices of Footballers

Thrombocyte Indices	Team ² (Mean ±SD)			Mean Total	Reference Ranges ¹
	LPFC	BUFC	BAFC		
PLT	232.32±49	249.26±66	239.33±81	237.22±63.31	145-450
PDW /fL	14.92±2.59	16.78±2.57	15.58±5.21	15.76±3.17	10.0-14.0
MPV /fL	11.05±0.95	11.69±0.66	12.76±4.37	11.58±3.17	7.4-10.4
P_LCR(%)	32.55±9.2	37.84±8.45	36.13±12.18	35.20±9.46	13-43

¹ Reference ranges were set by the local laboratory used for the study

² Bechem United Football Club (BUFC); Brong Ahafo United Football Club (BAFC); Liberty Professionals Football Club (LPFC)

4.3.3 Abnormal Haematological Indices

Eight percent of the footballers had haemoglobin levels below the reference range. These were also microcytic and hypochromic. In the absence of anaemia (Hb < 13.0 g/dL), some haematological indices did show abnormalities which are usually presented with anaemia or other pathologies associated with blood.

For MCV, 38.5% of footballers had values below the reference range in the absence of anaemia. In the case for MCH, 13.5% had low levels with normal haemoglobin concentration. Again 32.5% of footballers reported RBC count above the reference range. Red cell distribution width(RDW) had 19.% reporting values that were below the reference range and 11.5% reporting above the reference range. About 8% of footballers recorded platelet counts below the reference range. Platelet distribution width (PDW) and mean platelet volume (MPV) each reported 58% footballers to be above the reference range. (see table 14)

Table 14 Abnormal Blood indices

Parameter	Reference Range¹	% Below	% above
MCV	80-95fL	38.5	0
MCH	27-32pg	13.5	0
RBC	3.5-5.5×10 ⁶ /μL	0	32.5
RDW	39-46fL	19	11.5
PDW	10-14fL	0	58
MPV	7.4-10.4	0	58
WBC	4-10× 10 ³ /μL	25	0

¹ Reference Ranges were set by the local laboratories used for the study

4.4 BIOCHEMICAL ASSESSMENT

4.4.1 Creatinine

The mean serum creatinine levels for all footballers studied was 104.23± 16.35. There was a significant difference between the three teams ($p<0.05$). The mean creatinine level for LPFC was 113.79 ±13.93, that for BUFC was 100.65 ±9.39 and that for BAFC was 94.32 ±8.8 (Table 15). However, 17% of footballers had levels above reference value levels of creatinine (60-120μmol/L).

4.4.2 Ferritin

The mean serum ferritin levels for all footballers studied was 95.68±11.81 ng/mL. . There was a significant difference in ferritin levels between the 3 teams. Bechem United Football Club (BUFC) had a mean serum ferritin level of 100.10 ±9.39. Liberty

Professionals Football Club (LPFC) had a mean creatinine level of 90.44 ± 13.93 and BAFC had a mean ferritin level of 95.49 ± 8.80 . (Table 15)

Table 15: Biochemical indices of footballers in the 3 teams

Team ⁴	¹ Creatinine ($\mu\text{mol/L}$)	² Ferritin (ng/mL)
	Mean \pm SD	Mean \pm SD
LPFC	113.79 \pm 12.69	90.44 \pm 13.93
BUFC	100.65 \pm 17.80	100.10 \pm 9.39
BAFC	94.32 \pm 9.10	95.49 \pm 8.8
Mean Total	104.23 \pm 16.35	95.68 \pm 11.81
³Reference range	60-130	17.9-464.9

¹*p-value*=0.002

²*p-value*=0.027

³ Reference ranges were set by the local laboratory used for the study

⁴Bechem United Football Club (BUFC); Brong Ahafo United Football Club (BAFC); Liberty Professionals Football Club (LPFC)

4.4 ESTIMATED DAILY ENERGY EXPENDITURE

Daily energy expenditure according to the teams is presented in Table 16. Brong Ahafo United Football Club (BAFC) had the highest mean energy expenditure w (4384.42 ± 191.24 kcal/day) and lowest in LPFC (4296.12 ± 268.30 kcal/day). There was no significant difference between the teams ($p > 0.05$).

Table 16 Estimated daily energy expenditure of footballers according to their teams

Team ²	Est. Energy Expenditure/kcalday ⁻¹		
	Mean ±SD	Maximum	Minimum
LPFC	4296.12±268.30	4815.00	3787.50
BUFC	4332.88±303.97	4965.00	3888.75
BAFC	4384.42±191.24	4717.50	4012.50
Mean total	4329.36±269.69	3787.50	4965.50

² Bechem United Football Club (BUFC); Brong Ahafo United Football Club (BAFC);
Liberty Professionals Football Club (LPFC)

4.5 ASSOCIATION BETWEEN BIOCHEMICAL AND ANTHROPOMETRIC INDICES

4.5.1 Correlation between creatinine and anthropometric indices

Serum creatinine levels were correlated with weight, muscle mass and BMI. No significant correlations were found (All *ps* >0.05). Both weight and muscle mass showed a weak negative correlation ($r = -0.078$) with serum creatinine. There was a weak positive correlation ($r = 0.033$) between serum creatinine levels and BMI. .

4.5.2 Correlation between ferritin and anthropometric indices

No significant correlations were found (all $ps > 0.05$). There were weak negative correlations between ferritin and weight ($r = -0.083$), BMI ($r = -0.127$) and muscles mass ($r = -0.004$)



CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSIONS

5.1 DISCUSSION

The work assessed the energy expenditure, iron status and serum creatinine levels of premier league footballers in Ghana Fifty-two elite footballers from 3 different premier league teams were studied during the 2014/2015 competitive season.

The mean age (years) of the footballers in this study was 21.4 ± 2.7 years. This suggests that these footballers are still young and some may still be in their growing years. However, the ages could not be verified.

The relevance of anthropometry in soccer goes beyond knowledge of size and stature. Anthropometric status may predict soccer performance especially as the league season progresses. It gives information about muscle build in response to training and excess fat stores that may reduce performance (Medina *et al.* 2014). Height may be important in football especially in those positions where the footballer will need to win the ball in the air. These positions are the goalkeepers and the centre-backs. The mean height (m) of the footballers studied was 1.74 ± 0.7 m. Marco *et al.* (2015) reported that elite footballers in the Peruvian 1st National League had a mean height of 1.80 ± 0.1 m. Similarly, Michailidis (2013), reported the mean height of Greek elite footballers to be 1.80 ± 0.8 m and Luiz and his colleagues (2006) found a mean height of 1.80 m (no SD was provided) in elite Sao Paulo footballers (Brazil). It therefore appears that the Ghanaian footballers studied were shorter than their colleagues in Greece, Peru and Brazil.

Weight has an effect on the power of a footballer. It also determines his agility and strength. Ghanaian footballers in this study had a mean weight of 69.89 ± 7.4 kg. Footballers in equivalent leagues in other countries reported higher mean weights. In Greece, Michailidis (2013) reported a mean weight of 77.91 ± 6.72 kg. Peruvian footballers had a mean weight of 74.90 ± 5.60 kg (Marco *et al*, 2015). In Brazil, Maria and her colleagues (2013) reported a mean weight of 78.90 ± 6.90 kg. Ghanaian footballers therefore have lower weights. With all the advantages conferred on being taller and reasonably heavier, talented shorter smaller players can also perform well. Lionel Messi was 1.60 m tall and 70 kg when he won his 4th world best footballer award (FIFA, 2015b).

Body fat acts as a dead weight in sports though sufficient fat stores are essential for health. Low fat levels (<10%) have been implicated in sex hormone imbalances and reduced fertility in men and women (Brown, 2011). The mean percentage body fat for the premier league footballers was 15.56 ± 3.0 . Greek footballers were reported to have a mean body fat of 10.14 ± 1.92 (Michailidis, 2013). Again, Brazilian footballers mean body fat percentages between 11.60 (Maria *et al.*, 2013) and 10.80 ± 2.50 (Luiz *et al.*, 2006). The differences may be attributed to the fact that for the footballers in this study Ghana BIA was used whilst skinfold was used in the assessment of body fat in these other studies. Otherwise it suggests that Ghanaian footballers in this study may have more fat per weight than their counterparts in those other studies stated.

There were no significant differences (all $ps > 0.05$) between haemoglobin, haematocrit, mean cell volume, mean cell haematocrit and mean cell haematocrit concentration between the three teams in this study. The mean haemoglobin for the footballers studied

was 14.30 ± 0.88 g/dL. This was not very different from the 14.5 ± 1.0 g/dL reported by Reinke and his colleagues (2012) among Bundesliga (German top league) players. Players of Club Athletico Ponte Preta in Brazil also recorded a mean haemoglobin of 14.05 ± 0.6 g/dL (Maria *et al.*, 2013). In contrast, Valtuena and his colleagues (2006) reported higher mean haemoglobin levels in footballers of Real Madrid (15.48 ± 1.18 g/dL). In Turkey, Alper (2013) also found higher haemoglobin levels in elite 2nd division league players (15.7 ± 1.0 g/dL). Low levels of haemoglobin in footballers and other elite athletes could be due to haemodilution that results from intense training over a period of time (Ottomano and Franchini, 2012).

The mean haematocrit levels in the premier league footballers in this study was $39.4 \pm 2.0\%$. These were lower when compared to values of footballers elsewhere. Real Madrid players reported $45.5 \pm 3.324\%$ (Valtuena *et al.*, 2006), Greek footballers reported 46.06 (Michailidis, 2013) and footballers of Partizan Belgrade in Serbia had $46.2 \pm 0.05\%$ (Andelkovic *et al.*, 2015).

Mean cell volume values (79.98 ± 4.6) also showed that premier league footballers in this study had lower values compared to their counterparts in other countries. The mean value for Spanish footballers (Real Madrid) was 89.47 ± 3.24 fL (Valtuena *et al.*, 2006) and that for Serbian footballers was 86.81 fl (Andelkovic *et al.*, 2015). Though four players in this study had low haemoglobin levels (<13 g/dL) with low MCV, others had low MCV with normal haemoglobin levels (38.5%). In the absence of anaemia MCV is low during inflammation (WHO & CDC, 2005). Inflammation however transient may exist due to the high training load imposed on the professional footballers which leads to muscle soreness (Baird *et al.*, 2012). In those four footballers that showed low haemoglobin, MCV and MCH which were indicative of iron deficiency anaemia, ferritin levels were

normal. In marked anaemia, ferritin levels remain normal or elevated in the presence of inflammatory conditions (WHO 2011b). Therefore, low MCV in the absence of anaemia and high ferritin levels in the presence of marked anaemia indicate a high chance of marked injury or inflammation in the footballers albeit subclinical.

Exercise has been known to be associated with reduced WBC counts and the level of fitness resulting from exercise to be inversely related to WBC counts (Johannsen *et al.*, 2010). The reduced counts of circulating WBC in the 25% of footballers observed in this study, similar to that found in elite English footballers (Watson & Meiklejohn, 2001), could be exercise induced. Since the footballers in this study are involved in competitive matches, this finding was not surprising. High levels of PDW and MPV were observed in 58% of footballers in this study.

Low RBC counts in 32.5% of footballers studied in the absence of anaemia (Hb <13g/dL) could be due to foot strike haemolysis and intravascular haemolysis (Tobal *et al.*, 2003). As footballers kick the ball, run and get involved in collisions, there is the destruction of RBCs in the capillaries in contracting muscles. Haemodilution resulting from their intense training and competitive sessions may also reduce the numbers of circulating RBCs per microliter of blood (Ottomano and Franchini, 2012).

Creatinine levels between the teams studied was found to be significantly different. The mean serum creatinine levels for all footballers studied was $104.23 \pm 16.35 \mu\text{mol/L}$. This may not be very different when compared to that in professional Brazilian footballers found to be $143.21 \mu\text{mol/L}$ (Silva *et al.*, 2008). Creatinine levels were above the reference range in 9 footballers. This is in line with a finding that among professional Brazilian

footballers, soccer specific training did increase serum creatinine levels. Also as creatinine level rose, soccer specific performance decreased (Silva *et al.*, (2008).

Contrary to findings that BMI correlated positively with creatinine levels (Banfi & del Fabbro, 2006; Milic *et al.*, 2011), this study failed to find a significant correlation between creatinine with BMI, creatinine with muscle mass, and creatinine with weight. Although the Jaffe method was standardised with the reference method based on isotope dilution-mass spectrometry (IDMS), it is still possible that there could have been a significant margin of error in the values recorded (Peake and Whiting, 2006). Again the findings that reported these correlations studied other athletes, did not include footballers and the participants were of different races not African. An omnivorous diet will alter normal creatinine levels because meat contains creatine, a precursor of creatinine (Mahan *et al.*, 2012). As this study did not assess dietary intakes, such a possibility remains. Findings from this study did oppose the assertion made by Mahan and her colleagues (2012) that creatinine levels increased with increasing muscle mass. However, it was in line with the assertion that weight did not correlate with serum creatinine levels (Mahan *et al.*, 2012).

Ferritin showed very weak negative correlations with weight, BMI and muscle mass ($p>0.05$). This was because ferritin levels are indicators of iron stores and inflammation (Arthur and John, 2006). It was therefore not likely to be affected by anthropometric indices.

The mean energy expenditure of the footballers in this study was 4329.36 ± 269.69 kcal/day. A study of 7 professional Japanese footballers by Ebine *et al.*, (2002) using the doubly labelled water method reported mean daily energy expenditure to 3532 ± 408

kcal/day. Asians are smaller than Africans. However these footballers (Japanese) had a mean height and weights close to those of this study. The Japanese footballers had 1.75 m mean height and 69.8 kg mean weight. In this study mean height was 1.74 m and mean weight was 69.89 kg. Size and stature could therefore not be a factor for the observed difference. Japan is a developed country, physical activities levels in developed countries are lower than in developing countries such as Ghana (FAO, 2001). Thus the Japanese footballers probably did fewer activities apart from playing football as compared to the premier league players who sometimes cover miles walking to training grounds. Again, the predictive equation used has its shortcomings. It has been found to overestimate and underestimate BMR by about 20% (Muller *et al.*, 2004). In studies where energy intakes were found to be less than 3,200 kcal/day, investigators often suspected under reporting on the part of footballers (Maughan, 1997; Leblanc, 2002; Costas *et al.*, 2009; Luiz *et al.*, 2006). Therefore if the predictive equation overestimated daily energy expenditure the premier league footballers, it is reasonable to be certain that daily energy expenditure exceeds 3,200 kcal.

The limitation of the study were:

1. Sample size was small
2. Data collection was minimal because the study could apply FFQ and 24-hour diet recall on footballers.
3. Iron status assessment did not include serum iron, transferrin and total iron binding capacity which would have given a more detailed information.

5.2 CONCLUSION

The study found the mean height of premier league footballers to be 1.74m and the mean weight to be 69.89kg. For a game as football, higher weights and heights would have been expected. Surprisingly the footballers studied had a high percentage of body fat (15.56%). Mean ferritin levels was 95.68 ± 11.81 ng/mL. Iron deficiency as indicated by low ferritin levels was not found in the footballers studied. However 8% did record low Hb levels. Majority of footballers (58%, n=32) did record at least one haematological finding that indicated some form of pathology. However this could be a physiological adaptation. Seventeen percent (n=9) of footballers have elevated creatinine levels ($>160 \mu\text{mol/L}$). Mean daily energy expenditure of premier league footballers was 4329.36kcal. There was no significant correlation between serum creatinine levels and anthropometric indices but levels did differ significantly between the teams.

It is recommended that:

- i. Anthropometric measurements of footballers should be done at intervals throughout the season to monitor alterations in weight and body composition.
- ii. Literacy is almost universal in footballers. Literature on proper nutrition for sports performance should be simplified and used to educate them.
- iii. Haematological measurements should be carried out at intervals to assess iron status of footballers.
- iv. More research is needed to gather data on footballers for dietetic practice and for the setting of reference values and for creatinine.

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APPENDIX 1

Ethical Clearance Letter

**SCHOOL OF ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA
ACADEMIC AFFAIRS**

Phone: +233-0302-687974/5
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P. O .Box KB 143
Korle Bu
Accra
Ghana

7th July, 2014.

Mr. Richard Nabia,
Dept. of Dietetics,
SAHS,
Korle Bu.

Dear Mr. Nabia,

ETHICS CLEARANCE

Ethics Identification Number: SAHS – ET. /10396158/AA/12A/2013-2014.

Following a meeting of the Ethics and Protocol Review Committee of the School of Allied Health Sciences held on Monday 24th March, 2014, I write on behalf of the Committee to approve your research proposal as follows:

TITLE OF RESEARCH PROPOSAL: “Energy Balance and Iron Status of Premier League Footballers in Ghana”

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Committee on completion of the research. The Committee may observe the procedures and records of the research during and after implementation.

Please note that any significant modification of the research must be submitted to the Committee for review and approval before its implementation.

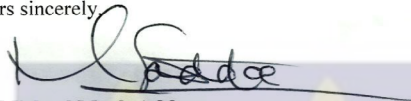
You are required to report all serious adverse events related to this research to the Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee’s duty to review the ethical aspects of any manuscript that may be produced from this research. You will therefore, be required to furnish the Committee with any manuscript for publication.

Please always quote the ethical identification number in all future correspondence in relation to this protocol.

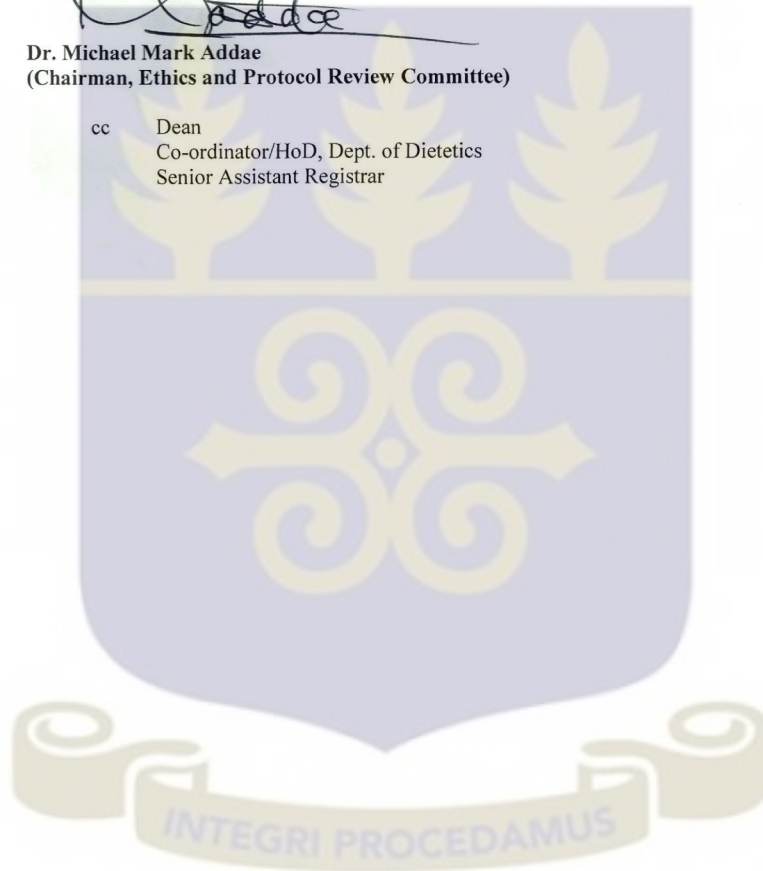
Thank you.

Yours sincerely,



Dr. Michael Mark Addae
(Chairman, Ethics and Protocol Review Committee)

cc Dean
Co-ordinator/HoD, Dept. of Dietetics
Senior Assistant Registrar



APPENDIX 2

Participant Information Form

RESEARCH SUMMARY.

1. Richard Nabia, Department of Nutrition and Dietetics, Univ. of Ghana. Tel. 0208562847
2. Charles Brown Ph.D., Department of Medical Laboratory Sciences, Univ. of Ghana
3. Nana Kofi MPhil R.D., Dietetics Department, Korle-Bu Teaching Hospital.

Title: Energy Balance and Iron Status of Premier League Footballers

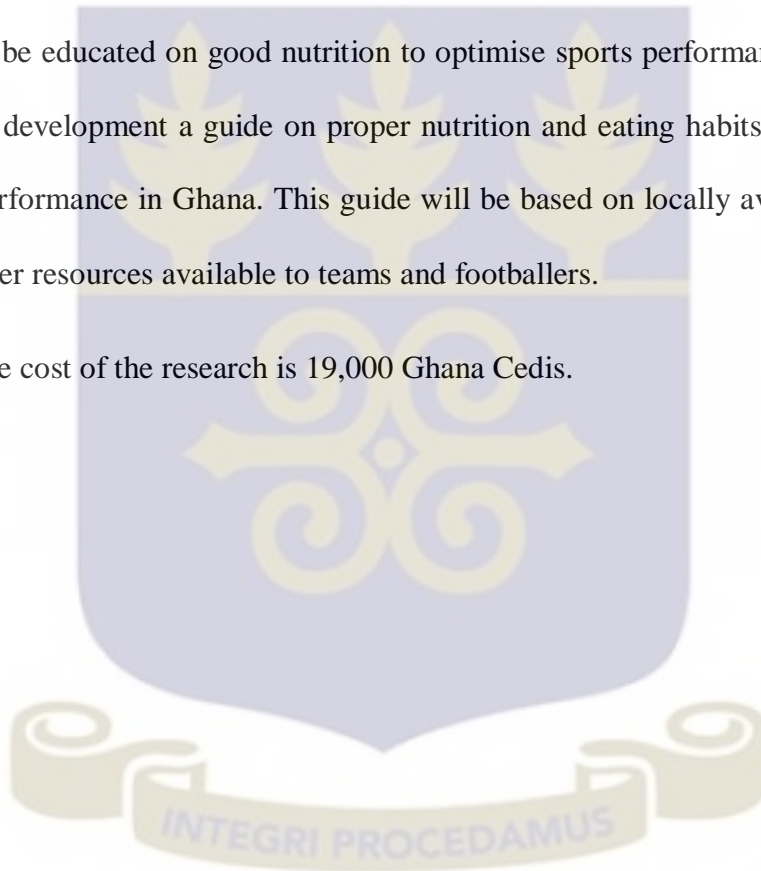
Brief Background: Sufficient energy intake is needed for sustained performance throughout the season. Adequate iron in tissues enables good oxygen to muscles which is required for energy release from food. Worldwide, iron is deficient in many people including footballers. Without an adequate energy intake and normal iron status, footballers fatigue easily, sustain injuries more frequently and loose concentration in the playing field.

Purpose of Research: The research will quantify the energy intake and energy expenditure of footballers .Based on that , it will calculate whether there is a deficit or excess intake in each individual footballer. It will also find out whether the amount of iron in the blood of footballers is up to standards required for normal healthy lives and sports.

Methods: The research will be done on footballers that have been registered to play in the premier league this season (2015). They will be asked to answer questions about their eating patterns only. A small amount of blood will be drawn for investigating their iron status. All findings will be confidential. Weight, height and body fat will be measured. No names will be used. Only codes and numbers. The research will start February, 2015 and will be completed by July, 2015

Outcome/Benefits: Footballers taking part will come to know their nutritional status and teams will be educated on good nutrition to optimise sports performance. Findings will be used to develop a guide on proper nutrition and eating habits that can improve football performance in Ghana. This guide will be based on locally available foods and will consider resources available to teams and footballers.

Budget: the cost of the research is 19,000 Ghana Cedis.



APPENDIX 3

Consent Form

University of Ghana College of Health Sciences

Research Title: **Energy Balance and Iron Status of Premier League Players in Ghana**

Team Name: Bechem United

We understand that the participation of our team in this research is voluntary and free. We have been assured that the footballers are not going to be subjected to any risk or danger.

We have been informed that the confidentiality of the information will be safeguarded and that the privacy and anonymity of the individual footballers will be ensured in the collection, storage and publication of the research material. We have the right to refuse to participate at any time we wish to.

We have read the information provided. All questions have been answered to our satisfaction. We consent voluntarily to participate in this study.

Team Representative Signature:.....

Date:.....

Signature of researcher:.....

Date:.....

APPENDIX 4

Team Information Sheet

THE UNIVERSITY OF GHANA COLLEGE OF HEALTH SCIENCES

QUESTIONNAIRE

RESEARCH TITLE: ENERGY BALANCE AND IRON STATUS OF PREMIER LEAGUE TEAMS IN GHANA

This form is to be completed to be completed by team management

Tick the space provided or write where applicable

Team Name ;

Location:

1 **No. Of Players registered for Premier League Competition.....**

2 **No. Of Players Recruited For Study.....**

3 **Specify professional titles of Health Professionals Assigned contracted/hired/employed if any. (Do not write names of persons)**

1

3

2

4

4 **Specify if Team Feeds Players** Yes () No ()

5 **If yes to number 4 above, Specify** Only on 'away'() Always ()

Others () Kindly Specify

.....
.....
.....
.....

6 **If yes to number 4 above, State number of times daily.**

7 **Has the team contacted Catering Service Provider(s)?** Yes ()
No ()

7 **Does the team provide accommodation for Footballers** Yes ()
No ()

8 **If yes to No. 7, are footballers living** together at one place? ()

Or at individual locations () Or in Groups but Different Locations ()