UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

NUTRITIONAL HABITS AND THE RELATIONSHIP WITH RELATIVE ENERGY DEFICIENCY IN SPORT SYNDROME (RED-S) OF FEMALE ENDURANCE RUNNERS IN JOHANNESBURG

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Declaration

I, Marizanne Pienaar, student number 1688958, declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature of student……………………………Date ……14 January……2023

Name of primary supervisor: Prof Demitri Constantinou

Abstract

Introduction: Many female runners limit their nutritional intake to maintain or achieve a specific body weight or goal. Calorie restriction and dieting may cause weight loss, which will inevitably reach a plateau after prolonged low energy intake. Some athletes will decrease energy intake even further to continue to lose weight and “this behaviour will lead to a downward spiral of caloric restriction, losing weight, and plateauing followed by another cycle — all of which will ultimately result in LEA and likely in the development of an eating disorder” (Wasserfurth et al., 2020, p. 5). Long-term energy availability (EA) can affect bone health negatively, putting these athletes at a higher risk of osteoporosis, incidence, and prolonged recovery from stress fractures, affecting participation and delaying their return to sport. Relative energy deficiency syndrome (RED-S) is well known in female athletes in various sports. RED-S includes and is not limited to, impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health. Although more common in females, RED-S occurs in both males and females. Low energy availability (LEA) is a big factor of RED-S, which is caused by insufficient nutritional intake. An appropriate diet would consist of an adequate intake of macro-and micronutrients that will match an athlete’s needs accordingly. Energy balance is achieved when energy intake matches total energy expenditure, therefore energy balance = dietary intake – exercise expenditure.

Aim of study: The aim of the study included determining nutritional habits, current nutritional status, energy balance, associations between nutrition and energy status, and risk of RED-S of female endurance runners in Johannesburg, South Africa.

Study design and methodology: The study design was quantitative, descriptive, and exploratory. The study population comprised 27 female endurance runners, including recreational and elite runners, residing in Johannesburg, South Africa. Ethical clearance was obtained from the University of Witwatersrand’s Medical Human Research Ethics Committee. Outcome measures included two questionnaires and venous blood sampling to test ferritin, magnesium, vitamin D and calcium. The data from the questionnaires were analysed along with the venous blood sample results. Statistical analysis was performed using Microsoft Excel, SPSS version 28.0 and the REDCap system version 11.4.3. Each participant was scored with the LEAF-Q scoring key system (risk for low energy availability).

Results: Eleven participants were at risk for the female athletic triad, which is also a risk for RED-S. Twenty-two (81%) out of 27 participants were under-eating or eating with a large daily calorie deficit. Ten runners (37%) had a sFer level (iron marker) of below 35 ng/mL and one participant as low as 9 ng/mL, indicating iron deficiency with anaemia. Vitamin D levels were normal in all but one participant, magnesium levels were in range for all participants, and seven out of 27 (26%) participants had low calcium levels.

Conclusion: The results showed that in our study most female runners do not balance their energy intake with energy expenditure, which leads to LEA and may cause a decrease in performance, which could have other health consequences. Numerous female athletes are still not well informed of RED-S and the risks associated with it, as many are unaware that they are at risk for RED-S. Dietary recommendations, education about RED-S and recommendations for minimising the risk of RED-S for female runners are put forward.

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25[OH]D – 25-hydroxyvitamin D

ALA – α-linolenic acid

ATP – Adenosine triphosphate

BMD – Bone mineral density

BMI – Body mass index

BMR – Basal metabolic rate

CAT – Clinical assessment tool

DHA – Docosahexaenoic acid

EA – Energy availability

EPA – Eicosapentaenoic acid

EU – Eumenorrheic

FFM – Fat-free mass

FHA – Functional hypothalamic amenorrhoea

FSH – Follicle-stimulating hormone

GI – Gastrointestinal

GnRH – Gonadotropin-releasing hormone

Hb – Haemoglobin

IGF-I – Insulin-like growth factor

IOC – International Olympic Committee

ITBS - Iliotibial band syndrome

LBM – Lean body mass

LEA – Low energy availability

LEAF-Q – Low Energy Availability in Females Questionnaire

LH – Luteinising hormone

MD – Menstrual dysfunction

OCPs – Oral contraceptive pills

PAL – Physical activity level

PTH – Parathyroid hormone

REDCap – Research Electronic Data Capture

RED-S – Relative energy deficiency in sports syndrome

RDA – Recommended daily allowance

sFer – Serum ferritin

TDEE – Total daily energy expenditure

TEE – Total energy expenditure

T3 - Triiodothyronine

CHAPTER 1. INTRODUCTION

1.1 Background

The female athlete triad is a well-known occurrence in female athletes in various sports. In more recent years, a broader and more comprehensive term, Relative Energy Deficiency in Sports Syndrome (RED-S), has been introduced. The female athlete triad consists of three elements, namely menstrual dysfunction (MD) or amenorrhoea, low energy availability (LEA) and low or altered bone mineral density (BMD) (Nazem & Ackerman, 2012). The traditional female athlete triad forms part of RED-S, alongside eight other components. RED-S also includes, but is not limited to, impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health (Mountjoy et al., 2018). Although RED-S occurs more commonly in females, it occurs in both females and males (same syndrome without the menstrual effects).

Many female runners limit their nutritional intake to maintain or achieve a specific body weight or goal. Low nutritional intake along with the high energy expenditure of training may lead to overall lower body weight and body fat and causes low calcium levels that may result in low bone density of the athlete. Low bone density could lead to stress fractures, prolonged recovery from injury, and decreased performance. Low energy availability A can furthermore negatively affect a runner’s muscle mass, cardiovascular function, neuromuscular function and recovery from injuries and illnesses (Melin et al., 2019).

Female endurance runners require sustainable nutrition for intense training and optimal performance in endurance events. Training for such endurance events also requires an athlete's body to recover swiftly from training to compete and vice versa. A well-balanced nutritional plan for an endurance runner would include sufficient vitamins, minerals, and macro- and micronutrients.

Many female endurance runners are unaware of RED-S and the effects it can have on their performance and recovery. This study explored the nutritional habits, running behaviour and biological marker measurements of ferritin, magnesium, vitamin D and calcium of female endurance runners and their association with RED-S in Johannesburg, South Africa.

1.2 Problem statement

Female endurance runners may be at risk of RED-S and the effects thereof. The risk and prevalence of RED-S are unknown in Johannesburg female endurance runners.

1.3 Research question

Do female endurance runners in Johannesburg have risks for or have RED-S, and do they have sustainable nutritional habits for their running behaviour?

1.4 Study aims

The aims of the study were:

- To determine the nutritional habits and status of Johannesburg female endurance runners;
- To determine the energy balance of Johannesburg female endurance runners;
- To examine associations between nutrition and energy status of female endurance runners in Johannesburg.

1.5 Study objectives

The objectives of the study were:

- To assess the nutritional habits and status using two self-administered questionnaires;
- To assess the energy balance of participants using a seven-day food diary;
- To determine blood levels of vitamin D and calcium, ferritin (as a marker for iron stores) and magnesium;
- To determine the risks of RED-S in the participants.

1.6 Significance of the study

Many female endurance runners are unaware of RED-S and the need for a well-balanced nutritional eating plan. This study aimed to provide information on the prevalence of RED-S and guideways to improve the performance and recovery of female endurance athletes through a better understanding of RED-S in an area where there is a gap in knowledge.

1.7 Outline of the research dissertation

1.7.1 Chapter 2 – Literature review

This chapter discusses the relevant literature for the study. It outlines the significance of RED-S, nutrition that is important for endurance runners, including macro- and micronutrients, how to screen for RED-S, and how to implement a treatment plan.

1.7.2 Chapter 3 – Research methodology

This chapter details the research methodology used to conduct the study.

1.7.3 Chapter 4 – Results

Chapter 4 provides the results and data analysis of the conducted study.

1.7.4 Chapter 5 – Discussion

This chapter discusses the results in relation to the study's aims and objectives and the limitations of the study.

1.7.5 Chapter 6 – Conclusion and recommendations

Chapter 6 concludes the study and puts forward recommendations.

1.8 Conclusion

RED-S is recognised in endurance running athletes, but many runners are still unaware of its signs, symptoms and consequences of it. The next chapter will discuss the literature on RED-S in endurance runners in more detail.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Female participation in ultra-marathon running has increased over time. The annual South African Comrades Marathon (90 km up-run and 87 km down-run) held from Durban to Pietermaritzburg (or reversed) may have a significant role in the faster marathon and ultra-marathon times. In contrast to other ultra-marathons or ultra-trail races around the world, the iconic South African Comrades Marathon requires a faster pace to complete the race in the cut off time of 12 hours (Comrades Marathon, 2021 and Ronto, personal communication, February 6, 2020). From 2016, the Comrades Marathon entries for females increased from 2959 to 3372 in the 2018 race (Comrades Marathon, 2021). In 2019, 3055 females crossed the finish line and accounted for 18,6% of the finishing field. Gerda Steyn from South Africa also become the first female to run under six hours (5:58:53) for the up-run in 2019 (Comrades Marathon, 2021). Five out of six of the world’s biggest ultra-marathon races with more than 1000 finishers are held in South Africa, with South African runners accounting for most entries (The Running Mann's Guide to April/May Marathons & Ultras - The Running Mann, 2020). The highest proportions worldwide out of the top 20 nations of ultra-runners are in France, the United States of America, and South Africa respectively (Ronto, 2020).

A study done in early 2020 by Paul Ronto at RunRepeat.com, in collaboration with the International Association of Ultrarunners, stated that over the last 23 years, South Africa had become the top ultra-running nation with an average pace of 10:36 min/mile (6:43 min/kilometre) followed by Sweden and Germany. South Africa also boasts the fastest female ultra-runners with an average pace of 11:11 min/mile (6:57 min/kilometre) (Ronto, 2020).

2.2 RED-S

Many endurance athletes seek an overall lower body fat percentage. Thinner morphology as the beneficial effects of lower body weight on athletic performance and running velocity are undeniable (Wasserfurth et al., 2020). Female endurance runners that do not follow a healthy balanced diet or have restricted-calorie eating habits are at risk of developing RED-S. An athlete’s performance can be heavily affected by RED-S, and health implications could include increased risk of injury, reduced coordination, decreased muscle strength, and decreased concentration (Briggs et al., 2020). RED-S consists of ten components as described by the International Olympic Committee (IOC) (Mountjoy et al., 2018) (Figure 2.1). Health concerns associated with RED-S, in addition to menstruation and bone health issues, may also include immunological, gastrointestinal (GI), cardiovascular, psychological, reproductive, skeletal, renal, central nervous system as well as endocrine disorders, leading to short-term and long-term complications (Briggs et al., 2020).

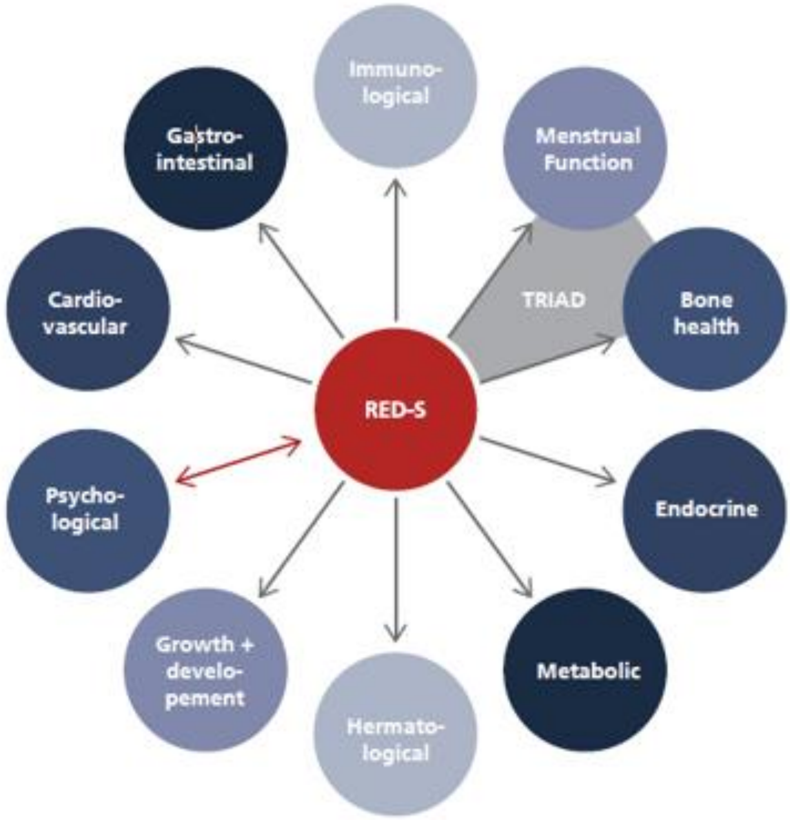

Figure 2.1. The ten components of RED-S and potential health consequences of RED-S as described by the IOC). Psychological consequences can either precede RED-S or be the result of RED-S. Growth + indicates expected growth development in adolescences. (Mountjoy et al., 2018, p. 2).

Relative energy deficiency can have a direct impact on an athlete's endocrine system, which can result in a chain reaction of the RED-S components (Kearney, 2016). Many hormones are affected by LEA, such as decreased levels of leptin, insulin, insulin-like growth factor-I (IGF-I), triiodothyronine (T3) and increased levels of cortisol and ghrelin (Kearney, 2016). These decreased levels of hormones will negatively affect any athlete's appetite. Hormonal and metabolic abnormalities caused by RED-S and if combined with carbohydrate deficiency, can result in a reduction in glucose utilisation, mobilisation of fat stores, slowing of metabolic rate and a decreased production of growth hormone

(Mountjoy et al., 2014). Unfavourable lipid profiles and endothelial dysfunction caused by LEA in athletes, increases cardiovascular risk (Mountjoy et al., 2014). Low levels of T3 and IGF-I can lead to reduced protein synthesis in the body, which means less muscle mass (Kearney, 2016). An increase in cortisol (due to stress physically or psychologically) will also lead to further protein breakdown and may negatively affect bone mineral density (Fuqua & Rogol, 2013). Athletes are at higher risk for bone injuries and fractures if bone mineral density is compromised (Mountjoy et al., 2014). Another factor of RED-S includes possible immune function suppression or impairment. Sarin et al. (2019) found that immunosuppression and energy restriction are related to the involvement of T- and B-cells. The risk of upper respiratory tract infections in female athletes is increased with LEA (Keaney et al., 2018).

Besides the multiple challenges associated with LEA and RED-S, their effects on bodily functions, psychological stress and sleep deprivation can also hinder performance (Wasserfurth et al., 2020). Overall health and exercise performance is negatively affected in the long term by having one component or multiple components of RED-S (Kearney, 2016; Mountjoy et al., 2014). The potential effects of RED-S are described by the IOC in Figure 2.2.

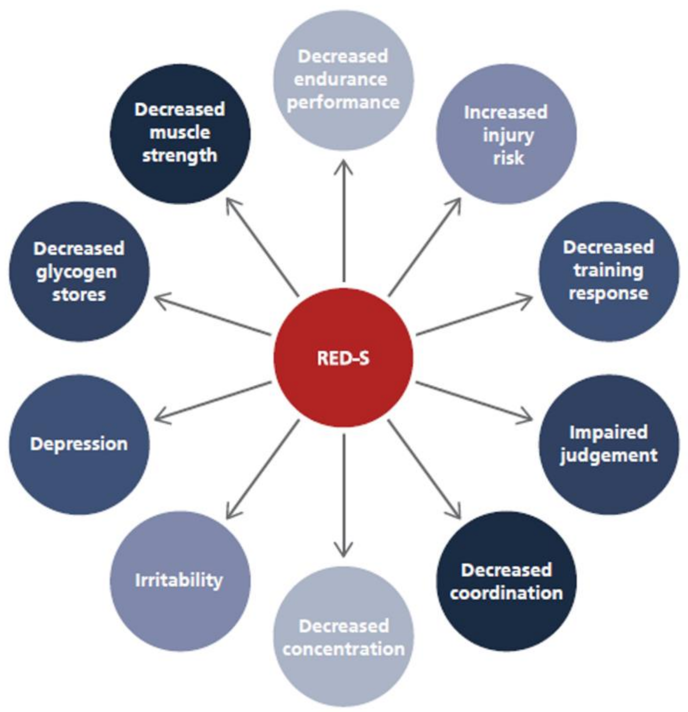

Figure 2.2. The potential effects of RED-S on an athlete and their sports performance as described by the IOC (Mountjoy et al., 2018, p. 2).

A study by Folscher et al. (2015) indicated that 16-78% of female runners that participated in the Comrades Marathon in 2014 had at least one out of the three components of the female athlete triad. This study was limited to the female athlete triad, which was the model reflecting energy deficiency at the time and has since been superseded by the RED-S model in 2018.

Relative energy deficiency is more common in elite athletes or athletes in a highly competitive environment that struggles with body dissatisfaction and can easily lead to eating disorders or deliberate inadequate energy intake (Wasserfurth et al., 2020). Athletes that compete in lean-focused sports or weight-sensitive sports are more prone to eating disorders, as found in a study by Torstveit et al. in 2008. Female athletes competing in leanness-focused sports had a higher prevalence of eating disorders (46,7%) in comparison with female athletes from non-leanness-focused sports (19.8%) (Wasserfurth et al., 2020). Athletes who have experienced chronic long-term LEA are at risk of developing nutritional deficiencies such as chronic fatigue, anaemia and compromised immune systems, making them more susceptible to infections and illnesses. Long-term LEA also affects bone health negatively, putting these athletes at a higher risk of stress fractures, prolonged recovery and osteoporosis, delaying their return to sport (Briggs et al., 2020).

RED-S is a syndrome, so diagnosing the condition can pose a challenge as symptoms are often subtle and vague, and athletes do not always know what signs and symptoms to look for (Briggs et al., 2020). Many athletes think that experiencing menstrual dysfunction, fatigue, low appetite, recurrent injuries and mood swings is not out of the ordinary (Briggs et al., 2020). Screening for RED-S can be done with the help of questionnaires such as the Low Energy Availability in Females Questionnaire (LEAF-Q) and RED-S Clinical Assessment Tool (CAT) as described by the IOC in 2018. “The LEAF-Q enables early recognition of active females at risk of low EA by evaluating the presence of symptoms associated with low EA, such as menstrual and GI dysfunction, injury history, as well as oral contraceptive use. The LEAF-Q is an easily administered questionnaire that has been validated for use in athletic endurance-trained females” (Logue et al., 2020). The LEAF-Q has a scoring key that measures each athlete’s score according to their answers (Appendix 7). A score of ≥ 8 is to be considered at risk for the female athletic triad. The RED-S CAT is based on the IOC consensus statement released in 2014 and is meant for medical professionals to use for evaluating and managing athletes who are at risk of RED-S (Mountjoy et al., 2018). The RED-S CAT specifies category descriptions for athletes, such as substantial weight loss, which is defined as 5-10% body mass loss in one month and MD. MD can be described as primary or secondary amenorrhoea, also known as functional hypothalamic amenorrhoea (FHA). Primary amenorrhoea is when no menstrual cycle is present by the age of 15 years, and secondary amenorrhoea occurs after a menstrual cycle has been absent for more than three months (Briggs et al., 2020). Screening should be considered for endurance athletes that present with disordered eating, an eating disorder, weight loss, long-term LEA, lack of normal growth and development, endocrine dysfunction, recurrent injuries, illnesses, decreased performance, performance variability or unusual mood changes (Briggs et al., 2020).

Treatment of RED-S is a multidisciplinary and interdisciplinary approach that includes education and support for the athlete, with the primary focus on correcting LEA for the maintenance of energy balance (Briggs et al., 2020). A multidisciplinary team could include doctors, psychologists, dietitians, gynaecologists, and others. Correction of LEA could include reduction in training and increased energy intake according to energy expenditure to achieve energy balance (Briggs et al., 2020). Coaches also need to be educated as they are often preoccupied with high performance and results, and the athlete’s long-term health is not taken into consideration (Wasserfurth et al., 2020).

2.2.1 Energy balance in female endurance runners

Energy balance is described as nutritional intake minus total energy expenditure (TEE) (Nikolaidis et al., 2018). Total daily energy expenditure (TDEE) is the energy cost required for essential processes of life, such as basal metabolic rate (BMR) and energy expended during physical activities (Heydenreich et al., 2017). Careful consideration should be given to the weekly amount of training for each athlete as well as their recovery to achieve energy balance (Tiller et al., 2019) as optimal body function is supported by sufficient energy intake (Heydenreich et al., 2017). Adequate energy intake is fuelled by an appropriate diet that consists of sufficient macro-and micronutrient intake that suits an individual athlete’s needs (Wasserfurth et al., 2020). Causes of low energy could result when nutrient or food intake is too low or when the energy expended through exercise is too high (or both), leading to an insufficient amount of energy for maintenance of normal physiological functions such as metabolic, immune function, bone health and menstrual cycle function in females (Wasserfurth et al., 2020).

Unfavourable health implications and performance consequences are highly associated with RED-S and LEA in athletes (Logue et al., 2020). Low energy availability over long periods of time leads to a negative energy balance and weight loss as increasing fuel needs are taken from the body's energy reserves (e.g., adipose tissue and body proteins) and not from the energy of foods consumed (Melin et al., 2019). "Long term LEA causes metabolic and physiological adaptations in order to reduce total energy expenditure to prevent further weight loss and promote survival, whereby the body obtains a new energy balance steady state" (Melin et al., 2019).

A study conducted by Thom et al. in 2020 showed that the Mifflin-St. JEOR equation for calculating BMR is used for females with healthy weight, underweight, overweight and obesity with an 80% accuracy. "The ± 10 % 'rule' for defining prediction accuracy is a pragmatic criterion which facilitates comparison between studies and is clinically relevant since chronic energy imbalances are likely to contribute to cases of malnutrition, or, conversely, to overweight and obesity" (Thom et al., 2020). Clinical studies have found that kcal/kg / fat-free mass (FFM) can be used as a threshold for achieving optimal energy balance (Wasserfurth et al., 2020). However, an EA of < 30 kcal/kg FFM serves as a threshold for serious health implications, with the effects visible after only five days of consuming < 30 kcal/kg FFM in young and healthy women (Wasserfurth et al., 2020).

Loucks et al. (2011) established threshold cut-offs for EA as follows:

- Low EA: EA < 30 $kcal \cdot kg^{-1}$ $FFM \cdot day^{-1}$
- Moderate EA: EA= 30-45 $kcal \cdot kg^{-1}$ $FFM \cdot day^{-1}$
- Optimal EA: EA > 45 $kcal \cdot kg^{-1}$ $FFM \cdot day^{-1}$

2.2.2 Nutrition in female endurance runners

Female endurance runners must adhere to specific daily nutritional needs to be able to manage high loads of intense training whilst keeping body fat levels and their body mass index (BMI) low for optimal performance and faster race times (Dedicque & Francoux, 2015; Knechtle & Nikolaidis, 2015). Nutritional imbalances and deficiencies can occur from this paradoxical complex (Dedicque & Francoux, 2015). Many female athletes with LEA do not consume the recommended daily fat intake to maintain specific body weight or to lose weight (Manore, 2017). Attempting to limit food intake for the purpose of achieving a specific body weight and goal has been named cognitive dietary restraint (Rideout et al., 2004; Barrack et al., 2008; Wood et al., 2021; Gibbs et al., 2013). Dietary restraints are often associated with increased intake of fruit, vegetables and fibre and lower intakes of carbohydrates, fats, and grains, with more athletes switching over to vegetarian diets (McLean & Barr, 2003; Moreira et al., 2005; Bardone-Cone et al., 2012; Zuromski et al., 2015; Wood et al., 2021). There have been only limited studies that have shown the association between micronutrient intake and cognitive dietary restraint. More studies are required in this field.

Female athletes that struggle with eating disorders can develop health issues such as altered nutritional absorption (Manore, 2017). Athletes that limit or avoid meat consumption and alternative protein sources, can develop iron deficiency (with or without anaemia) (Manore, 2017). A healthy balanced diet with macronutrients such as carbohydrates, proteins and fats as well as micronutrients such as iron, vitamin D, magnesium and calcium are essential. Adequate calorie consumption of carbohydrates, proteins and fats are important so that intake of amino acids can be used for protein synthesis and repair rather than being utilized as an energy source (Casazza et al., 2018). Nutrition is commonly overlooked despite the important role it plays in sporting performance, post-race or training recovery and prevention of injuries and illnesses (Citarella et al., 2019). Macronutrient requirements for a moderately active individual can be achieved by consuming a diet that consists of 45% to 55% of calories from carbohydrates (3 to 5 g/kg/day), 10% to 15% from protein (0.8 to 1 g/kg/day), and 25% to 35% from fat (0.5 to 1.5 g/kg/day) (Mahan & Raymond, 2016). When LEA is not a factor, exercise intensity, duration and frequency need to be considered alongside body mass to determine a female athlete's nutritional needs (Desbrow et al., 2019).

Endurance runners also often follow their own dietary restrictions to prevent GI disturbances before and during running, such as avoiding foods that are high in protein, fats and fibre. Foods such as meat and milk products, seafood, chocolate, legumes, coffee and tea, starchy vegetables and energy drinks are some examples. The prevalence of GI disturbances ranges from 30 to 90% of endurance runners (Parnell et al., 2020). Symptoms include "flatulence, bloating, diarrhea, urge to defecate, belching, reflux/heartburn, abdominal pain/cramping, nausea, vomiting, fecal blood loss, and bloody diarrhea" (Parnell et al., 2020). Dietary restrictions are mainly determined by the personal preferences of endurance runners through trial and error for pre, during and post exercising. It would be advisable for endurance athletes to do gut training with different carbohydrates and fluids to see what works best for them (Parnell et al., 2020). A study by Parnell et al. in 2020 showed that young females are more affected by exercise-induced GI symptoms pre-race than any other age or gender group, with high levels of stomach pain/cramps, bloating, heaviness/fullness, gas, and nausea/vomiting.

2.2.2.1 Macronutrients

Carbohydrates

Carbohydrates are an important fuel for any competing athlete (Mahan & Raymond, 2016), in this case, endurance running. With endurance running, energy is derived from glucose that is stored in the muscles as glycogen. Once this muscle storage has been depleted, the liver can produce glucose in two ways, namely glycogenolysis and gluconeogenesis, to maintain the glucose levels required to continue running (Mahan & Raymond, 2016). Glycogen is decomposed by glycogenolysis into glucose-1-phosphate and glucose in hepatocytes and myocytes (Paredes-Flores & Mohiuddin, 2021). In gluconeogenesis, new glucose is produced by converting precursors such as lactate, glycerol, pyruvate, and glucogenic amino acids into glucose (Gonzalez & Betts, 2019). Carbohydrates are equal to 4 kcal/g energy (Kelly et al., 2019), and the 45-55% recommended daily allowance (RDA) of carbohydrates should be enough for any female endurance runner for adequate energy and recovery (Mahan & Raymond, 2016). Daily requirements of carbohydrates vary according to the level of exercise for each athlete, with 5 to 7 g/kg/day (one hour daily of moderate exercise) likely suffice general training needs, for endurance athletes 6 to 10 g/kg/day (one to three hours daily exercise) is recommended to 8 to 12 g/kg/day (four hours daily exercise) for elite athletes (Mahan & Raymond, 2016; Vitale & Getzin, 2019). With an increase in exercise intensity from moderate (i.e., 65% VO_{2max}) to high intensity (85% VO_{2max}), carbohydrates provide energy when needed as glycogen is stored in the muscle or in the liver. Glycogen stores can, however, limit performance in prolonged intense training sessions (Hearris et al., 2018).

Carbohydrates can also be consumed strategically. Running performance may be enhanced by 2 to 3% if an athlete does “carbo-loading” 36 to 48 hours before running events that last more than 90 minutes (Vitale & Getzin, 2019). A carbohydrate-rich diet of 7 to 12 g/kg 24 hours prior is recommended to replenish muscle and liver glucogen lost on the day (Vitale & Getzin, 2019). Carbohydrates in high glycaemic foods would be preferable over low glycaemic foods to maximise glycogen storage (Thomas et al., 2016). A recommended 1 to 4 g/kg carbohydrate top-up should be consumed one hour (and up to four hours) prior to the race, as endurance events occur in the early morning after fasting during the night when liver glycogen is reduced (Vitale & Getzin, 2019). Pre-race feeding could also elevate muscle glycogen even further, which can only be advantageous (Hearris et al., 2018). During a running event lasting more than two and a half hours, an intake of 60 to 70 g/hour and up to 90 g/hours (if tolerated) can be consumed and has been associated with improved performance (Vitale & Getzin, 2019).

Many athletes rely on a combination of fuelling during a race that includes solids (e.g. bars), semi-solids (e.g. gels) and fluids (e.g. sports drinks), typically within the region of 30 to 90 grams per hour, depending on the duration of the exercise (Hearris et al., 2018). Smaller amounts of carbohydrates are recommended for shorter distances (such as half marathons), and a popular approach is the "mouth rinsing effect" used by many elite athletes, which activates the central nervous system simply with mouth exposure to carbohydrates, and may have performance benefits (Burke et al., 2019).

Athletes also use carbohydrate restriction deliberately as a strategy to enhance endurance performance by "training low" (McKay et al., 2020). By controlling carbohydrate availability on a meal-by-meal and day-by-day basis, the training-induced oxidative adaptation of skeletal muscle can be controlled, mediated by activating regulatory signalling pathways when exercise is performed whilst carbohydrates are restricted (Impey et al., 2018). However, long-term carbohydrate restrictions can lead to alterations in iron metabolism and could deplete the iron stores of the athlete (McKay et al., 2020). The low carbohydrate, high fat diet is also not ideal for endurance athletes. This diet restricts carbohydrates to less than 50 g/day, has low to medium intake of proteins (± 15% of total calorie intake per day), and requires the remaining consumption of energy to be consumed as dietary fats. Such diets appear to reduce muscle glycogen content at rest and reduce glycogen utilisation during exercise in studies of medium-term adherence (four weeks), which is undesirable for endurance athletes (McKay et al., 2020). A study done by Gunzer et al., 2012, found that low carbohydrate intake in endurance athletes is linked to weakening immune systems and increased risk for complications due to over-training (Gunzer et al., 2012). However, in 2020, Bailey and Hennessy released an article reviewing ketogenic diet for endurance athletes and if performance is enhanced or if it is simply a placebo effect. In theory, if endurance athletes are well adapted to and can tolerate a ketogenic diet, longer periods of training could be achieved with sustainable energy levels throughout (Bailey & Hennessy, 2020). With the reduction of needs to refuel, maximum aerobic benefits could be achieved. There is also some evidence that a steady supply of energy during exercise for the body and brain is a benefit of ketogenic diets, which also accelerates recovery afterwards (Bailey & Hennessy, 2020; Volek, Noakes & Phinney, 2015).

Protein

Proteins are important for muscle building, exercise training adaptations for athletes, protein synthesis, growth of lean body mass (LBM) and strength, contributing to metabolic pathways during exercise, maintaining and repairing of muscles and assisting with immune function (Rossi, 2017; Mercer et al., 2020). Daily protein intake for athletes depends on the exercise type, e.g. endurance vs resistance, and is recommended in the range of 1.2 to 2.0 g/kg, which is higher than the RDA of 0.8 g/kg for sedentary individuals (Mahan & Raymond, 2016; Vitale & Getzin, 2019). Protein provides energy of 4 kcal/g (Kelly et al., 2019). However, consuming more protein daily than an individual's requirements has not shown any further benefit to endurance athletes (Vitale & Getzin, 2019) and can influence carbohydrate status by indirectly compromising the ability to train and compete in races at optimal performance levels (Mahan & Raymond, 2016). Mercer et al. (2020) did a systematic review on protein requirements for pre-menopausal female athletes and found that the estimated average requirement for protein intake ranges from 1.28 to 1.63 g/kg/day for competitive endurance female athletes. During the review, the athletes completed multiple days of moderate to high-intensity cycling intervals or sprints with an exercise duration of up to 150 minutes (Mercer et al., 2020).

The adequate consumption of protein, as well as the timing of intake, is as much a critical factor to endurance athletes as it is to resistance-trained athletes (Vitale & Getzin, 2019). During an ultra-endurance race, ultra-marathon athletes are encouraged to consume 20 to 30 g every three hours where possible (Tiller et al., 2019).

Female endurance athletes who have LEA or low carbohydrate availability will likely have increased daily or acute protein requirements, as protein oxidation is increased during exercise. Depending on the training status of the female athlete, increased protein intake will attenuate the loss of LBM during enhanced training load, duration or intensity (Mercer et al., 2020). Therefore, protein requirements for a female endurance athlete should be considered carefully with training status, training load and nutritional status to determine the estimated average requirement (Mercer et al., 2020).

Post-exercise protein intake is likely to be beneficial for female endurance athletes and could be considered as a strategy to include more daily protein, but optimal or acute protein requirements for pre-menopausal female endurance athletes are yet to be determined (Mercer et al., 2020). According to the International Society of Sports Nutrition, a recommendation for aid in recovery after exercise is to consume proteins with carbohydrates at a carbohydrate to protein ratio of 3 to 4:1 (Potgieter, 2013). This will assist in glycogen resynthesis and ultimately enhance performance (Potgieter, 2013).

Fats

Dietary fats play a role in reducing inflammation, absorption of fat-soluble essential vitamins, cellular membrane formation, regulating hormone cycles and energy metabolism (Rossi, 2017). During LEA, oestradiol and progesterone are significantly reduced in female athletes with RED-S (Sale & Elliot-Sale, 2019). A function of oestradiol includes preserving BMD by increasing osteoclasts and decreasing osteoblast apoptosis, but oestradiol levels are very sensitive to fluctuations caused by LEA (Southmayd & De Souza, 2017; Fahrenholtz et al., 2018). High training load can lead to alterations in an athlete's hormonal profile, such as suppressing of gonadotropin-releasing hormone (GnRH), which plays an important role in controlling other sex hormones (Warren & Perlroth, 2001). In females, luteinising hormone (LH), follicle-stimulating hormone (FSH) and consequently oestrogen can be suppressed and could lead to secondary amenorrhoea (Sim et al., 2019). Recent studies in athletes with similar EA or daily energy balance have shown that large energy deficits during the day are associated with suppressed reproductive hormones (both males and females) and MD (female athletes only) (Fahrenholtz et al., 2018; Melin et al., 2019). The overall altered endocrine profile caused by LEA (including decreased anabolic hormones, e.g. leptin and testosterone) is harmful to BMD (Wasserfurth et al., 2020).

Fat consumption is crucial to ensuring optimal health, maintaining energy balance, consuming essential fats and fat-soluble vitamins, as well as replenishing intramuscular triacylglycerol stores (Potgieter, 2013). Intake of dietary fat by athletes should be assessed individually according to training levels and body composition goals (Thomas et al., 2016). Dietary fat intake recommendation depends on energy needs and expenditure; athletes that have a higher energy expenditure will require more fat in their diets (Thomas et al., 2016). The RDA for fats is 0.5 to 1.5 g/kg/day according to current literature and yields 9 kcal/g (Mahan & Raymond, 2016; Kelly et al., 2019). The International Society for Sports Nutrition recommends a moderate 30% consumption of total energy for dietary fats daily (Potgieter, 2013).

Omega-3 is a group of essential fatty acids that are building blocks for cell membranes and assist with immune regulation. Omega-3 consists of three types of essential fatty acids present in nuts, seeds and fish oil: α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA occurs naturally in nuts and seeds, whilst EPA and DHA are mainly found in fish oils (Gutiérrez et al., 2019). Omega-3 is anti-inflammatory reducing inflammation during training (Zhuravleva et al., 2019). Other indications are that it could aid in ailments of the menstrual cycle for women with FHA (Gibson et al., 2020). Research in previous years also suggests that omega-3 fatty acids could help prevent depression, a big consequence of RED-S (Gibson et al., 2020).

2.2.2.2 Micronutrients

This study focuses on a few micronutrients that female endurance runners must consume daily. Micronutrients are utilised in many metabolic pathways. Due to the additional stress and biological adaptations associated with exercise, athletes have increased micronutrient needs (Institute of Medicine, 1998). If a micronutrient is in deficit, it could lead to other deficiencies.

Vitamin D

Vitamin D is a vital micronutrient that is essential for athletes, and its production is dependent on sunlight exposure. This is influenced by geographical location and time spent training indoors or outdoors (de la Puente Yagüe et al., 2020). Females that spend most of their days indoors or train indoors may be at risk of vitamin D deficiency. Sun exposure, estimated between five to 30 minutes, to the arms and legs will produce adequate vitamin D daily, but this is also dependent on skin pigmentation, season, latitude and time of day (Lawley et al., 2020). Reduced sun exposure and the use of sunscreen can limit vitamin D absorption, and higher dietary through food or supplement intake will be required (Dedicque & Francaux, 2015).

Vitamin D facilitates calcium uptake for healthy bones and is therefore essential for healthy BMD (Mahan & Raymond, 2016). In some cases of LEA, particularly those involving intentional restricted calorie intake, athletes may not get sufficient calcium and vitamin D, both of which are essential to generating new bone (Charlton, Forsyth & Clarke, 2022). Clinical features of vitamin D deficiency in female athletes includes female with low energy availability, whether from amenorrhoea or hormonal contraceptives and can be used a guideline for further screening (Ip et al., 2022).

Vitamin D functions in the endocrine and autocrine systems by contributing to calcium homeostasis and regulating calcium and phosphorus levels in the blood (Neal et al., 2015; Lawley et al., 2020). Numerous benefits of vitamin D have been established, including influences on immune function, muscle function and injury, athletic performance, and bone health (Lawley et al., 2020). Vitamin D is required for normal bone formations, including bone trabeculae, callus formation and bone reabsorption. In the case of low serum calcium, vitamin D and parathyroid hormone (PTH) will increase intestinal absorption, in return elevating bone turnover through osteoclastogenesis to raise calcium levels (Lawley et al., 2020). However, if the rate of exercise load exceeds the rate of bone turnover (assists with bone remodelling, replacing damaged bone tissue), the risk of bone injuries such as stress fractures are greatly increased in many athletes (de la Puente Yagüe, 2018).

25-hydroxyvitamin D (25[OH]D) is often used for clinical or athlete trials as a measurement marker for vitamin D status due to its long half-life and relationship with vitamin D3 exposure (dermal synthesis or dietary uptake) (Owens et al., 2018). In this study, vitamin D was measured with the marker vitamin D 25[OH], and as such, 'vitamin D' is used throughout the study. A study done by Constantini et al. (2010) showed that 73% of athletes did not have sufficient levels of 25[OH]D concentration with a mean of 25.3 ± 8.3 ng/mL (sufficient level = 25[OH]D concentration of <30 ng/mL). A different study by Willis et al. (2012) found a possible link between decreasing vitamin D status and a particular biomarker of inflammation when vitamin D status and biomarkers of inflammation were measured in runners. Female endurance runners that have vitamin D deficiency should use supplementation and increase dosage beyond the RDA and use a minimum dose of 1500-2000 IU (Miraj et al., 2019) (Table 2.1).

Calcium

Calcium is the major component of strong and healthy bones, with a recommended daily intake of 1000 mg to 1200 mg per day (Mahan & Raymond, 2016) (Table 2.1). As much as 99% of total body calcium is stored in bones, with the remaining 1% circulating in the blood, extracellular fluid, muscles and other tissues (Flynn, 2003; Garner, 2020). Whilst the intensity or duration of running does not affect the amount of calcium required, it has been proposed that calcium loss through sweat and urine could increase the required amount of calcium (Dedicque & Francaux, 2015). Hormonal changes can be a side effect of calcium loss as well as impaired muscle function, but more research is required for direct markers of functional performance (Townsend et al., 2016). The calcium-dependent troponin complex and PTH homeostasis are highly dependent on cellular calcium, which assists with every skeletal muscle function in the body (Frontera & Ochala, 2015; Pu et al., 2016).

A study by Martin et al. (2007) showed that calcium supplementation had a positive effect on calcium homeostasis during exercise in healthy active pre-menopausal women that supplemented with 800 mg or more, which effectively reduced calcium loss due to exercise. However, Zorbas et al. (2000) concluded that additional supplementation of calcium over and above the RDA doesn't affect calcium stores or VO_2 peak performance for endurance runners, whether training was normal (around 67 km per week) or when runners were restricted to running no more than seven km/day for 12 months. VO_2 is a measurement of oxygen uptake during exercise, in this case it refers to a level of run intensity (Rogers et al., 2021). Calcium supplementation can reduce post-exercise decreases in serum calcium loss through oral (800 mg over eight days or 1352 mg prior to exercise in a single meal) or intravenous administration (156 mg prior to and during exercise) (Russel & Parnell, 2020).

Low calcium intake amongst female athletes could be the result of restriction of energy intake and disordered eating patterns or avoiding dairy products or other calcium-rich foods to maintain a certain body weight or body image (Thomas et al., 2016; Farrokhyar et al., 2017). LEA may worsen the issues and risks of low calcium intake that could include and is not limited to low BMD and stress fractures image (Thomas et al., 2016; Farrokhyar et al., 2017). Prevention of low BMD and stress fractures can be counteracted with dietary strategies of calcium and vitamin D supplementation (Ikedo et al., 2018).

Iron

For the female endurance runner, iron is one of the most essential micronutrients for training and recovery. Iron assists with oxygen transportation from the lungs to muscles (which ultimately enhances performance and endurance), energy production pathways in the body, and is a functional component of haemoglobin (Hb) and myoglobin (Dedicque & Francaux, 2015; Clénin, 2017; Dennis & Conway, 2019). Mitochondrial energy production is highly dependent on the activity of iron-dependent enzymes and proteins. This indicates that iron is vital for athletic performance (Buratti et al., 2015). However, a reduction in Hb levels limits the amount of oxygen consumption available for use during exercise and affects endurance performance (Dedicque & Francaux, 2015). Iron deficiency in endurance athletes, with or without anaemia, reduces the aerobic energy system of skeletal muscle (Passos et al., 2019). Archer and Brugnara (2015) referenced three stages of iron deficiency, namely:

1. “Storage iron depletion” – A decrease in serum ferritin (sFer) levels is noticed in the liver, spleen and bone marrow, but there are no significant haematological changes or effects present at this stage (ferritin levels < 35 ng/L)
2. “Iron deficiency non-anaemic” – A drop in Hb levels as erythropoiesis diminishes (iron supply to erythroid cells is reduced), and possibly other biochemical markers can be observed, but this could still be in normal referencing ranges, and no signs of anaemia are present (ferritin levels < 20 ng/L)
3. “Iron deficiency anaemia” – sFer and Hb, as well as possibly multiple other biochemical markers, are outside the normal ranges, and signs of anaemia are present (ferritin levels < 12 ng/L) (Archer & Brugnara, 2015; Sim et al., 2019).

Many women are known to have suboptimal iron status due to a net negative iron balance and thus are more prone to iron deficiency than males (Dennis & Conway, 2019). The negative iron balance mainly is contributed to by the loss of iron through menstruation and inadequate dietary intake. Other factors also include iron losses associated with sweating, exercise-induced acute inflammation, haemolysis and GI bleeding (Peeling et al., 2008; Auersperger et al., 2013; Ma et al., 2013). Hepcidin, an iron-regulatory hormone, has been identified as a protein of interest in the interplay of inflammation and iron absorption (Auersperger et al., 2012; Newlin et al., 2012; McClung et al., 2013). A single session of exercise increases not only inflammatory cytokines but also elevates oxidative stress (Newlin et al., 2012). Post-exercise inflammation has been shown to up-regulate hepcidin, which acts to inhibit the absorption of iron (Roecker et al., 2005; Di Santolo et al., 2008; Newlin et al., 2012; Peeling et al., 2009; Sim et al., 2012; Sim et al., 2013).

Low sFer is a valid marker for detecting iron deficiency (Jacobs & Worwood, 1975), as sFer is the cellular storage protein containing iron and is generally reflective of total body iron stores. Therefore, sFer is the measure most used to predict total body iron stores (Rubeor et al., 2018). However, a threshold for sFer that is indicative of iron deficiency has been inconsistent in the literature and can range from less than 12 ng/L to 35 ng/L. This can cause some confusion when results are being compared (Pate et al., 1993; Peeling et al., 2007; Di Santolo et al., 2008; Milic et al., 2011; Burden et al., 2015).

Iron deficiency is common amongst female endurance runners, but iron status can be improved with iron-rich foods and iron supplements. In Dennis and Conway's case study (2019), iron supplements showed improvement in the physical performance of an iron-deficient female endurance runner (Dennis & Conway, 2019). Increasing iron levels for an animal-based diet can typically include meat, liver, poultry, or fish at least five times a week, with a complementary intake of legumes and green vegetables is a recommended strategy (Clénin, 2017).

In 2016, Garvican-Lewis et al. found that athletes with pre-altitude iron stores (less than 35 μg/L) that supplemented with 210 mg of iron per day over a three week period, with moderate exposure to altitude, showed an increased haematological response in comparison with athletes that didn't take an iron supplement (Garvican-Lewis et al., 2016). Iron deficient non-anaemic athletes with significantly low sFer levels of less than or equal to 20 μg/L also reported having increased performance with iron supplementation (Rubeor et al., 2018). Intravenous iron supplementation is an option for increasing iron stores. After six weeks of usage, Archer and Brugnara (2015) found an increase in iron stores. Endurance capacity through erythropoiesis could be enhanced as well as improvement in the oxygen-carrying capacity of red blood cells (Archer and Brugnara, 2015).

Magnesium

Magnesium assists with energy production as well as fat and protein metabolism (Mahan & Raymond, 2016). Magnesium is required for glycolysis and oxidative phosphorylation to produce adenosine triphosphate (ATP), muscle contraction and nerve function (Shahsavar & Pourvaghar, 2010). Because it plays a significant role in the utilisation of glucose, exercise may cause a deficiency of magnesium as a result of sweating or urination (Chen et al., 2014). Magnesium helps with bone mineralisation, synthesis of active vitamin D metabolites, activation or stimulation of endogenous vitamin D and intestinal calcium absorption (Razzaque, 2018). Magnesium homeostasis helps to maintain the absorption of vitamin D, and without the maintenance thereof, the clinical benefits of vitamin D are significantly reduced (Lawley et al., 2020). Magnesium also co-factors with over 300 enzymatic reactions within the body daily (Gibson et al., 2020).

Current literature for endurance athletes with magnesium deficiency recommends that 300 to 500 mg per·day for short-term supplementation of one to four weeks will increase magnesium stores (Hefferman et al., 2019) (Table 2.1). Functional dynamic measures of muscle performance, exercise-induced inflammation, DNA damage, cortisol and immunological blood markers can all be positively influenced as a result (Setaro et al., 2014; Kass & Poeira, 2015; Petrović et al., 2016; Hefferman et al., 2019). Food products rich in magnesium are spinach, bread, fish, meat and dairy products (Olza et al., 2017).

Multiple symptoms of magnesium deficiency can lead to decreased performance as it is linked to many pathological conditions affecting the cardiovascular, skeletal and nervous systems (Williamson, 2016). Ultra-endurance athletes are at high risk of magnesium deficiency during prolonged intense activities where there are magnesium losses by urine and sweat, as magnesium is redistributed in the body (Williamson, 2016). If magnesium is deficient, it could amplify symptoms and susceptibility of stress as well as depression, a named component of RED-S (Gibson et al., 2020). The body's response to acute or chronic stress causes magnesium stores to be depleted, which leads to a vicious cycle of magnesium depletion and ongoing stress (Gibson et al., 2020). Deficiency of magnesium is closely associated with bone disorders, and in a study done by Matias et al. (2012), it was found that bone mass correlates with magnesium intake, and there is a substantial relationship between BMD and lean soft tissue (Matias et al., 2012). Regular weight-bearing exercises may be associated with a higher peak bone mass, suggesting that lean soft tissue increments serve as an important predictor of bone mineral content during growth (Matias et al., 2012).

There are many studies on magnesium and its effects on exercise performance, and whilst studies indicate that magnesium improves performance, it is still unclear how magnesium influences exercise performance (Chen et al., 2014). There is growing evidence that magnesium could potentially help maintain power, muscle mass and overall markers of systemic inflammation in the body (Hefferman et al., 2019). However, there is no evidence to support the benefit of magnesium (in general) in endurance performance despite the potential thereof (Hefferman et al., 2019).

In 2019, Hefferman et al. performed a study on elite Australian female athletes using a food frequency questionnaire. The study concluded that 15% of the athletes were deficient in magnesium. In a different study done in 2015, Kass and Poeira conducted a study on 13 recreational endurance athletes to investigate the effect of consuming an acute dose of magnesium (300 mg/day for one week) versus consuming a chronic dose over four weeks. The athletes were already consuming ~370 mg of dietary magnesium per day, and the results showed that the effects might vary depending on how long the supplementation is taken (Kass & Poeira, 2015).

Contraceptives and hormonal levels

In females taking oral contraceptive pills (OCPs), menstrual bleeding is typically reduced by 60%, and thus, the daily iron intake recommended for endurance female runners on oral contraceptives is reduced from 18 mg per day to 11 mg per day (Dedicque & Francaux, 2015). A study by Dedicque and Francaux (2015) measured eight different OCPs and their effect on running performance. The results showed that the female endurance runners that are using OCPs are prone to conserving the reserve of carbohydrates during prolonged times of running and do not fatigue as quickly as non-users. Although, prolonged use of OCPs can adversely affect BMD and can also mask the signs of LEA (Mountjoy et al., 2014). Recent studies have found that oral contraceptives should not be recommended for patients that have FHA (Gordon et al., 2017).

Functional hypothalamic amenorrhoea occurs when energy deficiency causes a disruption of GnRH from the hypothalamus, which leads to decreased secretions of LH and FSH from the anterior pituitary (Grosman-Rimon et al., 2019). In return, the reduced secretions of GnRH, LH and FSH can decrease or eliminate the stimulation of the ovaries for menstruation (Grosman-Rimon et al., 2019). Low oestrogen levels in young pre-menopausal athletes with amenorrhoea cause these athletes to be at higher risk for cardiovascular disease, contributed by endothelial dysfunction and adverse lipid profiles brought on by inflammation (Grosman-Rimon et al., 2019). Athletes that are training excessively and have low-calorie intake with amenorrhoea increases the severity of hypoestrogenism. Long-term hypo-oestrogenism could increase the risk of cardiovascular disease even more with long-term hormonal imbalance (Grosman-Rimon et al., 2019). FHA and LEA frequently occur together as athletes fail to adapt to increasing food consumption needs with increasing energy expenditure through physical activity (Gibson et al., 2020).

Oral contraceptives would therefore not be advisable for the treatment of RED-S as they can mask crucial clinical signs and symptoms such as amenorrhoea, and as a result, the BMD loss will be ongoing as an effect of RED-S (Briggs et al., 2020).

Table 2.1: Recommended daily intake of micronutrients for a normal active female (Mahan & Raymond, 2016).

Micronutrients	Recommended daily intake of normal active females:
Vitamin D	Age eight years and above 4000 IU
Ferritin (Iron)	Non-pregnant females: 10-150 ng/ml
Magnesium	Age 19 years and above 310-320 mg
Calcium	Age 19 years and above 1000 – 1200 mg

2.2.3 RED-S risk CAT

The IOC released a consensus statement in 2014 on RED-S and the scope of the condition beyond the female athletic triad. In 2015, the IOC presented the RED-S CAT, which included risk assessment categories for athletes in weight category sports, endurance sports and general athletic aesthetic or appearance (Mountjoy et al., 2015). The main purpose of the tool is for early detection of RED-S in athletes, for maintenance and improvement of performance, and to prevent long-term health consequences (Mountjoy et al., 2015). The tool is divided into two categories, a RED-S Risk Assessment Model for sport participation and steps for RED-S risk assessment decision-making determining readiness for returning to play (Mountjoy et al., 2015). The RED-S Risk Assessment Model for sports participation is further divided into three categories: low risk (green light), moderate risk (yellow light) and high risk (red light). Each category has criteria in which athletes are classified (Figure 2.3).

HIGH RISK: NO START RED LIGHT	MODERATE RISK: CAUTION YELLOW LIGHT	LOW RISK: GREEN LIGHT
- Anorexia nervosa and other serious eating disorders - Other serious medical (psychological and physiological) conditions related to low energy availability - Use of extreme weight loss techniques leading to dehydration induced hemodynamic instability and other life threatening conditions.	- Prolonged abnormally low % body fat measured by DXA* or anthropometry - Substantial weight loss (5–10 % body mass in one month) - Attenuation of expected growth and development in adolescent athlete	- Appropriate physique that is managed without undue stress or unhealthy diet/ exercise strategies
	- Low **EA of prolonged and/or severe nature	- Healthy eating habits with appropriate EA
	- Abnormal menstrual cycle: functional hypothalamic amenorrhea >3 months - No menarche by age 15 y in females	- Healthy functioning endocrine system
	- Reduced bone mineral density (either in comparison to prior DXA or Z-score <-1 SD). - History of 1 or more stress fractures associated with hormonal/menstrual dysfunction and/or low EA	- Healthy bone mineral density as expected for sport, age and ethnicity - Healthy musculoskeletal system
- Severe ECG abnormalities (i.e. bradycardia)	- Athletes with physical/ psychological complications related to low EA+/-disordered eating; - Diagnostic testing abnormalities related to low EA +/-disordered eating	
	- Prolonged relative energy deficiency - Disordered eating behavior negatively affecting other team members - Lack of progress in treatment and/or non-compliance	

* dual energy X-ray absorptiometry

**EA: Energy availability = Energy intake – Energy cost of exercise (additional energy expended in undertaking exercise).

Figure 2.3: RED-S CAT as set out by the IOC, categorised for high risk (red light), moderate risk (yellow light) and low risk (green light) (Mountjoy et al., 2015, p. 4).

Mountjoy et al. (2015) used EA, menstrual cycle, BMI, a food diary, relative history of injuries and blood tests to determine each participant's risk for RED-S.

The high risk (red light) category suggests no clearance for participation in sport. If an athlete falls in this category, continuation with the sport may result in severe health complications and could distract the athlete from suitable resting and recovery (Mountjoy et al., 2015). The moderate risk (yellow light) group suggests that the athlete can participate in sport with supervision and a medical treatment plan. The athlete should be re-evaluated at regular intervals every one to three months to assess compliance and improvements or deteriorations (Mountjoy et al., 2015). The low risk (green light) category suggests that an athlete can continue to participate in sport (Mountjoy et al., 2015).

If an athlete is categorised in red or yellow, medical evaluation and treatment are advisable, and the necessary healthcare professionals should be involved (medical doctor, dietitian, etc.). The main objective would be to correct the energy deficiency by either increasing EA to achieve energy balance or even decreasing energy output by decreasing training, or both (Mountjoy et al., 2015).

2.2.4 Return to play model

An athlete that is diagnosed with RED-S, should be re-evaluated with the RED-S CAT tool before returning to the sport, taking the athlete's health and sport requirements into consideration (Mountjoy et al., 2015). Steps for returning to play are summarised in Figure 2.4. When an athlete has been re-evaluated, the red-yellow-green categories can be re-used. If an athlete still falls under red, no training or competing is suggested. The yellow category suggests that an athlete can continue training but should follow a treatment plan to correct RED-S, and they may compete when medically cleared. The green category suggests full sports participation (Mountjoy et al., 2015).

STEPS	RISK MODIFIERS	CRITERIA	RED-S SPECIFIC CRITERIA
STEP 1 Evaluation of Health Status	MEDICAL FACTORS	- Patient Demographics - Symptoms - Medical History - Signs - Diagnostic Tests - Psychological Health - Potential Seriousness	- Age, sex - See Yellow Light column in RED-S Risk assessment model - Recurrent dieting, menstrual health, bone health - Weight loss/fluctuations, weakness - Hormones, electrolytes, electrocardiogram, DXA - Depression, anxiety, disordered eating/eating disorder - Abnormal hormonal and metabolic function - Cardiac arrhythmia - Stress fracture
STEP 2 Evaluation of Participation Risk	SPORT RISK MODIFIERS	- Type of Sport - Position Played - Competitive Level	- Weight sensitive, leanness sport - Individual vs. team sport - Elite vs. recreational
STEP 3 Decision Modification	DECISION MODIFIERS	- Timing and Season - Pressure from Athlete - External Pressure - Conflict of Interest - Fear of Litigation	- In/out of season, travel, environmental factors - Mental readiness to compete - Coach, team owner, athlete family, sponsors support - If restricted from competition

Figure 2.4: Steps for returning from RED-S to sport, as put forward by the IOC (Mountjoy et al., 2015).

The RED-S CAT tool has also been designed with flexibility for clinicians or sports coaches to be able to utilise the tool for many different sporting athletes (Mountjoy et al., 2015). For professional sporting athletes that are categorised in red or yellow zones, a treatment contract can be used to implement treatment of RED-S, but confidentiality of the athlete must always be maintained (Mountjoy et al., 2015).

Treatment for RED-S would include adjusting energy consumption versus energy expenditure to eliminate LEA. Energy consumption would have to increase to account for energy expenditure through exercise, even if it requires duration and frequency of exercise to be decreased to find a balance. Intake of nutrients and vitamins should follow established guidelines (Mountjoy et al., 2015). Once energy balance is acquired, amenorrhoea should resolve, but if it persists, further tests might be required to solve the problem. BMD can be assessed every six to 12 months, depending on the clinical presentation and initial values of each athlete (Mountjoy et al., 2015).

The IOC consensus group has made four recommendations to prevent RED-S (Briggs et al., 2020):

1. "Education using reputable sources of information, involving athletes, their families, coaches, medical providers and sports organisations. The education should cover healthy eating and nutrition, the concept of EA and the risks of LEA on performance and health associated.

2. Policies should be utilised by coaches on the development of healthy weight and body composition goals for athletes. The importance on weight and body composition to enhance performance should not be encouraged and the focus should be on nutrition.

3. The concept that good performance does not necessarily mean good health should be promoted. Education should be given on prolonged poor health and the inevitable impact on performance.

4. Multidisciplinary health support teams well educated in the detection and treatment of RED-S and the use of risk assessment and return to play models should be created".

2.3 Conclusion

Female endurance runners that do not follow a healthy balanced diet or have restricted calorie eating behaviours that are not aligned with their training habits are at risk for developing RED-S. As many athletes seek an overall lower body fat percentage, an eating disorder could be developed, and LEA can occur. Relative energy deficiency syndrome consists of ten components, and none are favourable. Athletes who have experienced chronic long-term LEA are at risk for developing nutritional deficiencies such as chronic fatigue, anaemia and compromised immune systems.

Female runners' weekly amount of training and their recovery should be considered to achieve energy balance. Adequate energy intake should consist of sufficient macro- and micronutrients for each athlete. A healthy balanced diet consisting of macronutrients such as carbohydrates, proteins, and fats as well as micronutrients such as iron, Vitamin D, magnesium, and calcium are essential. The BMR for each participant was calculated by the Mifflin-St. JEOR equation as described in chapter 3 under study design. Energy balance was also assessed, and these findings helped to determine if female endurance runners residing in Johannesburg have RED-S and, if they do, it is because of their nutritional habits. Screening for RED-S can be done with the help of the LEAF-Q and RED-S CAT. The return to play model can be utilised for athletes diagnosed with RED-S to assist them to return to their sport.

Methodology for the study is also described in chapter 3.

CHAPTER 3. RESEARCH METHODOLOGY

3.1 Introduction

This chapter discusses the methodology and study design used in this study. The methods chosen for this study stem from the literature review and research aims that were discussed in chapters 1 and 2. This chapter will be focused on the study design, ethical considerations, data collection and analysis of results.

3.2 Study design

The study used a descriptive and exploratory quantitative study design. Quantitative research focuses on answering the research question stated by the researcher. The data was collected in a systematic, objective and thorough manner, which enhances the accuracy of the research conducted. The researcher made use of data triangulation for data collection, as multiple means of data collection were used for this study (Brink et al., 2015). These included an online questionnaire, a seven-day food diary and a once-off blood test.

The study was also conducted in a descriptive manner. The researcher collected data from a representative sample of the population, in this case, female endurance runners in Johannesburg. The researcher did not manipulate any variables for the participants but merely searched for accurate information regarding nutrition and running habits (Brink et al., 2015).

3.3 Ethical considerations

Ethical clearance was obtained from the University of the Witwatersrand's Human Research Ethics Committee (Medical); ethical clearance number M201098 (Appendix 4). Once ethical clearance was obtained, the researcher commenced with the research. Ethical requirements, as stated by the University of the Witwatersrand's Human Research Ethics Committee, were followed throughout the research process. The research title was approved by the University of Witwatersrand (Appendix 9) before the researcher commenced the study. Each participant signed consent to answer the online nutritional questionnaire as well as give permission to proceed with the blood test (Appendix 5). Only participants who gave consent to proceed were included in the study. Participation in this study was voluntary, and refusal to participate did not involve any penalty or loss of benefits to the participant. Discontinuation from the study was permitted at any time (Appendix 6).

The confidentiality of the data collected was maintained throughout the research process. The researcher assigned each participant a participant number that was kept securely. The blood results were matched to the relevant participant afterwards when the results became available. The researcher allocated each participant a folder containing all the relative information and data. Only the researcher knew the identities of each numbered folder. All information was stored on a password-protected laptop accessible only to the researcher.

The information obtained from participants for the study was for research purposes only. Participants had the right to access all information pertaining to them at any given time during or after the study was concluded.

3.4 Research setting

The study was conducted at two locations in Johannesburg. Toga Laboratories (Germiston, Johannesburg, South Africa) was used for the blood sampling and analysis). Toga is a molecular biology and pathology laboratory that provides cost-effective tests for medical professionals. Alternatively, the researcher made use of the site of Testaro Laboratory (Dunkeld, Johannesburg, South Africa).

The online questionnaires were distributed through REDCap (version 11.4.3, Vanderbilt University, Ten, USA), an acronym for Research Electronic Data Capture. The REDCap application is a secure, web-based tool for creating and managing online surveys and databases (Harris et al., 2009). The researcher used the MyFitnessPal (San Francisco, California, 2015) application for the seven-day diary. MyFitnessPal is an electronic platform that consists of an online food database. Upon entering a food item, it is converted into nutrient values. It provides an easy way for consumers to record their food intake and track the number of calories and nutrients consumed (Evenepoel et al., 2020). Strava (San Francisco, California, 2009) was also utilised to collect data on running and other activities performed. Strava is an online application that allows users to track athletic activity or a sporting event through satellite navigation worldwide. Users can share their activity with location, activity type and duration (Camacho-Torregrosa et al., 2021).

3.5 Study participants

Participants, female endurance/ultra-runners residing in Johannesburg, were contacted via social media platforms and emails via registered running clubs that distributed the information to their members. Participants had run a marathon distance or ultra-marathon distance in the last two years or been training for one. They typically ran at least three times per week, at moderate to high intensity, for at least 40 minutes per session. All participants were over 18 years of age, pre-menopausal and not pregnant at the time of the study.

3.5.1 Sample selection and size

Sample size calculation for the study was done with the assistance of Raosoft and a biostatistician at the University of the Witwatersrand. The calculated sample size, based on an average population size of 200, was 51-91 participants. The sample focused on average female runners in Johannesburg that were still training as endurance runners with no marathon or ultra-marathon races scheduled for the months associated with the study. Qualifying participants had to respond by a certain date, and out of the 40 participants that responded by the set date, only 27 participants were able to participate in the study.

3.5.2 Sampling

Quota sampling was used for the selection of participants, as all participants had to qualify for the study by the criteria set out by the researcher. Convenience sampling was also used as many participants belonged to a running club in the area close to the laboratories where the venous blood samples were drawn.

3.6 Procedure of data collection

3.6.1 Preliminary test of study

A preliminary test was done for the online nutritional questionnaire. The questionnaire was previously validated (Appendix 1 & 3). The researcher tested the online questionnaire by filling it out to determine if it was configured correctly and to ensure proper working order. The researcher made use of REDCap version 11.4.3 (Vanderbilt University, Ten, USA) to distribute the questionnaire to the selected participants. The questionnaire consisted of 30 main questions with multiple sub-questions, but to reduce the burden on the participants, the researcher introduced skip-logic. Skip-logic made it possible for participants to skip over a question depending on their yes or no answer. For example, if a participant answered yes to a specific question, the next few questions that related to *yes* only were available for answering or if they answered no, only questions related to *no* would appear.

The researcher also tested the MyFitnessPal smart device application for three months before the commencement of the research to ensure that it could provide the required results for the study. This also ensured that the researcher could answer potential questions or queries participants had regarding the app.

3.6.2 Main study

The research commenced after ethics clearance was obtained from the University of the Witwatersrand's Human Research Ethics Committee (clearance number M201098). The researcher distributed information regarding the study to various running clubs through email and on social media platforms (on the Internet) and waited for willing participants to respond. After participants made contact and informed the researcher that they were willing to participate, the researcher sent each participant an informative email regarding the study. The email consisted of the information sheet (Appendix 6), consent form (Appendix 5), MyFitnessPal instructions (Appendix 7), available dates for the venous blood test and the address of the laboratory.

Participants were informed that participation in the study was voluntary and refusal to participate did not involve any penalty or loss of benefits. Discontinuation in the study was permitted at any time. If participants consented to go ahead with the study, the researcher scheduled a date with participants to have the venous blood sample drawn. The specific details of having the once-off venous blood sample drawn, as well as the seven-day food diary that had to be shared with the researcher, were explained to the participants in the email.

A single researcher conducted the research study, with assistance at the laboratories from a qualified nursing sister and Dr Tait to venesect participants for the blood sample. The main study was conducted at two laboratories: Toga Laboratories in Germiston and Testaro in Dunkeld.

3.7 Variables

The independent variables for the study included the seven-day food diary, weight, height, amount of training and running log.

Dependable variables included gender, age within the perimeters of non-menopausal and non-pregnant females, running at least three times per week at moderate to high intensity of 40 minutes and residence in Johannesburg.

3.8 Outcome measures

The primary outcome of the study was to assess the nutritional habits and statuses of female endurance runners of Johannesburg in terms of risk for RED-S.

The secondary objectives were to determine the energy balance of participants, examine associations between nutrition and energy status of female endurance runners in Johannesburg, and the overall risk of RED-S in female endurance runners in Johannesburg. The measurement tools used for the outcomes were two online questionnaires on REDCap, the MyFitnessPal and Strava apps, and blood sampling done at the designated laboratories.

To have achieved these objectives, the researcher implemented two online questionnaires for each participant to complete on REDCap, to log and share a seven-day food diary on the MyFitnessPal app, to share running and other activities through the Strava app, and to go for a blood sample test to establish levels of calcium, vitamin D, iron and magnesium (Figure 3.1).

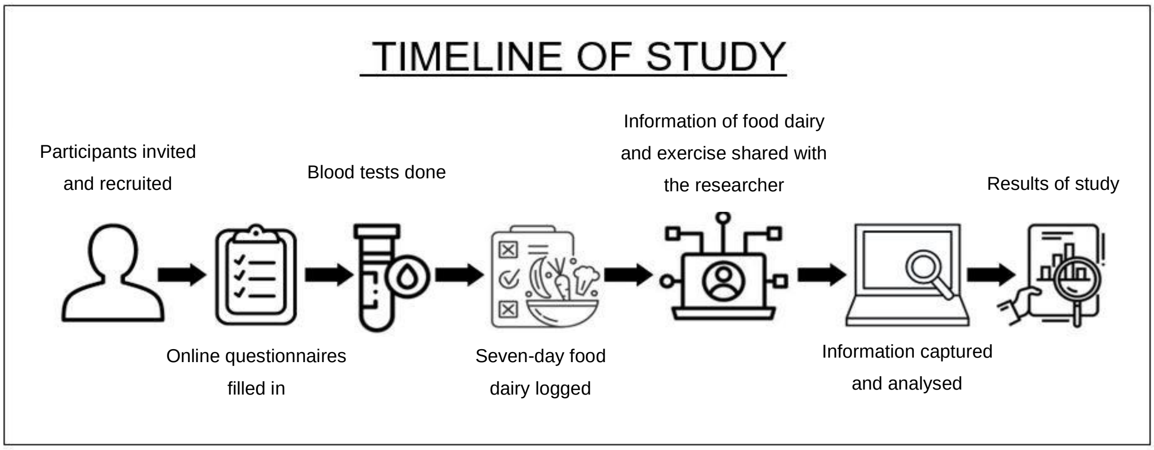

Figure 3.1: Study sequence

The online LEAF-Q provided a medical history for each participant, including smoking status, injuries, menstrual function, contraceptive usage and GI function. The online nutritional questionnaire provided information about overall nutrition: during base training, during competition season and on race day. The seven-day food diary was simply a record and therefore was not standardised or validated. Each participant had to self-report their daily food logs. The analysis focused on the total intake of calories, protein, carbohydrates, and fats. The seven-day food dairy was used to calculate energy balance and to determine the risk of RED-S. The completed online questionnaires were retrieved from the REDCap system. Each participant shared their food diary with the researcher to access all daily food diary information. All food items are broken down into nutritional values on the MyFitnessPal app, including macronutrients, micronutrients, total calorie intake, and calorie split between nutrients and calories per item.

The BMR for each participant was calculated using the Mifflin-St. JEOR equation. This equation uses age, height, and weight for calculating BMR:

Females: (10 x weight in kg) + (6.25 x height in cm) – (5 x age) - 161

as described in Krause's food and the nutritional care process (Mahan & Raymond, 2016).

However, to determine TDEE, the physical activity level (PAL) must be considered and can be classified as lightly active, moderately active and highly active. The PAL and set out values are illustrated in Table 3.1. According to the Food and Agricultural organization of the United Nations (2004), jogging or running is considered to be moderately active. Vigorously active includes leisure activities that are more than two hours per day (Food and Agriculture Organization of the United Nations, 2004).

Table 3.1: Physical activity levels (PAL) and estimated values (Food and Agricultural Organization of the United Nations, 2004).

Physical activity level	PAL value
Lightly active	1.40 – 1.69
Moderately active	1.70 – 1.99
Vigorously active	2.00 – 2.40

Energy requirement can then be calculated as (Food and Agriculture Organization of the United Nations, 2004):

$$TDEE = BMR \times PAL \text{ (mid-point)} = kcal/kg/day.$$

The outcomes are described in chapter 5 in section 5.1.

3.9 Data analysis

Data analysis established nutritional habits, statuses and demographic profiles for all participants. Demographic data are summarised in Table 4.1 with mean and standard deviation. Furthermore, all data were captured on an Excel spreadsheet and SPSS version 28.0.0 (IBM, NY, USA) for analysis which is discussed in chapter 4. The data from the questionnaires were all captured on the REDCap system (Vanderbilt University, Ten, USA). The REDCap system is an electronic data capturing platform that captures data that also provides "1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources" (Harris et al., 2009). Descriptive statistics interpreted the results. Statistical significance was set at an alpha level of 0.05. Pearson correlations were performed to evaluate associations between macronutrients, BMIs, and LEAF-Q scores. A one-sample t-test was used to determine the significance of total daily calorie intake over seven days and the TDEE of the participants.

The researcher scored each participant on the LEAF-Q score key card to determine the risk of female athlete triad and the RED CAT to determine the risk of RED-S. The REDCap system, Excel and SPSS were used to analyse the data.

3.10 Conclusion

Chapter 3 described the research methodology and the study design. Chapter 4 will discuss the results of the study, including how many participants are at risk for RED-S and the female athletic triad. Chapter 4 also discusses relationships and associations between the variables and reinforces the discussions of the data.

CHAPTER 4. RESULTS

4.1 Introduction

This study aimed to determine the nutritional habits and current statuses of female runners' nutrition associated with training and establish the energy balance of female runners in Johannesburg. The study's main objectives were to assess the participants' nutritional habits, status, energy balance and RED-S risk. Finally, it aimed to determine the effects of RED-S on the performance and recovery of female endurance runners.

This chapter contains the results from the questionnaires, the blood tests and the nutritional aspects of the food diary of all participants. The results were calculated with the aid of the REDCap system as recommended by a biostatistician.

Of the 40 participants who responded to participate, 27 met the requirements to take part in the study. These participants presented complete data and were used as the final sample for data analysis.

All participants (n=27) had the pre-requisites for the study, namely:

- Runners that typically run at least three times per week, at moderate to high intensity for at least 40 minutes per session
- Recreational runners as well as elite runners
- Over 18 years of age
- Pre-menopausal and non-pregnant
- Runners that had run a 42.195 km (standard) marathon in the last two years. Due to restrictions as a result of the COVID-19 pandemic, the researcher also accepted runners that were training for a marathon that may have been cancelled.

4.2 Demographic data

Participants were female runners who met the researcher's requirements, all residing in Johannesburg. The demographic data are summarised in Table 4.1.

Table 4.1: Demographic details of the study sample

Demographic details		Mean	Standard deviation (SD)
Age	25 – 48 years	35.89	7.23
Height	155 – 172 cm	152.66	43.79
Weight	49 – 64.8 kg	58.71	5.15
BMI	18.2 – 26.2	21.55	1.65

One participant was underweight with a BMI of 18.2, and one participant was overweight with a BMI of 26.2. All other participants (93%) were in a normal BMI range. Each participant's height and weight was self-reported and was used to calculate their BMI.

4.3. The LEAF-Q

Only one participant answered yes to being a smoker, whilst the rest were non-smokers. Twenty-two participants (81%) do other training besides running, such as strength training, cycling, swimming, cross-training or playing other sports, whilst five participants run only. Figure 4.1 shows the volume of training of participants weekly. An average of 11,5 km per run was achieved for the 27 participants with an average pace of 06:15 min/km (Figure 4.2). The researcher used the LEAF-Q scoring key designed by Dr Anna Melin (Appendix 2) to score participants on their risk for the female athlete triad and RED-S. Eleven out of 27 (41%) participants scored eight or more, with most points scored for irregular menstruation or amenorrhoea.

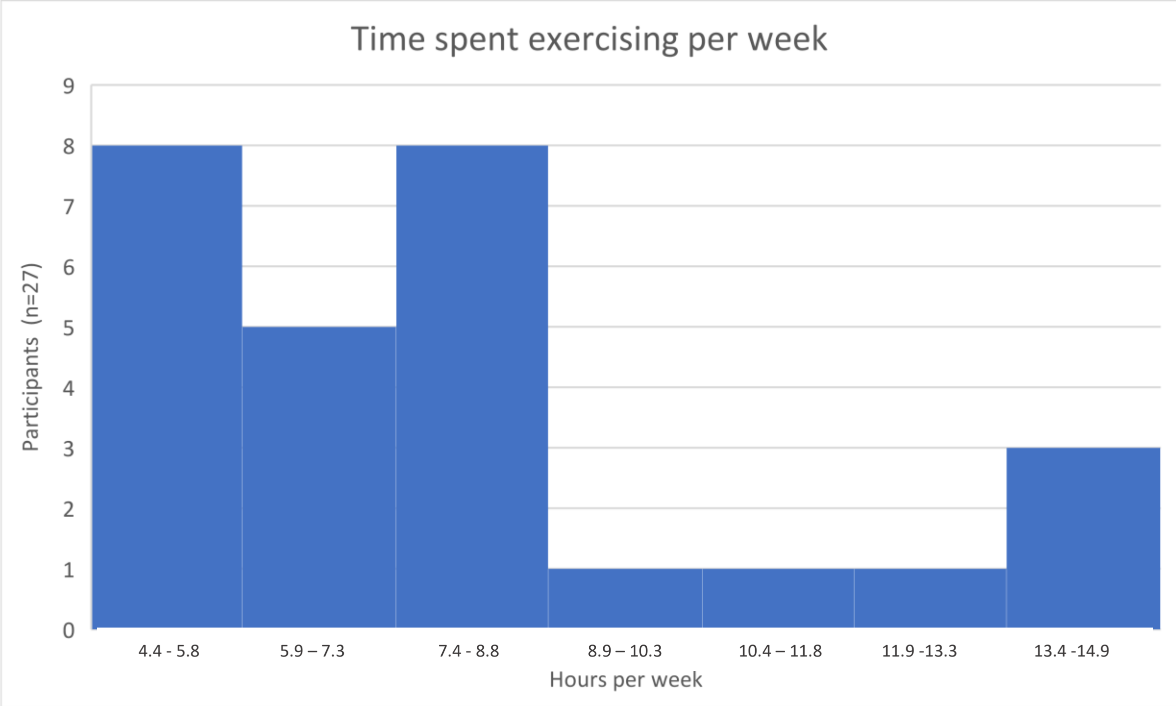

Figure 4.1: Weekly running training hours for each participant

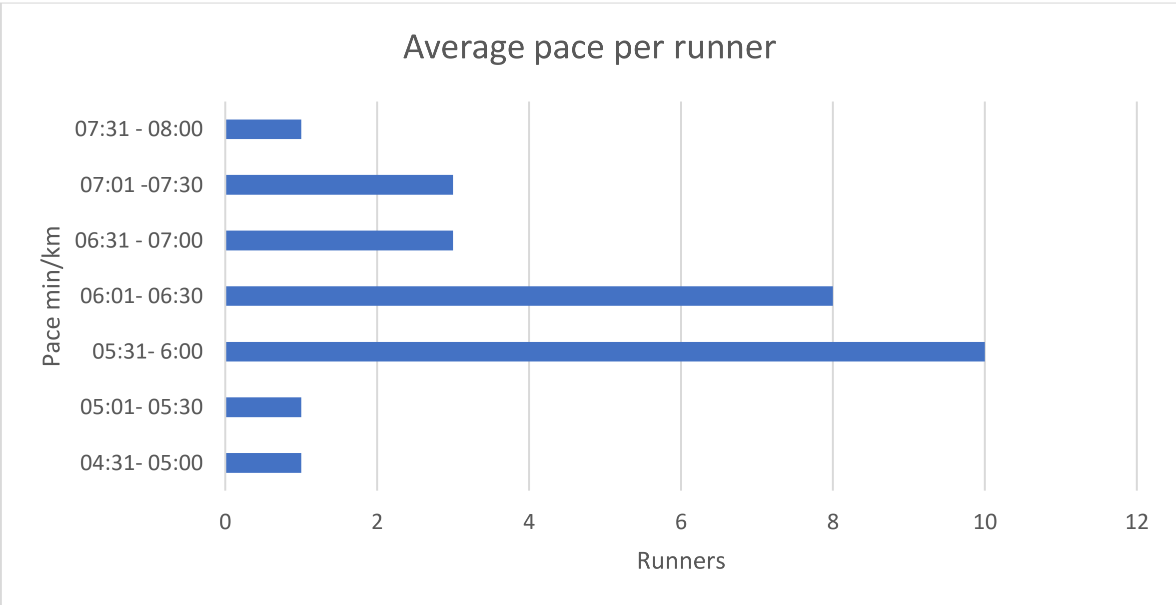

Figure 4.2: Average running pace per participant

The most frequent distance run per participant is between five and ten kilometres per run during the week, with longer runs over the weekend.

4.3.1 Injuries

Nine runners (33%) struggled with injuries related to lower body parts, such as the hip, knee and foot, in the last year. Three runners had iliotibial band syndrome (ITBS), hindering performance intermittently. These runners admitted taking time off every so often as required, or physiotherapy was considered instead. One participant had a hip injury, and a soleus strain that is muscle related. Four runners struggled with knee injuries such as collateral damage from ITBS, tendonitis in the knee, chondromalacia patellae ('runner's knee') and knee bone bruising from running or sustaining a fall during a run. Two of the runners had foot injuries, one had tendonitis in the foot, and the other had a current injury whilst the study was being conducted. This participant was in a moon boot at the time and had not had a diagnosis yet, but a bone injury was strongly suspected. The participant could not run and was walking with difficulty whilst wearing the moon boot. Six out of the nine injured runners took no more than seven days off before starting to run again. One participant who sustained a fall during a training run took 21 days off due to a bone bruise in the knee. The other runner with a bone bruise in the knee from running took 14 days off before starting exercise again. The runner in the moon boot only started slow running six weeks post-injury.

4.3.2 Gastrointestinal function

Ten out of 27 participants (37%) struggled with a bloated abdomen or cramps not related to menstruation. Many runners also struggled with abdominal issues when running long distances. Three runners indicated that they tended to have diarrhoea or constipation during a race, depending on what they ate before the race. Four runners had an existing condition: two had inflammatory bowel syndrome, one had abdominal polyps and one had a rectal prolapse that was corrected. These conditions play a role in their diets and the consistency of bowel movements before or during running.

4.3.3 Menstrual function and contraceptives

Seven participants (26%) reported using oral contraceptives. Eleven participants (41%) indicated that they have 12 or more periods during the year, whilst three (11%) specified that they only have 9 to 11 periods in a year. One participant indicated that she had not had a period in more than five months but had an intra-uterine device implanted. Ten participants (37%) reported having amenorrhoea for three consecutive months or more previously, and four (15%) of these runners were currently experiencing amenorrhoea (aged between 25 and 43). Seven (26%) participants reported that their menstrual cycle changes with less bleeding or bleeding for fewer days when intensity, frequency or duration of training increases. Participants were classified according to self-reported amenorrhoea in the MD group. If normal menstruation was self-reported, participants were classified as EU (eumenorrheic) for comparison. There is a significant difference between the MD versus EU participants' average calorie intake over seven days (P=0,001) versus their TDEE.

Table 4.2: Correlation between BMIs, macronutrient intakes and LEAF-Q scores.

	Menstrual dysfunction (n=16)	Eumenorrheic (n=11)	p-value
BMI	21,7 ±1,3	21,9 ± 2,0	0,5
Protein intake (mg)	299,4 ± 123,8	321,3 ± 126,2	0,3
Carbohydrate intake (mg)	683,2 ± 270,2	695,3 ± 261,1	0,08
Fat intake (mg)	557,6 ± 239,7	544,2 ± 236,8	0,08
LEAF-Q score	8,7± 2,8	5,8 ± 2,0	0,5
Daily average calorie intake over seven days	2024,3 ± 178,1	2014,3 ± 183,9	<0,001*
TDEE	2592,0 ± 360,2	2641,7 ± 386,0	

*BMI = Body mass index, LEAF-Q = Low Energy Availability for Females Questionnaire, BMR = Basal metabolic rate, TDEE = Total daily energy expenditure. Significance level was set at p=0.05. * Indicates significance. Daily average calorie intake over seven days per participant and TDEE significance was calculated with a one-sample t-test.*

4.4 Overall nutrition

Only nine runners followed a specific diet for their training; the reasons are listed in Figure 4.3. Some participants chose more than one reason for their specific diets. Options to choose from included:

- Vegan
- Vegetarian
- Very high energy/extra energy
- Restricted energy/low energy
- Paleo
- Paleo for athletes (more carbohydrates around training sessions)
- Low carbohydrate high fat

- High protein low carbohydrate
- Gluten free
- High carbohydrate
- Periodised carbohydrates
- FODMAP (Fermentable oligosaccharides, disaccharides, monosaccharides and polyols)

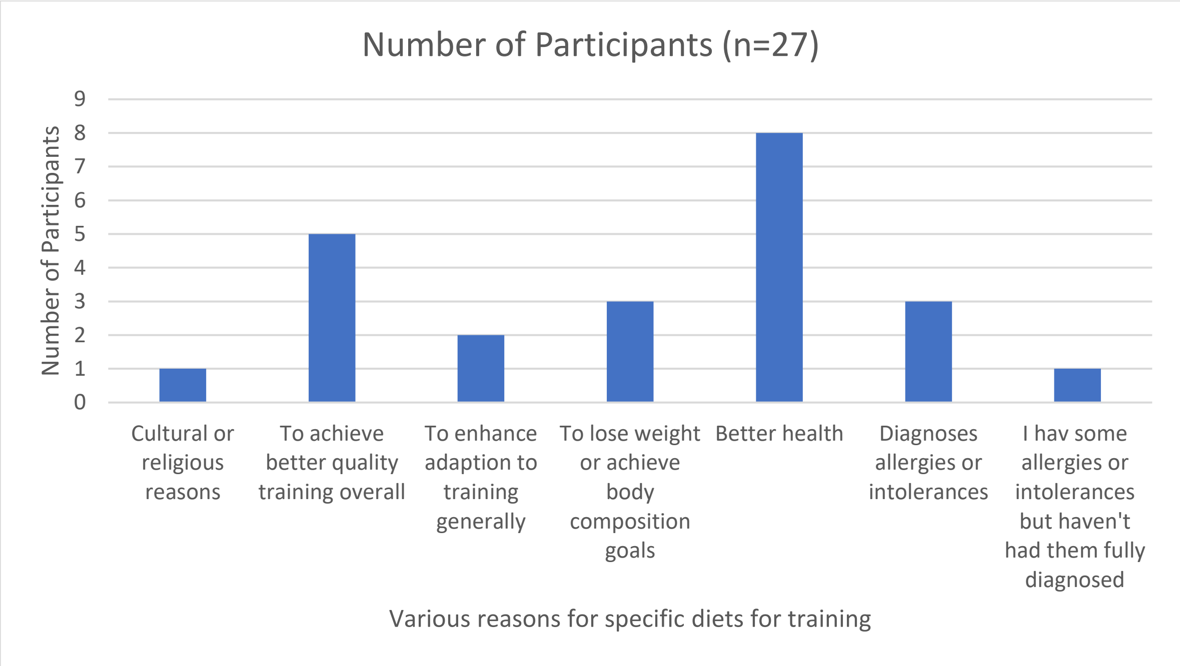

Figure 4.3: Various reasons for following specific diets for training

Only six runners (22%) follow a specific diet during certain training goals such as heat training, returning to running from the off-season and transitioning to race season.

4.4.1 Nutrition during base training

4.4.1.1 Hard training days

Fifteen (55%) runners indicated that they consume more food or calories for higher volume and intensity training sessions. These include more carbohydrate-rich foods (six participants), protein-rich foods (seven participants), fat-rich foods (three participants) and energy-containing drinks such as protein shakes or smoothies (five participants). Twelve runners (44%), of which four participants falls under the MD category, do not increase their calories for harder training sessions; reasons include not feeling hungry (two participants), do not feel like they need extra fuel (three participants), and loss of appetite because of the hard session (one participant), already eating to full calories budget for the day (one participant), monthly financial budget does not cater for extra food for training (one participant) and using these training sessions to lose weight effectively (one participant).

4.4.1.2 Easy training days

Sixteen (59%) runners do not adjust their eating for easier training days as many follow a specific diet plan, practise intermittent fasting or already eat at maintenance every day, whether training or not. Other reasons included fuelling up for harder training days and utilising food for recovery and adaptation to training. Six runners (22%) admitted to eating less on easy training days by cutting out more carbohydrates and fat-rich foods.

4.4.1.3 Key training sessions

Nearly half of the participants (13 participants, 48%) do not focus on adequate fuelling before high-quality/intensity training sessions. Many runners who do not fuel for these sessions also specified that eating before quality/high-intensity training makes them feel sick during the session. Many runners also have a threshold distance or run time (such as long runs over weekends), to consider eating before a run. It could otherwise interfere with intermittent fasting or eating plans during the week. However, 15 runners (55%) do fuel adequately by eating more protein or carbohydrate-rich foods and timing the meal optimally before the training session. These runners indicated that they believe it helps them train better, makes them feel more energised for the session and reduces the risk of illness or injury.

4.4.1.4 Recovery

Recovery after a key session is important for 16 (59%) participants, including more protein, carbohydrates, more food in general and timing of a recovery meal or drink within one-hour post-training. These participants do recovery eating as they feel hungry post-training, wish to build or retain muscle mass, or want to recover quicker after the session. The remainder of the participants never thought of recovery eating as a technique to help with their overall training.

4.4.1.5 Training in the fasted state

Twenty-two (70%) participating runners train whilst fasting. Only five (30%) runners do not fast for any runs. Many runners train fasted as they run early in the morning, and it is simply too early in the day for them to eat. An empty stomach helps with gut comfort during the run. Another reason is no time is wasted on eating between getting up in the morning and going for a run. Five (19%) of the runners also reduce carbohydrates during training to lose weight and feel less bloated for runs. Twelve of the participants (44%) do have carbohydrates during runs, but only if the run is longer than 90 minutes.

4.4.2 Nutrition during the competition preparation phase

Twelve (44%) runners train for more than eight weeks before a race, whereas fifteen (55%) runners train anything from two to eight weeks before the race. Twenty-two (70%) of the runners aim to run peak races one to four times a year, and only four runners race more than four times per year. In terms of nutrition during this phase, there is minimal difference compared to nutrition during base training.

4.4.2.1 Race week and race day nutrition

Twenty-four to 48 hours before race day, 16 of the runners (59%) reported eating more calories but also eating gluten free and focusing on low fibre foods. Reasons for specific diet plans include better performance on race day, improved recovery after the race, GI comfort during the race and aiming to be at race weight on the day. The remaining 11 (41%) runners prefer to keep their dietary intake the same throughout all phases for preparation and race week/day or indicated that they do not know if they should be following a specific race week/day plan.

Race day nutrition for many runners includes higher calories, carbohydrate loading and increased fluid intake. Reasons given include improved performance for race day, starting the race well hydrated and optimal recovery afterwards. Twenty-two (81%) of the runners increase fluid intake during the race, 16 (59%) ingest electrolytes and only 9 (33,3%) ingest performance supplements during the race, including:

- Gu chews
- 32Gi endure products
- Amino powder with caffeine
- Nuun caffeine
- Energy bars
- Gels
- Cramp ease

Runners who eat or drink during the race do so to improve performance, remain hydrated throughout the race and improve recovery afterwards. Twenty out of 27 runners (74%) indicated that they could mostly follow their eating and drinking plan during a race. It is sometimes more difficult for elite runners as they are not always allowed to have a seconding runner to help with fluid intake or eating during the race, or they could miss the water table when running at a constant fast pace. The remaining seven runners (26%) who indicated they could not follow their eating or drinking plan during the race, sometimes miss the aid stations on the way due to big groups of people or cannot carry their own food and fluids, also struggle with GI issues.

4.4.3 Nutritional status and energy balance

Each participant kept daily dietary records, including carbohydrates, protein and fats consumed for seven days, along with their daily training, using the MyFitnessPal app. Daily nutritional data were extracted from the MyFitnessPal app for macronutrients. Daily energy requirements were calculated using the BMR calculation (Mifflin-St. JEOR equation as described in chapter 3, section 3.8) and by BMR x PAL (mid-point) = kcal/kg/day (also described in chapter 3, section 3.8). After calculating daily energy requirements, calories per macronutrient were calculated as ranges for each participant: carbohydrates 5 to 7 g/kg, proteins 1,2 to 2.0 g/kg, fats 0,5 to 1,5 g/kg (Table 4.3).

Table 4.3: The average macronutrients measured in kcal versus each participant's RDA range of macronutrients

Participant	Protein (kcal) consumed	Range of protein consumption (kcal)		Fats (kcal) consumed	Range of fats consumption (kcal)		Carbohydrates consumed (kcal)	Range of carbohydrates consumption (kcal)	
1	266,3*	273,6	456	563,1	256,5	769,5	633,2*	912	1596
2	286,8	268,8	448	397,3	252	756	563,4*	896	1568
3	330,3	283,2	472	618,4	265,5	796,5	480*	944	1652
4	244,6*	278,4	464	618,4	261	783	717,2*	928	1624
5	181,7*	292,8	488	421,7	274,5	823,5	652*	976	1708
6	344,6	295,2	492	753,4	276,8	830,3	1056	984	1722
7	361,7	276	460	465,4	258,8	776,3	814,8*	920	1610
8	358,3	278,4	464	340,7	261	783	630,3*	928	1624
9	357,7	259,2	432	736,7	243	729	1094,3	864	1512
10	319,4	254,4	424	513	238,5	715,5	536*	848	1484
11	213,2*	287,0	478,4	290,6	269,1	807,3	602,3*	956,8	1674,4
12	348,6	244,8	408	793,3	229,5	688,5	1004	816	1428
13	293,2	235,2	392	533,6	220,5	661,5	475,4*	784	1372
14	280,6*	312	520	650,6	292,5	877,5	639,4*	1040	1820
15	404,6	293,7	489,6	511,7	275,4	826,2	720,6*	979,2	1713,6
16	395,4	297,6	496	727,7	279	837	777,7*	992	1736
17	176,6*	321,6	536	362,6	301,5	904,5	468,6*	1072	1876
18	134,8*	311,0	520	223,7*	291,6	874,8	447,4*	1036,8	1814,4
19	220*	268,8	448	425,6	252	756	334,8*	896	1568
20	412	302,4	504	885,9	283,5	850,5	858,8*	1008	1764
21	256,6	249,6	416	471,9	234	702	639,4*	832	1456
22	529,2	278,4	464	658,3	261	783	985,7*	928	1624
23	362,8	309,6	516	750,9	290,6	870,8	625,7*	1032	1806
24	254,3*	268,8	448	509,1	252	756	568*	896	1568
25	312,6	240	400	595,3	225	675	713,7*	800	1400
26	512	326,4	544	469,3	306	918	749,2*	1088	1904
27	233,2*	302,4	504	528,4	283,5	850,5	795,4*	1008	1764

RDA for protein = 1,2 – 2,0 g/kg (4 kcal for 1g), RDA for fats = 0,5 – 1,5 g/kg (9 kcal for 1g), RDA for carbohydrates = 5 – 7 g/kg (4 kcal for 1g) (Mahan and Raymond, 2016).

** Indicates insufficient macronutrient intake according to each athlete's calculated energy requirements as it is out of range of the RDA of Mahan and Raymond (2016).*

Ten participants (37%) have an insufficient protein daily; fat intake is in range for all except one participant; and 24 participants (89%) have too little carbohydrates on average over seven days. Out of all 27 participants, only one participant was in range for the seven days of daily tracking for protein, carbohydrates and fats. The calories burned during training, measured by Strava, was utilised to calculate each participant's TDEE (Figure 4.4).

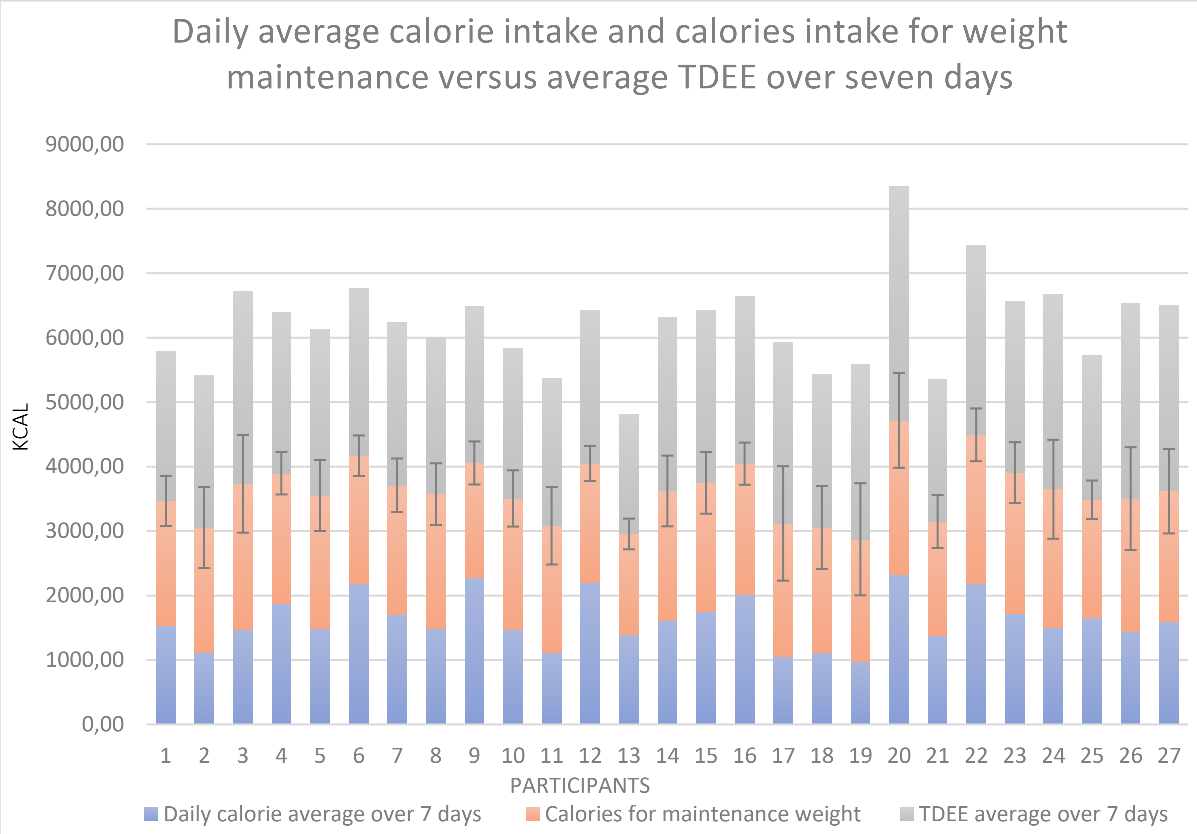

Figure 4.4: Daily intake of calories and calorie intake for maintenance weight versus TDEE over the measured seven days of food and exercise tracking for participants.

As seen in Figure 4.4, only three participants' daily calorie intake was sufficient for weight maintenance, three participants were slightly over, and one was slightly below. All the remaining participants (74%) were under-eating or eating in a large calorie deficit daily.

4.5 Blood sample results

4.5.1 Iron

Limited by resources, only ferritin was measured. The normal reference range for ferritin is between 6.5 to 147.1 ng/mL as used by the blood laboratory. All participants' ferritin levels were in range of the standard values used by the laboratory (Figure 4.5). However, current literature suggests that less than 35 ng/mL is deficient for sFer levels (Archer &

Brugnara, 2015; Sim et al., 2019). Ten runners (37%) had a level of below 35 ng/mL, and one participant as low as 9 ng/mL indicating iron deficiency with anaemia sFer levels less than 12 ng/mL (Table 4.4).

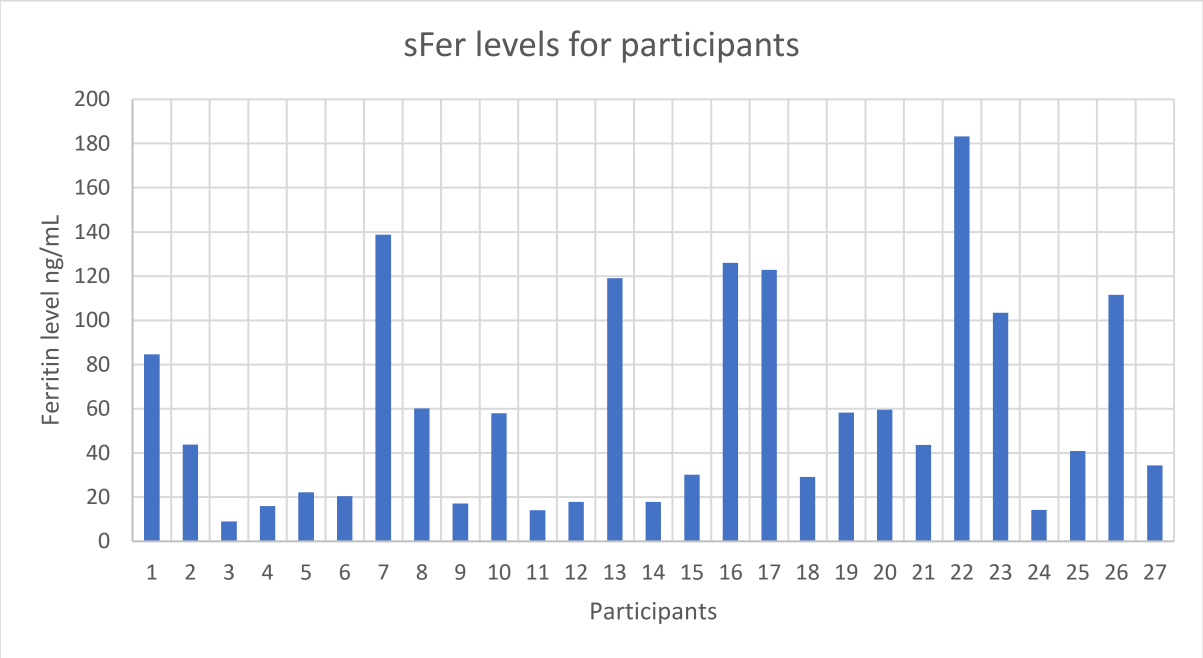

Figure 4.5: Participants' sFer levels

Table 4.4: sFer levels classification for participants with low serum ferritin levels

sFer level classifications			
		sFer levels: 12 – 20 ng/mL	sFer levels: 20 – 35 ng/mL
N = 11 (41%)	1	5	5
Deficiency level	*Iron deficiency with anaemia*	*Iron deficiency without anaemia*	*Iron depletion*

Only one participant had a higher-than-normal ferritin level, probably due to an intravenous iron infusion received the day before the blood test. The participant is an elite runner and receives iron infusions regularly to increase iron levels to improve her performance.

4.5.2 Vitamin D and magnesium

The sufficiency level for vitamin D is greater than 20 ng/mL, and all participants were in the normal range, except one participant that had a low level (15 ng/mL). All participants had a normal level of magnesium in the range of 0.65 to 1.05 mmol/L.

4.5.3 Calcium

Twenty participants tested in the normal range for total calcium levels, between 2.15 and 2.62 mmol/L. Seven participants had a low calcium score, which could be because of dietary choices or not taking a supplement (Figure 4.6). Low calcium levels could increase the risk of stress fractures, osteopenia and osteoporosis (NOFSA, 2017).

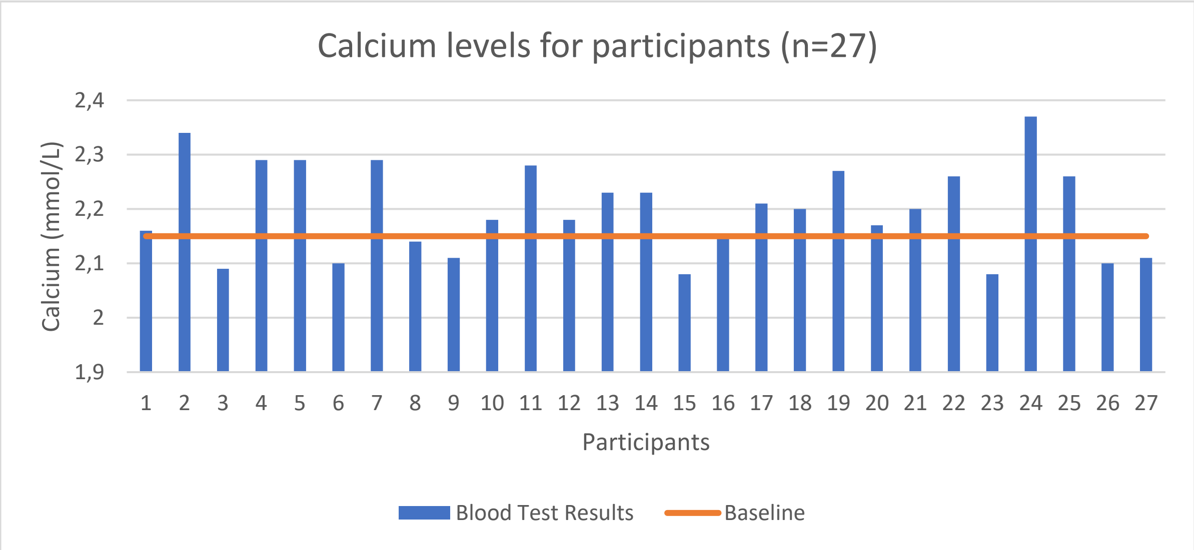

Figure 4.6: Total calcium level scores for all participants

4.6 Summary of key findings

The median age of participants was 36 years (interquartile range =13), with an average BMI score of 21.55. Twenty-two (81%) participants partake in sports other than running; with many including strength training to improve running performance.

Ten runners (37%) struggle with GI issues unrelated to menstruation, including eating before or after a run and even during long distance runs. Four participants (15%) reported amenorrhoea when the study was conducted, whilst ten participants (37%) previously struggled with amenorrhoea. Seven participants (26%) indicated that their menstrual cycle changes when higher intensity or increased volume of runs are incorporated into their training schedule. The researcher scored each participant with the LEAF-Q scoring key system, and 11 participants are at risk for the female athletic triad, which is also a risk for RED-S. Further, with seven days of food diary entries, 22 participants were at moderate risk for RED-S as scored with the RED-S CAT. Over the recorded seven days, 24 participants (89%) consumed insufficient carbohydrates, and ten participants (37%) did not consume enough protein. Dietary fat intake was in range for all participants except one recording slightly under RDA. Out of all 27 participants, only one participant was in range for the seven days of daily tracking for protein, carbohydrates and fats. Nineteen (70%) runners also train in the fasted state as many run in the morning. Fifteen runners (55%) did consume more food or calories for higher volume and intensity training sessions; however, 12 (44%) did not fuel for these sessions. Sixteen (59%) of the participants do eat more or higher calorie meals to fuel 24 to 48 hours before a running event, whereas the remaining 11 (41%) do not adapt their diets at all. Nine runners (33%) reported having injuries in the last year, mainly in the hips, knees, lower leg and feet.

Eleven participants had a sFer level of below 35 ng/mL and are iron deficient; one participant was found to be iron deficient with anaemia. Vitamin D and magnesium levels are normal in most participants, and seven participants have low calcium levels.

CHAPTER 5: DISCUSSION

Any female endurance runner requires sustainable nutrition for intense training and optimal performance in endurance events. Training for such endurance events also requires an athlete's body to recover swiftly to continue training and competing. A well-balanced nutritional plan for an endurance runner would include sufficient vitamins, minerals, macro- and micronutrients. The primary objective of the study was to establish the nutritional habits and statuses of female runners in Johannesburg. The secondary objectives included the determination of the energy balance of female runners, examination of associations between nutrition and energy status, and determining the risk of RED-S in participants.

This chapter will discuss each one of the named objectives of the study and compare the results obtained to existing literature.

5.1 Nutritional habits and statuses of female runners

A demographic profile was established for runners that reside in Johannesburg that train at least three times a week with moderate to high intensity of 40 minutes per session. The participant group (n=27) were all females that had run a marathon distance or more in the last two years or had been training for a marathon. Most participants (n=26) were not smokers.

Thirty-three percent of the participants follow a specific diet to support their training goals. Diets include vegan (one participant), restricted energy combined with a paleo for athlete diet (one participant), low carbohydrate high fat (4 participants) and a high protein low carbohydrate diet (three participants). The majority of runners who chose to follow a specific diet did so for better health. Many runners do not adjust their nutrition according to their training schedule, with one of the main reasons being gut comfort during a run.

The findings of the study on GI difficulties are similar to a study done in the United Kingdom and Ireland by Pugh et al., 2018, showed that GI symptoms arise as early as a week before the race for 42% of recreational runners. Twenty-seven percent of runners struggle with flatus during training (16%) and nausea during the race (8%); however, the study found that there is no correlation between nutritional intake and GI symptoms (Pugh et al., 2018). Several of the 27 participants indicated that they train in the fasted state for gut comfort; this could be due to stress and anxiety on the day, exercise intensity, dehydration, environmental temperatures and even time of day. Several runners indicated that they run in the fasted state unless it is a long run, a run longer than 90 minutes or a distance of ten kilometres or more. One participant indicated that she does most of her run training in the morning and finds it difficult to eat beforehand if a session starts at 5 am.

Eleven runners (41%) kept their diets the same for all preparation weeks (easy and hard training sessions such as hills or sprints included) as well as for race day. Sixteen runners (59%) do incorporate a recovery meal or drink post-running within one hour after running or training. These runners also indicated that they do not know what to change or what diet to follow. This is a sign that many female runners are not educated on nutrition for preparation and racing in running events. However, 15 (55%) of the participants adapted their nutrition for harder training days, whilst 12 (45%) kept their nutrition the same. Eleven (41%) of the participants eat less on easier training days, as their TDEE is less for shorter workouts or lower intensity training sessions. It should be noted that the train low strategy is popular among athletes, which entails exercising fasted and not refuelling between training sessions (Thomas et al., 2016). This practice is often used to enhance the cellular outcomes for endurance training for athletes, such as increasing the maximal mitochondrial density, mitochondrial enzyme activity and rate of lipid oxidation (Zourdos et al., 2015). However, this strategy should only be implemented for short periods of time in training plans as it could influence the carbohydrate availability athletes require for races or for key training sessions (Jeukendrup, 2017). Only six runners use a specific diet during heat and return to running from off-season mainly to incorporate it for enhanced adaptation to training during these blocks and better quality sessions. As for transition into race season, specific diets were followed for losing weight or achieving body composition goals, for better health, quality training and adaptation for running races.

Seventeen runners (63%) indicated they do not fuel adequately for key training sessions, and this could impact their performance for these important sessions. One of the participants indicated that she only eats dinner during the week and does intermittent fasting for breakfast and lunch, but usually only runs in the afternoon after work. A solution could include better timing of meals and training to have higher energy levels available for running.

Protein intake amongst 11 participants (41%) was not sufficient for their respective ranges. Nunes et al. (2018) concluded that 22% of female athletes were consuming below RDA of protein. Although, studies by Beerman et al. (2020) found that athletes consume enough protein or above RDA. Sixteen runners (59%) did consume enough protein on a daily basis. For participants in the study, 'more protein is better' could have influenced their notion of their food choices to rather have a higher protein snack or meal. Convenience and accessibility for higher protein levels could also play a role in food choices. Since an LBM is desired by long-distance runners, and if an athlete would like to increase their protein intake, options for animal-based diets include lean red meat or poultry, seafood and eggs, whilst plant-based sources include legumes, nuts, seeds, soy products or meat alternatives (Campbell, 2019).

The results showed that many participants do not consume enough carbohydrates. This is congruent with recent literature on female distance runners. Beermann et al. (2020) conducted a study on males (n=21) and females (n=20) and found that 73% consume fewer carbohydrates than the recommended amounts. In another study, 45% of female athletes were consuming below RDA for carbohydrates (Nunes et al., 2018). Low carbohydrate intake could also be utilised for maintenance, loss of weight, or to induce ketosis. Dietary practices that include low carbohydrates could lead to eating disorders amongst female runners striving for leanness and lower body weight. The relative energy balance of our cohort is discussed in the next section. Carbohydrates serve as an energy substrate during exercise over a large range of different intensities (Spriet, 2014). Compared to fats, carbohydrates provide a greater yield of ATP per volume of oxygen delivered to mitochondria (Spriet, 2014).

The fat consumption for participants was either in range or above (n=4); only one participant consumed too little fat daily. Only one participant of out the four that are above range, follows a high fat, low carbohydrate diet. These results are desirable to ensure sufficient intake of fat-soluble vitamins, essential amino acids and omega-3 fatty acids (Kerksick et al., 2017). Recent diet cultures have put emphasis on higher fat and lower carbohydrates, such as in a keto diet. This could influence athletes' choices to consciously opt for higher-fat meals with lower carbohydrate contents. One runner that follows a vegan diet supplements omega-3 daily.

Optimal nutrition during the training phase can also benefit female endurance runners during a race by training the gut to tolerate foods and fluids. This is also known as periodised nutrition, which is defined as a "strategic combination of nutrition and exercise to optimize training adaptations and competition performance" (Jeukendrup, 2017; Burke et al., 2019, p. 1). Fifteen runners (48%) do fuel for key training sessions, but 12 runners

(42%) do not adapt their nutrition at all to their training. It is suggested that high-quality training sessions should be performed with low carbohydrate availability, which simulates race conditions (Burke et al., 2019). A systematic review of 61 studies with 679 subjects concluded that 82% of studies showed consuming carbohydrates during an endurance race has significant benefits for the athlete (Burke et al., 2019). Earlier guidelines suggest consumption of 30 to 60 grams of carbohydrates during a race, but more recent recommendations include smaller amounts of carbohydrates for shorter races and larger amounts for races longer than 2.5 hours, depending on an athlete's tolerance (Burke et al., 2019).

Thong et al. (2000) reported that athletes that struggle with amenorrhoea have energy availabilities as low as 16 kcal·per kg of LBM·per day, resulting in decreased bone formation (Thong et al., 2000). Whilst it may appear practical to recommend to an athlete that preserving a power accessibility of 45 kcal·per kg LBM·per day in time is required to optimise their bone health and wellness and safeguard versus bony injuries, it is most likely an impractical target for lots of professional athletes (Sale & Elliott-Sale, 2019).

None of the participants reported having had stress fractures, but it is still a high risk indication for RED-S. One participant reported having a bone bruise on her left knee. Upon follow up with the participant, it was established that an MRI report indicated that the participant has a bipartite patella (kneecap is two bones instead of one) and would not be related to nutritional status.

5.2 Energy balance of female runners

Only five participants were consuming enough calories daily to maintain their current weight and replace energy utilised for running or other training. The logged physiological symptoms such as oligomenorrhoea or amenorrhoea in participants show a high prevalence of many athletes failing to balance their energy intakes with their energy expenditure, mainly due to eating under their daily BMR calorie target and over time, this causes low EA. No participant deliberately under ate, but what participants were consuming, was below their estimated TDEE. The results indicated that 22 participants are in a net negative energy balance. According to the IOC Relative Energy Deficiency in Sport CAT, these 22 runners are in the moderate risk category for RED-S. Determined by low EA or amenorrhoea, this is a total of 81% of the participants. The remaining five participants are in the green category, which is no risk for RED-S. Therefore, female runners should adapt their diets to TEE for optimal energy balance. If participants were to eat at their calculated BMR every day, daily nutrition could be adapted to eating 1.2 to 2 g/kg of protein, 0.8 to 1.0 g/kg of fats and utilising the remaining calories for carbohydrates (5 to 7 g/kg), depending on weight and height of each participant. For female runners to achieve energy balance, adaptations to diets to consume more calories or exercise less should be considered. It would be prudent that such female athletes should be followed up on to monitor their weight.

Eleven runners were found to be iron deficient, five of which were in the iron depletion category, five with iron deficiency without anaemia and one with iron deficiency with anaemia. In keeping with a study from 2020, female runners are at a potentially higher risk for menstrual and GI blood losses due to higher hepcidin levels post-exercise (McKay et al., 2020). One runner indicated she follows a vegan diet and is categorised in the iron depletion category with a ferritin level of 30.1 ng/mL. Iron-rich foods for a vegetarian or vegan diet would include fortified breakfast cereals, bread, textured vegetable protein, pulses (chickpeas, lentils, black beans), dried beans, soy foods and meat alternatives, nuts, and dried fruits (Brown, 2018).

Three participants indicated to only having nine to 11 menstrual periods in a year, which is abnormal (oligomenorhoea) and could be the start of amenorrhoea. Amenorrhoea was shown in ten participants, and four runners had amenorrhoea at the time of the study. MD occurs in female runners when LEA is coupled with exercise and inadequate energy intake (Gibbs et al., 2013). Seven participants also experienced menstrual changes when exercise is increased; this could indicate that their energy expenditure is more and their energy intake is not adjusted accordingly, which leads to LEA. Similarly, in a study done in 2015 after the London marathon, amenorrhoea and heavy menstrual bleeding seemed to be a common finding in one in three healthy runners, both recreational and elite runners included (Dugan et al., 2021). Though, runners with heavy menstrual bleeding are more prone to anaemia and iron deficiency (Dugan et al., 2021).

All participants except for one runner had sufficient vitamin D levels, yet seven participants had low calcium levels. In Beerman et al.'s study in 2020, participants that were deficient in calcium were also deficient in vitamin D. However, the results from this study do not correlate with studies showing deficiencies in both micronutrients. Low calcium levels can lead to greater health consequences later in life, especially bone health. Decreased bone health can be linked to EA, low carbohydrate availability, protein intake, vitamin D intake and dermal calcium and sodium losses (Sale & Elliott-Sale, 2019).

According to the South African Clinical guidelines for diagnosis and management of osteoporosis 2010, patients with low calcium levels should consider having a BMD scan to accurately assess risk levels (NOFSA, 2017). Female endurance runners in South Africa should have a sufficient vitamin D status if their running is done out of doors. Female runners that incorporate less dairy in their diets due to intolerance, such as following lactose-free diets, can also influence calcium levels. If there is no indication to avoid dairy in an athlete's diet, then calcium deficiencies could be counteracted with increases in milk, cheese, yoghurt, fortified soy products, nuts and seeds, green vegetables, and dried fruit (Brown, 2018). Further plant-based options also include soybeans, chickpeas, tofu, calcium-fortified citrus or vegetable juice, plant-based milk, and kale (Brown, 2018). Current research suggests an attempt to achieve EA above 30 kcal·per kg LBM·per day to minimise negative effects on the bone and increase calcium intake before exercise to minimise dermal calcium loss (Sale & Elliott-Sale, 2019). It is suggested that female runners that have LEA and MD might require more than just the RDA of calcium and vitamin D to optimise their bone health (Kerksick et al., 2017)

5.3 RED-S risk in female runners

The epidemiology of RED-S and the female athlete triad is not well known or well understood by female distance runners. A high volume of training as required by the sport and significant pressure for low bodyweight for increased performance continues to place female endurance runners at high risk (Currie, 2010). Although the researcher did not include BMD scans as a tool for screening for RED-S, a significant finding placed 81% of participants in the moderate risk category for RED-S. LEA and irregular menstrual cycles are the main causes of this finding. Many participants are not balancing their energy intake with energy expenditure as recorded on the seven-day food diary via the MyFitnessPal app and menstrual cycle disturbances as indicated on the LEAF-Q.

As a result of RED-S, injuries are also more prevalent in running, as low bone mass, the possibility of early onset of osteoporosis and constant LEA are all contributing factors. It should be recognised that appropriate nutritional strategies play a large role in injury prevention and recovery afterwards (Close et al., 2019). Runners that have struggled with injuries in the past did not take enough time off from running and exercising to fully recover from the sustained injury, which could affect their running in the future.

5.4 Limitations

The study does have potential limitations, including small sample size, location of study, and willingness and availability of participants to participate. The study had a small sample size, so the results are therefore limited. However, the sample size was large enough to draw valid statistical conclusions. The study was conducted only in Johannesburg, Gauteng, and the remaining representative population of South Africa's female endurance runners was thus excluded. Other tests such as different blood tests and BMD scans can also be beneficial to include.

Participants used the MyFitnessPal app to log their seven-day food diary, and there was an expectation that all participants used the app correctly. Incorrect quantities of food logged could also create an inaccurate result in diaries recorded. For example, an existing food entry that can be chosen could be conventional oats, but the quantity of the food entry is logged as 1g, and if the participant does not change the quantity to the amount that is consumed, the app will log the entry as 1g only, which is unrealistic. Underreporting or under-eating could have influenced the overall outcome of the study. Underreporting amounts of foods consumed on the MyFitnessPal app can happen when a food item is selected to be added to the food diary, but the amount isn't adjusted to what was actually consumed. For example, an entry for brown bread is selected, but the default selection is for one slice of bread and not two. Under-eating could be because of rest days and the need to eat less when no exercise is done. Some participants added that they eat the same amounts of calories every day for maintenance, and others don't want to eat more as they are very weight conscious. Under-eating could be appealing to female runners that are seeking to lose weight or stay within their calorie goal for the day, but this could lead to skipping meals and even an eating disorder.

Factors related to the low number of participants could include the COVID-19 pandemic and its effects on the runners and running events, as well as the date and times that runners had to commit to having their blood samples drawn. Many female runners have downscaled on their running as there were limited or no running events scheduled due to the pandemic.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study established the energy balance, nutritional habits and nutritional statuses of female endurance runners in Johannesburg.

There has been more recognition of RED-S, especially among female athletes since the release of the IOC statements on RED-S in 2015 and 2018. Much of the existing literature is more focused on male athletes, but more recent studies have included more females, as RED-S seems to be more common in female athletes. Countless female athletes are still not well informed of RED-S and the risks associated with it, as many are unaware that they are at risk for RED-S.

The study consisted of 27 participants. Of the 27 participants, 11 had a level of ferritin that is classified as iron deficiency, seven had low calcium scores, one participant had a vitamin D deficiency, and all had in-range scores for magnesium.

Using a LEAF-Q scoring key to determine the risk of the female athlete triad and with the RED-S CAT for risk of RED-S, an astonishing 81% of participants are at moderate risk for RED-S, including eight participants who also scored greater than eight points for the risk of the female athletic triad. Most points scored were for amenorrhoea or irregular menstrual function. Ten participants had reported having had amenorrhoea before or were currently experiencing amenorrhoea at the time of the study, which could indicate low energy balance. Nine participants have also struggled with injuries, including bone injuries and stress fractures. GI function also plays a factor in the risk for the triad as ten participants reported struggling daily with GI function. Many participants do not balance their energy intake with energy expenditure, which is indicated through their menstrual function, GI function and injuries, as BMI is within range for most participants. This may lead to long-term LEA and ultimately a decrease in performance and other health consequences. The participants that are at risk for RED-S can utilise the return to sport model to monitor themselves to reduce their risk for RED-S and, in due course, reach a stage of balanced nutrition and energy expenditure of running.

6.2 Recommendations

Further research in the field of RED-S is required in South African athletes and athletes at risk for RED-S should be monitored. Research should be expanded to determine health risk effects, including BMD studies, body composition and hormonal profiles.

The risk of RED-S in female endurance runners can be reduced by tracking daily intake of food to ensure sufficient calories are consumed for optimal energy balance. Female runners can also consider having a BMD scan to ensure good bone health. Irregular menstrual cycles should be investigated further by a general practitioner or gynaecologist. Interventions to reduce RED-S should include education programmes for athletes involving dietary knowledge, food diaries and personal health monitoring (like menstruation history) training volumes.

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APPENDIX 1 THE LEAF-Q QUESTIONNAIRE

(Supplemental Digital Content 1)

The LEAF-Q

A questionnaire for female athletes

Department of Nutrition, Exercise and Sports
Life Science
University of Copenhagen
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The low energy availability in females questionnaire (LEAF –Q), focuses on physiological symptoms of insufficient energy intake. The following pages contain questions regarding injuries, gastrointestinal and reproductive function. We appreciate you taking the time to fill out the LEAF-Q and the reply will be treated as confidential.

Age: ____________(years)

Height: ____________(cm) Weight: ____________(kg)

Your highest weight with your present height: ____________(kg)
(excluding pregnancy)

Your lowest weight with your present height: ____________(kg)

Do you smoke? Yes ☐ No ☐

Do you use any medication (excluding oral contraceptives)? Yes ☐ No ☐

If yes, what kind of medication?____

Your normal amount of training (average) – number of hours per week and what kind of exercise, such as running, swimming, bicycling, strength training, technique training etc.:

____

____

____

Comments or further information regarding exercise:______________________________

____

____

1. Injuries **Mark the response that most accurately describes your situation**

A: Have you had absences from your training, or participation in competitions during the last year due to injuries?

☐ No, not at all ☐ Yes, once or twice ☐ Yes, three or four times ☐ Yes, five times or more

A1: If yes, for how many days absence from training or participation in competition due to injuries have you had in the last year?

☐ 1-7 days ☐ 8-14 days ☐ 15-21 days ☐ 22 days or more

A2: If yes, what kind of injuries have you had in the last year? ______________________________

____

Comments or further information regarding injuries:______________________________

____

____

2. Gastro intestinal function

A: Do you feel gaseous or bloated in the abdomen, also when you do not have your period?

☐ Yes, several times a day ☐ Yes, several times a week

☐ Yes, once or twice a week or more seldom ☐ Rarely or never

B: Do you get cramps or stomach ache which cannot be related to your menstruation?

☐ Yes, several times a day ☐ Yes, several times a week

☐ Yes, once or twice a week or more seldom ☐ Rarely or never

C: How often do you have bowel movements on average?

☐ Several times a day ☐ Once a day ☐ Every second day

☐ Twice a week ☐ Once a week or more rarely

D: How would you describe your normal stool?

☐ Normal (soft) ☐ Diarrhoea-like (watery) ☐ Hard and dry

Comments regarding gastrointestinal function: ________________________________

____

3. Menstrual function and use of contraceptives

3.1 Contraceptives

Mark the response that most accurately describes your situation

A: Do you use oral contraceptives?

☐ Yes ☐ No

A1: If yes, why do you use oral contraceptives?

☐ Contraception ☐ Reduction of menstruation pains ☐ Reduction of bleeding

☐ To regulate the menstrual cycle in relation to performances etc..

☐ Otherwise menstruation stops

☐ Other ______________________________

____

A2: If no, have you used oral contraceptives earlier?

☐ Yes ☐ No

A2:1 If yes, when and for how long?______________________________

____

____

B: Do you use any other kind of hormonal contraceptives? (e.g. hormonal implant or coil)

☐ Yes ☐ No

B1: If yes, what kind?

☐ Hormonal patches ☐ Hormonal ring ☐ Hormonal coil ☐ Hormonal implant ☐ Other

____

3.2 Menstrual function

Mark the response that most accurately describes your situation

A: How old were when you had your first period?

☐ 11 years or younger ☐ 12-14 years ☐ 15 years or older ☐ I don't remember

☐ I have never menstruated (If you have answered "I have never menstruated" there are no further questions to answer)

B: Did your first menstruation come naturally (by itself)?

☐ Yes ☐ No ☐ I don't remember

B1: If no, what kind of treatment was used to start your menstrual cycle?

☐ Hormonal treatment ☐ Weight gain

☐ Reduced amount of exercise ☐ Other

C: Do you have normal menstruation?

☐ Yes ☐ No **(go to question C6)** ☐ I don't know **(go to question C6)**

C1: If yes, when was your last period?

☐ 0-4 weeks ago ☐ 1-2 months ago ☐ 3-4 months ago ☐ 5 months ago or more

C2: If yes, are your periods regular? (Every 28th to 34th day)

☐ Yes, most of the time ☐ No, mostly not

C3: If yes, for how many days do you normally bleed?

☐ 1-2 days ☐ 3-4 days ☐ 5-6 days ☐ 7-8 days ☐ 9 days or more

C4: If yes, have you ever had problems with heavy menstrual bleeding?

☐ Yes ☐ No

C5: If yes, how many periods have you had during the last year?

☐ 12 or more ☐ 9-11 ☐ 6-8 ☐ 3-5 ☐ 0-2

3.2 Menstrual function

Mark the response that most accurately describes your situation

C6: If no or "I don't remember", when did you have your last period?

- ☐ 2-3 months ago
- ☐ 4-5 months ago
- ☐ 6 months ago or more
- ☐ I'm pregnant and therefore do not menstruate

D: Have your periods ever stopped for 3 consecutive months or longer (besides pregnancy)?

- ☐ No, never
- ☐ Yes, it has happened before
- ☐ Yes, that's the situation now

E: Do you experience that your menstruation changes when you increase your exercise intensity, frequency or duration?

- ☐ Yes
- ☐ No

E1: If yes, how? (Check one or more options)

- ☐ I bleed less
- ☐ I bleed fewer days
- ☐ My menstruations stops
- ☐ I bleed more
- ☐ I bleed more days

APPENDIX 2 THE LEAF-Q SCORING KEY

(Supplemental Digital Content 2)

The LEAF-Q Scoring key

A total score ≥8 is to be considered at risk for the Triad

Department of Nutrition, Exercise and Sports
Life Science
University of Copenhagen
Denmark
Contact: Anna Melin, aot@life.ku.dk

1. A: 0 No, not at all, 1 Yes, once or twice, 2 Yes, three or four times, 3 Yes, five times or more

1. A1: 1 1-7 days, 2 8-14 days, 3 15-21 days, 4 22 days or more

2. A: 3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom,

0 Rarely or never

2. B: 3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom,

0 Rarely or never

2. C: 1 Several times a day, 0 Once a day, 2 Every second day, 3 Twice a week, 4 Once a week or more rarely

2. D: 0 Normal, 1 Diarrhoea-like, 2 Hard and dry

3.1 A1: 0 Contraception, 0 Reduction of menstruation pains, 0 Reduction of bleeding,

0 To regulate the menstrual cycle in relation to performances etc.., 1 Otherwise menstruation stops

3.2 A: 0 11 years or younger, 0 12-14 years, 1 15 years or older, 0 I don't remember,

8 I have never menstruated

3.2 B: 0Yes, 1 No, 1 I don't remember

3.2 B1: 1 Hormonal treatment, 1 Weight gain, 1 Reduced amount of exercise, 1 Other

3.2 C: 0 Yes, 2 No (go to question 3.2 C6), 1 I don't know (go to question 3.2 C6)

3.2 C1: 0 0-4 weeks ago, 1 1-2 months ago, 2 3-4 months ago, 3 5 months ago or more

3.2 C2: 0 Yes, most of the time, 1 No, mostly not

3.2 C3: 1 1-2 days, 0 3-4 days, 0 5-6 days, 0 7-8 days, 0 9 days or more

3.2 C4: 0 Yes, 0 No

3.2 C5: 0 12 or more, 1 9-11, 2 6-8, 3 3-5, 4 0-2

3.2 C6: 1 2-3 months ago, 2 4-5 months ago, 3 6 months ago or more

0 I'm pregnant and therefore do not menstruate

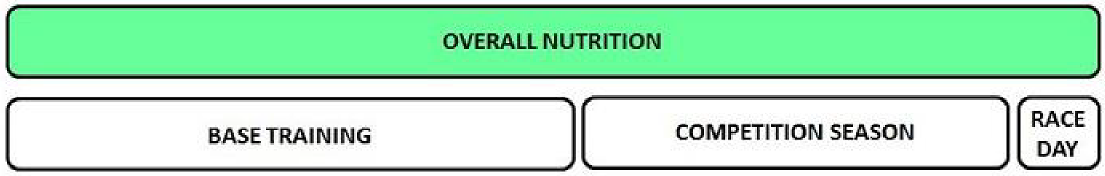
3.2 D: 0 No, never, 1 Yes, it has happened before, 2 Yes, that's the situation now

3.2 E: 1 Yes, 0 No

3.2 E1: 1 I bleed less, 1 I bleed fewer days, 2 My menstruations stops, 0 I bleed more, 0 I bleed more days

APPENDIX 3 NUTRITIONAL QUESTIONNAIRE

PART A: OVERALL NUTRITION PRINCIPLES ACROSS THE ANNUAL TRAINING/COMPETITION CYCLE



Next: Overall nutrition throughout the annual training/competition program

PART A. GENERAL PRINCIPLES OF YOUR ANNUAL TRAINING/COMPETITION DIET

6) Do you deliberately and consistently follow a special/unique overall general dietary plan (e.g. vegetarian) to support your training goals? [i.e. all year round during all training phases] (Tick all the answers that are important in describing what you do) *

[ ] Vegan/vegetarian

[ ] Very high energy/extra energy (high Calorie)

[ ] Restricted energy (low energy)

[ ] Paleo

[ ] Paleo for athletes (Paleo but with more carbs around training sessions)

[ ] Low Carb High Fat (LCHF)

[ ] High protein Low Carb

[ ] Gluten Free

[ ] High Carb

[ ] Periodised Carb (strategic periodisation on carbohydrate intake around training, i.e. intentionally training with high or low carbohydrate availability)

[ ] FODMAP

[ ] Other (please describe):

____

[ ] No, I do not follow any special dietary plan

If yes, what is the main purpose of following a specific diet chronically? (Tick all the answers that are important in describing what you do)

	Religious/ cultural reasons	**Ethical/ moral reasons**	**To achieve better quality training overall**	**To enhance adaptation to training generally**	**To lose weight/achieve body composition goals**	**Better health**	**Diagnosed allergies or intolerances**	**I have some allergies or intolerances but haven't had them fully diagnosed**	**Other (please specify in the comment box below)**	**Someone told me to follow this diet (who? Please specify in the comment box below)**
Vegan/vegetarian	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Very high energy/extra energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restricted energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo for athletes	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low Carb High Fat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Protein Low Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Gluten free	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Periodised carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
FODMAP	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments

2) Do you deliberately but periodically introduce a special dietary plan to support your training goals? [i.e. periodically following one or several nutrition strategies over the annual training/competition programme] If so, when do you follow a special dietary plan? (Tick all the answers that are important in describing what you do) *

[ ] Altitude training

[ ] Heat training

[ ] Return from off-season

[ ] Return from injury

[ ] Competition preparation phase/transition to race season

[ ] Other time period (please explain): ______________________________ *

[ ] No, I do not periodically follow a special dietary plan during the yearly training programme

What is the main purpose of following a special diet during altitude training? (Tick all the answers that are important in describing what you do) *

	Cultural or religious reasons	**Ethical/ moral reasons**	**Better quality training for a special training block**	**Enhanced adaptation to a special training block**	**To lose weight/achieve body composition goals**	**Better health**	**Diagnosed allergies or intolerances**	**I have some allergies or intolerances but haven't had them fully diagnosed**	**Other (please explain)**	**Cultural or religious reasons**
Vegan/vegetarian	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Very high energy/extra energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restricted energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo for athletes	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Low Carb High Fat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Protein Low Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Gluten free	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Periodised carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
FODMAP	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments

What is the main purpose of following a special diet during heat training? (Tick all the answers that are important in describing what you do) *

	Cultural or religious reasons	**Ethical/ moral reasons**	**Better quality training for a special training block**	**Enhanced adaptation to a special training block**	**To lose weight/achieve body composition goals**	**Better health**	**Diagnosed allergies or intolerances**	**I have some allergies or intolerances but haven't had them fully diagnosed**	**Other (please explain)**	**Cultural or religious reasons**
Vegan/vegetarian	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Very high energy/extra energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restricted energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo for athletes	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low Carb High Fat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Protein Low Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Gluten free	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Periodised carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
FODMAP	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments

What is the main purpose of following a special diet during recovery from off-season? (Tick all the answers that are important in describing what you do) *

	Cultural or religious reasons	**Ethical/ moral reasons**	**Better quality training for a special training block**	**Enhanced adaptation to a special training block**	**To lose weight/achieve body composition goals**	**Better health**	**Diagnosed allergies or intolerances**	**I have some allergies or intolerances but haven't had them fully diagnosed**	**Other (please explain)**	**Cultural or religious reasons**
Vegan/vegetarian	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Very high energy/extra energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restricted energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo for athletes	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low Carb High Fat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Protein Low Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Gluten free	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Periodised carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

FODMAP	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments

What is the main purpose of following a special diet during recovery from injury? (Tick all the answers that are important in describing what you do) *

	Cultural or religious reasons	**Ethical/ moral reasons**	**Better quality training for a special training block**	**Enhanced adaptation to a special training block**	**To lose weight/achieve body composition goals**	**Better health**	**Diagnosed allergies or intolerances**	**I have some allergies or intolerances but haven't had them fully diagnosed**	**Other (please explain)**	**Cultural or religious reasons**
Vegan/vegetarian	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Very high energy/extra energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restricted energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo for athletes	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low Carb High Fat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Protein Low Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Gluten free	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Periodised carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
FODMAP	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments

What is the main purpose of following a special diet during preparation for competition/transition to race phase? (Tick all the answers that are important in describing what you do) *

	Cultural or religious reasons	**Ethical/ moral reasons**	**Better quality training for a special training block**	**Enhanced adaptation to a special training block**	**To lose weight/achieve body composition goals**	**Better health**	**Diagnosed allergies or intolerances**	**I have some allergies or intolerances but haven't had them fully diagnosed**	**Other (please explain)**	**Cultural or religious reasons**
Vegan/vegetarian	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Very high energy/extra energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restricted energy	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Paleo for athletes	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low Carb High Fat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Protein Low Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Gluten free	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Periodised carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
FODMAP	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments

PART B. BASE TRAINING

OVERALL NUTRITION

BASE TRAINING | COMPETITION SEASON | RACE DAY

Next: Nutrition during base training (including competition preparation)

3) What is your weekly mileage during the base training phase (give ranges)? *

Kilometres per week: ____

4) How many key training sessions (hard, high-intensity sessions, or long per week during this training phase (give ranges, e.g. 2-3)?

____

Focus B1. Daily eating during the base training phase

5) HARD TRAINING DAYS Do you intentionally and purposefully eat more food/calories? [Hard training days = high volume and/or intensity days] (Tick all the answers that are important in describing what you do) *

[ ] Yes, I eat more in general

[ ] Yes, I eat more carbohydrate-rich foods

[ ] Yes, I eat more protein-rich foods

[ ] Yes, I eat more fat-rich foods or fat sources

[ ] Yes, I eat more sports foods

[ ] Yes, I eat more energy-containing drinks (e.g. protein shakes, smoothies, juice etc)

[ ] No, I do not eat more food/calories on hard training days

If yes, why do you eat more food/calories?

(Tick all the answers that are important in describing what you do)

[ ] I feel hungrier

[ ] I need more calories/fuel because of the higher training load

[ ] I don't want to lose weight/body fat

[ ] Other (please explain):
____*

If no, why do you not eat more food/calories on hard training days?

(Tick all the answers that are important in describing what you do)

[ ] I don't feel hungrier so I don't think I need it

[ ] I don't feel I need more calories/fuel on hard training days

[ ] Hard training actually makes me lose my appetite so it's hard to eat more

[ ] I am are already eating to full gut capacity so there's no room for more

[ ] My social or financial circumstances make it hard to get access to additional food (please explain): ____*

[ ] This is an effective way to lose weight or achieve body composition goals

[ ] Other (please explain):
____*

6) EASY TRAINING DAYS Do you intentionally and purposefully eat less food/calories (compared to eating in general)? (Tick all the answers that are important in describing what you do) *

[ ] Yes, I eat less in general

[ ] Yes, I eat less carbohydrate-rich foods

[ ] Yes, I eat less protein-rich foods

[ ] Yes, I eat less fat-rich foods or fat sources

[ ] Yes, I eat less sports foods

[ ] Yes, I eat less energy-containing drinks (e.g. protein shakes, smoothies, juice etc)

[ ] Yes, I eat more vegetables, salad or other low-energy foods/drinks to make up for the reduced volume of other foods

[ ] No, I do not eat less food/calories on easy training days

If yes, why do you eat less food/calories?

(Tick all the answers that are important in describing what you do)

[ ] I feel less hungry

[ ] I need fewer calories/less fuel on easy days

[ ] This is an effective strategy to help with weight/body composition goals (losing weight/body fat)

[ ] Other (please explain): ____*

If no, why do you not eat less food/calories on easy training days?

(Tick all the answers that are important in describing what you do)

[ ] I still feel hungry

[ ] I need calories to fuel up for future training days

[ ] I need the calories for recovery and adaptation

[ ] I don't want to get ill or injured

[ ] I don't want to lose weight

[ ] Other (please explain): ____*

Focus B2. Timing of meals around training sessions during the base training phase

7) KEY TRAINING SESSIONS Do you pay more attention to adequate fuelling in the 1 to 4 hours before these sessions? [Key training session = sessions > 90 min, high quality/intensity workouts, serious gym sessions; Note: fuelling = eating foods (carbohydrates foods, protein foods, sports foods, etc.) before training] (Tick all the answers that are important in describing what you do)*

[ ] Yes, I eat more carbohydrate- rich foods before training

[ ] Yes, I eat more protein-rich foods before training

[ ] Yes, I eat more sports foods before training

[ ] Yes, I eat more foods in general before training

[ ] Yes, I focus on the optimal timing of my pre-training meal

[ ] No, I do not pay more attention to adequate fuelling in the 1 to 4 hours before key training sessions

If yes, why do you pay more attention to adequate fuelling?

(Tick all the answers that are important in describing what you do)

[ ] It will help me to train better at that session

[ ] I feel more energised for the whole day when I focus on fuelling before key sessions

[ ] It will reduce the risk of illness or injury

[ ] I don't want to lose weight

[ ] Other (please explain): ____*

If no, why do you not pay more attention to adequate fuelling?

(Tick all the answers that are important in describing what you do)

[ ] I don't feel it's necessary to achieve better performance outcomes for that session

[ ] I'd like to, but eating food before higher intensity/quality sessions makes me feel sick

[ ] My finances or living arrangements make it difficult to access suitable options (please specify): ____*

[ ] My concerns around weight/body composition prevent me from eating extra foods
[ ] I haven't received any special advice about sports nutrition

[ ] It's difficult for me to implement any dietary changes due to my living situation (e.g. my partner/parents/roommates do most of the food preparation)

[ ] Other (please explain): ____*

Focus B2. Timing of meals around training sessions during the base training phase

8.) **KEY TRAINING SESSIONS** Do you pay more attention to adequate recovery after these sessions? [Recovery eating within 3hrs following a key session (sessions > 90 min, high quality/intensity workouts, serious gym sessions)] (Tick all the answers that are important in describing what you do)

[ ] Yes, I eat more carbohydrate-rich foods after training

[ ] Yes, I eat more sports foods after training

[ ] Yes, I eat more protein-rich foods after training

[ ] Yes, I eat more foods in general after training

[ ] Yes, I focus on timing my post-training meal within 1h after training

[ ] No, I do not pay more attention to adequate recovery after key sessions

If yes, why do you promote recovery eating?

(Tick all the answers that are important in describing what you do)

[ ] It will specifically enhance the benefits from the session I have just finished

[ ] I recover better for the whole day when I focus on recovery immediately after key sessions

[ ] It reduces the risk of illness and injury

[ ] It helps to build/retain muscle mass

[ ] I'm hungry and I feel like eating at this time

[ ] Other (please explain):
____*

If no, why do you not promote recovery eating?

(Tick all the answers that are important in describing what you do)

[ ] It's not necessary for that session

[ ] I'd like to, but I have no appetite after these sessions and/or eating food at this time makes me feel sick

[ ] My finances or living arrangements make it difficult to access suitable options

[ ] My weight/body composition concerns prevent me from eating extra foods

[ ] I haven't received any special advice about sports nutrition
[ ] It's difficult for me to implement any dietary changes due to my living situation (e.g. my partner/parents/roommates do most of the food preparation)

[ ] Other (please explain): ____*

Focus B3. Training with low carbohydrate availability during the base training phase

9) Do you intentionally train in the fasted state? [Fasted training = training first thing in the morning without having eaten any food or consumed any other carbohydrates, or training later in the day without having eaten any carbohydrates for at least 8hrs prior] (Tick all the answers that are important in describing what you do)

[ ] Yes, I complete some/all of the easy training sessions in the fasted state

[ ] Yes, I complete some/all of the hard training sessions in the fasted state

[ ] No, I do not train in the fasted state

If yes, when do you train in the fasted state? (Tick all the answers that are important in describing what you do)

[ ] In the morning after an overnight fast

[ ] In the afternoon after at least 8 hours of fasting

[ ] Other (please explain): ____*

If yes, how often do you train in the fasted state?

[ ] Once a week

[ ] Twice a week

[ ] Three times a week

[ ] More than three times a week

[ ] Other (please explain): ____*

If yes, why do you train in the fasted state?

(Tick all the answers that are important in describing what you do)

[ ] I find it helps my overall training

[ ] I can't actually tell, but I believe it's supposed to help my overall training

[ ] I like to train on an empty stomach in terms of gut comfort

[ ] It means I can get up later and then go straight to training without wasting time on eating

[ ] It helps with weight loss/body composition goals

[ ] Other (please explain): ____*

If yes, how long have you been doing fasted training?

[ ]

[ ] 1–3 years

[ ] 3–5 years

[ ] >5 years

If no, why do you not ever do fasted training?

(Tick all the answers that are important in describing what you do)

[ ] I feel terrible during the training session and perform so badly that it's not worth it

[ ] I find I get sick more easily

[ ] I find I get injured more easily

[ ] I do not like to train on an empty stomach/when I'm hungry

[ ] I do not feel it helps my training overall

[ ] It makes me lose weight that I don't need to lose

[ ] I don't know

[ ] This is the first time I have heard of this training / nutrition approach

[ ] Other (please explain): ____*

Focus B3. Training with low carbohydrate availability during the base training phase

10) Do you intentionally reduce your intake of carbohydrate foods periodically**?** [e.g restrict carb foods acutely or chronically during certain time periods] (Tick all the answers that are important in describing what you do)

[ ] Yes, I reduce carbohydrate intake for prolonged periods (e.g. days to weeks)

[ ] Yes, I reduce carbohydrate intake for a whole day

[ ] Yes, I reduce carbohydrate intake before easy training sessions

[ ] Yes, I reduce carbohydrate intake before hard training sessions

[ ] Yes, I reduce carbohydrate intake after easy training sessions

[ ] Yes, I reduce carbohydrate intake after hard training sessions

[ ] Yes, I reduce carbohydrate intake after the first session of the day and do the second (easy) session with low glycogen/low carb availability

[ ] Yes, I reduce carbohydrate intake after the first session of the day and do the second (hard) session with low glycogen/ low carb availability
[ ] Yes, I reduce carbohydrate intake after the second (key/hard) session of the day to sleep overnight with depleted glycogen and do the morning session with low carb availability

[ ] Yes, other (please explain): ____*

[ ] No, I do not reduce carbohydrate intake periodically

If yes, how often do you reduce carbohydrate intake during this phase?

[ ] Once a week

[ ] Twice a week

[ ] Three times a week

[ ] More than three times a week

If yes, why do you reduce carbohydrate intake periodically?

(Tick all the answers that are important in describing what you do)

[ ] I find it helps my overall training by making me adapt more

[ ] I can't actually tell, but I believe it's supposed to help my overall training

[ ] It is valuable for weight loss/body composition goals

[ ] I don't know

[ ] Other (please explain):
____*

If yes, how long have you been periodically reducing carbohydrate intake?

[ ] < 1 year

[ ] 1–3 years

[ ] 3–5 years

[ ] >5 years

If no, why do you not reduce carbohydrate intake periodically?

(Tick all the answers that are important in describing what you do)

[ ] I feel terrible during the specific training session and perform so badly that it's not worth it

[ ] I feel terrible during the whole period of training in which I am doing it, so it's not worth it

[ ] I find I get sick more easily

[ ] I find I get injured more easily

[ ] I get hungry when I restrict carb intake

[ ] I do not feel it helps my training overall

[ ] This is the first time I have heard of this training / nutrition approach

[ ] I don't know

[ ] Other (please explain):
____*

Focus B4. Training with high carbohydrate availability during the base training phase

11) Do you consume carbohydrates during workouts? (Tick all the answers that are important in describing what you do)

[ ] Yes, 1-2 times a week for key workouts

[ ] Yes, for all key workouts

[ ] Yes, 1-2 times a week, for a mixture of workouts

[ ] Yes, for all workouts, hard or easy

[ ] Yes, other (please specify):
____*

[ ] No, I do not consume carbs during workouts during the base training phase

If yes, how do you consume carbohydrates during the session? (Tick all the answers that are important in describing what you do)

[ ] I ingest carbohydrate -rich sports foods such as gels and sports drinks during training

[ ] I ingest carbohydrate -rich foods such as juice, bread, pasta, bananas or muesli bars during training

[ ] Other (please explain): ____*

If yes, why do you consume carbohydrates during the session?

(Tick all the answers that are important in describing what you do)

[ ] I find it helps me train better at that specific session

[ ] I find it helps me to practice for race fuelling strategies

[ ] I find it helps me train better for that training period
[ ] I can't actually tell, but I believe it is supposed to help me train better

[ ] It's part of consuming enough carbs and calories over the day when I am training hard

[ ] I don't know

[ ] Other (please explain): ____*

If yes, how long have you been doing this?

[ ] < 1 year

[ ] 1–3 years
[ ] 3–5 years

[ ] >5 years

If no, why do you not consume carbohydrates during workouts?

(Tick all the answers that are important in describing what you do)

[ ] I don't think it helps my training in any way

[ ] I don't need to practise fuelling as my race distance is so short

[ ] I'm trying to restrict carb and energy intake where possible

[ ] My stomach gets upset from eating/drinking anything during training sessions

[ ] This is the first time I have heard of this training / nutrition approach

[ ] I don't know

[ ] Other (please explain): ____*

PART C. OVERALL COMPETITION PREPARATION PHASE

Next: Nutrition during preparation for competition

12) How many weeks is the typical length of your preparation for competition period?

( ) 1

( ) 2

( ) 3

( ) 4

( ) 5
( ) 6

( ) 7

( ) 8

( ) More than 8

13) How many times do you peak (for competition) per year (for example, some marathon runners might peak twice, once early in the year and the second time later in the year)?

( ) 1

( ) 2

( ) 3

( ) 4

( ) More than 4

14) How many times do you typically race seriously within a peak (e.g. if you are peaking for World Championships, you might compete at both 10000m and the marathon (2 races within a peak) or 20km and 50km race walk (2 races within a peak), or just the marathon (1 race within a peak))?*

( ) 1

( ) 2

( ) 3

( ) 4

( ) More than 4

15) What is your weekly mileage during this phase (give ranges)? *

Kilometres per week: ____

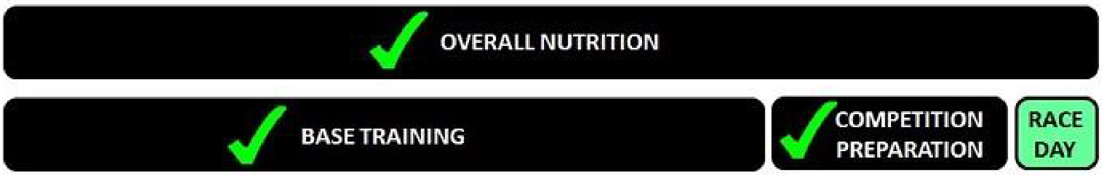
Focus C1. Periodized nutrition during preparation for competition

16) Compared to your practices during base training phase (part B questions), do you change your nutrition behaviour (see below) during preparation for competition? [Reflect back to base training nutrition strategies and report if/how your strategies change during preparation for competition] Choose the answer that best corresponds to your nutrition philosophy during preparation for competition (compared to base training): *less likely to focus on a particular strategy*, *equally likely to focus on a particular strategy*, *more likely to focus on a particular strategy**

	Less likely to focus on this strategy during preparation for competition	**Equally likely to focus on this strategy during preparation for competition**	**More likely to focus on this strategy during preparation for competition**
Do you eat more calories on hard training days?	( )	( )	( )
Do you eat less calories on easy training days?	( )	( )	( )
Do you focus on fuelling before key training sessions?	( )	( )	( )

Do you focus on recovery eating after key training sessions?	( )	( )	( )
Do you train in the fasted state?	( )	( )	( )
Do you intentionally reduce carbohydrate intake periodically?	( )	( )	( )
Do you ingest carbohydrates during training?	( )	( )	( )

PART D. ACUTE NUTRITION IMMEDIATELY BEFORE/ON THE RACE DAY



Next: Nutrition in the immediate 24-48h period around race day

Focus D1. Nutrition in the 24-48h before race day

17) Do you follow a specific diet plan in the 24-48 hours before the race day**?** (Tick all the answers that are important in describing what you do)

[ ] Yes, very high energy/extra energy (high Calorie)

[ ] Yes, restricted energy (low energy)

[ ] Yes, Low Carb High Fat (LCHF)

[ ] Yes, High Protein Low Carb

[ ] Yes, Gluten Free

[ ] Yes, High Carb/Carb loading

[ ] Yes, low residue (low fibre)

[ ] Yes, I focus on drinking more fluids

[ ] Yes, I restrict fluid intake

[ ] Yes, other (please explain): ____*

[ ] No, I do not follow any special diet plan during this period

18) If yes, why do you follow a specific dietary plan in the 24-48 hours before race day?

	I have heard it helps me perform better during the competition	**I feel more energised on the competition day**	**I have noticed that it improves my performance on race day**	**I want to make sure I am adequately before the competition**	**I have noticed that it improves my recovery after the race**	**I try to avoid GI upset by excluding certain foods/ingredients**	**I try to get down to race weight by limiting my intake of certain foods/fluids**	**I am tapering**	**I don't know**	**Other (e.g. someone told you to do it: please specify in the box below)**
Very high energy/ extra energy (high Calorie)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restricted energy (low energy)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low Carb High Fat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Protein Low Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Gluten Free	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Carb/Carb loading	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low residue (low fibre)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Focus on drinking more fluids	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restrict fluid intake	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments:

If yes, are you usually able to follow your desired diet plan(s) for the period in the 24-48 hours before the race day?

[ ] Yes, all the time

[ ] Yes, sometimes

[ ] No, mostly not

[ ] No, never

[ ] I don't know

What are the reasons for not being able to follow your diet plan in the 24-48 hours before the race day? (Tick all the answers that are important in describing what you do)

[ ] I am unable to bring food to the athlete village
[ ] I am unable to find appropriate foods at the race location to follow the plan

[ ] I am unable to travel with all the foods to satisfy my acute race diet

[ ] I am unable to find foods that fit my specific diet (which diet? Please specify): ____*

[ ] Other (please explain): ____*

If no, why do you not follow a specific diet plan in the 24-48 hours before the race day? (Tick all the answers that are important in describing what you do)

[ ] I don't think I need to follow any specific diet plan

[ ] I like to keep my dietary routines the same all the time

[ ] I would like to, but I am limited to foods and mealtimes that are available at the competition venue

[ ] I don't know if I should follow any specific plan for my event

[ ] I don't know

[ ] Other (please explain):
____*

Focus D2. Nutrition on the race day

19) Do you follow a specific diet plan on the race day**?** (Tick all the answers that are important in describing what you do)

[ ] Yes, very high energy/extra energy (high Calorie)

[ ] Yes, restricted energy (low energy)

[ ] Yes, Low Carb High Fat (LCHF)

[ ] Yes, high protein Low Carb

[ ] Yes, Gluten Free

[ ] Yes, High Carb/Carb loading
[ ] Yes, low residue (low fibre)

[ ] Yes, I focus on drinking more fluids

[ ] Yes, I restrict fluid intake

[ ] Yes, other (please specify):
____*

[ ] No, I do not follow any special diet plan during this period

20) If yes, why do you follow a specific dietary plan on the race day?

	I have heard it helps me perform better during the competition	I feel more energised on the competition day	I have noticed that it improves my performance on race day	I want to make sure I am adequately hydrated before the competition	I have noticed that it improves my recovery after the race	I try to avoid GI upset by excluding certain foods/ingredients	I try to get down to race weight by limiting my intake of certain foods/fluids	I am tapering	I don't know	Other (e.g. someone told you to do it: please specify in the box below)
Very high energy/ extra energy (high Calorie)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restricted energy (low energy)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low Carb High Fat	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Protein Low Carb	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Gluten Free	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
High Carb/Carb loading	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Low residue (low fibre)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Focus on drinking more fluids	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Restrict fluid intake	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments:

If yes, are you usually able to follow your desired diet plan(s) for the race day?

[ ] Yes, all the time

[ ] Yes, sometimes

[ ] No, mostly not

[ ] No, never
[ ] I don't know

What are the reasons for not being able to follow your diet plan on the race day? (Tick all the answers that are important in describing what you do)

[ ] I am unable to bring food to the athlete village

[ ] I am unable to bring my own foods/drinks to the competition venue/course or pack enough for the trip

[ ] The competition schedule sometimes changes which makes fuelling for the competition difficult

[ ] I am unable to find appropriate foods to follow the plan

[ ] I am unable to find foods that fit my specific diet (which diet?): ______________________________*

[ ] Other (please explain): ______________________________*

If no, why do you not follow a specific diet plan on the race day? (Tick all the answers that are important in describing what you do)

[ ] I don't think I need to follow any specific diet plan for my event

[ ] I like to keep my dietary routines the same all the time

[ ] I would like to, but I am limited to foods and mealtimes that are available at the competition venue

[ ] I don't know if I should follow any specific plan for my event

[ ] I don’t know

[ ] Other (please explain): ____*

Focus D3. Nutrition during the race

21) Do you follow a specific diet plan during the race**?** (Tick all the answers that are important in describing what you do)

[ ] Yes, I drink fluids during the race

[ ] Yes, I ingest electrolytes during the race

[ ] Yes, I ingest performance supplements (e.g. caffeine, beetroot juice) during the race (what supplements?): ____*

[ ] Yes, I ingest carbs during the race

[ ] Other (please explain): ____*

[ ] No, I do not follow any special diet plan during the race

22) If yes, why do you follow a specific dietary plan during the race?

	I have heard it helps me perform better during the race	**I feel more energised during the race**	**I have noticed that it improves my performance**	**I want to make sure I am adequately hydrated during the race**	**I have noticed that it improves my recovery after the race**	**I try to avoid GI upset**	**I don’t know**	**Other (e.g. someone told you to do it: please specify in the box below)**
Drink fluids during the race	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Ingest electrolytes during the race	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Ingest performance supplements (e.g. caffeine, beetroot juice) during the race	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Ingest carbs during the race	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Other diet plan	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Comments:

____

If yes, are you usually able to follow your desired diet plan(s) for during the race?

[ ] Yes, all the time

[ ] Yes, sometimes

[ ] No, mostly not

[ ] No, never

[ ] I don't know

What are the reasons for not being able to follow your diet plan for during the race? (Tick all the answers that are important in describing what you do)

[ ] I am unable to bring my own foods/drinks to the race venue/course

[ ] The competition schedule sometimes changes which makes fuelling for the race difficult

[ ] I am unable to find appropriate foods to follow the plan

[ ] Environmental conditions make it difficult for me to follow my plan

[ ] My GI tract gets upset very easily when I'm nervous which makes me unable to follow my plan

[ ] Sometimes I miss the aid station due to running/walking in a big group of people

[ ] Other (please explain):
____*

If no, why do you not follow a specific diet plan during the race? (Tick all the answers that are important in describing what you do)

[ ] I don't think I need to follow any specific diet plan for my event

[ ] I would like to, but I am limited to aid stations available during the race

[ ] I don’t know if I should follow any specific plan for my event

[ ] I don’t know

[ ] Other (please explain): ____*

ADDITIONAL COMMENTS

23) OPTIONAL: If you would like to receive information on study results, please type in your email address below.

____

24) OPTIONAL: Other comments

____

____

APPENDIX 4 ETHICAL CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Ms M Pienaar

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M201098

NAME: (Principal Investigator) Ms M Pienaar

DEPARTMENT: School of Therapeutic Sciences
Centre for Exercise Science and Sports
Medical School
University

PROJECT TITLE: *Nutritional habits and the relationship with relative energy deficiency in sport syndrome (RED-S) of female endurance runners in Johannesburg*

DATE CONSIDERED: 2020/10/30

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Professor D Constantinou

APPROVED BY: Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2021/02/25

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **«Missing mail merge field»** and therefore reports and re-certification will be due in the month of **«Missing mail merge field»** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

Date

APPENDIX 5 CONSENT FORM

Participant Informed Consent Form **Study Number:** …………………………

I, …………………………………………………………….., do hereby give consent to participate in the study entitled: **Nutritional habits and the relationship with relative energy deficiency in sport syndrome (RED-S) of female endurance runners in Johannesburg**

I have read the letter of information and confirm that the information has been explained to me in a language that I understand, and I am aware of this document's contents. I have asked all questions that I wished to ask, and these have been answered to my satisfaction. I fully understand what is expected of me during the study. By signing this informed consent declaration, I am not waiving any legal claims rights or remedies that I may have. An original copy of this informed consent declaration will be kept on record by the researcher.

I have not been pressurised in any way to participate in the study. By signing below, I voluntarily agree to participate in the above-mentioned study.

Participants Signature ………………………………………
Date ………………………………………

Witness's Signature………………………………………
Date ………………………………………

APPENDIX 6 INFORMATION SHEET

INFORMATION SHEET

NUTRITIONAL HABITS OF FEMALE ENDURANCE RUNNERS IN RELATIONSHIP WITH RELATIVE ENERGY DEFICIENCY IN SPORT SYNDROME IN JOHANNESBURG

INTRODUCTION

I, Marizanne Pienaar, am researching on female endurance runners, their nutrition and if they have relative energy deficiency syndrome (RED-S). RED-S is well known among female athletes of various sports. Any female endurance runner requires sustainable nutrition for intense training and optimal performance in ultra-endurance events. Training for such endurance events also requires an athlete's body to recover swiftly from training to compete in these events and vice versa. A well-balanced nutritional plan for an endurance runner would include sufficient vitamins, minerals, and macro- and micronutrients. This study will explore the nutritional habits and running behaviour of South African female endurance runners to discover if they are at risk for RED-S. Many female endurance runners are unaware of RED-S and the effects it can have on one's performance and recovery.

INVITATION TO PARTICIPATE

I would like to invite you, as a female endurance runner, to participate in this research study.

WHAT IS INVOLVED IN THIS STUDY?

This study will include a questionnaire for all participants to complete online and a venous blood sample will be drawn to check for any deficiencies in iron, calcium, magnesium, and vitamin D. Each participant will also have to track their nutritional intake for 7 days on the MyFitnessPal app and share the information with the researcher. All participants will have to reside in Johannesburg to participate in the study. The venous blood sample, no more than three testing tubes, will be drawn at Toga Pathology laboratory at the Alberton or Sunninghill branch, Johannesburg and will take about 5-10 minutes. It will only be required you to go for the blood test once for this study. The questionnaire will have multiple choice questions for you to answer as well as some open-ended questions. Parts of the questionnaire will be for low energy availability screening, your nutrition for your base training, before race day, on race day and afterwards. It should take about 20-30 minutes of your time to complete online. The questionnaire and the blood sample of each participant will be analysed anonymously. The nutritional intake tracking through the MyFitnessPal app will take 7 days to complete.

RISKS

There is truly little risk to having the blood sample drawn and should be a quick procedure. The blood test will require a tiny needle to be inserted into one of your veins to take an adequate sample for testing. This will be a bit painful, and you might have a small bruise on your arm where the blood was drawn, but it should subside within a few days.

BENEFITS

The participant will be given pertinent information on the study whilst involved in the project and after the results of the questionnaire and the blood tests are available.

PARTICIPATION IS VOLUNTARY

Participation in this study is voluntary and refusal to participate will involve no penalty or loss of benefits to which the participant is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the participant is otherwise entitled. Travelling expenses and other related costs will be paid for by the researcher. The participants will not receive any financial compensation but will be able to see the results upon completion.

CONFIDENTIALITY

Participants in the study will be anonymous and all information of participants will only be accessible by the researcher and will be kept confidential. A numbering system will be used to allocate the blood sample to each participant's questionnaire. The information shared on the MyFitnessPal app with the researcher will be allocated to each participant number.

CONTACT DETAILS OF THE RESEARCHER

Please feel free to contact me with any queries or questions by:

Researcher: Marizanne Pienaar
Contact details: 0725538152
Email: marizanne118@gmail.com

Study supervisor: Prof D Constantinou
Contact details: (011) 717-3372
Email: Demitri.Constantinou@wits.ac.za

This study has been approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg ("Committee"). A principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.

If you have any concerns over the way the study is being conducted, please contact the Chairperson of this Committee who is Professor Clement Penny, who may be contacted by telephone number 011 717 2301, or by e-mail at Clement.Penny@wits.ac.za. The telephone numbers for the Committee secretariat are 011 717 2700/1234 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za and Rhulani.Mukansi@wits.ac.za

APPENDIX 7 MYFITNESSPAL INSTRUCTIONS

MYFITNESSPAL APP INSTRUCTIONS

MyFitnessPal app

The researcher is using this app as it can track your macro- and micronutrient intake daily versus your energy expenditure during running or exercising. Each entry on the food diary can be done manually for reliability. This will help determine your daily energy balance (Energy consumed – Energy expenditure).

Installation of the app

You will be required to download the MyFitnessPal for the purpose of this research app on your phone or desktop. The app can be found on the Appstore on iPhones or the Playstore on Android devices. The app is free, and you don't require a premium account.

Create a profile

The app requires you to create a profile if you have not used it before. After setting up your profile, the researcher will add you as a friend on the app. The app has a feature where you can share your food diary with others that has the password. You will be required to share your food diary with the researcher and only the researcher will be able to access your food diary. This will be done for the confidentiality of each participant.

This can be done through the following steps:

“more” – “settings” – “sharing and privacy” – “share diary” – “share with a key” (choose a password and share with the researcher).

Log your foods and exercise for 7 days

It will be required of you as a participant to log your daily intake of nutrients for 7 days. You will add in the food eaten as well as the quantities e.g., 1 x brown toast, 1 x poached egg, 1 x coffee with milk. The MyFitnessPal has a feature to sync with other apps on your phone/desktop. Please sync your MyFitnessPal app with your app that you are using to track your exercise or running e.g., Garmin / Strava / Polar / Samsung health / Nike running / Suunto / Fitbit etc.

This can be done through the following steps:

“more” – “app and devices” – choose the app you would like to connect with and follow instructions - allow authorisation at the end.

APPENDIX 8 PLAGIARISM DECLARATION

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Marizanne Pienaar (Student number: 1688958) am a student registered for the degree of MSC MED SPORT AND EXERCISE SCIENCE in the academic year 2022.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: Pienaar Date: 28/03/2022

APPENDIX 9 TITLE APPROVAL

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

27 January 2021
Person No: 1688958
PAG

Miss M Pienaar
53 Portland Place, Thornvalley Estate
Stoneridge Drive
Greenstone Hill
1609
South Africa

Dear Miss Marizanne Pienaar

Master of Science in Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Nutritional habits and the relationship with relative energy deficiency in sport syndrome (red-s) of female endurance runners in Johannesburg* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

100 1922 2022

APPENDIX 10 TURNITIN REPORT

Nutritional habits and the relationship with relative energy deficiency in sport syndrome (red-s) of female endurance runners in Johannesburg

by Adj. Prof Demitri Constantinou

Submission date: 28-Mar-2022 01:26PM (UTC+0200)

Submission ID: 1794986148

File name:
1406_Adj._Prof_Demitri_Constantinou_Nutritional_habits_and_the_relationship_with_relative_energy_deficiency_in_sport_s_1210175902.docx (1.11M)

Word count: 17147

Character count: 93049

Nutritional habits and the relationship with relative energy deficiency in sport syndrome (red-s) of female endurance runners in Johannesburg

ORIGINALITY REPORT

2%	%	2%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Relative Energy Deficiency in Sport (RED-S)", British Journal of Sports Medicine, 2015 Publication	1%
2	Paulina Wasserfurth, Jana Palmowski, Andreas Hahn, Karsten Krüger. "Reasons for and Consequences of Low Energy Availability in Female and Male Athletes: Social Environment, Adaptations, and Prevention", Sports Medicine - Open, 2020 Publication	1%
3	Drew Mercer, Lilia Convit, Dominique Condo, Amelia J. Carr, D. Lee Hamilton, Gary Slater, Rhiannon M. J. Snipe. "Protein Requirements of Pre-Menopausal Female Athletes: Systematic Literature Review", Nutrients, 2020 Publication	1%
4	Hammond, Kelly M.. "The Effects of Macronutrient and Energy Availability on Metabolic Responses to Exercise: Implications	1%

for Training Adaptation.", Liverpool John Moores University (United Kingdom), 2021

Publication

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		

for Training Adaptation.", Liverpool John Moores University (United Kingdom), 2021
Publication

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