EFFECTS OF WEIGHT REDUCTION ON BODY COMPOSITION, HORMONE CONCENTRATIONS AND PHYSICAL PERFORMANCE IN FEMALE TRACK AND FIELD JUMPERS DURING A PREPARATORY AND COMPETITION SEASON

Sini Lällä

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Faculty of Sport and Health Sciences
University of Jyväskylä
Supervisor: Antti Mero

ABSTRACT

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Introduction. Weight reduction is common and generally accepted among obese people, but nowadays also a general practice among athletes. There are many reasons for athletes to reduce weight: to increase physical performance, to compete in a lower weight class or aesthetic reasons. The aim of this study was to follow the effects of a preparatory season (exercise, diet) on body composition, hormonal concentrations and physical performance in female track and field jumpers and follow those variables throughout summer's competition season.

Methods. Thirteen national level female Finnish track and field jumping event athletes volunteered to the study. The subjects were divided into a weight reduction (WR) group (n=7) (age 21.5 ± 2.0 years; height 1.74 ± 0.06 m; mass 61.6 ± 2.4 kg; fat 17.1 ± 3.0 %) or a control (C) group (n=6) (age 20.5 ± 1.6 years; height 1.72 ± 0.09 m; mass 60.9 ± 6.9 kg; fat 18.7 ± 3.7 %). All subjects underwent a medical screening done by a doctor. There were three measurement points in the study period. The subjects kept food, activity and training diaries five days before each measurement point and the menstrual cycle was followed throughout the whole study. Subjects' history of injuries was also ascertained. After the first measurement, the subjects were divided into the WR and C groups. After the first measurements, the WR group was advised to reduce their energy intake to achieve the target weight. From the mid to post measurements, the aim was to follow how body composition, hormonal concentrations and physical performance were affected. The C group was advised to keep their diet similar and correct any noted faults. The food diaries were analysed using the AivoDiet nutrient-analysis software and energy expenditure of physical activity assessed using MET-values by Ainsworth et al. (2011). Dual-energy X-ray absorptiometry (DXA) was used to evaluate fat percentage, fat mass, bone mineral content and bone mineral density. Blood samples were drawn from the antecubital vein after an overnight fast for determining serum total testosterone, free testosterone, estradiol, cortisol, insulin, insulin-like growth factor-1, triiodothyronine and ferritin concentrations. Physical performance tests included a 20 m sprint running test with a flying start, a 30 m sprint running test with a standing start position, counter movement jump, squat jump with and without extra weight and reactive jump. Statistical analysis was done with IBM SPSS Statistics 24.0 and Microsoft Office: mac Excel 2011. Group mean values and standard deviations were calculated with Excel. Within the groups pre versus mid, mid versus post and pre versus post significances were achieved by one-way ANOVA with repeated measures. Independent-samples t-test and Mann-Whitney U test were used to achieve significances WR versus C. Pearson's and Spearman's correlations were used to get correlation coefficients.

Results. Considering the WR group, the energy intake of the subjects during the weight reduction period was 1664 ± 251 kcal/day and they were in an energy deficit of 477 ± 218 kcal/day. Energy deficit was implemented by reducing the carbohydrate and fat intake. The protein intake remained similar throughout the study (about 2.0 g/kg/day). The body mass was slightly reduced by 1.3 ± 1.3 kg (p=0.179), the fat percentage by 2.2 ± 0.7 % (p=0.0003) and the fat mass by 1.5 ± 0.4 kg (p=0.001) without changes in lean mass, bone mineral content or bone mineral density. There were no changes in physical performance or hormonal variables. There were significant difference (13 days) between the groups in the menstrual cycle length (p=0.01) and the menstrual disturbances were more prevalent among the WR group. Bone stress injuries were also more prevalent among the WR group and there was a great drop out (n=4) in the physical performance tests in the post measurements due to that. The lower mid fat percentage led to faster 30 m sprint times (r=0.902, p=0.036). The reduced body mass correlated significantly with the increased cortisol concentrations during the weight loss period (r=-0.845, p=0.034) in the WR group.

Conclusion. According to this study, gradual weight reduction (-477 ± 218 kcal/day, 0.3 kg/week) has no negative effects on physical performance. Implementing weight reduction with moderate energy deficit by reducing carbohydrate and fat intake while maintaining high protein intake, would seem to be justified. Women athletes are at a greater risk of a menstrual disturbances and developing bone stress injuries as well as other health problems such as low energy availability and compromised iron status, especially, when reducing weight. The awareness of the risk factors of stress injury development and how to prevent them should be important among women track and field athletes, their coaches and nutritionists.

Key words: weight reduction, body composition, physical performance, hormone concentration, menstrual disturbances, female athlete

LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
BMC	bone mass content
BMD	bone mineral density
BMI	body mass index
C	control group
CBG	cortisol-binding globulin
CMJ	counter movement jump
DXA	dual-energy X-ray absorptiometry
EA	energy availability
ER	energy restriction
E%	percentage of total energy intake
E_2	estradiol
FFM	fat free mass
GH	growth hormone
GnRH	gonadotropin-releasing hormone
IGF-1	insulin-like growth factor-1
LBM	lean body mass
LH	luteinizing hormone
mTOR	mammalian target of rapamycin
RMR	resting metabolic rate
REE	resting energy expenditure
SHBG	sex hormone-binding globulin
TEF	thermic effect of feeding
T_3	triiodothyronine
WR	weight reduction group
1 RM	one repetition maximum

CONTENTS

1 INTRODUCTION

For health reasons, weight reduction is common and generally accepted among obese people (BMI > 30 kg/m). However, nowadays weight reduction is also a general practice among normal weighted people (BMI < 25 kg/m), especially among certain athletes. There are many reasons for athletes to reduce weight: to increase physical performance, to compete in a lower weight class (weight category events) or aesthetic reasons (gymnasts). (Fogelholm 1994; Helms et al. 2014.)

Body weight reduction, especially concerning fat, improves power-to-weight ratio and thus may be beneficial in weight-bearing efforts, such as jumping events for height and distance (Fogelholm 1994). It has been stated that a body mass reduction of 1 kg improves the elevation of the body centre of gravity by 2.3 cm in male high jumpers (Isolehto 2016, 433).

Reduction of body weight can be accomplished either rapidly or gradually. Mainly athletes in weight-class events use rapid weight reduction method. According to Fogelholm (1994), rapid weight reduction is carried out in less than one week principally by restriction of fluids, whereas weight reduction period over seven days is defined as gradual weight reduction and it is mainly accomplished with energy restriction (ER). This study focuses mainly on the gradual weight reduction.

In rapid weight reduction, several negative consequences both on body composition (Sagayama et al. 2014) and on physical performance (Viitasalo et al. 1987; Filaire et al. 2001) have been reported. However, minor improvements in the light of physical performance were found in Viitasalo's et al. study. Considering gradual weight reduction, both negative (Filaire et al. 2001; Umeda et al. 2004) and positive (Mero et al. 2010; Garthe et al. 2011a; Huovinen et al. 2015) consequences on physical performance have been reported. In the light of optimal body composition changes (loss of fat mass and maintaining lean mass), gradual weight reduction seems to be better option.

The purpose of this study was to follow the effects of a preparatory season (exercise, diet) on body composition, hormonal concentrations and physical performance in female track and field jumpers and follow those variables throughout summer's competition season.

2 GRADUAL AND RAPID WEIGHT REDUCTION

It is well known that to achieve body weight loss, a person must ingest less energy than they expend over some defined period of time (Phillips 2014). Fogelholm (1994) defined gradual weight reduction as a weight loss practise lasting one week or longer (≥ 7 days) and achieved mainly by negative energy balance. In athletes, energy expenditure is already at a high level so achieving weight loss by increasing physical activity is difficult. Therefore, weight reduction must be accomplished by energy restriction. (Fogelholm 1994.) From an athlete's point of view, maximal physical performance is desired and thus it is important to favour weight reduction that emphasizes fat loss and muscle preservation (Phillips 2014). Compared to rapid weight reduction, gradual weight reduction has not been studied much in athletes, not to mention in comparison with weight loss studies in obese people (Ramage et al. 2014).

2.1 Implementation of gradual weight reduction

According to previous literature the result of the weight loss is determined by the ratios of the macronutrients in the diet, the type and amount of physical training and the size of the energy deficit. Two of the most powerful variables that influence the outcomes of a weight reduction procedure seem to be the magnitude of energy deficit and protein intake (Helms 2014). It seems that about 550 kcal daily energy deficit together with about 0.5 kg (0.7 % of body mass) weekly weight loss rate produces best result from the point of view of body composition (Garthe et al. 2011a), explosive performance (Garthe et al. 2011a) and hormonal balance (Mero et al. 2010). In a study by Huovinen et al. (2015) with male track and field jumpers and sprinters the energy deficit was a little higher (about 750 kcal/day) but no negative changes in above-mentioned variables were observed. The protein intake was high (> 2.0 g/kg/day) in this study, which possibly explains the positive results despite the higher energy deficit. The same was also noticed in Volek's et al. (2002) and Mettler's et al. (2010) studies where protein intake was high (> 2.0 g/kg/day, > 30 % from total energy intake (E%)) and no negative changes in body composition (Mettler et al. 2010) or in hormonal balance (Volek et al. 2002) were detected.

According to previous studies, a high energy deficit (about 1000 kcal) and/or lower protein intake (0.6–1.4 g/kg/day) have harmful effects on body composition (Gornall & Villani 1996; Zachwieja et al. 2001; Layman et al. 2003; Alemany et al. 2008), physical performance

(Filaire et al. 2001; Umeda et al. 2004) and hormonal balance (Alemany et al. 2008; Mero et al. 2010). Like in the study by Huovinen et al. (2015) there were also 750 kcal daily energy deficit in Zachwieja's et al. (2001) study. However, since the protein intake was lower (1.4 g/kg/day, 19 E%) in Zahcwieja's et al. (2001) study, lean mass loss occurred. Thus, a high protein diet seems to prevent lean mass loss during energy deficit.

2.2 Implementation of rapid weight reduction

Rapid weight reduction is a practice that is used when an athlete needs to lose weight in a short period of time. It is defined as a weight loss practice lasting under seven days. (Fogelholm 1994.) Rapid weight reduction is usually done by combat sports athletes (i.e. wrestlers, judoists, boxers etc.) (Franchini et al. 2012). The official weigh-in in Olympic weight-class sports is typically held 6–24 hours before the competition, which allows most athletes to use aggressive weight-cut practices to lose weight in order to compete in a lower weight class (Pallarés et al. 2016). To achieve such a rapid weight reduction, athletes use a variety of methods. Most commonly used methods are fluid, energy and sodium restriction. Use of saunas, blouses and plastic suits are also used to lose weight. Therefore, the weight loss in rapid weight reduction is mainly achieved by the loss of water from the body through sweating (compared to gradual weight reduction, which is mainly accomplished with energy restriction). More aggressive methods such as diet pills and laxatives are also used. (Mero et al. 2016, 199–201.)

3 PHYSICAL EXERCISE AND DIET DURING WEIGHT REDUCTION

3.1 Effects of physical exercise

To achieve body weight loss, a person must ingest less energy than he or she is expended over some defined period of time (Phillips 2014). Weight reduction can be achieved by restricting energy intake, but also by increasing the volume/intensity of training or, most often, a combination of both of these strategies (Murphy et al. 2015). In athletes, energy expenditure is already at a high level so the weight loss by increasing physical activity is difficult (Fogelholm 1994).

Physical exercise increases energy expenditure both during and after training. Regular exercise bouts elevate the resting metabolic rate (RMR) and promote reaching the negative energy balance and thus assist in losing weight. The magnitude of resting metabolic rate is highly dependent on fat-free mass. Therefore, maintaining lean mass during weight loss is important, since if RMR lowers it also hinders the progress of weight loss. (Stiegler & Cunliffe 2006.)

The duration, type and intensity of training determine the effects of physical training on body composition. According to the literature in obese people, strength training seems to spare lean mass better than aerobic training but more research is required to clarify this topic. In addition, training at higher intensities also seems to produce better result in the light of body composition. (Stiegler & Cunliffe 2006.)

The lean mass sparing effect of strength training would seem to be true also among athletes. In a study by Garthe et al. (2011a), strength training together with a personally designed diet even increased the lean mass when the weight reduction rate was 0.7 kg per week. However, the lean mass increased mainly in the upper body, which is believed to come from better strength-training stimuli of upper body muscles. Besides, athletes already had a heavy load of leg muscles in their sport specific training, which may have reduced the training potential in these muscles. (Garthe et al. 2011a.) Even 11 weeks of energy restriction leading to a weight loss of 4.1 kg was not sufficient to degrade lean tissue when protein intake was high (2.4–2.6 g/kg/day) and hypertrophic strength training was included into the weight reduction regimen (Mäestu et al. 2010).

3.2 Effects of diet

According to Finnish dietary guidelines (VRN 2014), 45–60 % from total energy intake (E%) should come from carbohydrates, 10–20 E% from protein and 25–40 E% from fat. For athletes, these guidelines differ slightly. This study focuses on track and field jumping events for height and distance and those events can be considered as power events. Dietary guidelines for a power event athlete can be seen in tables 1 and 2.

TABLE 1. Dietary guidelines for a power-trained athlete (Ilander et al. 2014, 143–148) E%, percentage of total energy intake.

Macronutrient	Recommendation (g/kg/day)	Recommendation (E%)
Carbohydrates	4–6	40–65
Protein	1.4–2.0	15–20
Fat	1–2	25–40

TABLE 2. Dietary guidelines for a power-trained athlete (Mero et al. 2016, 202–203) E%, percentage of total energy intake.

Macronutrient	Recommendation Training season		Recommendation Competition season	
	g/kg/day	E%	g/kg/day	E%
Carbohydrates	4–6	50–60	2–3	30–40
Protein	2.0–3.0	15–20	1.5–2.0	20–30
Fat	0.8–1.2	15–20	0.5–0.9	20–30

As can be seen from the tables 1 and 2, there are slight differences in energy intake recommendations for a power-trained athlete between the references and also between the training and competition seasons. The main reason for the differences between the training and competition seasons is the training load, which is notably lower in competition season and thus the need for total energy intake is decreased (Mero et al. 2016, 202–203).

During the training season, the energy availability (the amount of ingested energy remaining for physiological functions, after the energy required for exercise has been removed) should be over 40 kcal/kgFFM/day in order to maximize the development of physical capacity (Ilander et al. 2014, 143–148). During a preparatory season some athletes are reducing their weight to improve their power-to-weight ratio and thus their physical performance (Fogelholm 1994). During this phase energy availability should be around 35 kcal/kgFFM/day so that negative effects on health can be avoided (Ilander et al. 2014, 143–148).

In regard to maximizing physical performance, a high lean-to-fat body weight ratio is desirable (Phillips 2006; Phillips & Van Loon 2011). According to previous studies, in order to get optimal body composition (losing fat mass / sparing lean mass), weight reduction is recommended to carry out with increasing protein intake and either decreasing carbohydrate (Tipton & Witard 2007; Phillips 2012; Ilander et al. 2014, 143–148) or carbohydrate and fat intake (Phillips et al. 2007; Phillips 2014; Murphy et al. 2015). The proportion of carbohydrate intake can temporarily be even under 40 E% and protein intake over 35 E% during a weight loss regimen in skill sport athletes. Fat intake should remain over 20 E%. (Ilander et al. 2014, 143–148.)

3.2.1 Effects of protein intake

It is important to understand the difference between protein requirement and optimal intake. The protein requirement is the minimal level of protein that will prevent deficiency. The optimal intake instead is advantageous, for example, in the light of performance. (Phillips 2012.)

General. It is believed that regular exercise increases the nutritional requirement for protein in athletes (Lemon 1998; Phillips 2006; Phillips 2007; Tipton & Witard 2007; Phillips & Van Loon 2011; Phillips 2014). This argument is based on nitrogen balance. In this technique, the protein requirement is defined by quantification of all protein that is consumed and all nitrogen that is excreted. In an anabolic situation, the nitrogen balance is positive and in a catabolic situation, it is negative. (Tipton & Witard 2007.) The increased protein demand in athletes has been explained with several aspects. Since training increases muscle damage, protein is needed for repairing and replacing damaged muscle proteins. (Phillips 2006; Phillips 2007; Tipton & Witard 2007). Protein is also needed for maintaining optimal function of all metabolic pathways and immune system (Phillips 2007; Phillips 2012). The proper amount of protein ingested per day differs between individuals and is dependent on several factors, therefore the right amount for maximizing performance is difficult to determine

(Tipton & Witard 2007). However, it has also been suggested that exercise does not increase the dietary requirement for protein. This argument has been based on the fact that exercise increases the efficiency of use of amino acids from ingested protein (Tipton & Witard 2007).

Effects on body composition. In comparison with dietary recommendation (VRN 2014) for protein intake (0.8 g/kg/day), a high-protein diet (25–30 E%, 1.6 g/kg/day) during a weight reduction period would seem to promote fat mass loss and prevent from lean mass losses. In a study by Garthe et al. (2011a), the athletes were even able to increase their muscle mass during energy restriction. Muscle mass increased by 2.1 ± 0.4 % when the energy deficit was about 550 kcal/day and protein intake 1.6 g/kg/day (25 E%). Subjects' weight was reduced 5.6 ± 0.8 % during 12 weeks period of energy restriction. However, it must be noted that subjects performed a resistance-training session four times per week along with their sport specific training.

Quality of protein and timing of ingestion. It is important to divide protein intake evenly throughout the day during energy restriction (Ilander et al. 2014, 209–214; Murphy et al. 2015). Approximately 8–10 g of essential amino acids, which translates into around 20–25 g of high quality protein, should be consumed immediately after resistance exercise and in every meal (Moore et al. 2009a). To preserve lean mass during ER, athletes may benefit from consuming four to five evenly spaced feedings containing 20–25 g of high quality protein throughout the day (Murphy et al. 2015). Animal-source proteins contain more high quality protein than plant protein sources and, therefore, it is recommended for athletes to consume animal proteins in the form of eggs, milk and whey protein during weight loss (Tipton & Witard 2007; Phillips & Van Loon 2011; Murphy et al. 2015). High quality protein also helps in maintaining lean mass (Murphy et al. 2015). When it comes to the time window of post workout protein consumption, there is no accurate consensus yet, so the literature instructs to consume a recovery drink straight after the training session ends (Phillips & Van Loon 2011). Munteanu et al. (2014) suggest that the post-exercise recovery drink should be consumed within 15 minutes after the end of a training session or competition.

According to the previous studies, branched-chain amino acids (BCAA) should be consumed to maximize muscle protein synthesis. Leucine is a branched-chain amino acid, which takes part in many metabolic processes in the human body. (Layman 2004.) Leucine can activate essential signalling proteins in the mTOR (mammalian target of rapamycin) pathway, which is responsible for translation initiation (Phillips & Van Loon 2011). Thus it has a unique role in sparing lean mass during weight loss with a high-protein diet (Layman 2004). Animal proteins are rich in leucine, which appears to stimulate effectively muscle protein synthesis (Phillips & Van Loon 2011). According to Koopman et al. (2009), protein should be consumed in a liquid form since it allows a more rapid digestion and absorption of the protein and thus more rapid rise in blood's leucine concentration leading to enhanced muscle protein synthesis. The spike in blood leucine appears to be significant in activating muscle protein synthesis (Phillips & Van Loon 2011).

Considering the absorption rate of the amino acids, Mero et al. (2008, 2009) observed that one-hour strength training session slowed the absorption of taurine, arginine, BCAA and leucine to blood when compared to the rest conditions in physically active men. There was smaller peak concentration of leucine in strength training session after leucine or BCAA treatment suggesting that leucine is possibly used as energy through oxidation and to stimulate the protein synthesis and anticatabolic processes. However, mechanical factors may affect gastric area when moving rapidly from one place to another and thus delay the gastric emptying process and, therefore, be a reason behind lower peak concentration. Another explanation might also be blood flow. During training the blood flow to the gastric region may be diminished, which may delay the transport of a single amino acid into the blood. (Mero et al. 2009.)

Protein and satiety. In comparison with other macronutrients, protein produces a greater satiety and thus high-protein diets are preferable in weight loss (Stiegler & Cunliffe 2006). This was observed in Weigle's et al. (2005) study. In that study 19 subjects were placed first on a weight-maintaining diet (15 % protein, 35 % fat and 50 % carbohydrate) and then on an isocaloric diet (30 % protein, 20 % fat and 50 % carbohydrate) for two weeks each. Finally, subjects were placed on an ad libitum diet (30 % protein, 20 % fat and 50 % carbohydrate) for 12 weeks but were instructed to eat only when hungry, stop eating when satisfied and avoid making any conscious effort to modify food intake, physical activity or body weight. During a 12-week intervention subjects' energy intake reduced by 441 ± 63 kcal/day, body mass decreased by 4.9 ± 0.5 kg and fat mass decreased by 3.7 ± 0.4 kg. The authors suggest that increased protein intake enhances leptin sensitivity in the central nervous system leading to a spontaneous decrease in energy intake.

Thermic effect of feeding (TEF). One benefit of a high-protein diet over other macronutrient rich diets is its ability to induce a greater increase in energy expenditure. When consuming a protein rich meal, the energy cost of nutrient absorption, processing and storage is 25–30 % of ingested energy. The same values for carbohydrates and fat are 6–8 % and 2–5 %, respectively. (Stiegler & Cunliffe 2006.) A study by Robinson et al. (1990) suggests that the greater increase in energy expenditure after a protein rich meal comes from postprandial increase in protein synthesis. In Robinson's et al. (1990) study, with the help of the theoretical estimates of the metabolic costs of the protein synthesis, it appeared that 36 % of the TEF response to feeding a carbohydrate rich (70 E%) meal may be accounted for by the postprandial increase in protein synthesis. For a high protein meal (70 E%), this value was 68 %.

3.2.2 Effects of carbohydrates intake

As has already been mentioned before, the energy restriction should be carried out mainly by decreasing carbohydrate intake. (Tipton & Witard 2007; Phillips 2012; Ilander et al. 2014, 143–148). The dietary guidelines of carbohydrate intake for power-trained athletes are about 3–7 g/kg/day (60–75 E%) depending on their training season (Mero et al. 2007, 182; Slater & Phillips 2011; Munteanu et al. 2014). It has been suggested, that a female's need for carbohydrates is a little less compared to males (Slater & Phillips 2011), since females utilize more fat and less carbohydrates as a fuel at the same relative exercise intensity as males, although, limited data is available. (Broad & Cox 2008.)

According to the previous literature, the proportion of carbohydrate intake can temporarily even be under 40 E% during a weight reduction period (Phillips et al. 2007; Phillips 2012; Ilander et al. 2014, 143–148). As stated by previous studies, a low-carbohydrate diet results in increased fat mass loss and lean mass preservation, which leads to a higher lean to fat mass -ratio and can translate into a competitive advantage (Phillips 2012). In events, where carbohydrates are not consumed much, carbohydrates restriction can be even higher (Ilander et al. 2014, 146–147). In weight loss studies, in which there have been positive changes considering body composition, the carbohydrates intake has been 3.0–3.7 g/kg/day (40–54 E%) on average. A study by Mero et al. (2010) is an exception, where carbohydrate intakes were 1.8 g/kg (115 g) and 2.5 g/kg (156 g) in high (-1100 kcal/day) and low (-550 kcal/day) energy deficit groups respectively. No negative effects on body composition, physical performance or hormonal balance were observed in this four-week study with healthy normal weighted young women, so according to this study, a lower carbohydrate diet is not harmful. However, according to Zachwieja et al. (2001), carbohydrate intake should stay above 3.0 g/kg/day during weight loss in order to maintain physical performance.

The effect of a very low carbohydrates diet on body composition was also examined in Volek's et al. (2002) study. Twelve healthy normal-weight non-athlete men reduced their carbohydrate intake to 8 % of their total energy intake for six weeks. Subjects were instructed to maintain their body weight similarly during the intervention. The energy intake remained unchanged (2334 vs. 2540 kcal/day) before and during the intervention, respectively. Carbohydrate intake was low (46 g vs. 306 g, 8 vs. 48 %), protein intake high (176 vs. 113 g, 30 vs. 17 E%), and fat intake high (157 vs. 91 g, 61 vs. 32 E%) during the intervention compared to the habitual diet, respectively. After the intervention body mass and fat mass were decreased significantly (-2.2 and -3.4, $p \leq 0.05$, respectively) and lean body mass was increased (+1.1, $p \leq 0.05$). The authors concluded that the low carbohydrate diet induced fat loss and a concomitant increase in lean body mass in normal-weight men may be partially mediated by the reduction in circulating insulin concentrations.

Even though a very low carbohydrate diet may be beneficial in the light of body composition (Volek et al. 2002), too little intake can be harmful. A single resistance exercise can reduce muscle glycogen stores by 23–44 % (Koopman et al. 2006) and the amount of depletion depends on the intensity, duration and overall work accomplished during the exercise session. The depletion of muscle glycogen stores can result in performance impairments (Slater & Phillips 2011; Phillips 2012), so it is important to take care of adequate carbohydrate intake during the energy deficit (Phillips 2012). During energy deficit athletes should pay attention to carbohydrate intake near the exercise sessions (before, during and after), in order to keep training capacity and recovery optimal (Slater & Phillips 2011; Ilander et al. 2014, 146–147). Muscle glycogen resynthesis is faster in the first two hours after exercise (Friedman et al. 1991) and thus, it is recommended to consume carbohydrates soon after the exercise session (Broad & Cox 2008). According to the literature, carbohydrates should be consumed along with protein after the exercise in order to maximize muscle protein synthesis and refill the muscle glycogen stores (Phillips et al. 2007; Slater & Phillips 2011). Carbohydrate of 0.8 g per kg body mass and 0.4 g per kg body mass protein are suitable amounts after exercise (Slater & Phillips 2011).

Glycaemic index. As stated by Brand-Miller et al. (2002), food with high glycaemic index (GI) enhance the accumulation of storage fats, whereas low-GI foods promote satiety, minimize postprandial insulin secretion and maintain insulin sensitivity. However, Sloth et al. (2004) conducted that weight loss is not dependent on GI of food. On the other hand, GI of ingested carbohydrates seems to modify the effect of the energy deficit on weight loss, but this requires further investigation (Stiegler & Cunliffe 2006).

3.2.3 Effects of fat intake

There are studies that recommend that weight loss should also implement by reducing fat intake together with carbohydrates (Phillips et al. 2007; Phillips 2014; Murphy et al. 2015). Fat has a high energy density. One gram fat contains about 9 kcal of energy, whereas the similar value for protein and carbohydrates is about 4 kcal. (e.g. Ilander et al. 2014, 234.) Therefore, reducing consumption of fat is an effective method to reduce energy intake. Ilander et al. (2014, 237–238) stated that among to power-event athletes, energy restriction is recommended to be carried out mainly by reducing fat intake. Thus, enough carbohydrates can be eaten since they are important for an athlete's training capacity, performance and recovery. However, in events where training does not consume that much carbohydrate, can carbohydrate intake also be decreased during weight reduction. (Ilander et al. 2014, 145–146; Ojala 2016, 172–173). Recommended fat intake during weight loss is 0.8–1.3 g/kg/day (Munteanu et al. 2014).

However, fat intake cannot be too low during the weight loss period. Lower dietary fat intakes are correlated with reduced resting serum testosterone and estrogen concentrations in women (Ingram et al. 1987). There are also negative effects on immune function with low-fat diet (Venktatraman et al. 2000). According to the literature, it is important to ensure that dietary fat intake is not lower than 20 E% in order to avoid the negative consequences of too low intake (Broad & Cox 2008; Ilander et al. 2014, 146–147). When the energy and fat intakes are low, should attention pay also to the quality of fat. A good rule of thumb is that two thirds of total dietary fat intake should consist of unsaturated fats (polyunsaturated and monounsaturated fatty acids). There are two essential fatty acids (EFA) for humans: alpha-linolenic acid and linoleic acid. They are essential for life, but must be obtained through diet since the body cannot synthesize them. EFAs are required, for example, for the proper structure and function of the cells in the body. They affect significantly to eicosanoids, immunity and inflammatory responses caused by the training. Fatty fish, nuts, vegetable oils, for instance, are good sources of essential fatty acids. (Ojala et al. 2016, 164–168.) It is also important to get enough micronutrients when the fat intake is low. (Mero et al. 2016, 199–206).

4 WEIGHT REDUCTION AND HORMONE LEVELS

Weight reduction affects hormonal functions in multiple ways. In this section, testosterone, estrogen, cortisol, insulin, insulin-like growth factor-1 (IGF-1) and triiodothyronine (T_3) are discussed. Ferritin and the Female Athlete Triad are also discussed in this section.

4.1 Testosterone

Testosterone is an anabolic steroid hormone. It is produced by the interstitial cells of Leydig of the testes and also in small amounts by the adrenal cortex and by the ovaries in females. (Guyton & Hall 2006, 1003–1006.) 98 % of testosterone is bound on carrier proteins in plasma. About 65 % of circulating testosterone is tightly bound to sex hormone-binding globulin (SHBG) and about 33 % loosely bound to albumin. Approximately 2 % of testosterone is freely circulating in the plasma. SHBG-bounded testosterone is classified unavailable, whereas albumin-bound and free testosterone are available for biological action. (Allen et al. 2002.) Free testosterone levels have shown to be inversely related to the levels of SHBG (Vermeulen 1988).

The hypothalamic-pituitary-axis mediates the secretion of testosterone. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates secretion of luteinizing hormone (LH) from the anterior pituitary gland, which is the most important stimulator of testicular testosterone secretion (Guyton & Hall 2006, 1003–1006.) Insulin has also been shown to regulate serum testosterone and SHBG concentrations. According to previous studies, insulin is negatively correlated with both serum testosterone and SHBG concentrations. Nevertheless, even though insulin decreases total serum testosterone levels, the biologically active free testosterone might be unaffected by insulin or could even be increased due to decreased SHBG levels. (Kraemer et al. 1998.)

According to previous studies, both total serum testosterone and free testosterone concentrations would seem to be reduced along with weight loss. The decrease in testosterone concentration would seem to correlate with reduced body weight (Roemmich & Sinning 1997; Mero et al. 2010). Considering lean body mass, decreased testosterone is positively related to lean mass (Roemmich & Sinning 1997). Changes in fat mass have also been shown to correlate with testosterone levels (Rossow et al. 2013). However, the amount of energy deficit would appear to have an effect on the reduction of testosterone concentration. In studies where the energy deficit has been bigger, both total serum testosterone and free testosterone levels are reduced. In a study by Mäestu et al. (2010) in male bodybuilders, the testosterone concentration decreased from 20.3 ± 6.0 to 17.2 ± 6.5 nmol/l during an 11-week weight reduction period where the energy deficit was -950 kcal/day at its greatest point. Similar results were also observed in Mero's et al. (2010) and Mettler's et al. (2010) studies where energy deficits were about -1100 kcal/day and about -1350 kcal/day, respectively. In a study by Alemany et al. (2008), total serum testosterone concentration decreased 49 % and free testosterone 60 % during eight day weight reduction period with -1150 kcal/day energy deficit.

A moderate energy deficit would appear to be less detrimental to testosterone levels. In Huovinen's et al. (2015) study, no statistically significant decrease in serum testosterone was observed in male track and field athletes during a four-week weight reduction period where the daily energy deficit was about 750 kcal. No changes in total serum testosterone concentration in recreational women athletes was observed either in a study by Mero et al. (2010) during four weeks of energy restriction (-550 kcal/day). However, the authors believed that the testosterone concentration would have decreased significantly, if the intervention period had been longer. Contrary finding has also been observed. In a study by Lällä (2016), the serum testosterone concentration decreased statistically significantly during a four-week energy deficit period (-530 kcal/day) in women high jumpers. Nevertheless, it must be taken into account that women have very low testosterone levels compared to men and that the sensitivity of the assay may not be accurate enough. In Koehler's et al. (2016) study, no reduction in testosterone levels were observed in exercising men with low energy availability (15 kcal/kgFFM/day). The authors supposed that low basal leptin concentration observed in those men acted to prevent changes in testosterone. The duration of the experiment was also short (4 days).

4.2 Estrogen

Estrogen is a steroid hormone, which is produced in the ovaries of female and also in small amounts in the adrenal cortices and male's testes (Guyton & Hall 2006, 1016–1018). Like with testosterone, the hypothalamic-pituitary-axis also mediates the secretion of estrogen. GnRH secreted by the hypothalamus stimulates the anterior pituitary gland to secrete gonadotropins, lutenizing hormone (LH) and follicle-stimulating hormone (FSH). In response to these hormones, the ovaries secrete estrogen and other female hormone, progesterone. These hormones mentioned above are not secreted in constant amounts, but depend on the current phase of the menstrual cycle. The concentrations of the gonadotropins and ovarian hormones during the normal female sexual cycle can be seen in figure 1. The secretion of estrogen is highest during the follicular phase. (Guyton & Hall 2006, 1011–1015.) In the blood, estrogen is transported mainly bound to albumin or specific estrogen-binding globulin (Guyton & Hall 2006, 1016–1018) and also, to a lesser extent bound to SHBG (Anderson 1974). Similar to testosterone, only a small fraction of estrogen is freely circulating in the plasma (Anderson 1974).

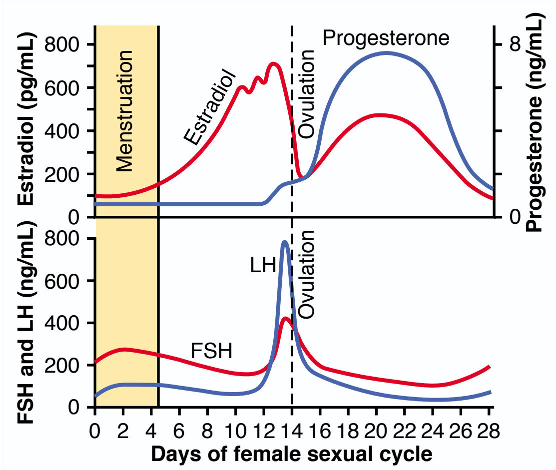

FIGURE 1. Plasma concentrations of the gonadotropins and ovarian hormones during the normal female sexual cycle. LH, lutenizing hormone; FSH, follicle-stimulating hormone. (Adopted from Guyton & Hall 2006, 1011–1015.)

There is not much date on the effects of weight loss and caloric restriction on estrogen levels. Westerlind and Williams (2007) conducted a study with normal weighted, sedentary premenopausal women. No change in estrogen metabolism was observed during four months of moderate-intensity exercise coupled with energy restriction. Subjects' body mass was reduced by 3.7 kg and fat percentage by 4.5 % during the intervention without changes in lean body mass. Similar was observed in Atkinson's et al. (2004) study, where the effects of a 12-month moderate intensity aerobic exercise intervention on estrogen metabolites in overweight postmenopausal women were studied.

4.3 Cortisol

Cortisol is a catabolic hormone and its secretion is also mediated by the hypothalamic-pituitary-axis. The hypothalamus secretes corticotropin-releasing factor (CRF) in response to physiologic or mental stress, which then induces the adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary gland. The ACTH then stimulates the adrenal cortex of the kidneys to secrete cortisol. (Guyton & Hall 2006, 950–957.) Most of the plasma cortisol (90–95 %) is bound to plasma proteins, particularly in cortisol-binding globulin (Guyton & Hall 2006, 944–947). Cortisol decreases protein synthesis and increases muscle catabolism. It also stimulates gluconeogenesis (carbohydrate formation from proteins and some other substances) by the liver and promotes the mobilization of fatty acids from adipose tissue. (Guyton & Hall 2006, 950–957.) Since cortisol secretion is stress-induced, the secretion increases with exercise intensity (Katch et al. 2011). Cortisol also supress the secretion of testosterone by depressing the release of GnRH (Kampmiller et al. 2013).

Gradual weight reduction does not seem to have an influence on cortisol levels in men (Mettler et al. 2010; Huovinen et al. 2015) or in women (Mero et al. 2010; Lällä 2016). Nevertheless, in a study by Proteau et al. (2006), a 7-day severe energy restriction led to a 4 % bodyweight lost with concomitant decreases in fat mass (8 %) and lean body mass (3 %) and significant increase in cortisol levels (81 %). In that study, the weight reduction was conducted with the rapid weight reduction method, which might explain the rise in cortisol concentration.

4.4 Insulin

Insulin is an anabolic hormone secreted by the islets of Langerhans of the pancreas. It circulates in the plasma almost completely in an unbound form. Insulin has many crucial roles in glucose, lipid and protein metabolism. (Guyton & Hall 2006, 961–970), and it is required for reproduction (Wade & Jones 2004). It affects reproductive physiology and behaviour by altering the availability of fuels for oxidation (Wade & Jones 2004).

According to the studies, plasma insulin concentration decreases, when the energy availability (EA) is low. In a study by Koehler et al. (2016), the insulin concentration decreased by 34–38 % during four days of energy restriction with EA of 15 kcal/kgFFM/day in recreational men.

The same was previously observed in sedentary women (Loucks et al. 1998) and in moderately active men (Volek et al. 2002), where the insulin concentration decreased also by 34 % during energy restriction period. Loucks & Thuma (2003) conducted that insulin levels decrease linearly with energy availability when the EA was below 30 kcal/kgFFM/day. Positive correlations between changes in insulin and fat mass as well as between changes in insulin and lean body mass have found (Mäestu et al. 2010).

4.5 Insulin-like growth factor-1

Insulin-like growth factor-1 (IGF-1) is an anabolic hormone that is mainly produced by the liver in an endocrine fashion and also in some peripheral tissues to act in an autocrine/paracrine fashion. Growth hormone from the anterior pituitary gland controls the synthesis of IGF-1. IGF-1 has an essential role in fetal development, adolescent growth and adult tissue homeostasis. It also controls body composition by regulating glucose and lipid metabolism together with insulin and growth hormone. (Yakar & Adamo 2012.)

Like in insulin, the IGF-1 concentration decreases when EA is low. Ihle & Loucks (2004) observed that most of the decline in the IGF-1 concentration occurred between EAs of 20 and 30 kcal/kgFFM/day. When EA further decreases the concentration did not become more extreme (Loucks & Thuma 2003). In the light of weight loss, decline in the IGF-1 levels have been shown. In a study by Mäestu et al. (2010), the IGF-1 concentration decreased significantly during 11 weeks of weight reduction. The same was also found in Mettler's et al. (2010) Koehler's et al. (2016) studies. In the study by Loucks et al. (1998), the IGF-1 concentration declined 26 % in sedentary women. Nevertheless, contrary finding has also been observed. In a study by Volek et al. (2002) no significant changes in the IGF-1 concentration were noticed during a 6-week carbohydrate-restricted diet. Low EA had no effect on IGF-1 concentration in Koehler's et al. (2016) study with exercising men either. However, in that study the energy restriction period lasted only four days.

Changes in IGF-1 concentration during WR have also been found to relate with changes in insulin concentration, so a low insulin concentration may hamper the compensatory role of IGF-1. Correlations between IGF-1 and fat mass, lean body mass and body mass have also been found, which may point out the significance of maintaining concentrations of key anabolic hormones to prevent muscle mass losses (Mäestu et al. 2010.) According to Mäestu et al. (2010) very low insulin and IGF-1 values will be followed by a rapid loss of muscle mass.

4.6 Triiodothyronine

The thyroid gland secretes two major hormones, thyroxine (T_4) and triiodothyronine (T_3). Thyroxine accounts for approximately 93 % of hormones secreted by the thyroid gland and triiodothyronine for 7 %. Nevertheless, almost all the thyroxine is finally converted to triiodothyronine in the tissues. (Guyton & Hall 2006, 931–934.) The hypothalamic-pituitary-axis mediates the secretion of these hormones. The hypothalamus secretes a hormone called thyrotropin-releasing hormone (TRH), which increases the secretion of thyrotropin (TSH) from the anterior pituitary gland. The TSH then causes the thyroid gland to secrete thyroxine and triiodothyronine. (Guyton & Hall 2006, 938–940.) Thyroid hormones have an important role in the body's metabolic rate regulation. Rises in circulating thyroid hormones are associated with an increase in the metabolic rate, whereas reduced thyroid levels result in decreased thermogenesis and overall metabolic rate. (Kim 2008.) This study will focus on T_3.

The T_3 concentration reduces when the EA is low (Thong et al. 2000; Ihle & Loucks 2004). Loucks & Thuma (2003) found that T_3 was considerably reduced when the EA was restricted to 30 kcal/kgFFM/day. When EA further declined to 20 kcal/kgFFM/day, there was an additional decrease in T_3, but when EA was decreased to 10 kcal/kgFFM/day no incremental effect on T_3 was observed (Loucks & Thuma 2003). Loucks & Callister (1993) conducted that T_3 levels depend on EA or more specifically on the carbohydrates availability associated with EA so that T_3 can be suppressed either by decreasing dietary intake or by increasing exercise energy expenditure without reducing dietary intake. Reed et al. (2013) investigated EA on female soccer players across a season. No differences in T_3 concentrations between subjects with low and higher EA during the pre, mid and post season were observed. Nevertheless, increases in T_3 were noticed in association with increases in EA when EA increased from under 30 kcal/kg/FFM/day to clearly above 30 kcal/kgFFM/day. (Reed et al. 2013). This indicates that T_3 levels are reversible when the EA is improved.

In a study by Loucks et al. (1998), T_3 concentration was decreased 18 % when the EA was 10 kcal/kgFFM/day. Reduced T_3 levels have also been reported in athletes who lost weight (Koehler et al. 2013). However, in Volek's et al. (2002) study, no significant changes in T_3

were observed during a 6-week carbohydrate-restricted diet in healthy men. Layman et al. (2003) found that substituting dietary protein for carbohydrates during weight loss maintained T_3 levels. Thong et al. (2000) observed that plasma leptin levels correlated significantly with plasma total T_3, which indicates that they probably function in a synergistic manner to suppress basal metabolic rate.

4.7 Ferritin

Iron has many essential biochemical roles in the body. It is a component of hemoglobin and myoglobin and plays a crucial role in oxygen delivery. Iron is also a central structural and functional component of the heme containing mitochondrial enzymes and cytochromes involved in the electron transport chain and in oxidative phosphorylation. Therefore, iron not only affects health and well-being but also athletic performance. (Peeling et al. 2008.) Iron deficiency has multiple detrimental consequences. It compromises immune function, impairs cognitive function and decreases metabolic efficiency. Iron deficiency combined with energy deficiency perturbs thyroid function, is associated with reproductive dysfunction and may play a role in bone loss. All of these points are related to the female athlete triad (discussed later). (Petkus et al. 2017.) The consequences of iron deficiency in exercising women can be seen in figure 2 and is discussed shortly below.

Iron deficiency affects thyroid metabolism by supressing basal serum T_4 and T_3 concentrations, which can lead to subclinical hypothyroidism and thus have also an influence on physical performance. Metabolic fuel availability may be decreased in exercising women via iron deficiency associated suppression in cortisol synthesis, and it may also further decline by iron deficiency associated growth hormone dysfunction. Iron deficiency restrains basal serum GH levels by suppressing serum ghrelin concentrations. Furthermore, IGF-1 concentration is declined in iron deficiency and thus contributes poor bone health in exercising women. Iron deficiency can probably hazard reproductive function since iron is needed in several key steps in follicular development and corpus luteal function. (Petkus et al. 2017.)

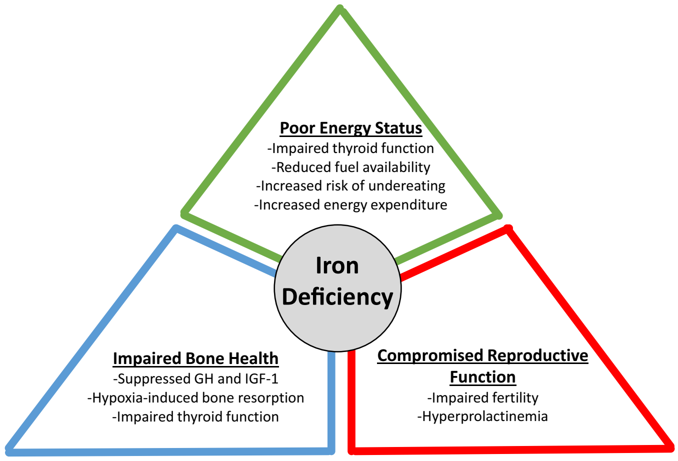

FIGURE 2. Consequences of iron deficiency in exercising women. Adopted from Petkus et al. (2017).

Hemoglobin is the most important pool of iron in the body. It contains up to 80 % of the total body iron (3–4 g) in young women and about 60 % in men. The majority of the remaining iron is found in ferritin and hemosiderin, and is referred to as “storage iron”. Serum ferritin levels correlate with body iron stores and the levels are normally within ranges 15–300 μg/l. (Worwood 2012.) Iron deficiency can be categorized into three degrees of severity (table 3). Oral iron supplementation is generally recommended, when an athlete’s serum ferritin is below 15 μg/l and haemoglobin below 120 g/l (Pedlar et al. 2018).

TABLE 3. Degrees of iron deficiency (Peeling et al. 2007).

Degree	Effect	Serum ferritin (SF) and hemoglobin (Hb) concetrations
Iron depletion	Depleted iron stores in the bone marrow, liver and spleen	SF < 35 μg/l Hb > 115 g/l
Iron deficient erythropoiesis	Erythropoiesis decreases as the iron supply to the erythroid marrow is reduced	SF < 20 μg/l Hb > 115 g/l
Iron-deficient anemia	Hb production falls, resulting in anemia	SF < 12 μg/l Hb < 115 g/l

4.8 The Female Athlete Triad

The term *the Female Athlete Triad* was first time officially described in 1997 by the American College of Sports Medicine (ACSM) as a syndrome with three different symptoms: disordered eating, amenorrhea and osteoporosis. These symptoms are often observed in physically active girls and women. In 2007, the ACSM changed terminology of these components, mentioned above, to energy availability, menstrual function and bone health. (Marcason 2016.) In 2014, the Female Athlete Triad coalition consensus statement was developed following the 1st and 2nd International Symposia on the Female Athlete Triad. The aim of this consensus is "to provide clinical guidelines for physicians, athletic trainers and other healthcare providers for the screening, diagnosis and treatment of the Female Athlete Triad and to provide clear recommendations for return to play." (De Souza et al. 2014.)

As mentioned earlier, the Female Athlete Triad is a medical condition observed in physically active girls and women. It involves any one of three components: (1) low energy availability (EA) with or without disordered eating, (2) menstrual dysfunction and (3) low bone mineral density (BMD). These components are inter-related since energy deficiency is associated with the development of menstrual disturbances and those are linked to play a causal role in low BMD. The continuum of the Female Athlete Triad from optimal health to the most severe presentation can be seen in figure 3. The risk of the triad is high in sports emphasizing leanness and requiring weight control. (De Souza et al. 2014.)

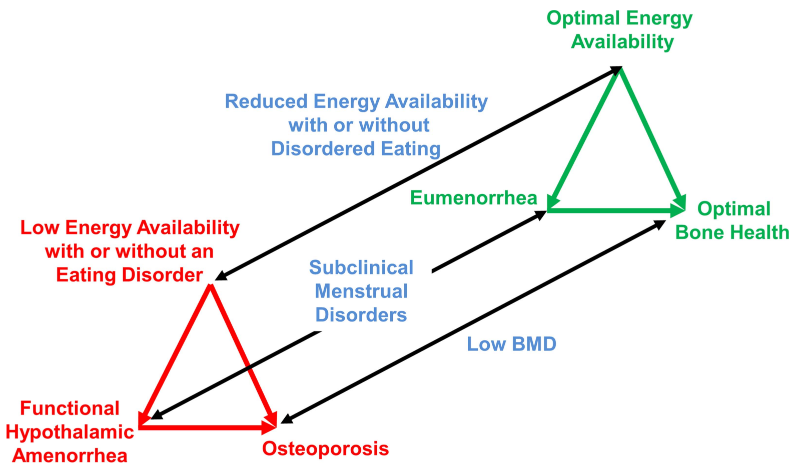

FIGURE 3. Spectra of the Female Athlete Triad (Adopted from De Souza et al. 2014).

Energy availability. The energy availability is defined as the amount of ingested energy minus the energy expended in exercise. The amount of energy remaining after exercise is available for physiological functions, for example locomotion, thermoregulation, immune function and growth. (Loucks et al. 2011.) Energy expended in one of the physiological functions is not available for the others. (Wade & Jones 2004). The calculation of EA can be seen in figure 4.

$$Energy\ availability = \frac{Energy\ intake\ (kcal) - Exercise\ energy\ expenditure\ (kcal)}{fat\text{–}\ free\ mass\ (kg)}$$

FIGURE 4. Energy availability formula (Logue et al. 2018).

Investigators have developed EA thresholds for physically active women. For growth and carbohydrate loading, EA of > 45 kcal/kgFFM/day is recommended (Manore et al. 2007). Optimal energy availability is about 45 kcal/kgFFM/day, which ensures weight maintenance, good training adaptations, athletic development and health (Loucks & Thuma 2003; Ilander et al. 2014, 22–27; Logue et al. 2018). With EA of 30–45, it is possible to reduce weight without negative consequences on health or performance (Manore et al. 2007; Ilander et al. 2014, 22–27; Logue et al. 2018). When EA reduces below 30 kcal/kgFFM/day detrimental consequences appears: the body suppresses reproductive function (Loucks & Thuma 2003) and bone formation (Ihle & Loucks 2004). According to Loucks & Thuma (2003), energy availability seems to dictate the hormonal changes rather than the exercise stress.

Menstrual dysfunction. There are different definitions for menstrual cycles, which are described in table 4. Low energy availability can inhibit the secretion of GnRh from the hypothalamus, which inhibits the LH pulsatility from the pituitary gland (Wade & Jones 2004). This can give a rise to hypoestrogenism and thus also for menstrual disturbances (De Souza & Williams 2005). This type of amenorrhea is called *functional hypothalamic amenorrhea* (Nattiv et al. 2007). Nevertheless, investigators have not found the effects of menstrual status on exercise performance. Still, little is known about the impact of menstrual disturbances on skeletal muscle function and strength. (Manore et al. 2007.)

TABLE 4. Definitions for different menstrual cycles (Nattiv et al. 2007).

Menstrual cycle	Definition
Eumenorrhea	Normal menstrual cycle (28 $\pm$ 7 days)
Oligomenorrhea	Prolonged cycle (> 35 days)
Amenorrhea	Absence of menstrual cycles > 90 days
• Primary	• Delay in the age of menarche (+15 years)
• Secondary	• Amenorrhea beginning after menarche

Bone mineral density (BMD). Estrogen plays a significant role in bone formation and maintaining bone mass. Therefore, chronic hypoestrogenism is the main cause of bone loss in exercising women. (Ott 1990.) Estrogen deficiency reduces the rate of bone formation and thus the bone mineral density decreases (Bennel et al. 1999). The more missed menstrual cycles, the more BMD declines. (Drinkwater et al. 1990.) The loss of BMD may not be fully reversible (Warren et al. 2002). Therefore, the risk of stress fractures increases being two to four times greater in amenorrheic than eumenorrheic athletes and can be lead to osteoporosis if left untreated (Bennel et al. 1999). The diagnosis of low BMD and osteoporosis can be made with DXA scan (Lewiecki et al. 2008). The Z-score compares individuals to age and sex-matched controls (Nattiv et al. 2007). A Z-score of -2.0 or lower is defined as "below the expected range for age" and a Z-score above -2.0 is "within the expected range for age (Lewiecki et al. 2008). According to the American College of Sports Medicine (ACSM), low BMD is defined as a Z-score that is less than -1.0 in female athletes in weight-bearing sports (Nattiv et al. 2007).

4.8.1 Other hormonal changes

When comparing menstrual dysfunction athletes to their eumenorrheic counterparts, higher cortisol levels have been observed in those with menstrual dysfunction (Melin et al. 2015.) In a study by Loucks & Thuma (2003) the effects of balanced (45 kcal/kgFFM/day) and restricted (10, 20, and 30 kcal/kg/FFM/day) energy availability treatments were studied in regularly menstruating, habitually sedentary, young women. It was noticed that when energy availability decreased the cortisol levels increased with progressively more extreme decrease with lower EAs. A similar finding observed in a study by Loucks et al. (1998), where cortisol levels increased by 11 % when energy availability was 10 kcal/kgFFM/day.

When the EA is chronically low, insulin has increased sensitivity (Logue et al. 2018). Lower insulin levels have been observed in amenorrheic people (Laughlin et al. 1998; Thong et al. 2000; Ihle & Loucks 2004). Amenorrheic athletes also display low levels of IGF-1 (Ihle & Loucks 2004). IGF-1 has an important role in modulating bone mineral density and bone turnover (De Souza & Williams 2005). Furthermore, T_3 levels have been found to decrease in female athletes with menstrual irregularities and in amenorrhea (Thong et al. 2000; Ihle & Loucks 2004; Loucks et al. 2004; Reinehr et al. 2008; Melin et al. 2015; Logue et al. 2018). T_3 also regulates bone formation (Nattiv et al. 2007). Ihle & Loucks (2004) suspected that about 25 % decrease in T_3 levels founded in amenorrheic athletes (Loucks et al. 1992 according to Ihle & Loucks 2004) may also impair bone formation and particularly bone mineralization. When compared to eumenorrheic sedentary women, luteally suppressed eumenorrheic athletes also display low concentrations of T_3 (Loucks et al. 2004; Vanheest et al. 2014).

5 WEIGHT REDUCTION AND PHYSICAL PERFORMANCE

Maximizing physical performance is one significant reason for weight loss in athletes (Fogelholm 1994; Mettler et al. 2010). Body weight reduction, especially fat, improves power-to-weight ratio and thus may be beneficial in weight-bearing efforts, such as jumping events for height and distance (Fogelholm 1994). From the point of view of performance, beneficial results have been noticed in gradual weight loss studies.

5.1 Maximum speed

In studies, no negative consequences on maximum speed after a weight reduction period have been noted. In studies by Fogelholm et al. (1993) and Garthe et al. (2011a) no changes were observed in maximum speed during a three week with -1000 kcal/day energy deficit (Fogelholm et al. 1993) or during 4–12 week 0.7–1.4 %/week weight reduction rate weight reduction periods. The same was observed in a study by Lällä (2016), where energy deficit was about -530 kcal/day for four weeks and no changes in maximum running speed was noticed. However, an improvement in maximum running speed was observed in Huovinen's et al. (2015) study, where the 20m sprint performance improved by 1.8 % in a higher energy deficit group (-750 kcal/day) during a four-week weight loss period. Sprint performance also improved in a lower energy deficit group (-300 kcal/day) in the same study, but the improvement did not reach statistical significance. Since the improvement in running speed after weight reduction has not been observed in other studies, further studies are needed to verify this finding.

5.2 Vertical jumps

Gradual weight loss studies have showed some beneficial effects in terms of weight-bearing athletic performance. Vertical jump performance and muscle strength expressed relative to body weight improved after three weeks of energy restriction of -1000 kcal/day in a study by Fogelholm et al. (1993). A significant improvement was shown in vertical jump height (+1.4 cm) with extra load (50 % of body weight) and also an increase (+0.5) with no extra load (Fogelholm et al. 1993). The same was observed in Mero's et al. (2010), Mettler's et al. (2010), Garthe's et al. (2011a) and Huovinen's et al. (2015) studies. It was observed, in a study by Garthe et al. (2011a) with athletes, a statistically significant improvement (7 ± 3 %) in the counter movement jump height (+2.0 ± 0.7 cm) in the slower weight loss rate group (0.7 % of body mass/week, -469 kcal/day), whereas the result remained unchanged in the faster weight loss rate group (1.4 % of body mass/week, -845 kcal/day).

In Mero's et al. (2010) study with recreational women athletes, the improvement in counter movement jump height was statistically significant only in the higher energy deficit group (-1100 kcal/day). There was also a trend for better CMJ performance with a lower energy deficit (-550 kcal/day), but this did not reach statistical significance. Also in a study by Huovinen et al. (2015), the improvement in the CMJ height was statistically significant only in the higher energy deficit group (-750 kcal/day) (+3.0 cm), but was increased in the lower energy deficit (-300 kcal/day) (+2.0 cm) too.

However, the vertical jump performance has not improved in all studies. In a study by Filaire et al. (2001), the performance remained unchanged after seven days of weight reduction when the energy deficit was -1000 kcal/day at the highest point. In a study with strength-trained males conducted by Mettler et al. (2010), the vertical jump performance did not change during a two-week period with low energy diet (60 % of subject's habitual energy intake) either. The peak force of the jump dropped down significantly over time, but the authors believed this was, at least, partially due to the lower body weight toward the end of the study, where less force was needed for the same jump height. The vertical jump performance remained at the same level after a four-week weight reduction period (-530 kcal/day) also in a study by Lällä (2016). However, in this study the performance tests were conducted a day after a high jump competition, which may have had an influence on the results.

In conclusion, vertical jump performance seems to improve together with reduced body weight. The improvement is bigger, when the energy deficit is higher. (Fogelholm ym. 1993; Mero et al. 2010; Garthe et al. 2011a; Huovinen et al. 2015.) However, according to Garthe's et al. (2011a), study the rate of weight loss (i.e. 0.7 %/bodyweight loss/week vs. 1.4 % bodyweight loss/week) seemed to dictate the outcome in performance. More improvements were found in the group of slower weight loss rate. Filaire's et al. (2001) study would seem to support this statement too.

6 RESEARCH QUESTIONS

Problem 1. Does gradual weight and fat reduction improve weight-bearing athletic performance (i.e. jumping, running)?

Hypothesis and rationale. Gradual weight reduction of 0.5 kg to 1.0 kg per week for three to twelve weeks improves jumping performance in athletes (Garthe et al 2011; Mero et al. 2010; Fogelholm et al. 1993).

Problem 2. Is lean tissue preserved after moderate gradual weight reduction (i.e. ≈ 0.5 kg/week) with high protein diet?

Hypothesis and rationale. Gradual weight reduction of 0.5 kg to 1.0 kg per week with a high protein diet for four to twelve weeks leads to fat catabolism but lean tissue is preserved (Garthe et al. 2011; Mero et al. 2010)

Problem 3. Does gradual weight reduction have an adverse effect on anabolic and catabolic hormones (i.e. testosterone, cortisol, insulin, IGF-1, T_3)?

Hypothesis and rationale. Gradual weight reduction with energy restriction leads to decrease in testosterone and increase in cortisol concentrations (Proteau et al. 2006; Mero et al. 2010). Low energy availability causes insulin, IGF-1 and T_3 levels to decrease (Loucks & Thuma 2003; Koehler et al. 2016).

Problem 4. Does energy restriction affect menstrual status?

Hypothesis and rationale. Low energy availability can inhibit the secretion of GnRh from the hypothalamus, which inhibits the LH pulsatility from the pituitary gland (Wade & Jones 2004). This can give a rise to hypoestrogenism and thus also to menstrual disturbances (De Souza & Williams 2005).

7 METHODS

7.1 Subjects

The subjects in the present study were 18–24-year old national level Finnish track and field women athletes from jumping events (long jump, triple jump, high jump and pole vault). All subjects were healthy and volunteered to participate in the study. The background information of the subjects is described in table 5. All subjects underwent a medical screening done by a doctor. The subjects signed a written consent to participate in the study, which involved the procedures, benefits and possible risks of the study. The study was approved by the Ethics Committee of the University of Jyväskylä. Subjects were divided into two groups, which were weight reduction (WR) and control (C) group. There were no significant body composition or energy expenditure (EE) differences between the groups, as seen on table 5.

TABLE 5. Characteristics of the subjects. WR, weight reduction group; C, control group.

	Age ± SD (years)	Height ± SD (m)	Body mass ± SD (kg)	BMI ± SD (kg/m^2)	Fat% ± SD	EE ± SD (kcal/day)
WR (n=7)	21.5 ± 2.0	1.74 ± 0.06	61.6 ± 3.0	20.5 ± 1.6	17.1 ± 3.0	2294 ± 134
C (n=6)	20.5 ± 1.6	1.72 ± 0.09	60.9 ± 6.9	20.6 ± 0.8	18.7 ± 3.7	2153 ± 263

7.2 Study design

The purpose of this study was to follow the effects of a preparatory season preparation (exercise, diet) on body composition, hormonal concentrations and physical performance in female track and field jumpers and follow those variables across summer's competition season. The progression of the study is shown in figure 5. The subjects kept food, activity and training diaries five days before each measurement. The menstrual cycle was followed throughout the whole study and subjects' history of injuries was also ascertained. After the first measurements (at the end of March, at the beginning of April) subjects were divided into either a weight reduction (WR) group or a control (C) group according to their individual needs. The weight reduction group was advised to reduce their energy intake by 500–600 kcal/day to induce a weekly weight loss of 0.5 kg. They were instructed to maintain a high protein intake (over 1.6 g/kg/day) and restrict carbohydrate and fat intake. The control group was advised to keep their diet similar and correct any noted faults. The summer's competition season started after mid measurements (at the end of May). From the mid to post measurements, the aim of the study was to follow, how body composition, hormonal concentrations and physical performance were affected. The post measurement was conducted as soon as possible after each subject's competition season ended (at the end of August, at the beginning of September).

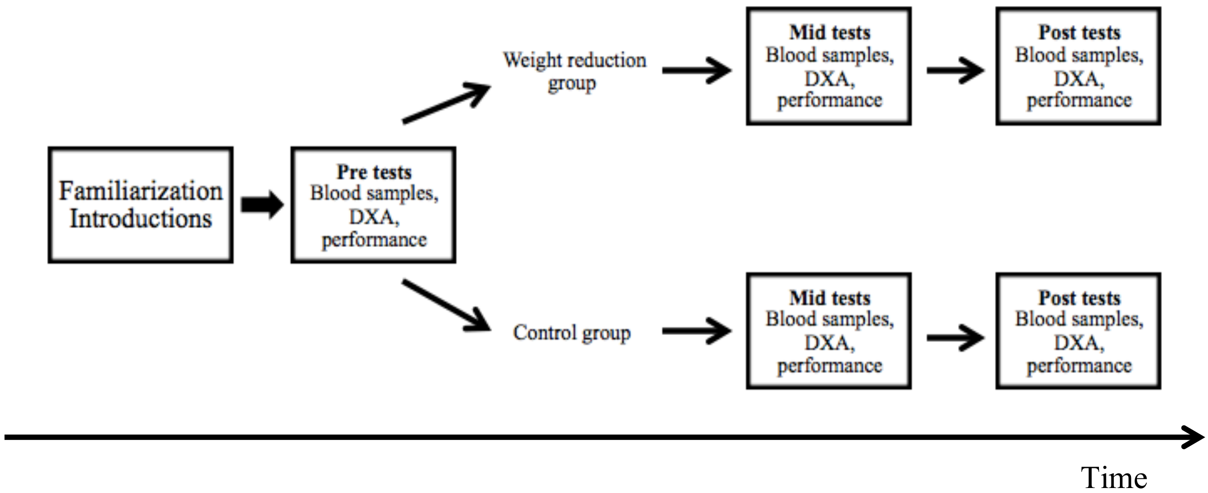

FIGURE 5. Study design.

All subjects continued their training regimen similarly than before the study. Each subject received individual nutritional guidance during the study and was responsible for following the planned diet. All the physical tests were already familiar to the subjects.

7.3 Data collection

The data collection in pre, mid and post measurements included a nutrition analysis, energy expenditure estimation, body composition measurement, blood samples and physical performance tests. Subjects' menstrual cycle was followed through the whole study.

7.3.1 Food, activity and training diaries, menstrual cycle and injury history

The subjects were asked to keep food, activity and training diaries for five days (three weekdays and two weekend days) before each measurement point and also to report their morning weight. The menstrual cycle was followed throughout the study. Subjects' history of injuries was also ascertained. The diaries were filled in specific forms, which can be seen in appendices 1, 2, 3, 4 and 5.

The food diaries included the timing and place of eating and consumed nutrients and their amounts as accurately as possible. Weighing the foods was recommended. The subjects were instructed to take pictures of their meals (especially when weighing was not possible) and write down recipes in order to facilitate the analysis and to increase its reliability.

The activity diaries involved daily living activities such as sleeping, watching TV, studying and doing household tasks reported with at least the accuracy of one hour. The training diaries included the duration and specific contents of the training performed. The menstrual cycle was monitored by writing down the beginning of bleeding, bleeding days and the end of bleeding. The use of hormonal contraception was also enquired. Subjects' history of injuries was charted with questions at the end of the study.

7.3.2 Blood samples and body composition

Blood samples were drawn in a sitting position from the antecubital vein in the morning of each measurement day after an overnight fast. Analysis of the blood samples included hemoglobin (Hb), hematocrit (HCT), serum total testosterone, free testosterone, estradiol (E_2), cortisol, insulin, insulin-like growth factor-1 (IGF-1), triiodothyronine (T_3) and ferritin.

Subjects' body composition was determined using a dual-energy X-ray absorptiometry (DXA). Analysis included fat mass (FM), fat percentage (fat%), lean body mass (LBM), bone mineral content (BMC), bone mineral density (BMD) and age matched Z-score of BMD. DXA measurement was conducted in a fasted state and wearing underwear, as follows. The subject was placed in a supine position on the scanning bed. The body was equally on each side of the scanning bed's median line and the head was 5 cm below from the upper line of the scanning bed. The spine of the subject was straightened by pulling one's legs. The position of legs was standardized using Styrofoam. Small pillows were used to standardize the position of the arms. They were placed in armpits and between palms and trunk. The same technician undertook all the scans. Body mass was in determined underwear before DXA measurement with the same digital scale each time.

7.3.3 Physical performance tests

Explosive power was measured in squat jump with (20 kg) and without extra weight, counter movement jump and reactive jump. All jumps were performed on a contact mat connected to a timer in order to measure contact and flight times. Running speed was evaluated with a 20 m sprint with a flying start and a 30 m sprint test with a standing start (accuracy of 0.01 s) using photocells. Warm-up was an individual routine for each athlete and included jogging, sprints and dynamic stretches. The warm-up was similar each time. The order of the performance tests was as follows: a 50 m sprint test with a standing start (30 m + 20 m tests together, start 70 cm behind the first photocell), squat jump, counter movement jump, squat jump with extra weight (20 kg) and reactive jump. The recovery between trials was 3–5 minutes. All performance tests were conducted in Hipposhalli in Jyväskylä according to the instructions of Keskinen et al. (2007).

7.4 Data analysis

7.4.1 Food, activity and training diaries and menstrual cycle

A 3–7-day diet record period is believed to give reasonably accurate and precise estimations of habitual energy and macronutrient consumption in athletes. Among athletes, under-reporting of habitual energy intake is prevalent, which originates from modification of the usual intake, imprecise reporting of food intake to enhance the perception of what the athlete is eating and erroneous description or quantification while reporting food intake. (Magkos & Yannakoulia 2003.)

The food diaries were analysed using the AivoDiet nutrient-analysis software (Aivo Finland Oy, Turku), which gave the amount of energy, macronutrients, vitamins and minerals and distribution of macronutrients in the diet. Both absolute and proportional (per body weight) intakes were calculated for each subject. Percentage portions were also determined, for instance as follows: the absolute intake of protein multiplied by four (carbohydrates/protein contain energy 4 kcal/g, fat 9 kcal/g and alcohol 7 kcal/g) and divided by total energy intake. Thereafter, the result received multiplied by 100.

As recommended by American College of Sports Medicine (ACSM), the equation of Cunningham (1991) (RMR (kcal/day) = 370 + 21.6*FFM) was used to estimate the resting metabolic rate (RMR). Cunningham's equation includes the fat-free mass, and therefore considers differences in body composition between athletes and non-athletes. In comparison to indirect calorimetry measurement of resting metabolic rate Cunningham's (1991) equation has been shown to give mean difference of 55.5 kcal/day in RMR in female athletes (Kim et al. 2015). A study by Johnstone et al. (2005) involving 150 adults in Scotland revealed that 62.3 percent of the variations seen in BMRs are related to fat-free mass. Kim et al. (2015) also concluded that a person's fat-free mass is the most demonstrative variable affecting the RMR. However, it has to be taken into account that genetic differences affect RMR (Ilander et al. 2014, 36) and it is also reduced in people with chronic energy deficiency (Melin et al. 2015).

Energy expenditure of physical activity assessed using MET-values by Ainsworth et al. (2011). MET-value (metabolic equivalent) is defined as the energy expenditure of physical activity divided by the energy cost of rest or resting energy expenditure (REE). There are individual differences in the REE and it can be reliably determined by measuring oxygen uptake under resting conditions. As a result of individual differences in REE, the value of one MET also differs individually. The standard value of one MET is 3.5 ml/kg/min, which is equal to the oxygen cost of sitting quietly and can be used if the accurate value is not known. (Ainsworth 2013.) "The 2011 compendium of Physical Activities" by Ainsworth et al. (2011) lists MET-values for selected exercise and sport activities, which can be used when estimating, for example, the energy expenditure of a single exercise or other physical activity. However, it must be noted that the MET-values do not take into account differences in body mass, adiposity, sex, age, efficiency of movement, and the geographic and environmental conditions in which the activities are performed. Therefore, individual differences in energy expenditure can be large and the true energy cost for a person may or may not be close to the stated mean MET level. (Ainsworth et al. 2011.)

According to the menstrual cycle length questionnaire, subjects' were divided into eumenorrheic (normal menstrual cycle 28 $\pm$ 7 days), amenorrheich (cycle length > 90 days) or oligomenorrheic (cycle length > 35 days) groups.

7.4.2 Blood samples

Two millilitres of blood was drawn from the antecubital vein in K2 EDTA tubes (Greiner-Bio-One GmbH, Kremsmünster, Austria) for measurements of hemoglobin and hematocrit. The samples were analysed immediately with Sysmex XP300 analyzator (SysmexCo., Kobe, Japan). The precision (CV%) was 2.4 % for hemoglobin and 2.3 % for hematocrit. For the determination of serum hormone concentrations, eight millilitres of blood was taken into serum gel tubes (Greiner-Bio-One GmbH, Kremsmünster, Austria). The samples were centrifuged 3600 rpm for 10 minutes with Mage 1.0 R centrifuge and kept frozen in -20 ℃ until analysed. The samples were analysed with Siemens Immulite 2000 Xpi analyzator (Siemens Healthcare Lianberis, United Kingdom).

Serum hormone concentrations were determined by an immunometric chemiluminescense method. The sensitivity of the assay is 0.5 nmol/l for testosterone, 5.5 nmol/l for cortisol, 55 ppmol/l for estradiol, 2 mIU/l for insulin, 0.26 nmol/l for insulin-like growth factor-1, 1.5 pmol/l for triiodothyronine and 0.4 μ/l for ferritin. Precision (CV%) for the assay was 7.3 % for testosterone, 8.3 % for cortisol, 13.5 % for estradiol, 5.6 % for insulin, 7.8 % for insulin-like growth factor-1, 7.3 % for triiodothyronine and 4.6 % for ferritin.

7.4.3 Body composition

A DXA-machine (GE Lunar Prodigy Advance, Madison, WI, USA) was used to measure body composition. Principally the DXA instruments are composed of a generator emitting X-rays of two energies, a scanning table, a detector, and a computer system. The measurement is quick (5 to 10 min), non-invasive, accurate and operator independent. (Genton et al. 2002.) The radiation doze is 2 to 5 μSv (Madden & Morgan 1997), which is low compared to the daily background radiation of 5 to 7 μSv (Laskey & Phil 1996).

The ultimate principle behind DXA is the measurement of the transmission of X-rays through the body at high- and low-photon energies. (Genton et al. 2002.) Rectilinear scanning of the supine body is performed, which divides the body into a series of pixels, within each of which the photon attenuation is measured at two different energies (Plank 2005). The attenuation depends on the photon's energy and the density and thickness of the tissues, which they pass. The DXA assumes that the body consist of three components that are distinguishable by their photon attenuation properties: bone mineral, fat and lean soft tissue. (Plank 2005.) The proportions of only two components (bone mineral and soft tissue) can be resolved by the differential absorption of two photon energies within any pixel. (Plank 2005.)

For Lunar Prodigy DXA device, which was used in this study, CVs % are reported to be 0.6–0.9 % for BMC (Aasen et al. 2006; Bilsborough et al. 2014), 0.7–2.5 % for fat mass (Aasen et al. 2006; Bilsborough et al. 2014), 0.3–2.5 % for fat percentage, 0.3 % for fat-free mass (Bilsborough et al. 2014), 0.7 % for lean mass (Aasen et al. 2006) and 0.02–0.2 % for total mass (Aasen et al. 2006; Bilsborough et al. 2014). The reproducibility of Lunar Prodigy DXA device was evaluated in Garthe's et al. (2011a) study where ten athletes did two repeated measurements within 24 hours. The coefficient of variation (CV%) was 3 % for fat mass and 0.7 % for fat-free mass.

In studies tracking body composition changes during preparation for competition or weight loss (Jebb 1997; Evans et al. 1999; Van Marken Lichtenbelt et al. 2004; Mahon et al. 2007; Santos et al. 2010), many researchers detected small, non-significant mean differences between percentage body fat changes measured via DXA and those obtained from the 4-C model. Regarding bone mineral density, it has been noted to decrease with Lunar Prodigy DXA device when individual undergoes weight loss, without any real change in the skeleton (Tothill 2005).

7.4.4 Physical performance tests

Both the 20 m sprint test with a flying start and the 30 m test with a standing start have been shown to be well reproducible. In a study with track and field sprinters (n=9), the correlation was 0.98 and coefficient of variation was 2 % for two successive efforts in the 30 m sprint. The same values for 20 m sprint were 0.99 and 1.2 % in a 20 m in a study with Finnish track and field sprinters (n=14). (Keskinen et al. 2007, 166–167.)

Vertical jumps are also highly reproducible performance tests. The correlation between two consecutive jumps has been shown to be 0.95 and coefficient of variation 4–5 %. Correlation between consecutive vertical jumps with extra weight has been shown to range between 0.79–0.96 and coefficient of variation between 2–10 %. (Keskinen et al. 2007, 153.) In comparison with other vertical jump tests the reproducibility of reactive jump test is not as good.

Measurements with Finnish track and field sprinters (n=11) correlation of two successive efforts have been shown to be 0.77 and coefficient of variation 7 %. (Keskinen et al. 2007, 156.)

7.5 Statistical analysis

Statistical analysis was done with IBM SPSS Statistics 24.0 (IBM Corp. Armonk, NY, USA) and Microsoft Office: mac Excel 2011 (Microsoft Corp. Redmond, USA). Group mean values and standard deviations were calculated with Excel. Within the groups pre versus mid, mid versus post and pre versus post significances were achieved by one-way ANOVA with repeated measures. Independent-samples t-test and Mann-Whitney U test were used to achieve significances WR versus C. Pearson's and Spearman's correlations were used to get correlation coefficients.

8 RESULTS

All thirteen subjects were able to complete the study. One subject did not participate in the last measurement session in Jyväskylä, because of the problems with the schedule. There was also a large drop out (n=4) in the physical performance tests in the last measurements in WR group because of injuries.

8.1 Menstrual status

According to the menstrual cycle none of the subjects' was amenorrheich (cycle length > 90 days). In WR, five out of seven subjects' had a prolonged menstrual cycle and were classified as oligomenorrheic (cycle length > 35 days). In C (n=6), only one subject was oligomenorrheic and remainder were eumenorrheic (normal menstrual cycle 28 $\pm$ 7 days). The menstrual cycle lengths were 43 $\pm$ 9 and 30 $\pm$ 6 days in WR and C, respectively. There was a statistically significant difference (p=0.01) between the groups in menstrual cycle length. The menstrual cycle lengths are shown in figure 6.

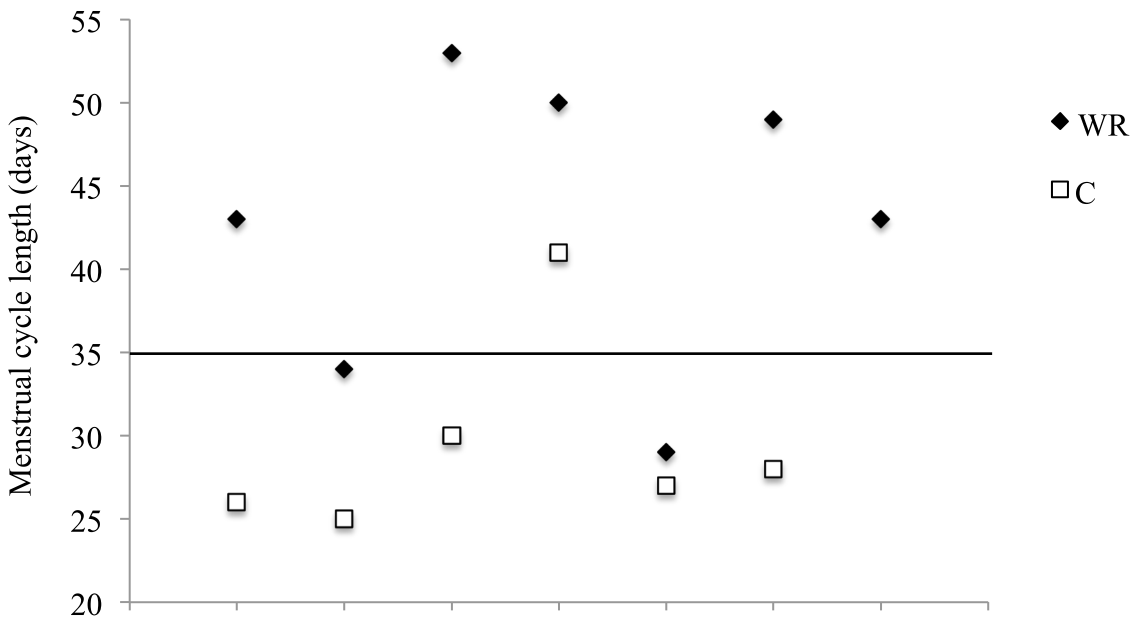

FIGURE 6. The lengths of menstrual cycles in WR and C. The black line represents the upper value of normal menstrual cycle classification.

8.2 Energy intake and expenditure

The estimated energy expenditure was 2302 ± 145 kcal/day before the study, 2137 ± 93 kcal/day during weight reduction and 2080 ± 114 kcal/day after competition season in WR. The same values for C were 2153 ± 263 kcal/day, 2137 ± 237 kcal/day and 1990 ± 273 kcal/day (figure 7). Energy intakes for both groups can be seen in table 6. Both energy intake and energy availability decreased statistically significantly in WR between pre and mid measurements (-448 ± 299 kcal/day, $p=0.022$ and -8 ± 6 kcal/kg$_{FFM}$/day, $p=0.044$, respectively). Between the mid and post measurements there was a trend for increased energy intake and energy availability, but those did not reach statistical significance ($p=0.08$ and $p=0.062$, respectively). Energy intake was similar in the pre and post measurements (table 6). In C, energy intake remained similar throughout the study. Energy intake was significantly higher in WR compared to C in the pre ($p=0.04$) and the post ($p=0.014$) measurements. The estimated energy deficit (the difference between energy intake and expenditure) during weight loss was -477 ± 218 kcal/day in WR. The C group was in a significant energy deficit throughout the study. Energy deficits were -541 ± 241 kcal/day, -451 ± 351 kcal/day and -353 ± 242 kcal/day in the pre, mid and post measurement, respectively.

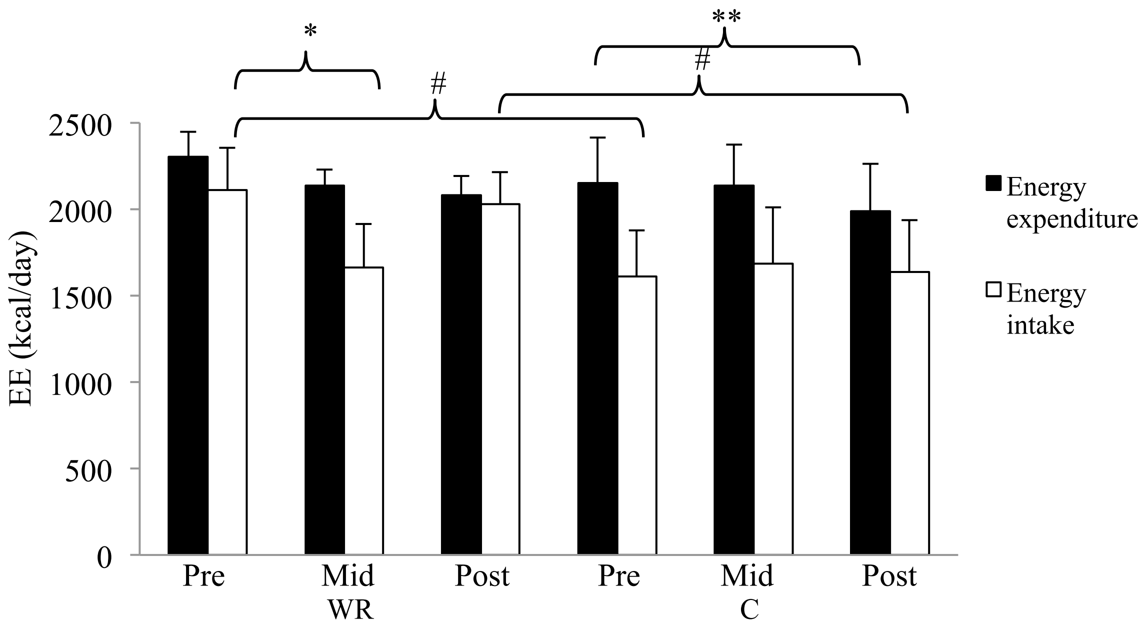

FIGURE 7. Energy expenditure (EE) and intake in the pre, mid and post measurements in WR and C (mean ± SD).

# $p<0.05$ significant difference WR vs. C

* $p<0.05$ significant difference pre vs. mid in WR

** $p<0.01$ significant difference pre vs. post in C

TABLE 6. Energy intake and energy availability in the pre, mid and post measurements. WR, weight reduction group; C, control group; EI, energy intake; EA, energy availability.

Variable	WR			C		
	Pre	Mid	Post	Pre	Mid	Post
EI (kcal/day)	2112 ± 243	1664 ± 251*	2030 ± 185	1612 ± 264 #	1686 ± 326	1637 ± 299 #
EA ($kcal/kg_{FFM}/day$)[1]	36 ± 5	28 ± 5*	37 ± 5	27 ± 5 ##	29 ± 6	30 ± 5 #

* p<0.05 significant difference pre vs. mid

# p<0.05; ## p<0.01 significant difference WR vs. C

[1]n=6 in WR

8.3 Nutritional intake

The nutritional intakes for both groups are shown in table 7. Macronutrient intake was significantly higher in WR before the weight reduction period. During weight reduction, fat intake in proportion to total energy intake was 5 E% higher (p=0.041) in C compared to WR. In the post measurements, protein and fat intake was higher in WR, while carbohydrate intake was similar.

During the weight reduction in WR, both absolute and in relation to body mass expressed carbohydrate intake reduced statistically significantly by -49 ± 31 g/day (p=0.016) and -0.7 ± 0.5 g/kg/day (p=0.015), respectively. There was also a trend for reduced fat intake (-24 ± 22 g/day), which did not reach statistical significance (p=0.084). Protein intake remained similar throughout the study. Both absolute and proportional fat intake increased statistically significantly between the mid and post measurements: 21 ± 16 g/day (p=0.039) and 5 ± 3 E% (p=0.035), respectively for absolute and proportional fat intake. The nutritional intake was similar in the pre and post measurements. No significant changes in the nutritional intake were observed in C apart from the proportional fat intake, which decreased 5 E% (p=0.021) between the mid and post measurements.

TABLE 7. Nutritional intake in the pre, mid and post measurements. WR, weight reduction group; C, control group; prot, protein; CHO, carbohydrates.

Variable	WR			C		
	Pre	Mid	Post	Pre	Mid	Post
Prot (g/day)	125 ± 14	115 ± 26	118 ± 14	96 ± 20 #	95 ± 25	89 ± 24 #
Prot (g/kg/day)	2.0 ± 0.3	1.9 ± 0.5	2.0 ± 0.3	1.6 ± 0.3 #	1.6 ± 0.4	1.5 ± 0.3 #
Prot (E%)	24 ± 4	28 ± 8	24 ± 3	25 ± 3	23 ± 3	23 ± 3
CHO (g/day)	222 ± 45	173 ± 45 *	210 ± 40	165 ± 43 #	175 ± 44	191 ± 34
CHO (g/kg/day)	3.6 ± 0.8	2.8 ± 0.8 *	3.5 ± 0.7	2.8 ± 0.8	2.9 ± 0.8	3.2 ± 0.5
CHO (E%)	43 ± 6	43 ± 7	43 ± 6	42 ± 6	43 ± 4	49 ± 4
Fat (g/day)	74 ± 13	51 ± 11	72 ± 10 §	56 ± 6 #	60 ± 8	51 ± 9 #
Fat (g/kg/day)	1.2 ± 0.2	0.8 ± 0.2	1.2 ± 0.2	0.9 ± 0.1 #	1.0 ± 0.1	0.8 ± 0.1 #
Fat (E%)	33 ± 4	29 ± 4	33 ± 4 §	33 ± 5	34 ± 3 #	29 ± 1 § #

* p<0.05 significant difference pre vs. mid

§ p<0.05 significant difference mid vs. post

# p<0.05; ## p<0.01 significant difference WR vs. C

Micronutrients. Considering the subjects' intakes of micronutrients (data not presented here due to the low reliability of the results), deficiencies compared to recommendations were most common in vitamin D, folate and iron. There were no differences between the groups in the intakes of micronutrients mentioned above except in vitamin D intake, which seemed to be higher in C in the mid measurements.

8.4 Body composition

The body composition results are presented in table 8. There were no significant differences between the groups in body composition values at any measurement point. The changes in body composition can be seen in table 9 and figure 8. The four-week weight reduction period resulted in weight loss of 1.3 ± 1.3 kg in WR, which did not reach statistical significance (p=0.179). Fat percentage and fat mass reduced significantly in WR (p=0.0003 and p=0.001, respectively). Lean body mass, bone mineral content and bone mineral density remained unchanged during weight reduction. In C, no significant changes were observed in body composition between the pre and mid measurements. No significant changes in body composition were observed between the mid and post measurement in either of the groups.

TABLE 8. Body composition in the pre, mid and post measurements. WR, weight reduction group; C, control group, BM, body mass; FM, fat mass; LM, lean mass; BMC, bone mineral content; BMD, bone mineral density.

Variable	WR			C		
	Pre	Mid	Post	Pre	Mid	Post
BM (kg)	61.6 ± 3.2	60.3 ± 2.7	60.2 ± 3.7	60.9 ± 6.9	61.1 ± 6.8	60.6 ± 7.1
Fat% (%)	17.2 ± 3.2	15.1 ± 3.3	16.1 ± 4.1	18.7 ± 3.7	18.8 ± 3.0	19.6 ± 4.0
FM (kg)	10.3 ± 2.3	8.8 ± 2.1	9.4 ± 2.8	11.0 ± 2.4	11.0 ± 1.8	11.4 ± 2.3
LM (kg)	49.3 ± 1.6	49.5 ± 1.9	48.8 ± 2.1	47.8 ± 5.6	47.9 ± 5.8	47.1 ± 6.3
BMC (g)	3031 ± 298	3012 ± 297	3026 ± 285	2980 ± 466	2998 ± 472	2984 ± 480
BMD (g/cm^2)	1.378 ± 0.053	1.371 ± 0.048	1.373 ± 0.046	1.339 ± 0.085	1.333 ± 0.082	1.339 ± 0.077
Z-score	3.3 ± 0.6	3.3 ± 0.6	3.3 ± 0.5	3.0 ± 1.0	2.9 ± 0.9	3.0 ± 0.8

TABLE 9. Body composition changes between the pre and mid measurements. WR, weight reduction group; C, control group.

Variable	WR	C	Δ WR vs. C
	Δ	Δ	p
Body mass (kg)	-1.3 ± 1.3	0.2 ± 0.9	0.042
Fat percentage (%)	-2.2 ± 0.7***	0.1 ± 1.3	0.007
Fat mass (kg)	-1.5 ± 0.4***	0.03 ± 0.9	0.005
Lean mass (kg)	0.2 ± 1.1	0.03 ± 0.6	0.776
BMC (g)	-19 ± 49	19 ± 24	0.123
BMD (g/cm^2)	-0.007 ± 0.009	-0.006 ± 0.008	0.866
Z-score	0.0 ± 0.1	-0.1 ± 0.1	0.172

* p<0.05; ** p<0.01 *** p≤0.001 statistically significant change

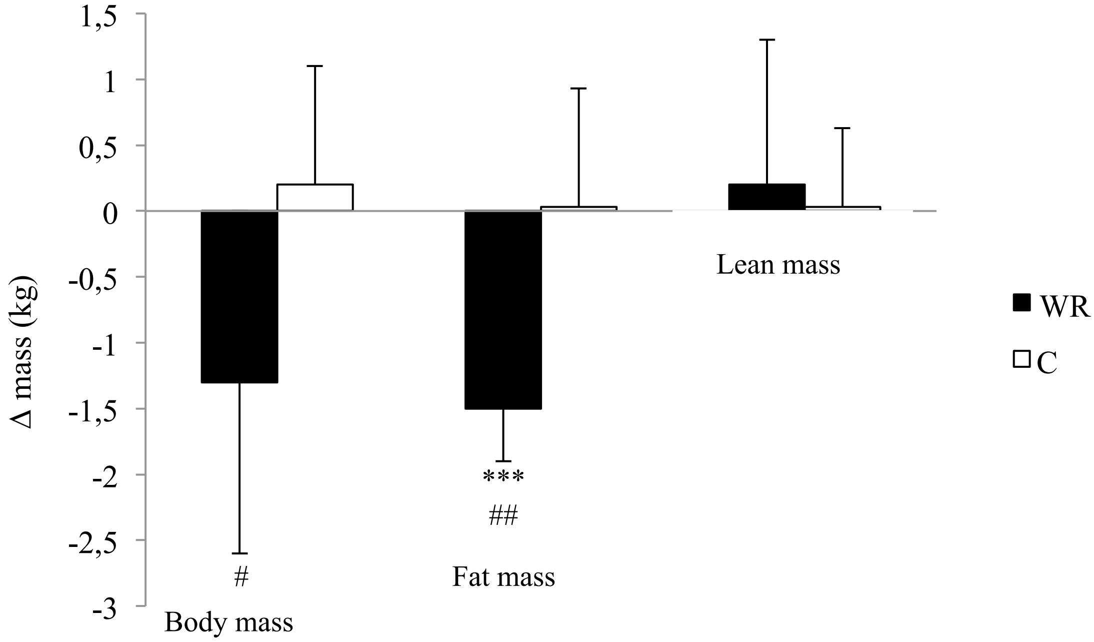

FIGURE 8. Body composition changes between pre and mid measurements.
*** p<0.001 statistically significant change pre vs. mid
# p<0.05; ## p<0.01 statistically significant change WR vs. C

8.5 Hemoglobin, hematocrit, hormone and ferritin concentrations

Hemoglobin, hematocrit, hormone and ferritin concentrations are presented in table 10. There were no differences between the groups in serum hormone concentrations at the measurement points, excluding the pre and mid testosterone, IGF-1 and T_3, which were significantly higher in C compared to WR (p=0.0003, p=0.011 p=0.05 p=0.038 p=0.005 p=0.022, respectively for pre and mid testosterone, IGF-1 and T_3 concentrations). Mid estradiol concentration also differed between the groups (p=0.041) being significantly higher in group C. No significant changes were observed in hemoglobin, hematocrit, hormone or ferritin concentrations during the study.

TABLE 10. Hemoglobin, hematocrit, hormone and ferritin concentrations in the pre, mid and post measurements.

Variable	WR			C		
	Pre	Mid	Post	Pre	Mid	Post
Hemoglobin (g/l)	134 ± 8	139 ± 7	137 ± 9	141 ± 11	142 ± 12	137 ± 14
Hematocrit (%)	39 ± 2	41 ± 1	40 ± 2	41 ± 2	42 ± 4	41 ± 3
Testosterone (nmol/l)	0.625 ± 0.176 ###	0.732 ± 0.227 #	0.721 ± 0.218	1.363 ± 0.292	1.244 ± 0.335	1.142 ± 0.416
Testosterone free (nmol/l)	2.0 ± 0.9	2.5 ± 1.6	1.5 ± 0.7	3.3 ± 1.6	2.4 ± 1.0	2.3 ± 1.0
E_2 (pmol/l)	157 ± 135	114 ± 34#	239 ± 233	286 ± 133	467 ± 318	331 ± 183
Cortisol (nmol/l)[1]	477 ± 162	493 ± 149	496 ± 67	521 ± 135	503 ± 60	565 ± 104
Insulin (mU/l)	1.50 ± 1.73	3.32 ± 2.80	3.55 ± 3.82	3.33 ± 2.38	4.22 ± 2.86	4.58 ± 2.28
IGF-1 (nmol/l)	29.1 ± 7.1 #	30.3 ± 6.2 ##	32.5 ± 8.4	39.7 ± 9.2	45.7 ± 13.5	38.7 ± 9.4
T_3 (pmol/l)	5.03 ± 0.40 ##	5.01 ± 0.82 #	5.45 ± 0.77	6.25 ± 0.72	6.28 ± 0.81	6.15 ± 1.03
Ferritin (ug/l)	47.7 ± 20.9	48.3 ± 31.5	55.7 ± 25.9	51.3 ± 49.2	62.9 ± 79.7	62.6 ± 67.2

$p \leq 0.05$; ## $p < 0.01$; $p < 0.001$ significant difference WR vs. C

[1] n=5 in WR

Eight out of thirteen subjects in the present study had iron deficiency at some degree. Below 12 μg/l ferritin levels was observed in three subjects, below 20 μg/l in one and below 35 μg/l in four subjects (figure 9).

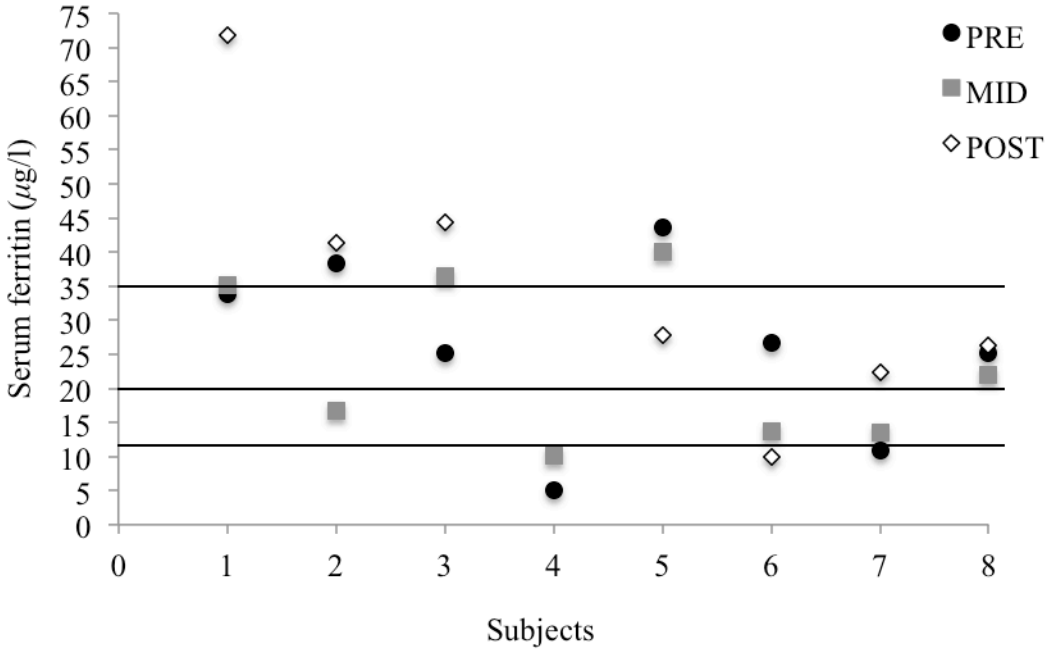

FIGURE 9. Serum ferritin concentrations in subjects' who were classified to have an iron depletion at least in one measurement point. Black lines represent the degrees of iron deficiency.

8.6 Physical performance

Physical performance variables can be seen in table 11. In the WR group, only two subjects were able to perform post measurements and thus statistical analysis could not be done. There were no significant differences between the pre and mid measurements between the groups. However, there was a trend for a better reactive jump result in the mid measurements in C (p=0.056) compared to WR. No significant changes in physical performance were observed during weight reduction in WR. In C, reactive jump result improved by 8.0 ± 3.2 w (p=0.015) between the pre and mid measurements and decreased by 10.4 ± 4.0 w (p=0.023) between the mid and post measurements.

TABLE 11. Physical performance variables in the pre, mid and post measurements. SJ, squat jump; CMJ, counter movement jump; SJ20, squat jump with 20 kg extra weight; F20, 20 m sprint with flying start; S30, 30 m sprint with standing start; RJ, reactive jump.

Variable	WR			C			
	Pre	Mid	n	Pre	Mid	Post	n
SJ (cm)	42.8 ± 4.1	44.3 ± 3.7	6	40.8 ± 3.7	40.9 ± 4.2	38.5 ± 4.1	6
CMJ (cm)	43.8 ± 4.4	44.3 ± 3.9	6	42.4 ± 3.5	41.8 ± 4.3	41.1 ± 4.8	6
SJ20 (cm)	29.9 ± 2.6	30.9 ± 3.1	5	28.6 ± 2.6	29.4 ± 4.5	27.5 ± 3.0	6
F20 (s)	2.45 ± 0.08	2.43 ± 0.12	5	2.45 ± 0.07	2.44 ± 0.08	2.48 ± 0.08	5
S30 (s)	4.35 ± 0.15	4.31 ± 0.14	5	4.40 ± 0.11	4.33 ± 0.10	4.43 ± 0.09	5
Preact (w)	52.2 ± 4.2	53.5 ± 5.2	4	53.6 ± 3.1	61.5 ± 5.2*	51.2 ± 8.4§	5

* $p<0.05$ significant difference pre vs. mid

§ $p<0.05$ significant difference mid vs. post

8.7 Associations between measured variables in all subjects

Relationships between measured variables in all subjects are presented as Pearson's correlations for normally distributed and as Spearman's correlations for not normally distributed variables. The correlations consider either all subjects from the weight reduction and control groups (WR+C) (to investigate relationships between variables among track and field jumpers in general), or the subjects from the weight reduction group (WR) alone (to investigate relationships between variables caused by weight reduction).

8.7.1 Menstrual status, body composition and hormones

When considering relationships in relation to the length of a menstrual cycle, correlations were found between the cycle length and body composition and hormones. The menstrual cycle length was determined as an average value of cycle length during the whole study. Those with lower body fat in the mid measurement had longer cycle ($r=-0.565$, $p=0.044$). The pre-mid change in BMC was also more negative in those with longer cycle length ($r=-0.574$, $p=0.04$). Significant correlations between the cycle length and the absolute hormone values are shown in table 12. Regarding the cycle length and the hormonal changes, those with the longer cycle length had more decrease in the IGF-1 concentration between the pre and mid measurements (r=-0.554, p=0.05).

TABLE 12. Correlations between menstrual cycle length and hormones. IGF-1, insulin-like growth factor-1; T_3, triiodothyronine.

	Menstrual cycle length	n
Testosterone pre	-0.763**	13
Testosterone mid	-0.667*	13
T_3 pre	-0.616*	13
T_3 mid	-0.536[1]	13
IGF-1 mid	-0.602*	12
IGF-1 post	-0.710*	13
Insulin post	-0.567[2]	13

* p<0.05; ** p<0.01, [1] p=0.059; [2] p=0.055

8.7.2 Body composition vs. nutritional intake

The body mass change correlated with the fat mass change both in the pre vs. the mid and the mid vs. the post measurements (r=0.781, p=0.02; r=0.823, p=0.01, respectively). The lean mass change showed no significant correlations with the nutritional intake changes at any measurement point. Significant correlations were observed in body mass, fat percentage and fat mass changes between pre and mid measurements, which are presented in table 13. No significant correlations in the above mentioned body composition and nutritional intake changes were found in the mid-post and pre-post measurements.

TABLE 13. Significant correlations between absolute body composition and nutritional intake changes (pre-mid). EI, energy intake; EA, energy availability; EB, energy balance; BM, body mass; FM, fat mass.

	ΔEI	ΔEA	ΔEB	Δfat	Δfat/kg
ΔBM	0.608*	0.562*		0.726**	0.694**
Δfat%		0.554*		0.601*	0.584*
ΔFM	0.595*	0.602*	0.623*	0.697**	0.675*

* p<0.05; ** p<0.01

The higher the energy intake was in the pre measurements, the bigger was the change (pre-mid) in body mass (r=-0.682, p=0.01) (figure 10), fat percentage (r=-0.609, p=0.027) and fat mass (r=-0.694, p=0.009), respectively. The same was also observed between energy availability in the pre measurements and the pre-mid body composition changes (r=-0.606, p=0.028; r=-0.583, p=0.037 and r=-0.610, p=0.027 for body mass, fat percentage and fat mass changes, respectively). The better initial energy balance led to the bigger pre-mid change in fat mass (r=0.559, p=0.047).

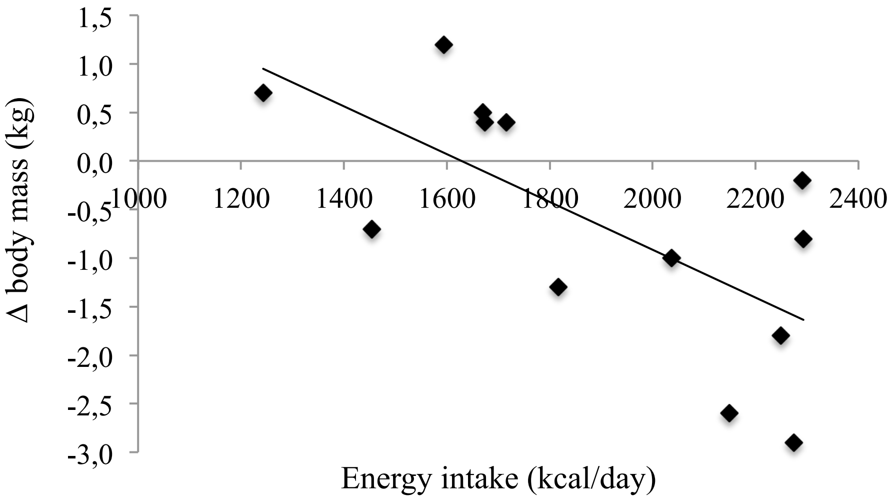

FIGURE 10. Correlation between initial energy intake (x-axis) and body mass change (pre-mid) (y-axis). r=-0.682; p=0.01.

The higher absolute and in relation to body mass expressed fat intake in the pre measurements also led bigger pre-mid changes in body mass, fat percentage and fat mass. The correlation coefficients were for absolute fat intake -0.786 (p=0.001); -0.710 (p=0.007); -0.822 (p=0.001) and the relation to body mass expressed fat intake -0.748 (p=0.003); -0.609 (p=0.027); r=-0.723 (p=0.005) for body mass, fat percentage and fat mass changes, respectively. The higher the protein intake was in the mid measurements, the bigger was the pre-mid change in the fat percentage (r=-0.568, p=0.043).

There was a significant correlation between the percentage changes in BMC and in energy intake in the mid-post measurement (r=0.581, p=0.048). The percentage mid-post change in BMC also correlated with the percentage change in the absolute and in the relation to body mass expressed fat intakes (r=0.645, p=0.024 and 0.713, p=0.009, respectively). No other significant correlations were observed with BMC or BMD and the nutritional intake variables in any measurement point.

8.7.3 Body composition vs. performance

The mid lean mass correlated negatively with percentage change in the standing 30 m sprint between the pre and mid measurements (r=-0.608, p=0.047). Fat mass correlated with the flying 20 m time in the post measurements (r=0.896, p=0.006) but not in other measurement points. The mid-post change in body mass correlated with the post flying 20 m times (r=0.829, p=0.021). The mid-post change in lean mass correlated with the post squat jump with the 20 kg extra weight (r=0.812, p=0.014).

8.7.4 Body composition vs. hormones

Significant correlations between the absolute values in body composition and hormones are presented in table 14. Significant correlations during the weight reduction period (pre-mid) were observed between the fat mass change and the testosterone concentration in the mid (r=0.573, p=0.041) and between the fat percentage change and T_3 in the mid (r=0.555, p=0.049). Significant correlations during the summer competition season (mid-post) can be seen in table 15. Testosterone in post correlated with the pre-post change in the fat percentage as well as in the fat mass (r=0.613 p=0.034 and 0.663 p=0.019), respectively.

TABLE 14. Significant correlations between absolute body composition and hormone values in the three measurement points. Test, testosterone; Cor, cortisol; E_2, estradiol; T_3, triiodothyronine; BMD, bone mineral density; BMC, bone mineral content.

	Test mid	Cor mid	Cor post	E_2 post	T_3 mid
BMD post			-0.810***		
Fat% mid	0.693**				0.561*
FM post				0.596*	
Lean post			-0.712**		
BMC mid		-0.625*			
BMC post			-0.647*		

* p<0.05; ** p<0.01; ***p<0.001

TABLE 15. Significant correlations between the changes in body composition and hormones (mid-post). Test, testosterone; E_2, estradiol; BMD, bone mineral density; FM, fat mass; BMC, bone mineral content.

	Test post	Δ E_2	%Δ E_2
ΔBody mass		0.641*	
Δfat%		0.594*	
ΔFM		0.682*	
%ΔBMC			0.660*
ΔBMD	0.647*		

* p<0.05

8.7.5 Nutritional intake vs. hormones

Decrease in energy and body mass expressed fat intake between the pre and mid measurements led to decrease in mid E_2 levels, (r=0.696, p=0.008; r=0.670, p=0.012, respectively). Pre-mid change in E_2 correlated with energy (r=0.594, p=0.032) and carbohydrate intake (r=0.560, p=0.047) changes.

Decrease in energy expenditure between the mid and post measurements led to rise in post T_3 concentrations (r=-0.593, p=0.042). Insulin post concentration correlated with mid-post change in energy intake (r=0.634, p=0.027), absolute and body mass expressed carbohydrate intake (r=0.730, p=0.007; 0.720, p=0.008, respectively). Correlations between E_2 and nutritional intake changes are presented in table 16.

TABLE 16. Correlations between mid-post nutritional intake and hormonal changes. E_2, estradiol.

	Δ E_2
Δ Energy intake	0.630*
Δ Energy balance	0.581*
Δ Carbohydrate	0.608*
Δ Carbohydrate/kg	0.598*
Δ Fat	0.612*

* p<0.05

8.8 Associations between measured variables in weight reduction group

8.8.1 Menstrual status, body composition, nutritional intake and hormones

When considering the correlations in relation to the length of the menstrual cycle, correlations were found between the cycle length and the body composition and the hormones. Those with the lower T_3 pre concentrations had longer cycle length (r=-0.813, p=0.026). The menstrual cycle length also correlated with the free testosterone concentration in mid (r=-0.777, p=0.04). Regarding to changes, it seemed that the free testosterone concentration decreased slightly more during weight reduction in those with longer cycle length (r=-0.693, p=0.084). Moreover, there was a trend that those with the longer cycle also gained more fat after weight loss during the summer competition season in comparison to subjects with shorter cycle (r=0.733, p=0.071). The correlations between the menstrual cycle length and the body composition and the hormonal changes throughout the study (pre-post) can be seen in table 17.

TABLE 17. The relationships between menstrual cycle length and body composition and hormonal changes (pre-post).

	Δ Body mass (kg)	Δ Fat mass (kg)	Δ Insulin (mU/l)
Menstrual cycle length (days)	0.796[1]	0.779[2]	-0.839*

* $p<0.05$

[1] p=0.058, [2] p=0.068

8.8.2 Body composition, body composition and nutritional intake

The only significant correlation between body composition variables during the weight reduction period (pre-mid) was found between the percentage changes in fat percentage and lean mass (r=-0.839, p=0.018). Considering the correlations between the body composition and the nutritional intake changes during weight reduction, the change in BMD correlated with the protein intake change (r=0.769, p=0.043) and the fat mass change correlated with the fat intake change (r=0.784, p=0.037) (figure 11).

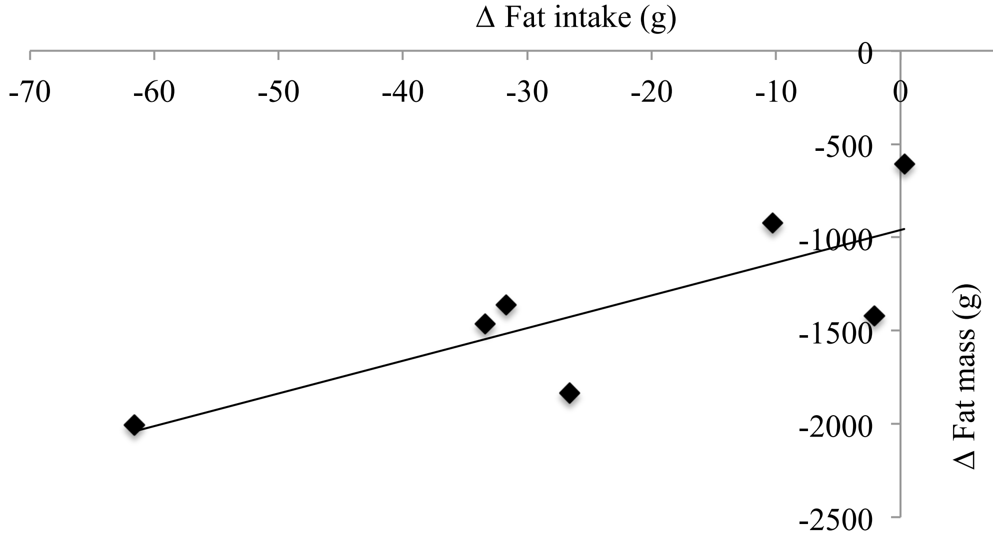

FIGURE 11. The relationship between fat intake and body fat mass changes (n=7). r=0.784; p=0.037.

Individuals with higher initial fat content reduced their fat intake more than those with lower initial values, (r=-0.839, p=0.018 and r=-0.821 p=0.024, respectively for initial the fat percentage and the fat mass). Fat percentage after weight reduction also correlated with the change in resting metabolic rate (RMR) (r=-0.827, p=0.022). Moreover, there was a significant correlation between the mid BMC and the pre-mid change in energy balance (r=0.838, p=0.019).

Considering the summer's competition season (mid-post), significant correlations were found between lean mass change and BMD in the post (r=0.982, p=0.0005). Also between the change in BMC and energy intake in the post (r=0.859, p=0.028) and the absolute and the body mass expressed carbohydrate intake in the post (r=0.950, p=0.004; r=0.982, p=0.0005, respectively).

8.8.3 Body composition vs. performance

Since only two subjects were able to perform the physical performance tests in the post measurements, statistical analysis could only be executed for the pre and mid measurements. The lower mid fat percentage led to the faster 30 m sprint time with the standing start position (r=0.902, p=0.036). The percentage changes in the lean mass and the 30 m sprint time with the standing start also correlated significantly (r=-0.916, p=0.029). Furthermore, there was a trend for improved squat jump result with the maintained or increased lean mass (r=0.798, p=0.057).

8.8.4 Body composition vs. hormones

Considering the weight reduction period, when body mass reduced the cortisol levels increased (r=-0.845, p=0.034) (figure 12). Those who had bigger negative change in the testosterone concentration had the lowest body mass after the weight reduction period (r=0.900, p=0.006). BMD change correlated with E_2 change (r=0.789, p=0.035). The percentage changes in the fat percentage and the T_3 also correlated, but this did not reach statistical significance (r=0.683, p=0.091).

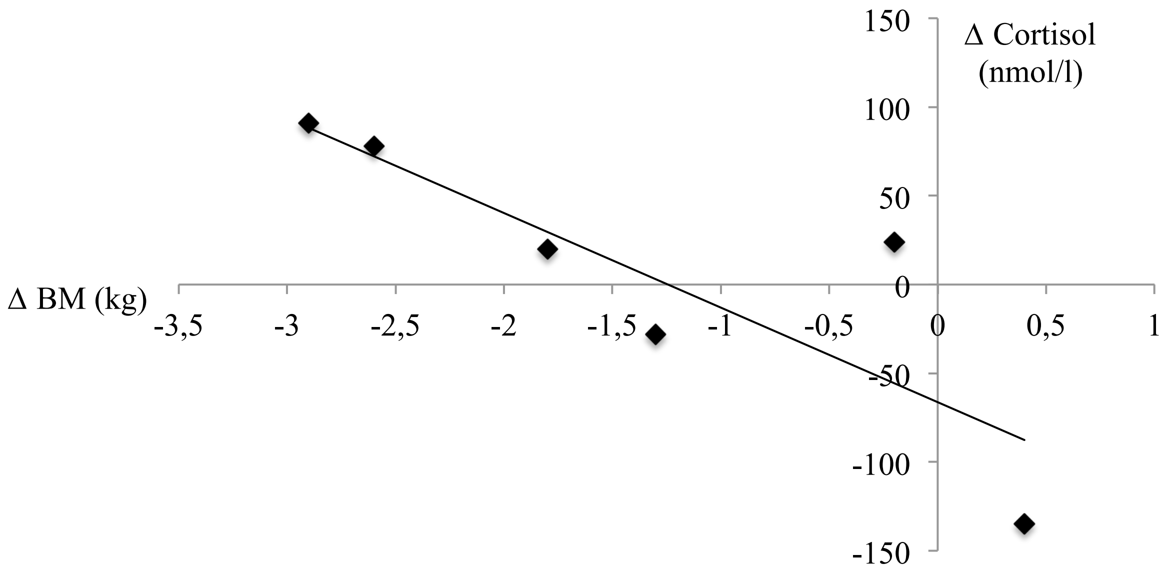

FIGURE 12. Correlation between body mass (BM) and cortisol changes during weight reduction (n=6). r=-0.845; p=0.034.

Regarding to the summer competition season (mid-post measurements), the change in body mass correlated with the testosterone level in the post (r=0.855, p=0.03), and there was also a trend for correlation with the cortisol in the post (r=-0.787, p=0.063). The percentage change in E_2 correlated with the fat percentage in the post (r=0.818, p=0.047) as well as with the fat mass in the post (r=0.820, p=0.046). The testosterone concentration in the post correlated both with the change in fat percentage (r=0.952, p=0.003) and the change in the fat mass (r=0.962, p=0.002). Reduction in the lean mass led to higher post measurement cortisol levels but this was outside of the limit of statistical significance (r=-0.759, p=0.08). The significant correlations between the changes are presented in table 18.

TABLE 18. Correlations with body composition and hormonal changes during the summer's competition season. E_2, estradiol; BMD, bone mineral density; BMC, bone mineral content.

	Δ Testosterone	%Δ Testosterone	Δ E_2
Δ Body mass	0.758[1]		
%Δ Fat%		0.828*	
Δ Fat mass	0.797[2]		
Δ BMC			0.732[3]
Δ BMD			0.901*

* p<0.05

[1] p=0.081; [2] p=0.057; [3] p=0.098

Regarding to the pre-post changes, the significant correlations can be seen in table 19. T_3 post concentration also correlated with both the fat percentage and the fat mass in the post, (r=0.843, p=0.035 and r=0.828, p=0.042, respectively).

TABLE 19. Significant correlations between body composition and hormonal changes (pre-post). E_2, estradiol; BMD, bone mineral density; BMC, bone mineral content.

	Δ Testosterone	Δ E_2	Δ Insulin
Δ Body mass	0.813*		
Δ BMD		0.881*	
Δ Fat%	0.933**		
Δ Fat mass	0.991***		
Δ BMC			0.886*

* p<0.05; p<0.01; *** p<0.001

8.8.5 Nutritional intake vs. hormones

The higher initial energy intake led to a bigger increase in the cortisol concentration between the pre and mid measurements (r=0.848, p=0.033). The same was also observed between the initial energy availability and the change in cortisol (r=0.819 p=0.046). The significant correlations considering the summer competition season are shown in table 20 and regarding the pre-post changes are in table 21.

TABLE 20. Significant correlations between nutritional intake and hormones (mid-post). E_2, estradiol; T_3, triiodothyronine.

	Insulin post	E_2 post	Δ T_3
Δ Energy intake	0.982***		
Δ Energy availability	0.930**		
Δ Energy balance	0.827*	0.836*	
Energy expenditure post			-0.849*
Δ Carbohydrate	0.928**		
Δ Carbohydrate/kg	0.928**		
Δ Fat	0.977***		
Δ Fat/kg	0.969***		

* p<0.05; p<0.01; *** p≤0.001

TABLE 21. Significant correlations between nutritional intake and hormones (pre-post). EI, energy intake; EA, energy availability; EB, energy balance; E_2, estradiol; T_3, triiodothyronine.

	Δ EI	Δ EA	Δ EB	Δ Carbohydrate	Δ Carbohydrate/kg
Δ E_2	0.841*	0.850*		0.928**	0.915*
Δ T_3			0.892*	0.871*	0.867*

* p<0.05; **p<0.01

9 DISCUSSION

In this study, subjects' energy deficit in the WR group was about -477 kcal/day during the weight reduction period, which produced a weight loss of 1.3 kg. Subjects' fat mass reduced significantly without changes in lean mass. The energy restriction was mainly implemented by decreasing carbohydrate intake, but fat intake was also reduced. Considering subjects' menstrual status, there was a significant difference (13 days) in the menstrual cycle length between WR and C. There were no changes in hormonal concentration during the study, but several noteworthy associations between hormones and nutrition and body composition variables were found. Decreased body weight related with reduced testosterone and increased cortisol concentration. The effect of low body fat on E_2 concentration and the association between E_2 and bone formation was also detected. Compromised iron status was also noticed since eight out of thirteen subjects had iron deficiency at some degree at some measurement point. All performance results changed positively in the light of better physical performance and beneficial effects of weight reduction in the shape of losing fat mass and sparing lean mass (improving power-to-weight ratio) were also noticed in this study. Noteworthy was also that many subjects suffered from bone stress reactions.

9.1 Menstrual status

Subjects' menstrual cycle was followed throughout the study. As regards subjects' menstrual status, five out of seven subjects' in WR and only one in C had prolonged menstrual cycle, and they were classified as oligomenorrheic (cycle length > 35 days). The remainders were eumenorrheic (normal menstrual cycle 28 ± 7 days). There was a significant difference between the groups in menstrual cycle length; the subjects in WR had approximately 13 days longer cycle than the subjects in C.

In WR, menstrual cycle length correlated positively with both pre-post changes in body mass and fat mass. This means that those with shorter cycle were able to reduce and/or maintain their weight and fat mass better than those with longer cycle. Moreover, there was a trend that those with longer cycle also gained more fat after weight loss during summer's competition season in comparison to subjects with shorter cycle. These findings support the importance of maintaining normal menstrual cycle during weight management.

When considering correlations in both groups (WR and C) combined, those with lower body fat in the mid measurement had longer cycle, which illustrates how body fat content affects menstrual cycle. Also pre-mid change in BMC was also more negative in those ones with longer cycle length. The relationship between menstrual cycle and bone status has also observed in the literature. Estrogen deficiency reduces the rate of bone formation (Bennel et al. 1999). The more missed menstrual cycles, the more it declines (Drinkwater et al. 1990). The subjects' in WR had the longest cycles during the pre-mid measurements (i.e. during weight loss period), which supports the reported findings (De Souza & Williams 2005; Nattiv et al. 2007). Menstrual cycle phases were not followed in this study but Loucks & Thuma (2003) conducted that women with slightly shorter luteal phase (11 vs. 12–14 days) may be of an increased risk of developing menstrual disturbances when exposed to energy deficiency.

Considering hormone variables, T_3 levels have been found to decrease in female athletes with menstrual irregularities and in amenorrhea (Melin et al. 2015; Logue et al. 2018). Also low insulin and IGF-1 levels have been associated with prolonged menstrual cycle and amenorrhea (Ihle & Loucks 2004; Logue et al. 2018). This was also observed in this study. In WR, those with longer menstrual cycle had lower T_3 levels in the pre measurements. Those with shorter cycle increased their insulin concentration during the whole study. Also it seemed that free testosterone decreased more during weight reduction in those with longer cycle length. Similar was observed also in whole group level, since those with longer cycle had lower testosterone pre/mid, T_3 pre/mid, IGF-1 mid/post and insulin post levels and also more reduced IGF-1 levels between the pre and mid measurements.

In order to summarize and conclude, there was a significant difference (13 days) in the menstrual cycle length between WR and C. The menstrual disturbances were more prevalent among the WR group, since five out of seven subjects were oligomenorrheic, whereas only one subject was oligomenorrheic in the C group. The declines in the concentrations of anabolic hormones were also associated with the longer cycle length as well as the unfavourable body composition changes in the light of weight management. This finding verifies that weight reduction affects menstrual cycle and that has to be taken into account when planning weight loss regimen in female athletes.

9.2 Energy intake and expenditure

As was planned for the WR group, energy intake decreased statistically significantly between the pre and mid measurements (21 $\pm$ 13 %). Between the mid and post measurement, there was a trend for increased energy intake but that did not reach statistical significance. Energy intake was similar in the pre and post measurements. Also energy expenditure remained similar in all the measurements points, even though there was a trend for decreased energy expenditure during weight reduction (pre-mid) and between the pre and post measurements. Energy expenditure was similar in both groups in all measurements points. Basic training season was underway during the pre measurements compared to the mid and post measurements when competition season was beginning or underway and thus explain the differences in energy expenditure.

In the control group energy intake remained similar throughout the study. However, they were in a significant energy deficit throughout the study. Energy intake was significantly higher in WR compared to C in the pre (31 %) and post (24 %) measurements. After the first measurements, the subjects in the group C were instructed to increase their energy intake since the objective was not to reduce weight and in the light of optimal training adaptations the energy intake was too low. However, most of the subjects did not increase their energy intake possibly because of the fear of gaining weight. Nevertheless, since subjects' menstrual and hormonal statuses were normal, the reason behind low energy intake may be underreporting and undereating during the periods of keeping food diaries, which is common among athletes (Magkos & Yannakoulia 2003).

As regard energy availability, it was under optimal (45 kcal/kgFFM/day) in the light of good training adaptations, athletic development and health in both groups throughout the study (28–37 kcal/kgFFM/day in WR and 27–30 kcal/kgFFM/day in C). With EA of 30–45, it is possible to reduce weight without negative consequences on health or performance (Manore et al. 2007; Ilander et al. 2014, 22–27; Logue et al. 2018). When EA reduces below 30 kcal/kgFFM/day detrimental consequences appears: the body suppresses reproductive function (Loucks & Thuma 2003) and bone formation (Ihle & Loucks 2004). However, it has to be taken into account that there are great inaccuracies when assessing EA, involving energy intake, exercise energy expenditure and fat free mass assessments. The recommended EA thresholds originated from experiments that determined the effects of EA and exercise stress on luteinizing hormone (LH) pulsatility and markers of bone turnover in a laboratory (Loucks & Thuma 2003; Ihle & Loucks 2004). However, in the light of health and physical performance, optimal EA thresholds have not been able to define in female athletes outside the laboratory (Koehler et al. 2013; Melin et al. 2015; Heikura et al. 2018) and that is why the need for further studies is essential.

According to previous studies, about 550 kcal daily energy deficit together with about 0.5 kg (0.7 % of body mass) weekly weight loss rate produces best results in terms of body composition (Garthe et al. 2011a), explosive performance (Garthe et al. 2011a) and hormonal balance (Mero et al. 2010). Higher energy deficit (about 1000 kcal) and/or lower protein intake (0.6–1.4 g/kg/day) have harmful effects on body composition (Gornall & Villani 1996; Zachwieja et al. 2001; Layman et al. 2003; Alemany et al. 2008), physical performance (Filaire et al. 2001; Umeda et al. 2004) and hormonal balance (Alemany et al. 2008; Mero et al. 2010). In this study, the energy deficit was about -477 ± 218 kcal/day during the weight reduction in the WR group, which is slightly lower than the energy deficit mentioned in the literature. Energy deficits in the pre and post measurements were -140 ± 242 kcal/day and -30 ± 176 kcal/day, respectively, and thus the subjects can be considered to be in energy balance.

Subjects' energy intakes were assessed with food diaries, which have been shown to underestimate the real energy intake in athletes, possibly due to underreporting and intentional undereating (Magkos & Yannakoulia 2003). Also the lack of different nutrients in the nutrient-analysis software may have affected the results. Energy expenditure was evaluated with activity diaries and training diaries. However, there are great inaccuracies when assessing the energy expenditure involving MET-values, mathematical formulas and subjects' reporting. Moreover, a five-day follow-up may not give a proper view of subject's regular dietary and training habits. Also individual differences have an effect on true energy expenditure. Thus the results of energy intake and expenditure can be considered more approximate.

In order to summarize and conclude, subjects' energy intake decreased as planned between the pre and mid measurements in WR. The energy deficit was about -477 ± 218 kcal/day during the weight reduction, which is slightly lower than the energy deficit in previous studies. In the control group energy, intake remained similar throughout the study, but they were in a significant energy deficit. Subjects' energy availabilities were under optimal in both of the groups. However, it has to be taken into account that there are great inaccuracies when assessing EA

9.2.1 Macronutrients

As can be observed from the results, energy deficit in the WR group was implemented by reducing total energy intake, mainly reducing carbohydrates and fat intake. As stated by a previous study (Phillips 2012), a low carbohydrates diet results in increased fat mass loss and lean mass preservation, which leads to a higher lean to fat mass -ratio and can translate into a competitive advantage. This was also supported by the present study, since subjects' fat mass reduced significantly without changes in lean mass.

Protein intake. Subjects' were instructed to keep protein intake high during weight reduction, since a high-protein diet (25–30 E%, 1.6 g/kg/day) during a weight reduction period has been found to promote fat mass loss and prevent from lean mass losses (Mero ym. 2010; Mettler ym. 2010; Mäestu ym. 2010; Huovinen ym. 2015). In this study, subjects' protein intake remained high throughout the study (1.9–2.0 g/kg/day). The proportion of protein from total energy intake was 28 ± 8 % during weight reduction. According to previous studies (Mero ym. 2010; Mettler ym. 2010; Mäestu ym. 2010; Huovinen ym. 2015), both in relation to body mass expressed and proportional protein intake in this study were sufficient to cause out positive changes during weight loss. Compared to the control group, protein intake was significantly higher in the pre and post measurements in WR.

Carbohydrates intake. As stated by previous studies, a low carbohydrate diet results in increased fat mass loss and lean mass preservation, which leads to a higher lean to fat mass -ratio and can translate into a competitive advantage (Phillips 2012). In this study, both absolute and in relation to body mass expressed carbohydrate intake reduced statistically significantly (22 ± 13 % and 20 ± 13 %, respectively for absolute and in relation to body mass expressed carbohydrate intake) during the weight reduction period in the WR group. The proportional carbohydrate intake remained similar throughout the study. In weight loss studies in which there have been positive changes considering body composition, the carbohydrates intake has been 3.0–3.7 g/kg/day (40–54 E%) on average. In this study, similar values were 2.8 ± 0.8 g/kg/day and 43 ± 7 E%. According to Zachwieja et al. (2001), carbohydrates intake should stay above 3.0 g/kg/day during weight loss in order to maintain physical performance. However, it has been suggested that female's need for carbohydrates is a little less compared to males (Slater & Phillips 2011), since females utilize more fat and less carbohydrates as a fuel at the same relative exercise intensity as males (Broad & Cox 2008.) In a study by Mero et al. (2010) with healthy normal weighted young women, where carbohydrate intake was 2.5 g/kg/day (156 g) in low (-550 kcal/day) energy deficit group, no negative effects on body composition, physical performance or hormonal balance were observed. Therefore, subjects' carbohydrates intake in this study was not probably too low. Considering summer's competition season, subjects' carbohydrate intake increased but the increase did not reach statistical significance. Carbohydrate intake was similar in the pre and post measurements. In comparison with the C group absolute carbohydrate intake was significantly higher in the pre measurements. In other measurements points carbohydrate intake was similar.

Fat intake. Fat has a high energy density. One gram fat contains energy about 9 kcal, whereas similar value for protein and carbohydrates is about 4 kcal. (Ilander et al. 2014, 234.) Therefore, it is an effective manner to reduce energy intake. Ilander et al. (2014, 237–238) states that as regards to power-event athletes, energy restriction is recommended to carry out mainly by reducing fat intake. Thus, carbohydrates can be eaten enough since they are important for athlete's training capacity, performance and recovery. Absolute and in relation to body mass expressed fat intake decreased during weight reduction by 29 $\pm$ 24 % and 27 $\pm$ 23 %, respectively for absolute and in relation to body mass expressed fat intake in WR. However, this did not reach statistical significance. Recommended fat intake during weight loss is 0.8–1.3 g/kg/day (Munteanu et al. 2014). In this study, fat intake was 0.8 $\pm$ 0.2 g/kg/day, which is the lowest end of the recommendation. According to the literature, it is important to ensure that dietary fat intake is not lower than 20 E% in order to avoid the negative consequences of too low intake (Broad & Cox 2008; Ilander et al. 2014, 146–147). In this study, the value was 29 E%. In the light of above mentioned, subjects' fat intake can be considered sufficient during weight reduction period. Considering the summer competition season subjects' fat intake increased significantly back to the pre measurement level. Compared to the control group, fat intake was significantly higher in the pre and post measurements and percentage fat intake significantly lower in the mid measurements in WR.

Micronutrients. Considering the subjects' intakes of micronutrients, deficiencies compared to recommendations were most common in vitamin D, folate and iron. It has also been observed that those micronutrients often require supplementation in athletes (Biesalski & Tinz 2018; Maughan et al. 2018). Many athletes are at risk of insufficiency of vitamin D. It is essential for bone health, immune function and inflammatory modulation, and it is also necessary for optimal muscle function and performance. Assessing vitamin D status by measuring serum 25(OH)D concentration may be useful for athletes. (Larson Meyer & Willis 2010.) Folate is particularly important in pregnancy for prevention neural tube defects. It plays an essential role in DNA synthesis, repair and methylation. (Liu et al. 2016.) Iron deficiency is the most common nutritional deficiency in athletes and, especially, among female athletes who emphasize low body mass and leanness (Ilander et al. 2014, 363–370). However, a significant number of days of diet records (3–44 days) are required to obtain a true representation of the athlete's intake of micronutrients (Basiotis et al. 1987), and thus reliable conclusion considering micronutrients cannot be made in this study.

In order to summarize and conclude, in the WR group, energy intake was significantly decreased from baseline by 477 kcal/day, and this decrease was caused mainly by significant decrease in carbohydrate intake, but fat intake also reduced. Protein intake remained the same throughout the study. During the weight reduction the diet consisted of 1.9 g/kg/day protein, 2.8 g/kg/day carbohydrates and 0.8 g/kg/day fat. Compared to recommendations for athletes, protein and fat intakes were sufficient, but carbohydrate intake low during the weight reduction. After the weight reduction period, carbohydrate and fat intake increased back to the pre-measurement level.

9.3 Body composition

The reproducibility of Lunar Prodigy DXA device was evaluated in Garthe's et al. (2011a) study where ten athletes did two repeated measurements within 24 hours. The coefficient of variation (CV%) was 3 % for fat mass and 0.7 % for fat-free mass. Thus, DXA is considered a suitable method to measure short-term changes in body composition in athletes. Based on the hemoglobin and hematocrit values, it can be noted that the subjects were not dehydrated and thus the change in lean mass cannot be attributed to that.

In the WR group, subjects' weight was reduced by 2 % (1.3 kg) during the weight reduction period but that was not statistically significant. Fat percentage and fat mass reduced significantly, percentage decreases being 13 $\pm$ 3 % and 15 $\pm$ 4 %, respectively for fat percentage and fat mass. Lean body mass, bone mineral content and density remained unchanged during weight reduction. When comparing to the control group, body weight, fat percentage and fat mass reduced significantly. In the control group, no significant changes were observed in body composition throughout the study.

In WR, there was a great individual variability in the amount of reduced weight (from -2.9 to +0.4 kg). One subject did not participate to the post measurements and, therefore, her results could not be use to statistical analysis with ANOVA. If analysing only the pre and mid results with T test the change in weight was statistically significant ($p=0.029$). One subject's weight increased but that was due to an increase in muscle mass. The weight reduction period lasted about four weeks. Energy deficit produced a weight loss rate of about 0.3 kg/week. The weight reduction rate was slightly slower than the optimal rate (-550 kcal/day, -0.5 kg/week) (Mero et al. 2010; Garthe et al. 2011a). However, since subjects' fat mass reduced significantly without changes in lean body mass, weight reduction succeeded. There was also a negative correlation between percentage changes in fat percentage and lean mass. Thus the more the lean mass increased, the more the fat percentage decreased.

Body fat. Studies have suggested that the minimum body fat for avoiding health issues would be within the range of 12–14 % in females (e.g. Meyer et al. 2013). In this study, one subject had initial fat percentage of 12.9 %, which reduced to 10.2 % and maintained being 10.9 % in the post measurement. Two other subjects also reduced their fat percentages to 13.2 % and 13.7 % and were able to sustain similar values to the post measurements. Subjects' fat percentages in both of the groups were within or over the minimum estimated body fat with the exception of that one subject described above. However, there are individual differences among athletes so a fat percentage that causes negative consequences on health and performance in one athlete may be appropriate for another (Meyer et al. 2013). Thus, if health problems (e.g. menstrual irregularities) will not arise, the fat percentage may not be too low.

Previous studies have observed that the initial fat percentage seems to dictate the proportion of fat and protein catabolism during energy deficit (e.g. Forbes 2000) so the individuals with higher initial fat percentage lose less protein and more fat in relation to lost weight during energy deficit. However, the same was not observed in this study. Nevertheless, better initial energy balance (the difference between energy expenditure and intake) led to bigger reduce in fat mass. An association was also found between mid fat percentage and resting metabolic rate. The amount of lean body mass affects considerably to RMR (Taguchi et al. 2011), so the increase in lean mass elevates RMR and, therefore, body uses more energy from body's fat deposits.

Lean body mass. Even though there was no change in lean body mass during weight reduction, the variability of change was from -1.9 to +1.4 kg. In Garthe's et al. (2011a) study, there were also increases in lean body mass during weight reduction period. Reasons behind the increase in lean body mass can be considered. There were about six to eight weeks between the pre and mid measurements. Most of the subjects did much strength training at that time which may have affected lean body mass gain together with lower energy deficit or, at least, be a reason behind lean mass maintaining. The reliability of the mid DXA measurement can also be considered. The measurement performed at the same time of the day in fasted state with the best practice protocol. Nevertheless, the DXA device was much used at that time in different studies with different settings, which may have affected the results. Also the muscle glycogen content may have had an effect, since it increases the fluid volume inside the cells (Osterberg et al. 2010) and, therefore, the lean body mass measured by DXA. In the desire of better results, subjects may have also restricted their eating before the first measurements.

When considering the summer's competition season no changes in body composition results were noticed in either of the groups. However, there was a trend for decreased lean body mass in the WR group. This can be explained mostly by lesser training and, especially, strength training during the competition season.

Bone status. According to previous studies, athletes participating in weight-bearing sport have approximately 10% higher BMD than non-athletes and athletes in high-impact sports have a higher BMD compared with medium- or low-impact sports (Torstveit & Sundgot-Borgen 2005). Track and field jumping events can be considered high-impact sport (Groothausen et al. (1997), according to Torstveit & Sundgot-Borgen 2005). According to the American College of Sports Medicine (ACSM) low BMD is defined as a Z-score that is less than -1.0 in female athletes in weight-bearing sports. In this study subjects' Z-score was 3.3 in WR and 3.0 in C, which are considerably higher than the value of low BMD.

Relationships between nutritional intake and body composition variables. In the WR group, fat mass change correlated with fat intake change, so the more the fat intake decreased the more was the fat mass reduced. The change in BMD correlated with protein intake change and there was a relationship between mid BMC and the change in energy balance. This would suggest that protein intake and energy balance have an effect on bone heath when reducing weight.

Regarding the summer competition season (mid-post), decrease in lean mass resulted in lower BMD post values. The change in BMC correlated with energy intake post as well as with absolute and body mass expressed carbohydrate intake. When considering all subjects together, there were a relationship between percentage change in BMC and percentage change in energy intake in the mid-post measurement. Percentage mid-post change in BMC also correlated with percentage change in absolute and in relation to body mass expressed fat intakes. Thus, it would seem that increase in the energy intake, especially, in the form of carbohydrates and fats, would lead to increase in bone mass. However, it has to be noted that the overall changes in BMD and BMC were minor. Considering bone mineral density, it has been noted to decrease with Lunar Prodigy DXA device when individual undergoes weight loss, without any real change in the skeleton (Tothill 2005).

When considering both of the groups, decrease in energy intake, especially in the form of fats, led to decreased body mass and fat mass. This confirms the recommended weight loss strategy in athletes stated by previous studies (Phillips et al. 2007; Phillips 2014; Murphy et al. 2015). Higher absolute and in relation to body mass expressed fat intake in the pre measurements led also to bigger pre-mid change in body mass, fat percentage and fat mass, which also verifies the above mentioned. The higher the protein intake was in the mid measurements, the bigger was the pre-mid change in fat percentage. This is also in line with previous studies since a high-protein diet (25–30 E%, 1.6 g/kg/day) during a weight reduction period would seem to promote fat mass loss (Garthe et al. 2011a; Helms 2014).

In order to summarize and conclude, in the WR group, subjects' weight was reduced by 2 % during the weight reduction period but that was not statistically significant. Fat percentage and fat mass reduced significantly, percentage decreases being 13 % and 15 %, respectively. Lean body mass, bone mineral content and density remained unchanged during weight reduction. The weight loss rate was about 0.3 kg/week, which was a little slower than the optimal (-550 kcal/day, -0.5 kg/week) (Mero et al. 2010; Garthe et al. 2011a). However, since subjects' fat mass reduced significantly without changes in lean body mass, weight reduction succeeded.

9.4 Hemoglobin, hematocrit, hormone and ferritin concentrations

No significant changes were observed in hemoglobin, hematocrit, hormone or ferritin concentrations. There were no differences between the groups in serum hormone concentrations in any measurement point, excluding pre and mid testosterone IGF-1 and T_3 concentrations, which were significantly higher in C compared to WR. Also mid estradiol concentration differed between groups.

9.4.1 Testosterone

Testosterone has been connected to body fat reduction and accumulation of lean tissue, as well as to bone status improvements (Isidori et al. 2005). According to previous studies, decrease in testosterone concentration would seem to correlate with reduced body mass (Roemmich & Sinning 1997; Mero et al. 2010) and this was also true in this study. In the WR group, those who had bigger negative change in testosterone concentration had the lowest body mass after weight reduction period. Regarding summer's competition season (mid-post measurements), there was a trend for decreased testosterone concentration with reduced body mass.

The connection between testosterone and body fat was also observed in this study. Considering all subjects (WR+C), lower testosterone mid concentration was associated with lower fat percentage. There was also a correlation with testosterone mid and pre-mid change in fat mass. In the WR group, testosterone post correlated both with the mid-post change in fat percentage and the change in fat mass. There was also a trend for a correlation between the mid-post changes in testosterone and fat mass. Regarding lean body mass, decrease in testosterone is associated with reduced lean mass (Roemmich & Sinning 1997). However, such a connection was not observed in this study. The association between testosterone and bone status was observed in a whole group (WR+C) level, since increase in testosterone post concentration was associated with improved BMD (mid-post).

According to the previous studies (Alemany et al. 2008; Mero et al. 2010; Mettler et al. 2010; Mäestu et al. 2010), the amount of energy deficit would appear to have an effect on reducing testosterone concentration. In studies, where the energy deficit has been bigger, both total serum testosterone and free testosterone levels have reduced. The WR group's energy deficit was about -480 kcal/day, which is much lower than in studies where the testosterone concentration has been decreased (about -1000 kcal/day). Therefore, the energy deficit in the present study may not have been enough to produce adverse effects on testosterone. This finding is also in line with the findings in previous studies that a moderate energy deficit would appear to be less detrimental to testosterone levels (Mero et al. 2010; Huovinen et al. 2015). Testosterone concentrations were higher in the control group in every measurement point. It can be considered, was it due to natural reasons or did weight reduction have an effect? Could there be an association between testosterone and injuries, since testosterone is an anabolic hormone involved in several physiological functions. Nevertheless, it must be taken into account that women have very low testosterone levels compared to men and that the sensitivity of the assay may not be accurate enough and differences can be partly due to that.

In order to summarize and conclude, decrease in testosterone concentration correlated with reduced body weight and body fat, which is in line with the literature. According to the previous studies moderate energy deficit would appear to be less detrimental to testosterone levels and this was also true in this study since there were no correlations between energy status and testosterone levels.

9.4.2 Estradiol

In a study by Westerlind and Williams (2007) with normal weighted, sedentary premenopausal women no change in estrogen metabolism was observed during four months of moderate-intensity exercise coupled with energy restriction. Subjects' body mass reduced by 3.7 kg and fat percentage by 4.5 % during the intervention without changes in lean body mass. No change in estradiol concentration was observed in this study either. Nevertheless, the WR group has significantly lower E_2 concentration compared to group C in the mid measurements.

Decreased E_2 concentration led to lower BMD both during the weight reduction and during the summer competition season in WR. There was also a trend for lower BMC with reduced E_2 during the summer's competition season. This has also been observed in the literature since estrogen deficiency reduces the rate of bone formation (Klein-Nulend et al. 2015). Low body fat has been associated with reduced estradiol levels (Ziomkiewicz et al. 2008). This was also true in this study since in the combined whole group level (WR+C) mid-post changes in estradiol and body mass correlated as well as E_2 and fat percentage and fat mass. There was 3.6 % absolute difference in fat percentages between the groups in the mid measurements. It can be considered was the above-mentioned statistical difference between the groups in E_2 concentration in the mid measurements due to the lower body fat accomplished by the weight reduction or due to other reasons (discussed in the last paragraph).

Considering nutrition, lower dietary fat intake has been found to decrease estrogen concentration (Goldin et al. 1994; Wu et al. 1999). In low-fat diet, the supply of essential fatty acids can remain too low and thus interfere the hormone production through the eicosanoids and, therefore, induce the decrease of estrogen concentration (Ilander et al. 2014, 237). This was also observed in the present study considering both of the groups since reduced dietary fat intake (pre-mid) led to lower E_2 mid concentration, and there was also a relationship between the mid-post changes in estradiol and fat intake.

Considering both of the groups together relationships were found between pre-mid and mid-post changes in E_2 and energy and carbohydrate intake and also mid-post changes in E_2 and energy balance. In the WR group, post estradiol concentration correlated with the mid-post change in energy balance. Pre-post changes in E_2 and energy intake, energy availability and carbohydrate intake also correlated. Thus, it would seem that there are also other nutritional aspects, which have an effect on E_2 levels. Negative effects of low energy availability on hormonal function have been observed also in a study by Loucks & Thuma (2003). However, it has to be taken into account that there are great inaccuracies when assessing EA.

All in all, the results considering estradiol must be taken with caution since several factors affect the concentration. The secretion of estrogen by the ovaries depends on the current phase of the menstrual cycle (Guyton & Hall 2006, 1011–1015) (see plasma estrogen concentration during the normal female sexual cycle from figure 1). The use of hormonal contraception also affects the concentration. The menstrual cycle was followed in the present study but due to practical reasons (all subjects did not live in the same city) blood samples were not able to take at the same phase of the cycle throughout the study. Subjects were divided according to their menstrual phase but no differences in estradiol or other hormone levels were observed. However, there were very long cycle lengths especially in the WR group, which made the separation difficult.

9.4.3 Cortisol

No significant changes were observed in cortisol levels in either of the groups. According to the previous studies, gradual weight reduction seems not to have an influence on cortisol levels in men (Mettler et al. 2010; Huovinen et al. 2015) or in women (Mero et al. 2010; Lällä 2016). Nevertheless, even though no changes were observed in cortisol concentration in this study, decreased body mass led to increased cortisol levels during the weight loss period in the WR group. Thus, it would seem that weight reduction would have an influence on cortisol levels, which is opposite to the previous studies as regards gradual weight loss studies.

Nevertheless, it has to be taken into account that there can also be several other reasons behind the increased cortisol concentration. Since cortisol secretion is stress-induced the secretion increases with exercise intensity (Katch et al. 2011). Between the pre and mid measurements, the subjects were preparing for the competition season and, therefore, the training intensity was higher than in pre measurement, which might also explain the rise in cortisol levels. Other noteworthy finding was that higher initial energy intake led to bigger increase in cortisol concentration and the same was also observed between initial energy availability and the change in cortisol. There was a statistically significant decrease both in energy intake (21 %) and in energy availability (21 %) between the pre and mid measurements. In a study by Loucks & Thuma (2003), the effects of balanced (45 kcal/kgFFM/day) and restricted (10, 20, and 30 kcal/kg/FFM/day) energy availability treatments were studied in regularly menstruating, habitually sedentary, young women. It was noticed that when energy availability decreased the cortisol levels increased with progressively more extreme decrease with lower EAs. There was also a negative correlation between cortisol concentration and BMC in mid measurements as well in the post measurements when considering both of the groups. Cortisol is a catabolic hormone (Guyton & Hall 2006, 950–957), so the increase in cortisol could explain the decrease in BMC.

In the WR group, there was a trend for a negative correlation between cortisol post and mid-post body mass change. Since there was also a trend for a higher cortisol concentration with bigger mid-post lean mass change the decrease in body mass was due to lean mass reduce. As a catabolic hormone, cortisol decreases protein synthesis and increases muscle catabolism (Guyton & Hall 2006, 950–957) and thus might explain the lean mass reduction. However, lean mass reduction can also be explained by lesser training and specially strength training during the competition season. All in all, the effect of small sample size must be taken into account when considering the above-mentioned results and studies with bigger sample size must be conducted to verify these findings.

9.4.4 Insulin and insulin-like growth factor-1 (IGF-1)

No change in insulin concentration was observed during the study in either of the groups. In the WR group, rise in insulin concentration may have resulted in increased BMC between pre and post measurements, which relates the anabolic effect of insulin. There were several significant correlations between insulin post concentration and mid-post changes in energy and nutritional intake in WR. The more energy intake increased during summer's competition season the higher was the post insulin concentration and the rise in energy intake was in a form of fat and carbohydrates. When considering both of the groups together significant correlations between insulin post concentration and mid-post changes in energy and nutritional intake were also observed. The relationship between insulin and energy availability has also been observed in other studies (Loucks et al. 1998; Volek et al. 2002; Loucks & Thuma 2003; Koehler et al. 2016).

No change in IGF-1 concentration was observed either during the study. IGF-1 levels were significantly lower in WR compared to C in the mid measurements (34 %). This could be considered as an effect of weight reduction but since the same was observed also in the pre measurements, the difference is probably explained by natural differences. IGF-1 concentration has been noticed to decrease when the EA is low. Ihle & Loucks (2004) observed that most of the decline in IGF-1 concentration occurred between EAs of 20 and 30 kcal/kgFFM/day. No relationship between EA and IGF-1 was found in this study since the EA was near 30 kcal/kgFFM/day throughout the study.

9.4.5 Triiodothyronine

No changes were observed in triiodothyronine (T_3) concentration in this study in either of the groups. Control group had significantly higher T_3 levels in the pre and in mid measurements. Considering the WR group, it seemed that a decrease in fat percentage led to reduce in T_3 during the weight loss period. T_3 post correlated also strongly with fat percentage and fat mass in post. When considering all subjects together the more fat percentage reduced between the pre and mid measurements, the lower was the mid T_3 concentration. Thus it would seem that weight loss, especially, fat mass loss would reduce T_3 concentration. In Koehler's et al. (2013) study, reduced T_3 levels were also reported in athletes who lost weight.

It has been noted that the T_3 concentration reduces when the EA is low (Thong et al. 2000; Loucks & Thuma 2003; Ihle & Loucks 2004). No relationship between the EA and T_3 was observed in this study but it has to be noted that there are several inaccuracies when defining EA, which might cover the relationship. Loucks & Callister (1993) conducted that T_3 levels depend on EA or more specifically on the carbohydrates availability associated with that EA so that T_3 can be suppressed either by decreasing dietary intake or by increasing exercise energy expenditure without reducing dietary intake. Even though no relationship between EA and T_3 was found in this study, a positive change in the energy balance led to bigger positive change in T_3 concentration and pre-post change in T_3 correlated with carbohydrate change in WR. A relationship between energy expenditure and T_3 was also found in the present study since a decrease in energy expenditure during the summer competition season would seemed to led to higher T_3 post concentration (when considering all subjects (WR+C) together). The same was detected when considering the WR group alone since mid-post change in T_3 correlated negatively with energy expenditure in post measurement. These findings are in line with Loucks & Callister (1993) and would seem to verify that T_3 can be suppressed either by decreasing dietary intake or by increasing exercise energy expenditure without reducing dietary intake. However, small sample size must be taken into account.

9.4.6 Ferritin

Iron has many essential biochemical roles in the body. It not only affects health and well-being but also athletic performance. (Peeling et al. 2008.) Iron deficiency has multiple detrimental consequences. Hemoglobin is the most important pool of iron in the body. It contains up to 80 % of the total body iron (3–4 g) in young women. The majority of the remaining iron is found in ferritin and hemosiderin, and is referred to as “storage iron”. Serum ferritin (SF) levels correlate with body iron stores and the levels are normally within ranges within 15–300 μg/l. (Worwood 2012.) Iron depletion is considered when serum ferritin levels are below 35 μg/l, iron deficient erythropoiesis when below 20 μg/l and iron deficient-anemia when below 12 μg/l.

According to the above-mentioned classification eight out of thirteen subjects in the present study had iron deficiency at some degree. Below 12 μg/l ferritin levels was observed in three subjects, below 20 μg/l in one and below 35 μg/l in four subjects. However, none of the subjects with serum ferritin concentration below 12 μg/l had hemoglobin levels below 115 g/l so low serum ferritin levels have not impacted on hemoglobin levels yet.

According to the literature, female athletes are at a greater risk of compromised iron status since there are a number of factors in addition to diet that affects on iron status in female athletes. Blood losses from menstruation can be considered the major route of iron excretion. (McClung et al. 2014.) Other affecting factors are represented in figure 13.

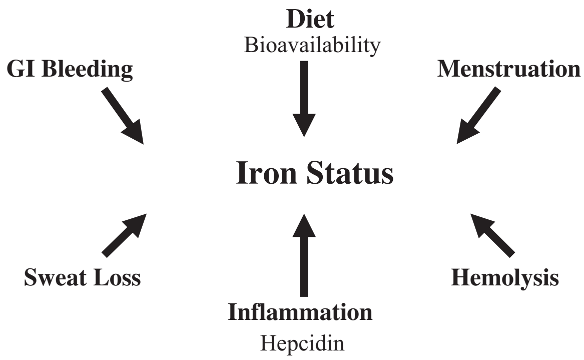

FIGURE 13. Factors affecting iron status in female athletes. GI, gastrointestinal. Adopted from McClung et al. (2014).

9.5 Physical performance

Considering physical performance, only two subjects were able to perform the post measurements in the WR group, so statistical analysis could not be done. Regarding the effects of weight reduction, no statistically significant changes was observed between the pre and mid measurements. Nevertheless, all performance variables changed positively in the light of better physical performance, so at least weight reduction did not have harmful effects on physical performance in this study.

In this study, squat jump result improved by 3.6 % and counter movement jump by 1.3 % in WR. In previous studies, improvements in vertical jumps due to weight reduction have been observed. Nevertheless, it has been noticed that the improvement is bigger when the energy deficit is higher. (Fogelholm 1993; Mero ym. 2010; Mettler ym. 2010; Garthe ym. 2011a; Huovinen ym. 2015.) In this study the energy deficit was about 480 kcal/day, which is the same as in Garthe's et al. (2011a) study in slower weight loss rate group (0.7 % of body mass/week, -469 kcal/day). However, the duration of Garthe's et al. (2011) study was much longer (8.5 weeks), which might explain the improvement.

There were no differences between the groups at any measurement points except pre versus mid change in reactive jump, which was significantly higher in the group C. In C, reactive jump result improved significantly between pre and mid measurements, which can be considered to be a result of successful preparatory season training. The reactive jump result also decreased significantly during the summer competition season (mid-post), which can be explained by lesser training during the summer and decreased motivation to perform the last measurements. No other significant changes were observed in the control group.

Relationships between body composition and physical performance. In the WR group, lower mid fat percentage led to faster 30 m sprint with standing start times. Percentage changes in lean mass and 30 m sprint with standing start time also correlated negatively. Furthermore, there was a trend for improved squat jump result with maintained or increased lean mass. These findings support the beneficial effects of weight reduction in the shape of losing fat mass and sparing lean mass, which improves power-to-weight ratio and thus may be beneficial in weight-bearing efforts, such as jumping events for height and distance (Fogelholm 1994; Phillips 2006; Phillips & Van Loon 2011). This was also true in whole group (WR+C) level since mid lean mass correlated negatively with percentage change in standing 30 m sprint time between the pre and mid measurements.

In the whole group level (WR+C), fat mass correlated with flying 20 m time in post measurements but not in other measurement points. Mid-post change in body mass was in a relationship with post flying 20 m times. Mid-post change in lean mass correlated with post squat jump with 20 kg extra weight. These findings also confirm the benefit of a high lean-to-fat body weight ratio in the light of better physical performance.

In order to summarize and conclude, only two subjects were able to perform post measurements in the WR group, so statistical analysis could only be done for pre and mid measurements. There were no statistically significant changes between the pre and mid measurements in WR. Nevertheless, all performance variables changed positively in the light of better physical performance, so at least weight reduction did not have harmful effects on physical performance. Beneficial effects of weight reduction in the shape of losing fat mass and sparing lean mass (improving power-to-weight ratio) were also noticed in this study, which verifies the findings of previous studies that a high lean-to-fat body weight ratio is advantageous in the light of better physical performance (Fogelholm 1994).

9.5.1 Injuries

There was a great drop out in the WR group in the post measurement performance test mainly due to injuries. One subject did not participated to the post measurements. Three subjects were able to perform post measurement, however, one of those could not perform the mid measurements due to an injury and one subject performed only vertical jumps without extra weight. So only one subject was able to perform all the physical performance tests in all the measurement points. Many subjects suffered from bone stress reactions and only one subject had acute injury (torn ligaments). In the group C, all subjects were able to perform all the performance tests except one subject who did not perform running tests in post measurements. In the control group, only one subject had symptoms of stress reaction but was able to return normal training after few weeks. Other subjects were injury free throughout the study.

Reasons behind injuries can be considered. Bone stress injuries are more prevalent among track and field athletes and military recruits than in other sports (Goolsby & Boniquit 2017). Diet, hormone levels, training and biomechanics for instance affect bone health. It has been noticed that women participating in sports that emphasize a low body mass are more likely to restrict their energy intake (Manore et al. 2007). Stress injuries have been observed to be more frequent in female athletes with menstrual disturbances (Goolsby & Boniquit 2017). In the present study, menstrual disturbances were more prevalent among the WR group. Declines in the concentrations of anabolic hormones were observed with longer cycle length as well as unfavourable body composition changes. Subjects' fat percentages cannot be considered to be too low and also subjects' Z-scores were high. However, it has been reported that weight-bearing activities can protect against the negative effects of menstrual disturbances on bone density (Torstveit & Sundgot-Borgen 2005). Training habits and biomechanical factors can also be discussed. Since track and field jumping events are classified to be high impact sport, the mechanical loading is high. If the energy intake is low and training volume and mechanical loading is high the body is in a catabolic state and cannot repair the exercise induced muscle damage, which increases the risk of stress injuries. Biomechanical factors (for example the foot pronation) can also contribute the exposure to stress reaction and stress fractures (Torstveit & Sundgot-Borgen 2005). Above-mentioned can be considered to be risk factors for stress injuries and they can be classified as intrinsic and extrinsic ones and are presented in figure 14. In conclusion, since bone stress injuries have a high prevalence in track and field athletes and were also common in the present study, should the awareness of the risk factors of stress facture development and how to prevent them increase among athletes and coaches.

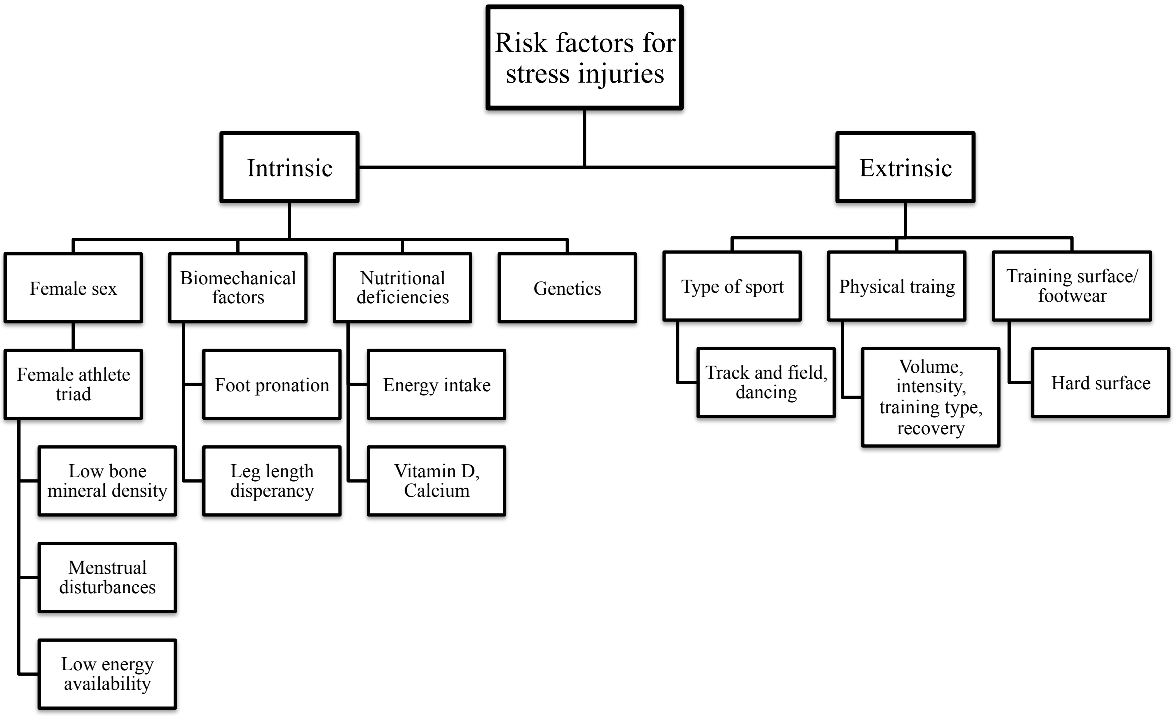

FIGURE 14. Risk factors for stress injuries (Bennel et al. 1999; Behrens et al. 2013 Goolsby & Boniquit 2017).

9.6 Conclusion

The aim of this study was to follow the effects of a preparatory season (exercise, diet) on body composition, hormonal concentrations and physical performance in female track and field jumpers and follow those variables throughout summer's competition season.

Subjects' energy deficit was -477 $\pm$ 218 kcal/day during the weight reduction period in the WR group, which produced a weekly weight loss rate of 0.3 kg. Subjects' fat mass was reduced significantly without changes in lean mass. There were no negative changes in physical performance or hormone concentration either. Subjects were also able to maintain the reduced weight throughout the summer's competition season. Therefore, implementing weight reduction with moderate energy deficit by reducing carbohydrate and fat intake while maintaining high protein intake would seem to be verified.

One of the main findings of this study was that menstrual irregularities were more prevalent and the menstrual cycle length was longer among the weight reduction group. Declines in the concentrations of anabolic hormones were also associated with longer cycle length as well as unfavourable body composition changes in the light of weight management. Other noteworthy finding was that only one subject in the WR group was able to perform all the physical performance tests in all the measurement points. Many subjects suffered from bone stress reactions, which have observed to be common among track and field athletes compared to other sports. Stress injuries have been noticed to be more frequent in female athletes with menstrual disturbances (Goolsby & Boniquit 2017.) This study also supports the finding of the previous studies. Bone stress injuries have a high prevalence among track and field athletes and they were also common in the present study. Thus the awareness of the risk factors of stress injury development and how to prevent them should increase among athletes, coaches and other support team. The effect of weight reduction on menstrual cycle has to be also taken into account when planning weight loss regimens in female athletes.

Even though there were no changes in hormonal concentration during the study, several noteworthy correlations between hormones and nutrition and body composition variables were found. Decreased body mass led to increased cortisol levels during the weight loss period ($r=-0.845$, $p=0.034$) in the WR group. Considering testosterone, those who had bigger negative change in testosterone concentration had the lowest body mass after weight reduction period (r=0.900, p=0.006). Regarding E_2, reduced dietary fat intake (pre-mid) led to lower E_2 mid concentration (r=0.670, p=0.012). Female athletes are at a greater risk of compromised iron status (McClung et al. 2014), and this was also true in this study since eight out of thirteen subjects had iron deficiency at some degree at some measurement point.

There were limitations in this study. First, the sample size of this study was small (n=13) and therefore reliable universal conclusions cannot be made. Subjects' motivation and commitment to the study can be also considered especially in the last measurements when the competition season was over. Inaccuracies of the research methods and external factors such as medication and stress factors have to take into account. Weight reduction among women athletes have been studied little and the need for further studies with bigger sample size is essential. Studies where blood samples are taken at the same phase of the menstrual cycle are needed. According to the present study, the awareness of the risk factors considering women athletes (factors of the female athlete triad) should increase among women track and field athletes, their coaches and support team in the future in order to avoid the development of stress injuries.

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APPENDICES

APPENDIX 1. Filling instructions and an example of food diary.

NÄIN KÄYTÄT PÄIVÄKIRJAA

1. Kirjaa jokainen syömäsi ruoka, juoma ja ateria ruokapäiväkirjaan HETI aterian jälkeen tai sen yhteydessä
2. Kerro myös pakattujen tuotteiden paino (esim. Leader so low-carb proteiinipatukka suklaa 61 g)
3. Merkitse aterian kellonaika ja paikka (koti, ravintola, jne.). Muista merkitä harjoituksen aikana syömäsi/juomasi ravinto. Muista merkitä myös harjoituksen kellonaika, esim. juoksu 9.15–10.30
4. Kuvaile ruoka-aine, ateria tai juoma mahdollisimman tarkasti, esim. täysjyväpasta tai Saarioisen lihapullat, ja kerro onko tuote valkoisesta/täysjyväviljasta, rasvaton/vähärasvainen/täysrasvainen, sokeriton/lisättyä sokeria sisältävä silloin kun vaihtoehtoja on monta (esim. spagetti, jogurtti, maito, kerma, margariini). Esim. Arla Natura kermajuusto 17%, Keiju Catering 40%, Kalkkunaleikkele 1,5%, Maustamaton jugurtti 2,3%.
5. Kerro, miten ruoka on valmistettu (esim. uunissa, hauduttamalla, paistettu) ja mainitse valmistuksessa käyttämäsi rasva ja/tai neste ja niiden määrä
6. Pyri arvioimaan mahdollisimman tarkasti ruoan tai juoman määrä joko painona (g), kappaleina (kpl), tilavuusyksikköinä (rkl, dl, l) tai esim. viipaleina tai lasillisena ilmaistuna. Jos mahdollista, ilmoita syödyn ruoan määrä kuivapainona tai desilitroina, esim. kaurahiutaleita 1dl kuivana
7. Mikäli mahdollista, punnitse kaikki ruoka-aineet erikseen keittiövaa´alla (maksaa tavarataloissa 10-20e). Jos et tiedä painoa, kuvaile “kiinteiden” ruoka-aineiden määrä ulottuvuuksina, käyttäen alla olevaa mittanauhaa (esim. grillipihvi, rasvaton, 20 cm x 8 cm x 4 cm paksu, tai vihreä omena 10cm halkaisija)
8. Jos olet valmistanut itse ruokaa tai leiponut, kirjoita resepti (=ainesosat) kyseisen päivän ruokapäiväkirjan loppuun/kääntöpuolelle/reunoille ja ilmoita, kuinka suuren osan annoksesta söit.
9. Jos söit ulkona, arvioi ruoka-annoksen sisältö (esim. kanarisotto, 2dl valkoista riisiä, muutama kanapala, herneitä, vaikuttaa öljyiseltä/kermaiselta)
10. Muista merkitä myös lisäravinteet.
11. Muista kertoa jos olet lisännyt ruokaan jotain – voita leivän päälle, maitoa muroihin, sokeria teehen/kahviin. Jos sitä ei ole kerrottu, oletan ettet ole lisännyt mitään.
12. Voit myös ottaa kuvan aterioistasi, jos et osaa arvioida (täytä joka tapauksessa arvioisi päiväkirjaan).
13. Ole rehellinen, merkkaa myös napostelut. Pyri syömään mahdollisimman normaalisti päiväkirjan täytöstä huolimatta!
14. Jos epäröit, miten jokin ruoka-aine tulisi raportoida päiväkirjaan, ole yhteyttä.

Nimi: **ESIMERKKI** Päivämäärä: **18.12.2015** Kehonpaino: **59,2 kg**

Aika klo	**Paikka**	**Ruoat ja juomat (valmistustapa, ruoanvalmistuksessa käytetty rasva ja neste)**	**Määrä (kpl, dl, g, viipale, lasillinen jne.)**
8:30	Koti	Kaurahiutaleet, Kotimaista	36 g, vesi: 2 dl
		Kananmuna, keitetty, punnittu kuorittuna	54 g
		Banaani, punnittu kuorittuna	51 g
		Mustikka, pakaste	34 g
		Rainbow, maustamaton, rasvaton jugurtti	69 g
		Berocca	1 tabletti
		Vesi	3 dl
		D-vitamiini	1 tabletti
		Möller omega3 + A,D,E	1 kapseli
10:00	Yliopisto	Omena, ulkomainen	153 g
12:00	Yliopisto	Perunamuusi, veteen tehty	2,2dl
		Jauhelihakastike, tomaattipohjainen	1,5 dl
		Porkkanaraaste	100 g
		Jäävuorisalaatti	70 g
		Auringonkukansiemenet	1 rkl
		Valkosipulisalaattikastike (rasvapitoisuus 28g/100g)	1 rkl
		Herneet	3/4 dl
		Ruisreal	1 pala
		Pågen, aurinkopalat	1 pala
		Oivariini, normaalisuolainen	8 g
		Rasvaton maito	2 dl
		Vesi	2 dl
12:40	Yliopisto	Kahvi kevytmaidolla	2,5 dl
17:30	Koti	Peruna, keitetty	202 g
		Intialainen kanakastike (RESEPTI)	186 g
		Porkkana	81 g
		Kurkku	61 g
		Parsakaali, keitetty	31 g
		Vesi	3 dl

Aika klo	**Paikka**	**Ruoat ja juomat (valmistustapa, ruoanvalmistuksessa käytetty rasva ja neste)**	**Määrä (kpl, dl, g, viipale, lasillinen jne.)**
20:45	Koti	Reissumies tosi tumma	23 g
		Flora kulta 80%	4 g
		Omena, punnittu kuorineen	151 g
		Satsuma, kuorittu	87 g
		Ananas, tuore	78 g
		Rahka, rasvaton, Rainbow	126 g
		Vadelma	14 g
		Juusto, Arla Natura loputon kermajuusto 17%	10 g
		Kurkku, kotimainen	25 g
		Tee	2,5 dl
		Kananmunan valkuainen	39 g

INTIALAINEN KANAKASTIKE
400g maustamattomia kanasuikaleita, Kotimaista
1 rkl rypsiöljyä
87 g sipuli
17 g valkosipuli
200 g paseerattua tomaattia
2 dl ruokakermaa 4%
2 rkl japanilainen soijakastike, Rainbow

APPENDIX 2. Filling instructions and an example of activity diary.

ARKIAKTIIVISUUSPÄIVÄKIRJA

Ohje arkiaktiivisuuspäiväkirjan täyttöön

Merkitse päiväkirjaan mahdollisimman tarkka päivän kulku jokaiselta päivältä, jolloin täytät ruokapäiväkirjaa (auttaa energiankulutuksen analysoinnissa). Täytä päiväkirjaa päivän edetessä, jotta tapahtumat ovat tuoreessa muistissa. Merkitse uni, arkiaskareet, opiskelu, harrastamasi liikunta, ruokailu ja muu päivän aikana tapahtunut. Ruokailun sisältöä sinun ei tarvitse tähän päiväkirjaan merkitä. Päiväkirjaan merkattu kellonaika viittaa alkavaan tuntiin, esimerkiksi 6 = klo 6-7. Alla on esimerkkipäivä.

klo	**Mitä olet tehnyt? (ruokailu, peseytyminen, autolla ajo, ulkoilu, uni, istuminen sohvalla…)**
0	Uni
1	Uni
2	Uni
3	Uni
4	Uni
5	Uni
6	Uni
7	Uni, herätys 7.15, aamutoimet
8	Pyöräily kouluun (15 min), luennolla istuminen (45 min)
9	Luennolla istuminen
10	Luennolla istuminen
11	Luennolla istuminen/lounaan syöminen
12	Luennolla istuminen
13	Pyöräily kotiin (15 min), opiskelua istuen (45 min)
14	Opiskelua istuen
15	Ruuanlaitto/tiskaaminen seisten (35 min), syöminen istuen (25 min)
16	Pyöräily treeneihin (15min), treenit 16.15–17.30
17	Treenit, pyöräily kotiin (15 min), treenitavaroiden purkua yms. (15min)
18	Suihkussa käynti (20 min), sohvalla makoilu (40 min)
19	Iltapalan syönti, sohvalla makoilu
20	TV:n katselu sohvalla makoillen
21	TV:n katselu sohvalla makoillen
22	Kirjan lukeminen sängyssä, nukkumaan 22.30
23	Uni

APPENDIX 3. An example of training diary.

PÄIVÄ	SISÄLTÖ	SARJAT (kpl)	TOISTOT (kpl)	KUORMA / MATKA	SI-yksikkö	LIIKKEIDEN TOTEUTUSTAPA
15.11.15	Hyppelykestävyys			75	min	
ESIMERKKI	Vauhtileikittelyä (10min)					
	Venyttely (10min)					
	Submax (15 min)	2	3	60	m	rentoa 80% / 2 min (kävelypalautus) /5 min
	Hyppelyt/loikat (30 min)					
	kinkka-ponnistus (2 askel liuútus)	2	4	40	m	
	vuoroloikka	2	4	40	m	1. sarja vuorokäsin, 2. tuplilla
	vaihtokinkka	2	4	40	m	VVOOVVOOVV..
	isovuorohyppely	2	4	40	m	1. sarja vuorokäsin, 2. tuplilla
	Loppuverryttely, hölkkää (10min)					

APPENDIX 4. Menstrual cycle follow-up form.

Kuukautiskierron seuranta

Käytätkö hormonaalista ehkäisyä? En____ Kyllä____, mitä?________
Merkitse oheiseen taulukkoon rastilla kuukautisten alkaminen, päättyminen ja vuotopäivät. Aloita päiväkirjan täyttö edellisten kuukautisten alkamisesta.

Pv	Esim.	Helmikuu	Maaliskuu	Huhtikuu	Toukokuu	Kesäkuu	Heinäkuu	Elokuu	Syyskuu
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14	X								
15	X								
16	X								
17	X								
18	X								
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

APPENDIX 5. Subjects' history of injuries questionnaire.

Terveydentila ja vammat kaudella 2016-2017

Nimi:

Terveydentilaa ja vammoja koskevat kysymykset. Kysymykset koskevat kautta 2016-2017 (lokakuu 2016-lokakuu 2017). Vastaa alla oleviin kysymyksiin ja palauta mahdollisimman pian Sinille.

1. Onko sinulla ollut edellisen kauden aikana **vammoja**, jotka ovat vaikuttaneet harjoitteluusi tai kilpailemiseesi? Anna alle kuvaus vammasta/vammoista, **milloin** vamma tapahtui/alkoi, **miten** se vaikutti harjoitteluun ja **minkä ajanjakson** vaikutukset harjoitteluun / kilpailemiseen kestivät.

2. Onko sinulla ollut edellisen kauden aikana **sairastelua** tai muita **terveyteen liittyviä ongelmia**, jotka ovat vaikuttaneet harjoitteluusi tai kilpailemiseesi? Anna alle kuvaus sairaudesta/ongelmasta, koska se **alkoi**, miten se **vaikutti** harjoitteluun/kilpailemiseen ja **minkä ajanjakson** vaikutukset harjoitteluun/kilpailemiseen kestivät.

3. Onko sinulla ollut edellisen kauden aikana **kipua tai arkuutta akillesjänteissä** tai **polvijänteessä** (patellajänne)? Kuvaa alle tarkemmin **millaista** kipua tai arkuutta, **kuinka kauan** kipu/arkuus kesti ja **kummassa jalassa** ja **missä jänteessä** kipu/arkuus oli.