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博士（スポーツ科学）

Energy Availability and Metabolic Suppression in Korean Male Collegiate Soccer Players

アジア人男性アスリートにおける エナジー・アベイラビリティと代謝抑制

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List of Abbreviations

ACSM = American College of Sports Medicine

ACTH = Adrenocorticotropic hormone

BAP = Bone alkaline phosphatase

BMD = Bone mineral density

BTMs = Bone turnover markers

BW = Body weight

CHO = Carbohydrate

CRH = Corticotropin-releasing hormone

CTx = Carboxy-terminal collagen cross-links

DE = Disordered eating

DIT = Diet-induced thermogenesis

DXA = Dual-energy X-ray absorptiometry

EA = Energy availability

EAT-26 = Eating Attitude Test 26

EB = Energy balance

ED = Eating disorder

EE = Energy expenditure

EHMC = Exercise-hypogonadal male condition

EI = Energy intake

EPOC = Excessive post-exercise oxygen consumption

FAT = Female Athlete Triad

FFM = Fat-free mass

FHA = Functional hypothalamic amenorrhea

FM = Fat mass

FOR = Functional overreaching

FSH = Follicle-stimulating hormone

GH = Growth hormone

GnRH = Gonadotropin-releasing hormone

H-P-G = Hypothalamic-pituitary-gonadal

HR = Heart rate

IGF-1 = Insulin-like growth factor-1

IOC = International Olympic Committee

LH = Luteinizing hormone

METs = Metabolic equivalents

NEAT = Non-exercise activity thermogenesis

NFOR = Non-functional overreaching

NTx = Amino-terminal cross-linking telopeptide

OTS = Overtraining syndrome

POMS-2 = Profile of Mood States 2

RED-S = Relative Energy Deficiency in Sport

REE = Resting energy expenditure

REE_m = Measured resting energy expenditure

REE_p = Predicted resting energy expenditure

REE_{ratio} = Ratio between measured and predicted resting energy expenditure

RER = Rate of exchange ratio

RMR = Resting metabolic rate

SEE = Sleeping energy expenditure

T_3 = Triiodothyronine

T_4 = Thyroxine

TNF = Tumor necrosis factor

TRH = Thyrotropin-releasing hormone

TSH = Thyroid-stimulating hormone

VCO_2 = Carbon dioxide production

VO_2 = Oxygen uptake

VO_2 max = Maximal oxygen uptake

WDEB = Within-day energy balance

WDED = Within-day energy deficiency

Chapter 1. Introduction

1.1 Background

Over the last few decades, the importance of exercise and regular activities to prevent metabolic disorders and obesity has been emphasized. With numerous positive health benefits, there has been an increased interest in sports, and a rise in national and international events promoting sports and exercise participation. With an increased value placed on sports and exercise, the study of sports science has been advanced substantially. The positive aspects of exercise and performance enhancement of athletes has been studied by various disciplines within sports science, including physiology, psychology, biomechanics, and nutrition. Meanwhile, many studies have observed an increased prevalence of stress and injury among athletes undergoing excessive training and competing under strenuous schedules (Soligard et al., 2016). A biological understanding of training adaptation emphasizes the importance of proper balance between appropriate training load and adequate recovery for the prevention of injury and illness in athletes (Soligard et al., 2016).

Early studies investigating the exercise-recovery imbalance among athletes identified that energy deficiency can result in physiological and psychological alterations, such as changes in reproductive functions, bone health, psychological status, and performance (Hackney et al., 1988; Nattiv et al., 1994). In particular, among female athletes, there is a high risk of disordered eating (DE) driven by the pressure to achieve resulting in menstrual dysfunction and impaired bone health (Nattiv et al., 1994). Previous studies demonstrated the effect of energy availability (EA) on reproductive functions and bone metabolism (Ihle & Loucks, 2004; Loucks, 2004). These studies also contributed to the development of the Female Athlete Triad

(FAT) model which presents the interrelationships between EA, reproductive functions, and bone mineral density (BMD) (Nattiv et al., 2007). In contrast, the initial studies examining the exercise-recovery imbalance among male athletes presented a dissimilar approach to energy deficiency. Exercise-hypogonadal male condition (EHMC) has been identified in male athletes. Specifically, male athletes under endurance training have low levels of resting testosterone (Hackney et al., 2005). Overtraining syndrome (OTS) has also been observed in athletes who experienced a decline in performance, due to an imbalance between training and recovery (Meeusen et al., 2013). Subsequent studies on energy deficiency and physiological functions in athletes has led to a broadened FAT model; the expanded term 'Relative Energy Deficiency in Sport (RED-S)' was proposed by the International Olympic Committee (IOC) in 2014 and updated in 2018 (Mountjoy et al., 2014; Mountjoy et al., 2018). The expanded concept emphasized an understanding of the importance of an athletes' energy status. However, energy deficiency in male athletes and non-Caucasians remain unclear (Mountjoy et al., 2018).

Accumulated studies investigating endocrine responses to physical stresses have resulted in guidelines and reviews on energy deficiency in athletes. Unfortunately, the interactions between environmental stresses and the endocrine systems among athletes have not been fully elucidated due to individual variabilities and the complexity relating to the numerous conditions influencing sports and exercise (Hackney & Constantini, 2020; Kraemer & Rogol, 2005). There are numerous factors limiting research into the scientific evidence supporting the current guidelines in specific sports and population groups (Melin et al., 2019). To enhance athletes' well-being, it is essential to investigate and understand the current knowledge gaps.

1.2 Assessment of energy deficiency in athletes

Most of the relevant research in the field of dietetic and nutrition, the concept of energy balance (EB) was studied to identify the energy status. EB is estimated by subtracting total energy expenditure (TEE) from energy intake (EI) which is the energy remaining after all the physiological processes of the day, which is considered an output from the energy system (Loucks et al., 2011). EA, which is originally proposed in the field of bioenergetics, identifies the energy for fundamental physiological processes, and when the expended energy for one of these processes is not available for other processes. Therefore, in the field of sports nutrition, EA is defined as the amount of energy remaining for other physiological processes after exercise, which is considered as an input to the physiological system (Loucks et al., 2011). The contrasting aspects of EB and EA have been presented in a previous study showing a return to the balanced energy status under constant negative EB conditions without changes in EA (Loucks et al., 2011). The adaptations that occur as a response to prolonged negative EB result from the suppression of various physiological processes, thereby preventing critical weight loss and maintaining the healthy state. However, without changes in weight and body composition, the damaging factors are difficult to monitor and prevent, resulting in negative health and performance because of the physiological impairments by energy deficiency (Burke et al., 2018b). Furthermore, the estimation of EB requires the measurements EI and TEE, which is usually calculated by the sum of resting energy expenditure (REE), diet-induced thermogenesis (DIT), non-exercise activity thermogenesis (NEAT), and exercise energy expenditure (EEE). The advancement in the elucidation of the energy metabolism processes provides an array of measurement methods for each aspect of TEE. However, multiple factors limit the accuracy and practical measurement in the field of sports science.

Therefore, EA has been widely studied to prevent the underestimation of energy requirements, and the possible health problems resulting from energy deficiency among athletes. Loucks et al. (1998) is the first study to examine the effect of short-term low EA on physiological functions in women. Koehler et al. (2016) examined the effect of low EA in exercising males. The following interventional research based on these studies provided the scientific basis for the current EA recommendation values for female and male athletes (Hilton & Loucks, 2000; Ihle & Loucks, 2004; Loucks & Thuma, 2003; Murphy et al., 2021; Papageorgiou et al., 2017). Clinically low EA is recommended at EA <30 kcal/kg fat-free mass (FFM)/d in female and male athletes, and subclinical low EA at 30-45 kcal/kg FFM/d and 30-40 kcal/kg FFM/d in female and male athletes, respectively (Melin et al., 2019). However, observational studies that examined low EA of free-living athletes could not associate the current recommended EA values with the alterations in physiological functions (Koehler et al., 2013; Melin et al., 2015; Reed et al., 2015; Taguchi et al., 2020). A recent review paper suggested that metabolic and physiological adaptations to prolonged low EA can diminish the symptoms of energy deficiency in free-living athletes (Melin et al., 2019). Furthermore, individual variability influencing clinical EA is related to a dose-response continuum relationship between EA and the impairments in physiological functions. This explains the difference between laboratory-controlled studies and monitoring studies in free-living individuals (De Souza et al., 2019b).

To reduce the methodological difficulties in monitoring energy deficiency in free-living athletes, the use of EA analysis in conjunction with other methods, such as the ratio between measured and predicted REE (REE_{ratio}) and within-day energy balance (WDEB) analysis method, has been recommended (De Souza et al., 2019a; Logue et al., 2020). Adaptive response to prolonged energy deficiency can result in

alterations in metabolic and endocrine system without decrease in body weight (BW), and a comparison between measured and predicted REE can help to detect the metabolic suppression by energy deficiency (De Souza et al., 2007). In female participants, low REE_{ratio} was related to alterations in endocrine system and low EA (De Souza et al., 2008; Melin et al., 2015). In male athletes, energy deficiency after 4-week training intervention resulted in REE_{ratio} decrease (Stenqvist et al., 2020). Daily analysis of energy status has limitations to understand hourly changes of endocrine responses to energy deficiency, but hourly analysis of energy status using WDEB can help to understand the real-time effect of energy deficiency on endocrine system (Arroyo et al., 2018; Fahrenholtz et al., 2018). Recent studies on male and female athletes presented that energy deficit status using WDEB analysis was related to alterations in the endocrine system (Fahrenholtz et al., 2018; Torstveit et al., 2018).

1.3 Energy deficiency in exercising females

Among the components of FAT model, EA is a key factor directly affecting menstrual function and also has direct and indirect effects on bone health (Nattiv et al., 2007). Low EA is caused by low EI and excessive EEE in athletes. Restriction of EI in female athletes, especially those competing in weight-sensitive sports (endurance, esthetic, and weight-categorized) is common. The female athletes focus on low body fat for performance improvement and the desire to be thin can be promoted by the social environment (Nattiv et al., 1994). Abnormal eating patterns can increase the risk of DE and eating disorder (ED), which are regarded as clinical mental disorders (Nattiv et al., 2007).

The proposed mechanism for the effect of low EA on endocrine systems suggests that limited brain glucose availability affects glucose-sensing neurons in the hindbrain which transmit the signals to the hypothalamus in the forebrain via

catecholamines, neuropeptide Y, and corticotropin-releasing hormone neurons, resulting in gonadotropin-releasing hormone (GnRH) pulse alterations (Loucks, 2005). Additionally, changes in substrate availability can reduce fat mass (FM), and hence the secretion of adipokines (leptin and adiponectin) and metabolic hormones (ghrelin, peptide YY, cortisol, insulin, and insulin-like growth factor-1 (IGF-1)), which can alter the hypothalamic-pituitary-gonadal (H-P-G) axis responses (Misra, 2014). These alterations driving the disturbances in the H-P-G axis also disrupts menstrual function in female athletes (Nattiv et al., 2007). Moreover, low EA has direct and indirect endocrine influences on bone health. The decreased negative feedback from energy deficiency can directly alter the responses of the growth hormone (GH) and IGF-1, which regulate bone remodeling. A decrease in metabolic hormones, such as triiodothyronine (T_3), leptin, and insulin, can influence bone formation and resorption (Areta et al., 2020; Papageorgiou et al., 2018; Southmayd et al., 2019). Low EA indirectly affects bone metabolism through decreased estrogen suppressing of the H-P-G axis. Estrogen deficiency increases interleukin-7 production and thus reduces antiapoptotic influences on osteoblasts; antioxidant pathways that promote receptor activator of nuclear factor kappa-B ligand and tumor necrosis factor (TNF), which drive osteoclasts formation, are also upregulated (Southmayd et al., 2017; Weitzmann & Pacifici, 2006).

With an understanding of the interrelationship between the FAT components, highlights the importance of monitoring and screening for FAT risk factors, and the screening tools and analysis methods have been developed (De Souza et al., 2014; Melin et al., 2014). Moreover, non-pharmacological (optimizing energy status and micronutrient supplementation) and pharmacological (hormone administration) treatments for the FAT conditions have also been studied and provided for female

athletes (De Souza et al., 2014).

Research on energy deficiency, reproductive function, and bone health in males has not been as widely conducted. However, comparable studies investigated the effect of the imbalance between training and recovery on the physiological functions in males.

1.4 Energy deficiency in exercising males

1.4.1 Mechanisms of the status of the exercise-hypogonadal male condition

Compared to the accumulated studies and current understanding of energy deficiency in exercising females, a relatively small number of studies have investigated energy deficiency in exercising males. Early studies have focused on the effect of excessive and prolonged exercise on the physiological functions in male athletes. Hackney et al. (2005) proposed the term EHMC, for referring to the condition of exercise-induced low testosterone concentrations in exercising males, with ≤50%-75% basal testosterone concentration of the reference value. Hackney et al. (2005) followed up on previous research from the 1980-1990s, which detected reproductive dysfunction among males undergoing chronic endurance exercise. In these studies, prolonged endurance exercise was associated with alterations in prolactin, luteinizing hormone (LH), and testosterone concentrations. These were related to the disruption of the H-P-G axis, which has been previously mentioned as contributing to the effect of low EA on endocrine functions (Hackney et al., 1988; Lane & Hackney, 2014; MacConnie et al., 1986; McColl et al., 1989; Wheeler et al., 1991). Furthermore, the central (H-P) and peripheral (G) dysfunction in EHMC have been investigated. The central mechanisms exhibit alterations of GnRH responses in the pituitary and abnormalities of LH and prolactin, causing a suppressed testosterone concentration (Hackney et al., 2005; Hackney et al., 1990; MacConnie et al., 1986). In addition, testis

dysfunction was associated with the changes in either testicular receptor sensitivity or testosterone synthesis, or both (Hackney et al., 2005; Hackney et al., 1990; Hackney et al., 2003; MacConnie et al., 1986).

Low testosterone concentrations by EHMC disrupt androgenic and anabolic functions leading to detrimental effects on spermatogenesis and libido while anabolic dysfunction can result in decreased muscle synthesis and bone growth (Arce et al., 1993; Hackney et al., 2005; McGrady, 1984). However, there are conflicting study results and insufficient evidence showing different consequences of low testosterone (Hackney et al., 2005; Lane & Hackney, 2014; Lucía et al., 1996; MacDougall et al., 1992). Therefore, further research of the pathophysiological mechanism and consequences of EHMC is warranted. The disturbances in the H-P-G axis in EHMC share a similar mechanism to the FAT, hence suggesting a detrimental effect of exercise stress on reproductive function in both exercising males and females.

EHMC can be diagnosed by resting basal testosterone concentrations (≤50%-75% of the reference values), but its characteristics are limited to prolonged endurance training and exercising males (Hackney et al., 2005). In addition, as aging gradually reduces testosterone concentrations in blood circulation, the testosterone concentration of normal, healthy, age-matched sedentary males should serve as a reference value for EHMC diagnosis (Hackney, 2020).

1.4.2 Mechanisms of the overtraining syndrome

The symptoms of EHMC were commonly reported in endurance athletes, and many of them were involved in overtraining or OTS (Hackney et al., 2005). The term “overtraining” was related to cognitive studies in the 1960s, was adopted in physiological research in the 1970-1980s (Cadegiani, 2020b), and recently redefined

during the International Conference on Overtraining in Sport, before the 1996 Olympic Games (Kreider et al., 1998). Table 1.1 lists definitions of OTS and its conditions, including overreaching (functional overreaching (FOR) and non-functional overreaching (NFOR)) and overtraining (Kreider et al., 1998; Meeusen et al., 2013).

The central mechanism of OTS can be explained by either physiological or psychological maladaptation to a hostile environment, or both, and this maladaptation can trigger various dysfunctions in the endocrine, autonomic, muscular, neuromuscular, and psychological systems, which result in performance decrease and mood disturbances (Cadegiani, 2020a). OTS includes a wide range of physiological and psychological aspects, and therefore, a single theory cannot explain the pathophysiology of OTS. Many studies investigated the cascades of dysfunctions triggered by OTS, and central fatigue induced by insufficient energy (glycogen depletion) and imbalance of H-P axes share similar mechanisms with the FAT and EHMC (Cadegiani, 2020a).

OTS is related to the effect of acute excessive exercise, and it presents gradual alterations in performance and mood (Hackney, 2020). OTS can result from mismanaged training or the monotony of exercising, excessive competition, and personal and occupational stresses (Meeusen et al., 2013). Pathological mechanisms of OTS are characterized by persistent sport-specific performance decrease and mood disturbances, and importantly, other possible triggers, such as organic disease and disordered eating behaviors are excluded during diagnosis (Cadegiani & Kater, 2019; Hawley et al., 2015; Meeusen et al., 2013).

1.4.3 Prevalence and management of the exercise-hypogonadal male condition and overtraining syndrome

Limited studies have investigated the prevalence of EHMC in athletes. A recent small sample study presented that more than 50% of endurance-trained athletes had low baseline testosterone concentrations, suggesting possible EHMC (Hooper et al., 2019). In a study of 196 male endurance-trained runners, 15.3% of them had low testosterone (≤300 ng/dl) and the resting testosterone concentrations decreased ~30% after 5 years of training without further reduction in 10-15 years (Hackney & Lane, 2018; Hackney & Lane, 2020). The unclear classifications of OTS symptoms make it difficult to investigate its exact prevalence in athletes. Raglin and Wilson (2000) reported that 7%-21% collegiate athletes had NFOR/OTS. Morgan et al. (1988) found that 64% of elite male distance runners had experienced more than one episode of NFOR/OTS throughout their careers. In multinational studies, the prevalence of NFOR/OTS across various age-groups and sports is 29%-37% (Kenttä et al., 2001; Lienhard et al., 2013; Matos et al., 2011; Raglin et al., 2000). These previous studies highlight the risk of OTS in various population groups in sports and indicate the need for a more accurate OTS screening method.

EHMC represents an adaptive response of the H-P-G axis to prolonged endurance training and exercise, and the adaptive response can have beneficial physiological effects on the cardiovascular system (Blair et al., 1996; Hackney et al., 2005; von Eckardstein et al., 1997). In contrast, the low testosterone concentrations associated with EHMC should be carefully monitored because of a high risk of undesirable side effects resulting in disturbances in reproductive function and bone health (Hackney, 2020; Hooper et al., 2018). Pharmacological treatment using exogenous testosterone or anabolic agents to stimulate testosterone production is the

medical standard for male hypogonadism. However, for athletes, the administration of testosterone or anabolic agents is strongly prohibited by World Anti-Doping Agency except in severe cases (Hackney, 2020; Hooper et al., 2018). Therefore, non-pharmacological treatment including nutritional support and training modification are recommended for male athletes with EHMC (Hooper et al., 2018). The various suggested triggers and mechanisms related to OTS are not well understood and there is insufficient evidence to support their roles reducing the symptoms and risk of OTS. A recent review paper suggested that the early prevention and diagnosis of NFOR and OTS must be considered and appropriate management of training with recovery, including sufficient rest, sleep, and nutrition, is recommended as treatment strategies of OTS (Meeusen et al., 2013).

Despite the different triggers for the condition, as well as complex mechanisms and symptoms, the disruption in the H-P-G axis induced by the imbalance of training and recovery is the shared pathophysiological mechanism of FAT, EHMC, and OTS. Furthermore, prevention and treatment strategies for these conditions all emphasize the balance between training and recovery. This balance can be considered in terms of the assessment of EA and energy deficiency in athletes.

Table 1.1 Stages of training, overreaching and overtraining syndrome (Meeusen et al., 2013)

Training load				
Stage	Acute fatigue	Overreaching: an accumulation of training and/or non-training stress resulting in short-term decrement in performance capacity with or without related physiological and psychological signs and symptoms of maladaptation in which restoration of performance capacity may take from several days to several weeks.		Overtraining: an accumulation of training and/or non-training stress resulting in long-term decrement in performance capacity with or without related physiological and psychological signs and symptoms of maladaptation in which restoration of performance capacity may take several weeks or months
		Functional overreaching (short-term overreaching)	Non-functional overreaching (extreme overreaching)	Overtraining syndrome
Performance outcome	Increase	Temporary decrease	Stagnation or decrease	Decrease
Recovery	Day(s)	Days-weeks	Weeks-Months	Months

1.5 Relative energy deficiency in sport

1.5.1 Introduction and components of the relative energy deficiency in sport

In 2007, the American College of Sports Medicine (ACSM) Position Stand provided the scientific background and clinical prevalence of the FAT. The imbalance between EI and energy expenditure (EE) was identified as the etiological factor, resulting in endocrine dysfunctions and impaired bone health (Nattiv et al., 2007). The relative energy deficiency resulting from the imbalance of energy has also been identified in males with EHMC and OTS and is also involved in altering various physiological functions. In 2014, the IOC consensus statement introduced the RED-S, which refers to the physiological dysfunction caused by relative energy deficiency. The physiological dysfunction induces disturbances in homeostasis resulting in alterations of metabolism, reproductive functions, immune function, anabolic action (bone and protein synthesis), cardiovascular and psychological health, in both female and male athletes (Mountjoy et al., 2014).

Low EA is the etiological factor of RED-S. Previous studies have suggested that the threshold for low EA is 30 kcal/kg FFM/d (Mountjoy et al., 2018). The risk of low EA is higher in female than male athletes, possibly due to greater concerns about body image and composition among females. Male athletes also have a high risk of low EA, resulting from weight cycling, mismanagement of EI during training and competition, and inadequate food availability (Burke et al., 2018a; Mountjoy et al., 2018). In the RED-S model, the consequences of relative energy deficiency are the expansion of the FAT, and include menstrual function, bone health, psychological, endocrine, metabolic, hematological, growth and development, cardiovascular, gastrointestinal, and immunological problems (Mountjoy et al., 2018). The supporting evidence of RED-S mainly focused on female athletes. Moreover, there are limited

studies and understanding on the effect of energy deficiency on male athletes in the 2018 consensus statement. Despite limited studies in male athletes, the RED-S model proposed the importance of clear insight on physiological impairment in male athletes with the risk of energy deficiency, and related studies which can help to understand it are presented in Table 1.2 and Table 1.3.

1.5.2 Effects on the endocrine system

GH-IGF-1 axis

The GH-IGF-1 axis is involved in the regulation of metabolic and repair processes, growth and development, and aging, starting with the stimulation of the hypothalamus to synthesize GH-releasing hormones and somatostatin, which regulate GH synthesis and release from the anterior pituitary (Eliakim et al., 2005). GH is a peptide hormone that stimulates IGF-1 synthesis in the liver regulates the metabolism of macronutrients and tissue differentiation, and IGF-1 has a negative feedback effect on GH secretion (Shimon & Melmed, 2007). IGF-1 is an insulin-related GH-dependent peptide that enhances the anabolic and growth effects of GH (Eliakim et al., 2005). The GH-IGF-1 axis is dependent on the aging process related to reproductive hormones, and the nutritional status related to the receptor responses, and thus its regulation is also associated with physical activities and environmental factors (Eliakim et al., 2005). A laboratory-controlled EA study of healthy females presented that low EA (10 or 20 kcal/kg FFM/d) conditions resulted in GH increase and IGF-1 decrease (Loucks & Thuma, 2003). An observation study on male adolescent wrestlers also detected increases in GH and decreases in IGF-1 after ~4 months of the competitive season involving energy restriction and weight reduction (Roemmich & Sinning, 1997). Energy deficit after the 11-week of dietary restriction in male bodybuilders resulted in

IGF-1 reduction without GH alterations (Mäestu et al., 2010). After 54 hours of the ultra-endurance cycling race, male cyclists experienced approximately 45% reduction of IGF-1, which presented a positive correlation with EB during the race (Geesmann et al., 2017). Additionally, a cross-sectional study in Swedish male Olympic athletes reported that the lean athletes with an expected low body mass for performance had lower IGF-1 bioavailability than the less lean athletes (Hagmar et al., 2013). Further research on various sports groups can help to provide clear evidence of the effect of energy deficiency on the GH-IGF-1 axis in athletes.

H-P-G axis

The regulation of the gonads (ovary and testis) depends on the H-P-G axis. In the hypothalamus, GnRH is secreted in pulsatile and modulated by the subgroups of neurons and peripheral hormone feedback (Lanfranco & Minetto, 2020). LH and follicle-stimulating hormone (FSH) are glycoprotein hormones synthesized and secreted in the anterior pituitary gland, and regulated by size and time-pattern of the GnRH pulsatile secretion (Stratakis & Chrousos, 2007; Veldhuis & Weltman, 2005). In males, LH stimulates the synthesis and secretion of testosterone in the testis, and FSH promotes spermatogenesis with testosterone (Veldhuis & Weltman, 2005). Testosterone is a steroid hormone produced at the Leydig cells in the testis (>95%) and the adrenal cortex, which regulates the H-P-G axis by a negative feedback loop (Veldhuis & Weltman, 2005). The androgenic function of testosterone is responsible for spermatogenesis, secondary sexual characteristics, and libido (Bhasin, 2005). The anabolic function of testosterone is responsible for protein synthesis in muscle tissues. The anti-catabolic effect of testosterone by blocking the glucocorticoids receptor also influences protein synthesis (Viru & Viru, 2005). Additionally, testosterone can directly promote bone formation by influencing the androgen receptor, and indirectly through

cytokines and growth factors (Shigehara et al., 2021). Disruptions of the H-P-G axis in females with energy deficit have been studied with the development of the FAT model. Laboratory-controlled studies in healthy females reported that short-term low EA resulted in disruption of the LH pulsatility (Loucks & Thuma, 2003; Loucks et al., 1998). A recent cross-sectional study comparing female athletes with menstrual dysfunction with controls demonstrated that the hours of energy deficiency were negatively associated with estradiol (Fahrenholtz et al., 2018). In male athletes with EHMC, disruptions of the H-P-G axis have been studied, as previously noted, but the following studies presented inconsistent results. A cross-sectional study reported lower testosterone concentrations and LH pulsation in endurance athletes than controls (McColl et al., 1989). However, similar cross-sectional studies presented lower testosterone in endurance athletes but no differences in LH and FSH (Hackney et al., 1988; Hooper et al., 2017). Effects of energy deficiency induced by excessive exercise or EI restriction on testosterone have been investigated. Pre- and post-race testosterone analysis during ultra-endurance events showed that excessive exercise resulted in testosterone decrease (Geesmann et al., 2017; Kraemer et al., 2008). A long-term (11-week) EI restriction in bodybuilders led to testosterone decrease, and relatively short-term (7-day) EI restriction in judo athletes also resulted in testosterone decrease (Abedelmalek et al., 2015; Mäestu et al., 2010). There are limited studies investigated the effects of EA on the H-P-G axis in males. A laboratory-controlled study analyzing the effect of short-term low EA in exercising males presented no alterations in testosterone after the intervention (Koehler et al., 2016). However, a cross-sectional study in endurance athletes indicated that low EA (21 ± 6 kcal/kg FFM/d) athletes had lower testosterone than moderate EA (37 ± 4 kcal/kg FFM/d) athletes (Heikura et al., 2018). Different study designs may have contributed to the inconsistent results. Future

studies with a clear analysis of energy deficit status and hormonal pulsatility to understand the effect of energy deficiency on the H-P-G axis in male athletes are recommended.

Thyroid hormones

Thyroid hormones (thyroxine (T_4) and T_3) are synthesized and secreted from the thyroid gland which is regulated by the H-P axis (Kogai & Brent, 2007). Thyrotropin-releasing hormone (TRH) from the hypothalamus stimulates the pituitary to secrete thyroid-stimulating hormone (TSH). TSH regulates the synthesis and secretion of the thyroid hormones in the thyroid, and thyroid hormones regulate TRH and TSH via a negative feedback mechanism (Kogai & Brent, 2007). Thyroid hormones are amino acid derived amine hormones affecting various processes in the body (Kogai & Brent, 2007). Thyroid hormones regulate REE via stimulation of Na+/K+ ATPases synthesis, increase enzymes related to cellular respiration, and increase the number and activity of mitochondria, which is called the calorigenic effect. Thyroid hormones increase the catecholamine's actions promoting sympathetic responses and regulates the development and growth of nervous tissues and bones (Tortora & Derrickson, 2018). Energy deficiency induces alterations in the H-P-thyroid axis resulting in low T_3 as an adaptive response to low REE and preserving the energy for essential physiological functions (Misra & Klibanski, 2014). A laboratory-controlled intervention study in healthy females found that low EA (8 kcal/kg BW/d) resulted in 15% lower T_3, and 18% lower free-T_3 than moderate EA (30 kcal/kg BW/d) without the effect of exercise (Loucks & Callister, 1993). In contrast, a cross-sectional study on female endurance athletes concluded that there was no T_3 difference between the low EA (24 ± 6 kcal/kg FFM/d) and moderate EA (38 ± 8 kcal/kg FFM/d) groups (Heikura et al., 2018). The effect of energy deficiency on T_3 in males is also inconsistent. A 4-day intervention on

short-term low EA (15 kcal/kg FFM/d) did not alter free-T_3 in exercising males (Koehler et al., 2016). However, a recent 4-week training intervention among trained cyclists reduced T_3 by 4.8% (Stenqvist et al., 2020). A cross-sectional study comparing elite runners and sedentary males presented lower TSH and TSH:free-T_3 in the runners, but there were no differences in free-T_3 and T_4 between the groups (Perseghin et al., 2009). Another recent cross-sectional study in endurance athletes presented no T_3 difference between the low EA (21 ± 6 kcal/kg FFM/d) and moderate EA (37 ± 6 kcal/kg FFM/d) groups, but the low total testosterone group (15.1 ± 3.0 mmol/L) had lower T_3 than the normal total testosterone group (25.0 ± 7.1 mmol/L) (Heikura et al., 2018). Different study designs and severity of energy deficiency may have contributed to the inconsistent results of thyroid hormones alterations. Disruption of T_3 can affect various physiological factors related to health and performance of athletes. The analysis of T_3 can serve as a clinical marker of relative energy deficiency and suppressed metabolism. Further research is required to understand the mechanisms underlying energy deficiency on T_3 in athletes.

Cortisol

Cortisol is synthesized and secreted from the adrenal cortex which is regulated by the H-P axis (Rämson et al., 2008). Alterations of homeostasis stimulate the hypothalamus to secrete corticotropin-releasing hormone (CRH), and CRH induces adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary. ACTH stimulates cortisol secretion from the adrenal cortex, and a negative feedback mechanism regulates the cortisol secretion process (Tortora & Derrickson, 2018). Cortisol is a glucocorticoid that regulate metabolisms and stress responses. It increases the protein breakdown in muscle fibers, lipolysis from adipose tissue, and gluconeogenesis in the liver (Tortora & Derrickson, 2018). Furthermore, cortisol

regulates the resistance to stress, including exercise, energy deficiency, extreme environments, and injuries via additional glucose supply by the liver. It also influences the anti-inflammatory and immune responses (Tortora & Derrickson, 2018). The H-P-adrenal axis regulates macronutrients metabolism and external stress, and energy deficiency in athletes can increase cortisol secretion. Controlled low EA (12.7 ± 1.1 kcal/kg FFM/d) in exercising females resulted in an increase in 24 h mean cortisol of 11% compared to baseline values (Loucks et al., 1998). In a study investigating ACTH stimulation in females demonstrated that amenorrheic runners presented a blunted cortisol response compared to eumenorrheic runners and controls, and suggested that this may be the result of mild hypercortisolism (De Souza et al., 1994). Several cross-sectional studies on female athletes have shown higher cortisol concentrations in amenorrheic athletes than eumenorrheic athletes (Rickenlund et al., 2004; Tornberg et al., 2017). In male triathletes, a 6-day exercise intervention indicated a significant cortisol increase after exercise in the control group (2818 ± 825 kcal/d) and no change in the high carbohydrate (CHO) diet (4246 ± 641 kcal/d) group (Costa et al., 2005). Pre- and post-race hormone analyses during an ultra-marathon found that cortisol increased in nine male athletes (Kraemer et al., 2008). In another study of 7-day calorie restriction in 11 male judo athletes, cortisol increases was noted (Abedelmalek et al., 2015). However, a cross-sectional study on low EA (27.2 ± 12.7 kcal/kg FFM/d) athletes with EHMC and normal EA (45.4 ± 18.2 kcal/kg FFM/d) controls presented no group difference in cortisol concentrations (Hooper et al., 2017). The H-P-adrenal axis is associated with physiological and psychological stress, and the complex response processes may have contributed to the inconsistent results of energy deficiency in athletes. Therefore, further research is recommended to understand the physiological and psychological influences of energy deficiency on athletes.

Leptin

Leptin is a peptide hormone and an adipokine secreted from adipose tissue (Misra, 2014). Its secretion is regulated by the glucose flux in muscles and the adipose tissue (Loucks, 2005). It is an anorexigenic hormone that suppresses appetite and has an impact on LH pulsatile secretion by affecting GnRH secretion (Lanfranco & Minetto, 2020; Misra, 2014). Laboratory-controlled low EA (10 kcal/kg FFM/d) in healthy females reduced the 24 h mean and amplitude of leptin (Hilton & Loucks, 2000). A cross-sectional study in female athletes versus controls found that amenorrheic athletes had lower leptin pulsatile secretion than eumenorrheic athletes and controls over 8 h (Ackerman et al., 2012). In trained male rowers, a 3-week intensified training resulted in a reduced fasting leptin concentration (Jurimae et al., 2003). The pre- and post-race analyses during ultra-endurance events demonstrated a significant reduction in serum leptin in male endurance athletes (Geesmann et al., 2017; Karamouzis et al., 2002; Roupas et al., 2013). A short-term (4-day) interventional study that investigated four different EA conditions (low EA of 15 kcal/kg FFM/d by diet and diet with exercise, high EA of 40 kcal/kg FFM/d by diet and diet with exercise) showed that low EA conditions resulted in a reduction in fasting leptin by 53%-56% (Koehler et al., 2016). Increased training volume resulted in energy deficiency-induced reduction of leptin response to exercise in trained male rowers (Rämson et al., 2008). A cross-sectional study of Swedish male Olympic athletes presented that the athletes emphasizing leanness had lower serum leptin than the less lean group (Hagmar et al., 2013). However, a longitudinal observational study of female and male swimmers during the training season showed that an increased training volume and a decreased EI in males did not result in leptin alterations (Noland et al., 2001). Leptin analysis in athletes may help to monitor and diagnose the energy deficiency. However, more

research studies with various study designs and populations groups are needed.

Insulin

Insulin is a polypeptide hormone synthesized and secreted from the beta cells of pancreas islets (Tortora & Derrickson, 2018), regulated by the blood glucose concentration via a negative feedback mechanism. Insulin release is also stimulated by various neurotransmitters and hormones including glucagon, GH, and ACTH (Tortora & Derrickson, 2018). Insulin is an anabolic hormone inducing the glucose uptake of muscle and liver, triglyceride synthesis of adipose tissue, protein synthesis by stimulating intracellular amino acids uptake (Yu et al., 2007). Insulin also affects the GnRH activity resulting in alteration of LH pulsatile secretion (Misra, 2014). A cross-sectional study on female athletes and controls presented that amenorrheic athletes had lower 24 h insulin than normal athletes and controls, and insulin was positively associated with LH pulse frequency (Laughlin & Yen, 1996). Laboratory-controlled low EA (<30 kcal/kg FFM/d) in healthy females resulted in a decrease in the 24 h mean insulin (Loucks & Thuma, 2003). A previously mentioned EA intervention study in exercising males showed that low EA (15 kcal/kg FFM/d) with or without exercise resulted in a reduction of insulin (Koehler et al., 2016). In a study of male bodybuilders, energy restriction for 11-weeks to reduce body fat decreased insulin, which was related to alterations in FM and FFM (Mäestu et al., 2010). Other cross-sectional studies in athletes have shown no difference in insulin concentration between the low EA and moderate EA groups and no association between EA and the insulin concentration (Heikura et al., 2018; Koehler et al., 2013). The accumulated evidence from various studies suggests that further research on the prolonged effect of energy deficiency on insulin in athletes is indicated.

1.5.3 Effects on bone

Bone metabolism

Bone is a dynamic organ consisting of trabecular and cortical bone, which maintains the body's physical structure as well as calcium homeostasis for physiological processes (Rosen, 2005). Bone tissues are constantly remodeled via the mechanisms of bone resorption by osteoclasts and bone formation from osteoblasts, and maintained by osteocytes (Dipla et al., 2021; Seibel, 2005). Major factors affecting bone remodeling are nutrition and hormones, and mechanical loading. The key nutrients including protein, calcium, phosphorus, and vitamin D, are essential for bone formation, and adequate EA is important for inducing anabolic status and hormone secretion for bone formation (Dipla et al., 2021). Gonadal and metabolic hormones are closely related to bone remodeling in athletes. In females, increased estrogen affects calcium absorption in the intestine and decreases osteoclast activities, while progesterone increases osteoblast activity. (Duff & Chilibeck, 2020). Therefore, disturbances of estrogen and progesterone by energy deficiency increase the risk of impaired bone health in female athletes, which is discussed in the FAT model. In males, testosterone affects calcium absorption and osteoblasts activities, resulting in bone formation. Disruptions in the H-P-G axis due to energy deficiency in male athletes can impair the testosterone balance, resulting in bone mass reduction and increased risks of bone injuries (Duff & Chilibeck, 2020). IGF-1 is the most important growth factor for bone formation, by promoting synthesis of the proteins and cell proliferation, and GH indirectly influences bone remodeling by stimulating the secretion of IGF-1 (Tortora & Derrickson, 2018). Thyroid hormones and insulin also affect bone remodeling through the stimulation of osteoblasts and protein synthesis (Tortora & Derrickson, 2018). As leptin affects GnRH secretion, H-P-G axis disruption can suppress testosterone

production in males. Therefore, leptin also can influence bone remodeling indirectly in athletes (Duff & Chilibeck, 2020). Mechanical loading leads to the adaptive process of bone mediated by cellular mechanotransduction (Santos et al., 2017). This process stimulates the secretion of cellular factors activating the bone formation of osteoblasts, and various hormonal and growth factors respond to the mechanical loading, which results in dynamic bone metabolism (Rosen, 2005).

Bone turnover markers and bone mineral density

Diagnosis of bone health in athletes is critical for the prevention of bone injuries and diseases. Various methods have been developed to screen and monitor the bone status of athletes. The metabolic processes of bone remodeling are controlled by endogenous and exogenous factors, as previously discussed, and various bone turnover markers (BTMs) are produced and released during the processes (Banfi et al., 2010). Alkaline phosphatase is a membrane-bound enzyme originating from various organs, and the differences in isoforms of alkaline phosphatases help to distinguish bone alkaline phosphatase (BAP) specifically (Seibel, 2005). BAP is related to bone mineralization, and the analysis of BAP can indicate osteoblast activity and bone formation (Banfi et al., 2010). Carboxy-terminal collagen cross-links (CTx) and amino-terminal cross-linking telopeptide (NTx) are the cross-linked telopeptides produced by the degradation of bone type 1 collagen (Banfi et al., 2010). CTx and NTx are cross-linked by peptides, and they are released into the circulation after proteolytic degradation (Banfi et al., 2010). The growth in length and thickness of bone until adolescence are influenced by humoral substances, muscle activity, and various local factors (Tortora & Derrickson, 2018). Accumulation of bone mass peaks during late teens to early twenties, and then, bone mass and BMD are maintained or regulated by bone remodeling processes as discussed earlier. Therefore, BTM measurements

can be used to detect the current dynamics of bone metabolism, and BMD analysis can provide the consequences of long-term bone metabolism, especially in bone mass and structure (Banfi et al., 2010).

Effects of energy deficiency on bone health

Energy deficiency in athletes can, directly and indirectly, influence bone health. Bone remodeling processes are closely related to the endocrine responses associated with energy metabolism and the sympathetic nervous system (Banfi et al., 2010). Studies on athletes with menstrual dysfunction and the FAT model provide accumulated evidence of impaired bone health which is related to amenorrhea and energy deficiency. A cross-sectional study of runners versus sedentary controls found that there were lower concentrations of bone formation markers, estradiol, T_3, IGF-1, and EB in amenorrheic runners compared to eumenorrheic runners and controls (Zanker & Swaine, 1998). A laboratory-controlled study also detected that a low EA (15 kcal/kg FFM/d) resulted in a higher concentration of bone resorption markers and a lower concentration of bone formation markers, leptin, and insulin, compared to a normal EA in females (45 kcal/kg FFM/d) (Papageorgiou et al., 2017). Studies on the BMD of female athletes demonstrated that amenorrheic athletes had a lower BMD and a higher fracture risk than eumenorrheic athletes (Ackerman et al., 2015; Heikura et al., 2018; Southmayd et al., 2017). Laboratory-controlled energy restriction in male distance runners found decrease in the bone formation markers and IGF-1 concentrations and the correlation between them (Zanker & Swaine, 2000). An observation study on male professional jockeys presented low EI (~1785 kcal/d) and high CTx within the clinically normal range (Wilson et al., 2013). In a study of Japanese male distance runners, low EA (18.9 ± 6.8 kcal/kg FFM/d), low BMD Z-score (-1.1 ± 0.6), and higher NTx than the normal ranges were also found (Taguchi et al., 2020).

However, interaction between bone metabolism and energy deficiency and exercise may differ depending on the gender and types of physical activity. A short-term (5-day) cross-over study with low EA (15 kcal/kg FFM/d) and normal EA (45 kcal/kg FFM/d) presented that low EA resulted in changes of BTMs only in females. Previous cross-sectional studies of various sports groups and controls have demonstrated that weight-bearing-related sports can result in higher BMD and positive BTMs than other sports and controls (Maimoun et al., 2004; McCormack et al., 2019; Prouteau et al., 2006; Schipilow et al., 2013). Therefore, in the future, longitudinal studies to examine the bone metabolism and hormonal status with energy deficiency in various sports should be considered, with the aim of providing further scientifical evidence for preventing impaired of bone health in athletes.

1.5.4 Effects on energy metabolism

REE or resting metabolic rate (RMR) refers to the EE for maintaining the basic metabolisms of the body. In the general population, REE accounts for 60%-75% of TEE which is affected by body composition, hormones, age, gender, and genetic factors (Van Zant, 1992). In athletes, the REE can account for 20%-55% of TEE, which results from excessive EEE (Ebine et al., 2002). Energy deficiency resulting in alterations in the endocrine system and body composition of athletes can impair the metabolism with a decrease in REE. Studies in female athletes presented that amenorrheic runners had lower RMR and worse eating patterns than eumenorrheic runners, and a low EA was associated with a low RMR in female endurance athletes (Melin et al., 2015; Myerson et al., 1991). A comparison study between low EI (2981 ± 762 kcal/d) and adequate EI (4471 ± 969 kcal/d) in male endurance athletes found that low EI athletes had lower RMR than adequate EI athletes without differences in body composition (Thompson et al., 1993). A laboratory-controlled 10-day intervention

on healthy males found that an increased EI led to an increased RMR (Goran et al., 1994). Another study investigating the effect of 4-week of intensified training on RMR without changes in EI demonstrated absolute and relative RMR decrease in trained male rowers and cyclists (Stenqvist et al., 2020; Woods et al., 2017). A similar observational study of a 6-week training program in trained male cyclists also demonstrated a decreased RMR when training was increased, and a positive association between relative RMR and EI (Woods et al., 2018). Accumulated studies provide evidence to support an association between energy deficiency and REE alterations. However, as previously discussed, prolonged energy deficiency can result in physiological adaptations with suppressed metabolic functions, which makes it difficult to interpret the metabolic status of athletes. Therefore, further research with enhanced diagnostic methods for the metabolic status of athletes and a longitudinal study design to understand the effect of energy deficiency on REE of athletes is necessary.

Table 1.2 Summary of intervention studies investigating the effect of energy deficiency on physiological function in males

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Costill et al. (1988)	10-day intervention Group comparison	Trained swimmers 12 M	Group A 3631 kcal/d Group B 4682 kcal/d	↑ 200% 1142→2293 kcal/d	A: EA ~20 k/k/d B: EA ~36 k/k/d	Glycogen ↓: A ↓ > B ↓ Distance/Stroke: A ↓ vs. B ↔	Group categorized by subjective fatigue
Goran et al. (1994)	4 EB group 10-day intervention	Healthy young 19 M	EI ↔ EI ↑ 50% EI ↑ 50% EI ↔	PA ↔ PA ↑ 50% PA ↔ PA ↑ 50%	EB ↔ EB ↑ EB ↔ EB ↓	RMR ↔ RMR ↑ RMR ↑ RMR ↔	Significant effect of EI on RMR
Zanker & Swaine (2000)	2 EB × 3-day intervention	Well trained 8 M	EI ↔ EI ↓ 50%	Prescribed exercise	EB ↔ EB ↓	P1NP ↓ & IGF-1 ↓ in EB ↓ condition	Significant relation between P1NP & IGF-1 reduction
Costa et al. (2005)	6-day intervention Group comparison	Trained triathletes 32 M	HCHO 2949→4246 kcal/d CON 2818 kcal/d	↑ 1 h running 70% VO_2 max	N/A	HCHO s-IgA ↑ CON cortisol ↑, glucose ↓	Effect of EI on immune function during training

(*continued*)

Table 1.2 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Rämson et al. (2008)	4-week intervention	Trained rowers 8 M	Ad libitum EI ↑ 4488→4880 kcal/d	EEE ↑ 150-160%	EB -455 kcal/d	RESTQ-Sport ↓ Response to exercise leptin↓ TNF-α↓	Effect of energy deficiency on recovery and leptin
Areta et al. (2014)	4 EA × 5-day intervention	Resistance trained 7 F, 8 M	N/A	N/A	EA 45 k/k/d + rest EA 30 k/k/d + rest EA 30 k/k/d +exercise + protein or no protein	Myofibrillar protein synthesis EA 45 > 30 EA 45 ≤ 30 + exercise	Effect of low EA on MPS and exercise and/or protein restore MPS
Abedel malek et al. (2015)	7-day intervention	Judo 11 M	EI ↓ 3475→2192 kcal/d	N/A	N/A	Body composition ↓ Performance ↓ Baseline testosterone ↓ Hormone response to exercise cortisol, GH ↑ testosterone ↓	Effect of energy deficiency on endocrine system and performance

(*continued*)

Table 1.2 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Koehler et al. (2016)	4 EA × 4-day intervention	Exercising 6 M	EI 15 k/k/d EI 30 k/k/d EI 40 k/k/d EI 55 k/k/d	EEE 0 k/k/d EEE 15 k/k/d EEE 0 k/k/d EEE 15 k/k/d	EA 15 k/k/d EA 15 k/k/d EA 40 k/k/d EA 40 k/k/d	Leptin ↓ & insulin ↓ in 15 k/k/d conditions	Effect of energy deficiency on endocrine system, but no effect on testosterone, T_3, IGF-1
Svendsen et al. (2016)	2 condition × 8-day intervention	Cyclists 13 M	LCHO 3513 kcal/d HCHO 4159 kcal/d	EEE ~725 kcal/d	N/A	Intensified training results in Performance, ACTH ↓ cortisol ↑ Inflammatory markers ↑ LCHO ↑ > HCHO ↑	Modest effect of HCHO on physiological and immunological function
Papageorgiou et al. (2017)	2 EA × 5-day intervention	Healthy 11 F, 11 M	EI 60 k/k/d EI 30 k/k/d	EEE 15 k/k/d EEE 15 k/k/d	EA 45 k/k/d EA 15 k/k/d	No significant changes in males	Significant effect of low EA on BTM, leptin, and insulin in females, but no effect on males

(*continued*)

Table 1.2 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Killer et al. (2017)	2 condition × 9-day intervention	Cyclists 13 M	LCHO 3501 kcal/d HCHO 4148 kcal/d	↑ 153%	N/A	Performance and sleep efficiency ↓ Mood ↓ LCHO ↓ > HCHO ↓	Effect of intensified training on performance and mood
Poffé et al. (2019)	3-week intervention Group comparison	Healthy 18 M	Ad libitum Ketone EI ↑ CON EI ↔	EEE ↑ 120% →165% →300%	Ketone EB ↔ CON EB ↓	Performance Ketone > CON GDF15 ↑ Ketone ↑ < CON ↑	Effect of energy deficiency on performance and overtraining
Hackney et al. (2019)	18-week intervention	Recreational runner 9 M	Ad libitum	EEE ↑ 25% →50% →75%	N/A	Body mass, free testosterone, T:C ratio ↓ Performance ↑	T:C ratio is not always marker for OTS

(*continued*)

Table 1.2 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Kojima et al. (2020)	2 EA × 3-day intervention	Long distance runner 7 M	EI 38 k/k/d EI 72 k/k/d	EEE 19 k/k/d	LEA 19 k/k/d NEA 53 k/k/d	BW, FFM, skeletal muscle volume, muscle glycogen, IGF-1 ↓ in LEA	Effect of low EA on body composition and IGF-1
Murphy and Koehler (2020)	3 condition × 3-day intervention	Recreational weightlifters 2 F, 5 M	CEA: EI 15 k/k/d + CHO PEA: EI 15 k/k/d +protein CON: EI 40 k/k/d +CHO	N/A Resistance exercise response	CEA 15 k/k/d PEA 15 k/k/d CON 40 k/k/d	Body mass ↓ CEA, PEA ↓ > CON ↓ GH ↑ CEA, PEA ↑ > CON ↑ IGF-1 ↓ CEA, PEA ↓ > CON ↓	Effect of low EA on endocrine response to exercise
Areta et al. (2020)	2 condition × 2-day intervention	Endurance athletes 9 M	EI 70 k/k/d EI 49 k/k/d	EEE 30 k/k/d	NEA 40 k/k/d LEA 19 k/k/d	p53 gene expression ↑ NEA < LEA Response to recovery drink after exercise Glucose, insulin AUC NEA > LEA	No effect of EA on gene expression related to skeletal muscle adaptation

(*continued*)

Table 1.2 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Stenqvist et al. (2020)	4-week intervention	Trained cyclist 20 M	Ad libitum EI ↔	↑ 3 times/week	N/A	Performance ↑ Testosterone, cortisol ↑ T_3, RMR ↓	Effect of energy deficiency on metabolism

Abbreviations: AUC=area under the curve, BTM = bone turnover marker, CON = control, GDF15 = growth differentiation factor 15, HCHO = high carbohydrate, k/k/d = kcal/kg FFM/d, LCHO = low carbohydrate, LEA = low energy availability, M = males, MPS = myofibrillar protein synthesis, N/A = not available, NEA = normal energy availability, PA = physical activity, RESTQ-Sport = recovery stress questionnaire for athletes, T:C = testosterone:cortisol, TNF-α = tumor necrosis factor alpha.

Table 1.3 Summary of observation studies investigating the effect of energy deficiency on physiological function in males

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Noland et al. (2001)	Observation during training season 9-week	Trained swimmers 12 F, 9 M	Ad libitum	↑ 147%	N/A	Females BM, EI, leptin ↔ BF ↓ Males BM, BF, leptin ↔ EI ↓	No effect of energy deficiency on leptin
Karamouzis et al. (2002)	Pre- & post-race comparison	Trained swimmers 16 M	Ad libitum	Swimming 6.5-10.5 h	N/A	Leptin ↓	Effect of acute energy deficiency on leptin
Jurema e et al. (2003)	Observation 3-week heavy training & 2-week tapering period	Trained rowers 12 M	Ad libitum	↑100%	N/A	Leptin, insulin ↓	Effect of acute energy deficiency on hormones
Kraemer et al. (2008)	Pre- & post-race comparison	Endurance athletes 9 M	N/A	160 km Ultra-marathon	N/A	BM ↓ Cortisol, GH, IL-6 ↑ Testosterone ↓	Effect of acute energy deficiency on hormones

(*continued*)

Table 1.3 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Cangemi et al. (2010)	Cross-sectional Observation	CR 24 M ER 24 M CON 24 M	CR 1350-2415 kcal/d ER 2564-4345 kcal/d CON 2145-3537 kcal/d	N/A	N/A	Testosterone and free androgen index: CR < ER, CON 17β-estradiol and estradiol:SHBG: CR, ER < CON SHBG: CR > CON	Effect of long-term (3-20 years) calorie restriction on reproductive hormones
Mäestu et al. (2010)	Cross-sectional Observation 11-week	Bodybuilders CR 7 M CON 7 M	EI ↔	EE CR > CON	EB CR < CON	CR: BM, fat % ↓ Testosterone, IGF-1, insulin ↓ Cortisol ↑	Effect of energy deficiency on hormones
Wilson et al. (2013)	Cross-sectional Observation	Professional jockeys Flat 19 M Jump 18 M	Flat 1459 kcal/d Jump 1784 kcal/d	N/A	N/A	CTx, PTH higher than average in both groups Mood state: Flat < Jump	Effect of low EI on bone metabolism and mood

(*continued*)

Table 1.3 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Koehler et al. (2013)	Cross-sectional Observation	Athletes 185 F, 167 M	Males LEA 31.6 k/k/d NEA 48.2 k/k/d	Males LEA 10.9 k/k/d NEA 9.0 k/k/d	Males LEA 21.7 k/k/d NEA 38.2 k/k/d	No significant group differences in hormone concentrations	Lack of associations between EA and hormones
Woods et al. (2017)	Pre- & post-comparison 4-week training	Elite rowers 5 F, 5 M	EI ↔	↑ 21±7%	N/A	Absolute RMR ↓ RMR/kg FFM ↓ Performance ↓ Fatigue, mood/sleep disturbance ↑	Effect of energy deficiency on metabolism, performance, and mood
Geesmann et al. (2017)	Pre- & post-race comparison	Trained cyclists 14 M	EI 6070-12620 kcal/d	EE 9689-13585 kcal/d	EB -5271-+1597 kcal/d	Testosterone, IGF-1, leptin ↓ Positive correlation between EB and IGF-1	Effect of energy deficiency on hormones
Hooper et al. (2017)	Cross-sectional Observation	Distance runners with EHMC EHMC 9 M CON 8 M	EHMC 2623 kcal/d CON 2743 kcal/d	EHMC 914 kcal/d CON 0 kcal/d	EHMC 27.2 k/k/d CON 45.4 k/k/d	Testosterone: EHMC < CON Aging male symptoms questionnaire score: EHMC > CON	Low EA and impaired reproductive function in EHMC runners

(*continued*)

Table 1.3 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Torstveit et al. (2018)	Cross-sectional Observation	$NRMR_{ratio}$ 11 M $SRMR_{ratio}$ 20 M	N/A	$NRMR_{ratio}$ 662 kcal/d $SRMR_{ratio}$ 675 kcal/d	$NRMR_{ratio}$ 41 k/k/d $SRMR_{ratio}$ 37 k/k/d	Largest single-hour energy deficit: (-) association with cortisol (+) association with T:C ratio	Effect of energy deficiency on hormone
Woods et al. (2018)	Multiple time point observation	Trained cyclists 13 M	EI ↔	↑ 120% →141% →147% →79% →73%	N/A	RMR, BM, BF, HR variability, performance ↓ Mood disturbance ↑ (+) associations between relative RMR and EI	Effect of EI on metabolism, but no effect of energy deficiency on hormone
Cadegiani and Kater (2018)	Cross-sectional Observation	OTS 14 M ATL 25 M CON 12 M	OTS 2673 kcal/d ATL 4114 kcal/d CON 4473 kcal/d	N/A	N/A	EI, sleep quality, libido, mood, BMR: OTS < ATL EI, libido: OTS < CON	Association between OTS and EI

(*continued*)

Table 1.3 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Heikura et al. (2018)	Cross-sectional: testosterone concentration or EA Observation	ER 35 F, 24 M Testosterone LTS 10 M NTS 14 M EA LEA 6 M NEA 18 M	EI: kJ/kg/d LTS 212 NTS 212 LEA 185 NEA 222	Running: km/week LTS 117 NTS 110 LEA 130 NEA 107	LTS 31 k/k/d NTS 35 k/k/d LEA 21 k/k/d NEA 37 k/k/d	Total testosterone, T_3: LTS < NTS Total testosterone, EA: LEA < NEA Negative associations between fracture history and testosterone	Association between testosterone and EA
Keay et al. (2018)	Observation	Road cyclists 50 M	N/A	Average hours on bike/week 12.5	LEA N=14 Chronic LEA 10/14 ED/DE 5/10	EA determine lumbar spine BMD Z-score Testosterone: chronic LEA < NEA	Effect of low EA on BMD and testosterone (Low EA assessed using SEAF-I)
McCormack et al. (2019)	Cross-sectional Observation	Cross-country runners 33 F, 27 M CON 24 F, 23 M	Males CCR 2662 kcal/d CON 2257 kcal/d	Males CCR 1189 kcal/d CON 192 kcal/d	Males CCR 35.6 k/k/d CON 41.6 k/k/d	Femoral neck, total hip, whole body BMD: CCR < CON Eating concern: CCR > CON 42% of male CCR with low EA	Effect of low EA on BMD

(*continued*)

Table 1.3 (*continued*).

Author	Study design	Participants	EI	EE	Energy status	Outcomes	Comments
Taguchi et al. (2020)	Observation	Long-distance runners 6 M	2482 kcal/d	1516 kcal/d	18.9 k/k/d	REE_{ratio}: 0.90±0.05 NTx: 28.5±7.1 (normal value: 9.5-17.7)	Suppressed metabolism and increased bone resorption in low EA runners

Abbreviations: ATL = healthy athletes, BF = body fat mass, BM = body mass, CCR = cross-country runners, CON = control, CR = calorie restriction, ER = endurance runners, F = females, k/k/d = kcal/kg FFM/d, LEA = low energy availability, LTS = low testosterone, M = males, N/A = not available, NEA = normal energy availability, $NRMR_{ratio}$ = normal RMR_{ratio}, NTS = normal testosterone, PTH = parathyroid hormone, SEAF-I = sport-specific questionnaire and clinical interview, $SRMR_{ratio}$ = suppressed RMR_{ratio}, T:C = testosterone:cortisol.

1.6 Aims of the research

As previously discussed, the RED-S model was originally developed from the FAT model, and therefore, most of the studies supporting the RED-S model have focused on female athletes. The recommendation of further research of the RED-S model in male athletes in the IOC consensus statement helped to increase research interests and connect related perspectives. A recent consensus statement on the female and male athlete triad coalition has suggested three interrelated conditions in male athletes parallel the FAT (Nattiv et al., 2021). Moreover, it shared the pathophysiology of EHMC, except for the adaptation to prolonged stress. A recent narrative review on the OTS and RED-S suggested that the conditions have common triggers, pathways, symptoms, and complex diagnoses (Stellingwerff et al., 2021).

Previous consensus statements and reviews on energy deficiency of athletes mainly discussed endurance and weight-sensitive sports, such as cyclists, jockeys, combat sports, and distance runners (Burke et al., 2018a; De Souza et al., 2019b; Mountjoy et al., 2018; Nattiv et al., 2021). The athletes competing in these sports have high risks of low EA and energy deficiency due to acute and chronic weight control and excessive EEE. However, studies on the athletes competing in different sports also presented excessive EE and risk of energy deficiency. A study on self-reported EA of various sports groups demonstrated that ball and racquet sports groups exhibited a low EA (<30 kcal/kg FFM/d) (Koehler et al., 2013). Among various ball sports, soccer is the world's most popular sport played by more than 265 million players (Herrero et al., 2014), but there are limited studies on the energy deficiency of soccer players. The monitoring of EA on female soccer players presented reduced EA (<45 kcal/kg FFM/d) during the competition season (Moss et al., 2020; Reed et al., 2013). In male soccer players, negative EB was observed during the training and

match season (Hannon et al., 2021; Rico-Sanz et al., 1998; Russell & Pennock, 2011), but these studies did not analyze the EA status and energy deficiency of male soccer players which is remained to be elucidated.

The influence of different ethnicities on the pathophysiology of energy deficiency in athletes has not been clearly investigated yet. The difference in ethnicities may not affect human biology and health, but the interactions between the genome and different environments can result in various phenotypes and increase the biological diversity (Fuentes et al., 2019). Social-environmental factors can also affect the balance of exercise and recovery. Previous studies on the race and ethnicity associations with nutrition knowledge presented that there was lower sport nutrition knowledge in adolescents Latino soccer players than White and adolescents from low socioeconomic backgrounds could have inadequate food intake patterns (Manore et al., 2017; Neumark-Sztainer et al., 1998). Furthermore, the number of hours for working and studying in OTS athletes were higher than normal athletes which could be related to the effect of excessive cognitive activity on recovery (Cadegiani & Kater, 2018). These studies presents that the socioeconomic aspects can affect EI and recovery of the athletes, which may result in energy deficiency. Previous studies on energy deficiency in athletes have mainly been conducted in Caucasians and western countries. The number of studies in different ethnic groups, especially in Asians, relating to the RED-S model is insufficient. To date, there are few studies analyzing the EA and investigating the effect of low EA on Asian athletes (Kojima et al., 2020; Quah et al., 2009; Taguchi et al., 2018; Taguchi et al., 2020). This highlights the need for further research in Asian athletes, using various study designs.

There is insufficient research on the energy deficiency of Asian male athletes, including soccer players, who also could have high risks of energy deficiency and

related health issues. Different food habits, training cultures, and different social-environmental factors can modify the triggers of energy deficiency, physiological and psychological responses, treatments, and the prevention of energy deficiency in athletes. Therefore, it is important to better understand the current EA status and possible physiological alterations resulting from energy deficiency in Asian male athletes. In addition, analysis of energy deficiency in free-living athletes using EA analysis in conjunction with REE_{ratio} and WDEB analysis can help to detect the energy deficiency and its associations with physiological functions.

This dissertation aimed to understand of the pathophysiology of energy deficiency and the assessment of energy status with biochemical analysis in Asian male collegiate soccer players.

Aim 1

To assess the EA, hormones, and bone status in Korean male collegiate soccer players. This will be described in Study 1 (Chapter 3).

Aim 2

To evaluate the metabolic suppression by energy deficiency using the WDEB in Korean male collegiate soccer players. This will be described in Study 2 (Chapter 4).

Chapter 2. General methodology

The data for Study 1 and 2 were obtained by conducting an observational and cross-sectional study involved the university soccer team in Korea during their pre-competition training period in October 2018. Anthropometric assessments, dual-energy X-ray absorptiometry (DXA) scans, maximal oxygen uptake test, REE measurements, blood and urine sampling, and the questionnaires were conducted in Korea. Condition check forms, food diary sets, physical activity record sets were given to the participants for self-recording.

2.1 Study design and ethical approval

All data collection was completed in one month: one day for initial profile recording and orientation, two laboratory visits for the assessments, 7-consecutive days of self-recording, and one day for the interview and questionnaires. During the initial profile recording and orientation day, participants were informed of the study procedures and the risks of assessments. Informed consent forms were signed. A 4-week condition check form was given to each participant. During the two laboratory visits, anthropometric assessments, body composition analysis, maximal oxygen uptake (VO_2 max) test, REE measurements, and blood and urine sampling were conducted. After the laboratory visits, participants were instructed to record the 7-consecutive day food diary and document physical activities by wearing heart rate (HR) sensors and accelerometers. Following the self-recording period, participants were interviewed for data checking and completed the questionnaires. This study was approved by the Human Research Ethics Committee of Waseda University for use of human subjects in accordance with the Declaration of Helsinki (2018-082).

2.2 Participants

The recruitment was conducted in the university soccer team competing in a national university league in Korea. Fifteen male soccer players aged 18-21 years were recruited. During the initial meeting for orientation, basic profiles were recorded including the date of birth, position, exercise history, current training goal, residence type, meal preparation, food preference and allergy, use of supplements, and smoking and alcohol consumption. All participants were non-smokers without any health issues and have been training soccer for 7-12 years. One participant dropped out due to personal reasons before the self-recording period. Two participants sustained injuries, and one participant only completed 3-consecutive days of the recording during the one-week self-recording period.

2.3 Anthropometric and body composition assessments

During the first visit to the laboratory, participants removed their watch and jewelry, and wore light half sleeves and shorts for assessment of height and body mass. Height was measured to the nearest 0.1 cm using a digital stadiometer (BSM 330, Biospace, Seoul, Korea), and body mass was measured to the nearest 50 g using a digital scale (UC-321, A&D Medical, Tokyo, Japan). BMI (kg/m^2) was calculated using the collected height and body mass data.

After the anthropometric measurements, the participants completed body composition assessments using a DXA scanner (Lunar Prodigy Advance with enCORE software version 16, General Electric, Madison, Wisconsin, USA) by a certified technician. From the DXA scan results, total BMD, bone mineral content, percent body fat, FM, FFM, skull area, and lean tissue in extremities data were collected. BMD was evaluated by the Z-score.

2.4 Maximal oxygen uptake

VO_2 max was determined by an incremental test using a bicycle ergometer (Ergomedic 828E, Monark, Varberg, Sweden) after the DXA scans. Participants were asked to refrain from strenuous exercise 24 h before the incremental test. After 3 min rest on the ergometer, participants performed a 3-min warm-up with 90 W workloads to become accustomed to the cadence. After the warm-up, participants performed an initial workload of 120 W, which was increased by 30 W every 2 min until exhaustion. During the test procedure, expired air was continuously monitored, and oxygen uptake (VO_2), carbon dioxide production (VCO_2), and rate of exchange ratio (RER) were measured by a breath-by-breath gas analyzer using Quark b version 10.0 (Cosmed, Rome, Italy). HR was continuously monitored by a heart rate sensor using H7 and A300 fitness tracker (Polar Electro Oy, Kempele, Finland). When the incremental test ended, VO_2 max was determined when the participants showed the following criteria: unable to maintain 60 rpm, RER >1.1, VO_2 plateau, HR max (220 - age), and rate of perceived exertion >19.

2.5 Energy intake

EI was assessed from the analysis of 7-consecutive days (5 training days + 2 rest days) food diary with digital photography and nutrition information on food packets during the self-recording period. Participants were instructed to record all consumed foods and beverages using the provided cooking scale (SD-004, Tanita, Tokyo, Japan), as well as time of day and location. Photographs of consumed foods and beverages were taken with a 15-cm ruler as a reference. The food records and photos were checked during an interview to clarify any ambiguous information. EI was analyzed using the Computer-Aided Nutritional Analysis Program (CAN-Pro 5.0, The Korean Nutrition Society, Seoul, Korea) by a registered dietitian (PhD candidate of the thesis).

2.6 Resting energy expenditure

During the second visit to the laboratory, REE was measured by open-circuit indirect calorimetry using the Douglas bag method between 7:00 a.m. and 9:00 a.m. Participants were asked to refrain from caffeine and alcohol consumption, and avoided exertion 24 h before the measurements (Compher et al., 2006). Participants arrived at the laboratory at 7:00 a.m. after an overnight fast. After a 20 min rest in the supine position to acclimatize to the room temperature, participants rested wearing a mask (Hans Rudolph, Kansas, Missouri, USA) and were familiarized with the equipment. Resting HR and body temperature were measured to confirm a resting status. Participants were asked to remain awake, quiet, and relaxed without motion to prevent possible skeletal muscle contractions during the measurements. After confirming the rested status, 10 min expired gas samples were collected in Douglas bags. VO_2 and VCO_2 were assessed by a gas analyzer using AE-100i (Minato Medical Science, Osaka, Japan), and expired gas volume and temperature were assessed by a dry gas volume meter using DC-5A (Shinagawa, Tokyo, Japan). REE_m was determined using the Weir equation: $3.94 \times (VO_2) + 1.1 \times (VCO_2)$ (Weir, 1949). The gas collection was continued until less than 5 % of REE_m differences between the two samples among the collected samples. The mean of intra-individual variance in REE_m was 3.2 ± 1.6%.

2.7 Exercise energy expenditure

During the self-recording period, participants were instructed to wear HR sensors and trackers (H7 and A300 fitness tracker, Polar Electro Oy, Kempele, Finland) during the scheduled team training and individual training. EEE was determined by the FLEX-HR method (Leonard, 2003). From the VO_2 max test, FLEX-HR (the average of highest rest HR and lowest exercise HR) and maximum HR of participants were recorded, and individual HR-VO_2 and HR-VCO_2 regression equations were obtained.

Minute-by-minute HR data of exercise, between the FLEX-HR and maximum HR, was applied to the regression equations, and VO_2 and VCO_2 during exercise were estimated. VO_2 and VCO_2 of the HR data below the FLEX-HR or above the maximum HR were applied to the VO_2 and VCO_2 of rest HR and maximum HR during the VO_2 max test, respectively. From the VO_2 and VCO_2, EE was calculated using the Weir equation (Weir, 1949). The net EEE was calculated by subtracting REE_m of exercise time.

2.8 Energy availability

EA was calculated using the following equation: EA = (EI - net EEE)/FFM (Loucks et al., 2011; Taguchi et al., 2020).

2.9 Ratio between measured and predicted resting energy expenditure

To evaluate the metabolic suppression of participants, predicted REE (REE_p) was determined, and the ratio between REE_m and REE_p (REE_{ratio} = REE_m/REE_p) was calculated. REE_p was determined using the tissue-organ level models (Hayes et al., 2002). The major tissues and organs mass affecting REE were predicted using the data from DXA scans. The detailed prediction models and the calculation of REE_p are explained in Table 2.1. The REE_{ratio} 0.94 was used as a cut-off value to evaluation of the metabolic suppression of participants (Staal et al., 2018; Strock et al., 2020).

Table 2.1 The calculation of REE_p using the tissue-organ level models (Hayes et al., 2002)

Tissue & Organ	Mass (kg) prediction model	Energy (kcal/d) prediction model
Brain	$Brain_m$ = 0.005 × skull area (cm^2) + 0.2 × sex + 0.24	$Brain_{EE}$ = 240 × $Brain_m$
SM	SM_m = LST (kg) × 1.13 - 0.02 × age + 0.61 × sex + 0.97	SM_{EE} = 13 × SM_m
Bone	$Bone_m$ = 1.85 × bone mineral (kg)	$Bone_{EE}$ = 2.3 × $Bone_m$
AT	AT_m = 1.18 × fat mass (kg)	AT_{EE} = 4.5 × AT_m
Residual	$Residual_m$ = BW - ($Brain_m$ + SM_m + $Bone_m$ + AT_m)	$Residual_{EE}$ = 43 × $Residual_m$
Total	BW = $Brain_m$ + SM_m + $Bone_m$ + AT_m + $Residual_m$	REE_p = $Brain_{EE}$ + SM_{EE} + $Bone_{EE}$ + AT_{EE} + $Residual_{EE}$

Note. Sex = 0 for females, 1 for males. m = mass, EE = energy expenditure, SM = skeletal muscle, LST = appendicular lean soft tissue, AT = adipose tissue.

2.10 Blood analysis

During the second visit to the laboratory, fasting blood samples were drawn from the participants after the REE measurements via an antecubital vein in a sitting position by a certified nurse. Eight and a half milliliters of blood were dispensed into a Vacuette gel serum tube (Greiner Bio-One GmbH, Kremsmünster, Austria). After 30 min clotting, the samples were centrifuged for 10 min at 3000 rpm to collect serum. Collected serum samples were stored frozen until analysis. Three milliliters of blood were dispensed into a Vacuette EDTA whole blood tube (Greiner Bio-One GmbH, Kremsmünster, Austria) and gently mixed. Collected whole blood samples were refrigerated until analysis. Urine samples were taken from the midstream of the first urine after wake-up. Five milliliters of urine were dispensed into a conical tube (Falcon, Los Angeles, California, USA) and stored frozen until analysis. All the blood and urine sample analyses were conducted in the clinical laboratory (GC Labs, Yongin, Korea). The reference ranges for the biochemical analyses were provided from the clinical laboratory (Table 2.2).

Table 2.2 Reference ranges for biochemical analysis

	Reference value		
	Lower	Upper	Unit
Hormones			
FSH	1.5	12.4	mIU/mL
LH	1.7	8.6	mIU/mL
Estradiol	5.2	43.9	pg/mL
T_3	0.8	2.0	ng/mL
Cortisol	3.7	19.4	μg/dL
Insulin	2.6	24.9	μU/mL
IGF-1	247.3	481.7	ng/mL
GH	0.97	4.7	ng/mL
Testosterone	2.40	8.71	ng/mL
Bone turnover markers			
s-CTx	0.05	0.45	ng/mL
s-BAP		20.1	μg/L
u-NTx	21	83	nM BCE/mM Creatinine

Note. FSH = follicle-stimulating hormone, LH = luteinizing hormone, T_3 = triiodothyronine, IGF-1 = insulin-like growth factor-1, GH = growth hormone, s-CTx = serum carboxy-terminal cross-linked telopeptide of type 1 collagen, s-BAP = serum bone alkaline phosphatase, u-NTx = Urine amino-terminal cross-linked telopeptide of type 1 collagen

2.11 Questionnaires

During the initial profile recording and orientation day, participants were asked to record the 4-week daily condition check form which includes the 7-point subjective condition assessment after wake-up (body temperature, HR, weight, condition, and fatigue), after training (training time, intensity, volume, and fatigue), and before sleeping (fatigue).

During the last meeting for the interview and questionnaires, participants completed the Eating Attitude Test 26 (EAT-26) and Profile of Mood States 2 (POMS-2) questionnaires. The validated Korean version of EAT-26 was used to screen DE/ED in participants (Kang et al., 2006; Logue et al., 2018). A total score of 19 or above was considered as DE. A score of 15 to 18 was considered to be at risk of early-stage of DE (Rhee et al., 1998). The Korean version of POMS-2 was developed for this study using the Japanese edition of POMS-2 and the original POMS-2 (Heuchert & McNair, 2012; Heuchert et al., 2015). Seven subscales (anger-hostility, confusion-bewilderment, depression-dejection, fatigue-inertia, tension-anxiety, vigor-activity, and friendliness) and Total Mood Disturbance (the sum of five negative subscales score minus vigor-activity score) were assessed to analyze mood and emotional disturbance of participants.

2.12 Statistical analysis

IBM SPSS statistics (Version 26, IBM Japan, Tokyo, Japan) was used for statistical data analysis. All data were initially tested for normality using the Shapiro-Wilk test. In Study 1 (Chapter 3), normally distributed data were presented as mean ± SD, and non-normally distributed data were presented as medians and interquartile ranges (IQ 25 and 75). In Study 2 (Chapter 4), all data were presented as mean ± SD. An independent *t*-test was used for normally distributed data. The Mann-Whitney *U*

test was used for non-normally distributed data. Pearson's correlation analysis was used for normally distributed data and Spearman's rho was used for non-normally distributed data in Study 2. For all tests, statistical significance was set at $p < 0.05$.

Chapter 3. Study 1

Association of low energy availability and suppressed metabolic status in Korean male collegiate soccer players

3.1 Objective

EA is defined as the amount of energy remaining after exercise for physiological functions. The fundamental physiological functions for cellular maintenance, thermoregulation, growth, and reproduction can be suppressed because of insufficient EA, which can result in health issues (Nattiv et al., 2007). The IOC published a consensus statement on RED-S using this etiological background, and it promoted interest and research on the effects of EA in various athletic populations, including male athletes with a high risk of low EA (Mountjoy et al., 2018). Most of the previous studies of EA, however, have been conducted on females, weight-class, and endurance athletes of Caucasian descent, with only a limited number of studies involving subjects of males, different ethnicities, and other sports populations (Mountjoy et al., 2018). In males, alterations in the hypothalamic-pituitary-testicular axis were reported in the subjects who had intensive and chronic exercise training (Hackney, 2008). This condition was termed “Exercise-hypogonadal male condition (EHMC)” and has been studying in exercising males. Hackney (2020) suggests that the mechanisms of physiological alterations by low EA in females can support the development of EHMC. EA studies in males presented the effects of low EA on the alteration of the endocrine system, and there were similar responses of low EA in BTMs in females and males (Koehler et al., 2016; Papageorgiou et al., 2017). A previous study on Japanese male runners (19-21 years) has identified that a high risk of having low EA in this population exists, leading to metabolic suppression and

increased bone resorption (Taguchi et al., 2020). Studies on professional female and male soccer players during the training period provided evidence that energy deficiency and low EA can be found in different sports (Moss et al., 2020; Reed et al., 2014; Russell & Pennock, 2011). These studies can support the IOC consensus statement on RED-S in male athletes with different ethnicities and sports backgrounds. However, it still requires more evidence, and there have been no studies on EA status and physiological factors of Korean male athletes. The purpose of this study was to evaluate the EA status of Korean collegiate male athletes during the training period and to investigate its association with metabolic status, bone metabolism, and hormonal status.

3.2 Material and methods

Participants

Fifteen male Korean collegiate soccer players aged 18-21 years were recruited from a local university team competing in a national university league. During the assessment period, three participants were excluded because of injury and personal reasons. Data of twelve participants (age: 19.1 ± 0.7 years, height: 175.8 ± 5.1 cm, body mass: 69.61 ± 5.79 kg) were analyzed for this study.

Blood analysis

Blood samples were drawn after REE measurements via the antecubital vein in a sitting position by a certified nurse. S-CTx, FSH, LH, estradiol, T_3, and cortisol were assessed via an electrochemiluminescence immunoassay method, and serum BAP (s-BAP), IGF-1, GH were assessed via a chemiluminescent immunoassay method. Leptin was assessed via a radioimmunoassay, and testosterone was assessed via a chemiluminescent microparticle immunoassay. All blood sample

analyses were conducted in the clinical laboratory (GC Labs, Yongin, Korea). Reference values were provided from the analysis laboratory.

Energy intake

EI was assessed from 7-day food records with digital photography and nutrition information on food packets. In this study, 3-day (3 training days + 1 rest days) data of 12 participants were analyzed.

Energy availability

EA was determined as EI minus EEE relative to FFM in kilograms: (EI kcal/d - net EEE kcal/d)/FFM kg (Taguchi et al., 2020). Participants who exhibited <30 kcal/kg FFM/d of average EA were considered to have low EA (Melin et al., 2019). Participants were then categorized into two groups with low EA (LEA, n = 5) and high EA (HEA, n = 7).

Questionnaires

During their last visit to the laboratory, participants answered the EAT-26 and POMS-2 for the evaluation of mood disturbances, ED, and DE (Mountjoy et al., 2018).

Statistical analysis

IBM SPSS statistics (Version 26, IBM Japan, Tokyo, Japan) was used for statistical data analysis. Normally distributed data were presented as mean ± SD, while non-normally distributed data were presented as medians and interquartile ranges (IQ 25 and 75). An independent t-test (normally distributed data) and Mann-Whitney U test (non-normally distributed data) were used to evaluate the differences between LEA and HEA groups. For all tests, statistical significance was set at p <0.05.

3.3 Results

The descriptive characteristics of the 12 participants who completed the study are presented in Table 3.1. There were no differences in age, height, body mass, BMI, BMD, body fat, and VO_2 max between the LEA and HEA groups. The LEA group showed a significantly higher FFM than the HEA group (LEA: 63.2 ± 4.5 kg, HEA: 57.9 ± 3.2 kg, $p = 0.037$).

The mean EA and REE-related parameters are presented in Table 3.2. The mean EA of all participants (31.9 ± 9.8 kcal/kg FFM/d) was above the cut-off value (30 kcal/kg FFM/d), but the LEA group showed a significantly lower EA than the HEA group (LEA: 22.4 ± 2.9 kcal/kg FFM/d, HEA: 38.7 ± 6.6 kcal/kg FFM/d, $p < 0.001$). There were only two participants who had an EA above 45 kcal/kg FFM/d in the HEA group. The HEA group showed a significantly higher EI (LEA: 3114 ± 297 kcal/d, HEA: 3701 ± 348 kcal/d, $p = 0.012$), REE/FFM (LEA: 26.0 ± 1.7 kcal/kg/d, HEA: 28.8 ± 1.4 kcal/kg/d, $p = 0.011$), and REE_{ratio} (LEA: 0.91 ± 0.06, HEA: 1.01 ± 0.05, $p = 0.008$) than the LEA group. The participant with the lowest REE_{ratio} (0.84) had the lowest EA (18.7 kcal/kg FFM/d). There was no difference in EEE between the groups.

The blood analysis results are presented in Table 3.3. Both groups showed increased BTMs compared to the reference values, but there was no significant difference between the groups. The LEA group had higher FSH concentrations than the HEA group (LEA: 5.50 ± 1.01 mIU/mL, HEA: 3.64 ± 1.41 mIU/mL, $p = 0.031$), but there was no significant difference in LH concentrations between the groups. Both groups showed increased testosterone and GH concentrations without any difference between the groups. The LEA group had lower IGF-1 concentrations than HEA (LEA: 248.6 ± 51.2 ng/mL, HEA: 318.9 ± 43.4 ng/mL, $p = 0.028$). There were no significant differences in cortisol, T_3, and leptin concentrations between the groups.

The POMS-2 and EAT-26 results are presented in Table 3.4. No significant difference was presented in the total scores of the POMS-2 result between the groups as well as in the subscales except friendliness. None of the participants showed ED and DE according to EAT-26 results, and there was no significant difference between the groups.

3.4 Discussion

In this study, the mean EA of all participants was 31.9 ± 9.8 kcal/kg FFM/d, with 5 out of 12 participants (41.7%) showing <30 kcal/kg FFM/d and only 2 participants showing an EA higher than 45 kcal/kg FFM/d. Participants with low EA exhibited metabolic suppression and lower REE_{ratio} (<0.94) as well as REE/FFM, but there were no significant alterations of the endocrine system and bone metabolism. Metabolic suppression and low EA status can affect bone metabolism and hormonal status, especially in endurance, weight category, and aesthetic athletes. To the best of our knowledge, this study is the first study to investigate EA status and related parameters in Korean male soccer players. The result of this study shows that the effects of low EA can vary depending on the sport and population group.

Energy availability status

In this study, 83% of the participants had an EA lower than 45 kcal/kg FFM/d, with more than 40% having an EA lower than 30 kcal/kg FFM/d. Although the prevalence of low EA was high, the mean EA of all the participants was 31.9 kcal/kg FFM/d, which was slightly higher than 30 kcal/kg FFM/d. Previous studies have reported that endurance athletes were identified to have a higher prevalence (63%) of low/reduced EA (<45 kcal/kg FFM/d) than other athletes in different sports (36%) (Melin et al., 2015). The participants of this study were team sport (soccer) players

and the prevalence of low EA was 83%, which was higher than the previous study. Studies on male athletes with a high risk of low EA reported that the mean EA of male cyclists were 8 and 20 kcal/kg FFM/d while that of jockeys was 12 kcal/kg FFM/d (Dolan et al., 2011; Viner et al., 2015; Vogt et al., 2005). A comparison study among various sports groups has reported that the mean EA of male athletes in ball sports was 27.5 kcal/kg FFM/d, but there was no difference compared to endurance sports (26.9 kcal/kg FFM/d) and weight-class sports (24.9 kcal/kg FFM/d) (Koehler et al., 2013). The mean EA of this study was 31.9 kcal/kg FFM/d, which was a relatively higher EA than the previous studies involving male athletes. All the participants stayed in the University dormitory and had the same food menus and training schedules. Individual differences may have been due to the participants' additional individual training and snack consumption. The high prevalence of low EA can be explained by the high amounts of scheduled team training. On the other hand, the low severity of reduced EA can be explained by the scheduled meals and the additional intakes from 24/7 delivery foods and snacks in the convenience store of the dormitory. Athletes' energy intakes and expenditures are largely affected by their environments (Mountjoy et al., 2018). These environmental factors should be taken into consideration when either monitoring or controlling the energy status of athletes in free-living conditions, or both.

Energy availability and metabolic suppression

The IOC consensus statement on RED-S introduced the risks of low EA in athletes, with many studies presenting dysfunction of physiological systems due to metabolic suppression by low EA (Mountjoy et al., 2018). In this study, the LEA group showed lower REE/FFM (26.0 kcal/kg/d vs 28.8 kcal/kg/d) and REE_{ratio} (0.91 vs. 1.01) than the HEA group. Many studies on metabolic suppression in athletes have reported

its relationship with insufficient energy intake and excessive exercise. In female athletes, the participants with low EA (19.1 kcal/kg FFM/d) exhibited suppressed metabolisms compared to the participants with optimal EA (51.7 kcal/kg FFM/d); having an RMR_{ratio} 0.87 vs. 0.93, respectively (Melin et al., 2015). Research on male cyclists reported a reduction of absolute and relative RMR after 6-week of being in an energy deficit status (Woods et al., 2018), while the previous study on Japanese male endurance runners reported low EA (18.9 kcal/kg FFM/d) with suppressed REE/FFM (26.4 kg/FFM/d) (Taguchi et al., 2020). These studies support the association between low EA and metabolic suppression, which was also presented in this study. Metabolic suppression resulting from low EA can be explained by adaptive mechanisms for preserving essential energy, with these possibly resulting in various physiological dysfunctions which can increase risks of negative health and performance consequences in athletes (De Souza et al., 2019b; Mountjoy et al., 2018). Monitoring and diagnosis of athletes' metabolic status using REE and EA are important to prevent physiological dysfunctions. For the evaluation of metabolic suppression, De Souza et al. (2007) was the first to present the concept of REE_{ratio} in athletes and calculated predicted REE using the Harris-Benedict equation (Harris & Benedict, 1918). Other studies, however, assessed REE_{ratio} using different methods, such as organ tissue assessment or the Cunningham equation (Taguchi et al., 2020; Torstveit et al., 2018), with the accuracy and validity of the methods differing depending on the population groups (Kim et al., 2015; Taguchi et al., 2011). A study on exercising women suggested that RMR_{ratio} can be an alternative method to assess the energy status (Strock et al., 2020).

Energy availability and bone markers

In this study, the participants showed high bone absorption (s-CTx: 0.93 ng/mL)

and bone formation markers (s-BAP: 23.9 μg/L) without low BMD (1.384 g/cm^2), and there was no association between bone markers and EA. Previous studies on athletes reported decreased bone metabolism and BMD related to low EA. In exercising women, energy deficiency was associated with decreased osteocalcin (De Souza et al., 2008), while a study on male athletes reported a 15% decrease in N-terminal pro-peptide of type 1 collagen concentrations after 3-day of 50% energy restriction (Zanker & Swaine, 2000). Prolonged energy deficiency can result in either hypothalamic or hypogonadal status, or both, as well as altered hormonal concentrations, which can increase abnormal bone remodeling (Hackney, 1996; Nattiv et al., 2007). Bone quality and structure, however, are can vary according to the type of exercise done by athletes (De Souza et al., 2019c). In a comparison study between different sports groups, skiers and soccer players exposed to loading environments showed 28%-38% higher failure load indicating stronger bone at the distal tibia than swimmers (Schipilow et al., 2013), which identified that osteogenic stimuli such as weight-bearing activity can have protective effects on bone. This may explain the results of this study, which found that high BMD co-occurred with low EA in male soccer players. A high prevalence of bone stress injuries in male collegiate runners has been reported (De Souza et al., 2019c), but the effects of energy deficiency on bone metabolism can be clearly understood with consideration of the continuous and complex nature of bone remodeling.

Energy availability and endocrine markers

In this study, there was no suppression of reproductive hormone concentrations regardless of EA status, and among the metabolic hormones, only IGF-1 concentrations were lower in the LEA group than in the HEA group. Previous studies in men and women reported that an energy deficit could result in a suppressed endocrine system, leading to suppressed hypogonadal conditions and metabolic

hormone concentrations (Geesmann et al., 2017; Hackney, 2008; Kraemer et al., 2008; Loucks & Thuma, 2003; Loucks et al., 1998). A cross-over study, involving males using EA of 15 and 40 kcal/kg FFM/d for interventions, reported that there were no differences in reproductive and metabolic hormones between the groups except for leptin (Koehler et al., 2016). A similar study using EA of 15 and 45 kcal/kg FFM/d did not identify any differences in the hormone concentrations of male participants (Papageorgiou et al., 2017). In this study, there were no significant alterations of the endocrine system except the lower IGF-1 concentrations in the participants with low EA. Other studies have reported male athletes to be at high risk for endocrine system suppression (De Souza et al., 2019b). Notably, male athletes have different reproductive mechanisms and susceptibilities to energy deficiency and thus, the current cut-off value (<30 kcal/kg FFM/d) may not be appropriate to identify the clinical signs of reproductive suppression as well as the symptoms of suppressed metabolic status due to either low EA or excessive training, or both (De Souza et al., 2019b; Hackney, 2020).

This study is the first study to investigate the EA status of male athletes in Korea using recognized methodologies and biochemical analysis, but there are several limitations. The study involved a small number of participants given that it was observational research. The limited experimental period restricted the frequency of biochemical analysis. Estimation of EI relied on the analysis of food records which can have under- and over-reporting of the participants, and the complexity and variety of food ingredients in Asian culture make it difficult to access the EI of the free-living participant. To reduce the error in EI estimation, the present study implemented the weighed dietary records with photo records using a smartphone. Despite the limitations, this study can provide evidence to understand low EA in male athletes and

help promote heightened interest in EA research in Asian countries.

The main finding of this study was a high prevalence of low EA and inadequate mean EA levels, which were associated with metabolic suppression in Korean male athletes. However, low EA had no effect on bone metabolism, reproductive hormones, or psychological parameters. In conclusion, low EA can be related to metabolic suppression without leading to changes in bone metabolism and hormonal status in Korean male soccer players. In future studies, to identify the cut-off value and the effects of energy deficiency on physiological functions, the inclusion of a larger number of participants and longitudinal monitoring of energy deficiency of male athletes involved in various sports types using a precise monitoring method is needed.

Table 3.1 Descriptive characteristics of the participants

	Total (*n* = 12)	LEA (*n* = 5)	HEA (*n* = 7)	*p* value
Age (years)	19.0 (19.0-19.5)	19.0 (19.0-19.0)	19.0 (18.0-20.0)	0.647
Height (cm)	175.8 ± 5.1	178.4 ± 6.7	173.9 ± 2.9	0.212
Weight (kg)	69.61 ± 5.79	72.70 ± 7.10	67.40 ± 3.78	0.122
BMI (kg/m^2)	22.5 ± 1.2	22.8 ± 1.2	22.3 ± 1.3	0.488
Body fat (kg)	9.5 ± 2.3	8.7 (8.0-8.9)	9.1 (8.1-11.6)	0.685
Body fat (%)	13.6 ± 2.6	12.0 (11.4-12.3)	13.3 (13.0-16.2)	0.254
FFM (kg)	60.1 ± 4.5	63.2 ± 4.5	57.9 ± 3.2	0.037
BMD (g/cm^2)	1.384 ± 0.075	1.396 ± 0.091	1.376 ± 0.068	0.677
VO_2 max (ml/kg/min)	54.9 ± 5.7	57.7 ± 6.8	52.9 ± 4.2	0.152

Note. Normally distributed data were presented as mean ± SD, and nonnormally distributed data were reported by median (interquartile range). BMI = body mass index, FFM = fat-free mass, BMD = bone mineral density.

Table 3.2 EA and REE-Related parameters of the participants

	Total (n = 12)	LEA (n = 5)	HEA (n = 7)	p value
EI (kcal/d)	3456 ± 435	3114 ± 297	3701 ± 348	0.012
EEE (kcal/d)	1747 ± 334	1890 ± 154	1645 ± 400	0.226
EA (kcal/kg FFM/d)	31.9 ± 9.8	22.4 ± 2.9	38.7 ± 6.6	<0.001
REE				
REE_m (kcal/d)	1654 ± 129	1636 ± 112	1668 ± 147	0.691
REE/FFM (kcal/kg/d)	27.6 ± 2.1	26.0 ± 1.7	28.8 ± 1.4	0.011
REE_p (kcal/d)	1715 ± 114	1805 ± 82	1651 ± 88	0.012
REE_{ratio} (REE_m/REE_p)	0.97 ± 0.07	0.91 ± 0.06	1.01 ± 0.05	0.008

Note. Normally distributed data were presented as mean ± SD. EA = energy availability, EI = energy intake, EEE = exercise energy expenditure, REE = resting energy expenditure, REE_m = measured resting energy expenditure, REE_p = predicted resting energy expenditure.

Table 3.3 Bone turnover markers and hormone concentrations of the participants

	Total (n = 12)	LEA (n = 5)	HEA (n = 7)	p value	Reference value
Bone metabolism					
s-BAP (μg/L)	23.9 ± 6.5	21.4 ± 4.9	25.7 ± 7.3	0.289	<20.1
s-CTx (ng/mL)	0.93 ± 0.18	0.93 ± 0.16	0.92 ± 0.20	0.920	0.05-0.45
Hormone					
FSH (mIU/mL)	4.42 ± 1.54	5.50 ± 1.01	3.64 ± 1.41	0.031	1.5-12.4
LH (mIU/mL)	5.08 ± 1.93	5.96 ± 1.05	4.46 ± 2.24	0.197	1.7-8.6
Testosterone (ng/mL)	8.06 ± 2.19	8.72 ± 1.81	7.58 ± 2.44	0.401	2.40-8.71
Estradiol (pg/mL)	40.0 (33.5-42.0)	34.0 (33.0-40.0)	42.0 (38.0-42.0)	0.251	5.2-43.9
GH (ng/mL)	7.77 ± 7.31	7.16 ± 7.26	8.20 ± 7.89	0.819	≤3.0
IGF-1 (ng/mL)	289.6 ± 57.3	248.6 ± 51.2	318.9 ± 43.4	0.028	247.3-481.7
Cortisol (μg/L)	11.2 ± 3.2	10.6 ± 4.4	11.6 ± 2.2	0.676	3.7-19.4
T_3 (ng/mL)	1.06 ± 0.16	1.02 ± 0.13	1.09 ± 0.18	0.500	0.8-2.0
Leptin (ng/mL)	2.2 ± 0.9	1.9 ± 0.8	2.3 ± 1.0	0.484	

Note. Normally distributed data were presented as mean ± SD, and nonnormally distributed data were reported by median (interquartile range). s-BAP = serum bone alkaline phosphatase, s-CTx = serum C-terminal telopeptide of type 1 collagen, FSH = follicle stimulating hormone, LH = luteinizing hormone, GH = growth hormone, IGF-1 = insulin-like growth factor 1, T_3 = triiodothyronine.

Table 3.4 POMS-2 and EAT-26 scores of the participants

	Total (n = 12)	LEA (n = 5)	HEA (n = 7)	p value
POMS-2	3.4 ± 8.3	0.6 ± 5.1	5.4 ± 9.9	0.345
Anger-Hostility	1.7 ± 1.4	2.6 ± 1.5	1.0 ± 1.0	0.051
Confusion-Bewilderment	2.9 ± 2.2	2.2 ± 1.6	3.4 ± 2.5	0.363
Depression-Dejection	0.5 (0.0-2.0)	0.0 (0.0-2.0)	1.0 (0.0-2.0)	0.718
Fatigue-Inertia	7.1 ± 3.3	6.8 ± 2.5	7.3 ± 4.0	0.817
Tension-Anxiety	3.0 ± 2.3	2.4 ± 2.1	3.4 ± 2.5	0.471
Vigor-Activity	12.2 ± 3.4	14.2 ± 3.4	10.7 ± 2.6	0.073
Friendliness	11.5 (11.0-13.0)	13.0 (12.0-14.0)	11.0 (10.0-11.0)	0.013
EAT-26	3.8 ± 1.8	4.2 ± 1.3	3.4 ± 2.1	0.482

Note. Normally distributed data were presented as mean ± SD, and nonnormally distributed data were reported by median (interquartile range). POMS-2 = profile of mood states 2, EAT-26 = eating attitude test 26.

Chapter 4. Study 2

Within-day energy balance and metabolic suppression in male collegiate soccer players

4.1 Objective

EB is important for maintaining appropriate body weight and preventing the negative effects of excessive and insufficient energy. In athletes, excessive EE from training and matches is inevitable, but EI is often inadequate to achieve an appropriate energy status. The imbalanced energy status of athletes has been widely studied, and the concept of RED-S suggested the effect of low EA, which can result in altered health and performance in both male and female athletes (Mountjoy et al., 2014).

EA is calculated by subtracting EEE from EI and normalized to FFM. Monitoring EA is important to prevent various physiological impairments, including endocrine, metabolic, and psychological problems (Mountjoy et al., 2018), but assessing precise EA in free-living athletes is difficult because of limitations resulting from measurement errors and burden in athletes (Burke et al., 2018b).

Recent studies have presented the concepts of WDEB and the ratio between measured and predicted REE (REE_m:REE_p = REE_{ratio}) to understand energy deficiency and metabolic suppression (Fahrenholtz et al., 2018; Staal et al., 2018; Strock et al., 2020; Torstveit et al., 2018). Assessment of WDEB measures EI and EE in 1 h intervals, and real-time changes in energy status help identify the imbalanced energy within a day (Deutz et al., 2000). Traditional multiple-day EB and EA assessments could mask the energy deficiency by the compensation effect, while WDEB can detect the hidden energy deficiency with more physiological relevance,

showing association with liver glycogen and brain glucose availability (Benardot, 2007; Benardot, 2013; Torstveit et al., 2018). The results of previous studies on WDEB have shown associations between negative WDEB status and metabolic suppression in male and female athletes (Fahrenholtz et al., 2018; Torstveit et al., 2018). Reduction in REE can be interpreted as the alterations in metabolically active organs and tissues, and reduced REE has been reported in clinical energy deficiency, which is 60%-80% of the predicted REE (De Souza et al., 2007). The difference between measured and predicted REE can identify the metabolic suppression by energy deficiency, and REE_{ratio} is significantly associated with the conditions related to energy deficiency in exercising women (De Souza et al., 2019a; De Souza et al., 2008; Reed et al., 2015).

In addition to EA measurement, the concepts of WDEB and REE_{ratio} can provide options to monitor the energy deficiency of athletes in free-living status. However, there are limited studies on the WDEB and REE_{ratio} of male athletes, as this has been mainly studied in female participants (Arroyo et al., 2018; Fahrenholtz et al., 2018). To the best of our knowledge, the research by Torstveit et al. (2018) is the only study to present the relationship between energy deficiency and metabolic suppression in male athletes by evaluating the WDEB and REE_{ratio}. Nutritional analysis of soccer players clearly showed that there is a high risk of energy deficiency, but there is a lack of studies examining the association between energy deficiency and the markers of RED-S (Moss et al., 2020; Reed et al., 2013; Rico-Sanz et al., 1998; Russell & Pennock, 2011).

In Study 1, there was a relation between low EA and decreased IGF-1 concentration, but there was no other physiological dysfunction related to low EA. Previously mentioned limitations of EA analysis in free-living athletes may affect exhibiting energy deficiency and its associations with physiological functions of the

participants.

Therefore, the purpose of this study was to evaluate and analyze WDEB and REE_{ratio} in male soccer players in the free-living status and to investigate the relationship between the markers of energy deficiency and metabolic suppression.

4.2 Material and methods

Participants

Among the 12 participants who completed the data collection in Study 1, two participants were excluded because of non-compliance with wearing devices and absence from training which resulted in incomplete data for 7 consecutive days. Data of 10 participants (mean ± SD; age 19.1 ± 0.6 years; height, 175.8 ± 5.5 cm; weight, 69.81 ± 6.14 kg) were analyzed in this study.

Resting energy expenditure measurements and blood analysis

REE_{ratio} was calculated as the REE_m/REE_p, and a participant with less than 0.94 REE_{ratio} was considered to be metabolically suppressed (Staal et al., 2018; Strock et al., 2020). Participants were then categorized into two groups with the normal REE_{ratio} (n = 5) and the suppressed REE_{ratio} (n = 5).

After the REE measurements, blood samples were collected in the fasting state. T_3, cortisol, and insulin were assessed via an electrochemiluminescence immunoassay method, and IGF-1 and GH concentrations were assessed via a chemiluminescent immunoassay method. All blood sample analyses were conducted in a clinical laboratory (GC Labs, Yongin, Korea). Reference values were obtained from the analysis laboratory.

24 h energy intake

Participants recorded all consumed foods and beverages for one week (5 training days + 2 rest days). Validity of the dietary records was analyzed using the Goldberg cut-off, and all participants met the criteria for plausible reporters (EI:REE_p = 1.17-2.78) (Black, 2000).

24 h energy expenditure

TEE can be divided into REE, DIT, and activity thermogenesis (Levine, 2005). In this study, activity thermogenesis was divided into EEE and NEAT, and they were assessed separately. To prevent overestimation of EEE, only the purposeful exercise of participants, such as individual and team training, was analyzed for estimation of EEE. Non-purposeful daily activities including >4.0 metabolic equivalents (METs) of intensity were analyzed for estimation of NEAT. In addition, excessive post-exercise oxygen consumption (EPOC) was calculated to consider the elevated REE after exercise (Phelain et al., 1997). TEE was calculated using the following equation: TEE = REE_m + DIT + net EEE + EPOC + net NEAT.

Resting energy expenditure and sleeping energy expenditure

The hourly REE was calculated from REE_m, and during the sleeping hours, sleeping energy expenditure (SEE) was calculated as 90% of REE_m (Torstveit et al., 2018). To monitor the current metabolic status of the participants, adapted REE_m was used instead of unadapted REE_p.

Diet-induced thermogenesis

DIT was calculated as 10% of EI and calculated hourly after the meal or snack using the following equation: $175.9 \times T \times e^{-T/1.3}$ (Reed & Hill, 1996; Torstveit et al.,

2018).

Exercise energy expenditure and excessive post-exercise oxygen consumption

The net EEE was calculated by subtracting the REE_m during training. EPOC was determined as 5% of net EEE in the first hour and 3% of net EEE in the second hour post-exercise (Phelain et al., 1997; Torstveit et al., 2018).

Non-exercise activity thermogenesis

NEAT was determined by analyzing the data from the 7-consecutive day physical activity record and accelerometer (Active Style Pro HJA-750C, Omron, Kyoto, Japan). Participants were instructed to wear the accelerometer on the waist from wake-up to sleep, and were only allowed to remove it during showering, swimming, and training. METs of participants were obtained from the accelerometer data, and it was confirmed by the physical activity records and interview. NEAT was calculated using the following equation: NEAT = duration (min) × METs × 3.5 (mL/kg/min) × 0.005 (kcal/mL) × weight (kg) (Ainsworth et al., 2011; Gifford et al., 2020). The net NEAT was calculated by subtracting REE_m of activity time.

Within-day energy balance

To evaluate the within-day energy deficiency (WDED) of participants, 24 h EB, total hours with energy deficit, total hours spent in exceeding 400 kcal of energy deficit, and the largest single-hour energy deficit during the day were calculated using the WDEB analysis (Benardot, 2007; Benardot, 2013; Torstveit et al., 2018). 24 h EB was calculated as the sum of hourly EB of the day, and the hourly EB was calculated using the following equation: EB = EI - TEE; REE_m (or SEE) + DIT + net EEE + EPOC + net NEAT. Total hours with energy deficit (WDEB <0 kcal) was determined by the sum of total hours of hourly EB <0 kcal, and total hours spent in exceeding 400 kcal of energy

deficit (WDEB <-400 kcal) was determined by the sum of total hours of hourly EB <-400 kcal (Torstveit et al., 2018).

Within-day energy availability

To prevent the masking effect of EA calculation method, the hourly EA was also calculated by subtracting the net EEE from EI relative to FFM in kilograms for every hour, and 24 h EA was calculated as the sum of hourly EA (Torstveit et al., 2018).

Statistical analysis

IBM SPSS statistics (Version 26, IBM Japan, Tokyo, Japan) was used for statistical data analysis with a statistical significance level of p <0.05. All data were tested for normality using the Shapiro-Wilk test and are presented as mean ± SD. Differences between the normal (REE_{ratio} ≥0.94) and suppressed (REE_{ratio} <0.94) groups were analyzed using the independent *t*-test (normal distribution) and Mann-Whitney *U* test (non-normal distribution). The associations of REE_{ratio} and WDED variables with metabolic markers were analyzed using Pearson's correlation (normal distribution) and Spearman's rho (non-normal distribution).

4.3 Results

The descriptive characteristics of the 10 participants are presented in Table 4.1. There were no differences in the descriptive characteristics between the normal and suppressed groups.

The REE_{ratio} and WDEB characteristics with the total 7-day, training, and rest day variables are presented in Tables 4.2-4.4. There were significant differences in the REE_{ratio}, REE_m/FFM, EI, and DIT between the groups. Table 4.2 shows that the normal group had higher REE_{ratio} and REE_m/FFM than the suppressed group (REE_{ratio} 1.03 ± 0.05 vs. 0.90 ± 0.04, p = 0.002 and REE_m/FFM 29.4 ± 1.0 vs. 25.7 ± 1.4 kcal/kg/day,

p = 0.001), and total 7-day EI and DIT were significantly higher in the normal group than in the suppressed group (EI 3660 ± 347 vs. 3024 ± 491 kcal/day, *p* = 0.046 and DIT 364 ± 33 vs. 301 ± 49 kcal/day, *p* = 0.043). There were no significant group differences during training days (Table 4.3). Table 4.4 shows that the rest day EI was significantly higher in the normal group than in the suppressed group (3772 ± 463 vs. 2796 ± 800 kcal/day, *p* = 0.046).

The associations between the REE_{ratio} and WDED variables and metabolic markers are presented in Table 4.5. The REE_{ratio} was significantly positively associated with the IGF-1 concentration (*r* = 0.771, *p* = 0.009). There were no other significant associations between the WDED variables and metabolic markers.

Hourly changes in the WDEB during the training days are presented in Figure 4.1. The lowest hourly EB was -1505 ± 246 kcal at 17:00, which was after 2 h of scheduled training spending 1103 ± 134 kcal, and after 1 h of morning training at 8:00 a.m., EB was -976 ± 33 kcal, which was spending 403 ± 32 kcal. The hourly EI was high at 8:00-9:00, 11:00-12:00, and 17:00-18:00, consuming 711 ± 82 kcal, 546 ± 256 kcal, and 689 ± 196 kcal, respectively. At the end of the day, the energy deficit was -957 ± 503 kcal.

Hourly changes in the EA during the training days are presented in Figure 4.2. The EA decreased after scheduled (7:00-8:00 and 16:00-17:00) and individual (10:00-11:00) training. The hourly EA increased, except during sleeping (0:00-6:00) and training (7:00-08:00, 16:00-17:00, and 10:00-11:00) hours, and there were large increases at 8:00-9:00, 11:00-12:00, and 17:00-18:00 spending 11.8 ± 1.2, 9.0 ± 4.6, and 11.5 ± 3.9 kcal/kg FFM, respectively. The 24 h total EA was 25.6 ± 9.8 kcal/kg FFM/day.

4.4 Discussion

The present study analyzed the WDEB and investigated its relationship with metabolic status in Korean male collegiate soccer players. In this study, half of the participants (5 out of 10) had suppressed REE with lower total EI, rest day EI, and total DIT compared to the normal participants. The REE_{ratio} had a significantly positive relationship with IGF-1 concentrations, but there was no association between the WDED variables and metabolic markers. Hourly changes in the WDEB during training days showed a severe energy deficit after training with insufficient compensation of EI, resulting in negative EB at the end of the day.

In this study, 50% of the participants showed metabolic suppression (REE_{ratio} <0.94) and had significantly lower REE_{ratio} than normal participants (1.03 ± 0.05 vs. 0.90 ± 0.04, p = 0.002). The participants in the normal group had higher total and rest day EI than those in the suppressed group (total EI 3660 ± 347 vs. 3024 ± 491 kcal/day, p = 0.046, rest day EI 3772 ± 463 vs. 2796 ± 800 kcal/day, p = 0.046), but there were no other significant differences in the WDEB variables. In a previous study on male endurance athletes, the participants with suppressed RMR had significantly lower measured RMR (kcal/h), time in severe negative EB (WDEB <-400 kcal), and larger single-hour energy deficit than the normal participants, but there was no significant difference in 24 h EA and 24 h EB (Torstveit et al., 2018). Another WDEB study on female endurance athletes showed that athletes with menstrual disturbance spent a longer time in WDEB <0 kcal than eumenorrheic athletes (Fahrenholtz et al., 2018). In contrast to the findings of previous studies, the present study found no significant difference in the WDED variables between the normal and suppressed groups, which might be due to the different prediction equations of REE, group categorization REE_{ratio} level, and WDEB data collection methods in this study. Previous studies on EB and

EA status of athletes reported differences in the EI, EEE, and EA between the training (or match) and rest days (Heikura et al., 2019; Moss et al., 2020; O'Brien et al., 2019; Vogt et al., 2005). Improved energy status of athletes on rest days may help to reduce the risk of physiological alterations, and studies on females showed that the addition of rest day with EI intervention could decrease the risk of menstrual dysfunction (Dueck et al., 1996; Guebels et al., 2014; Mountjoy et al., 2014). Despite the negative energy status in both groups on training days, the suppressed group had significantly lower EI on rest days than the normal group. Limited recovery of energy status during rest days may result in metabolic suppression

A significant association was found between the REE_{ratio} and IGF-1 (r = 0.771, p = 0.009) in this study, but there was no other association between energy status and endocrine markers. The association between the endocrine markers of metabolic suppression and energy status variables has been studied to diagnose and monitor the health of athletes. Previous studies on male athletes have reported that negative EB due to undernutrition and excessive exercise results in a decrease in IGF-1 concentration (Geesmann et al., 2017; Roemmich & Sinning, 1997), but low EA (15 kcal/kg FFM/day) did not affect metabolic hormones, except leptin and insulin (Koehler et al., 2016). Recent studies on WDEB in athletes reported associations of WDED with alterations in the cortisol concentrations and reproductive hormones (Fahrenholtz et al., 2018; Torstveit et al., 2018). Additionally, the measured-to-predicted RMR ratio of exercising female participants was associated with menstrual dysfunction and low T_3 concentrations (Strock et al., 2020). The present study could not identify a relationship between energy deficiency and reproductive hormones, but this study supports the findings of previous research that reported the association of REE_{ratio} with alterations in metabolic hormones. Most studies on energy deficiency have examined the effects

of low EA on the health and performance of athletes (Logue et al., 2020). Low EA results in metabolic suppression to preserve EE required by essential processes for survival, which can cause physiological dysfunction of less critical processes; previous studies on female and male participants suggested the threshold and optimal levels of EA to prevent the adverse effects of low EA (Ihle & Loucks, 2004; Koehler et al., 2016; Loucks & Thuma, 2003; Melin et al., 2019). Many studies have applied the suggested thresholds, but the results vary depending on the participant characteristics, measurement, and analysis methods (Cooper & Ackerman, 2020; Elliott-Sale et al., 2018). A recent review on the role of EA in athletes suggests the consideration of various assessment methods of energy status and of the application of the EA thresholds (De Souza et al., 2019b). The REE_{ratio} and WDEB analysis may provide supporting evidence to understand the negative effect of energy deficiency on metabolic suppression and endocrine alterations.

Due to severe energy deficit during training days (-957 ± 530 kcal/day), all participants showed negative 7-day EB (-561 ± 529 kcal/day), despite positive EB during rest days (429 ± 693 kcal/day). Analysis of WDEB during training days clearly showed the most significant energy deficit after the scheduled team training (-1505 ± 246 kcal, at 17:00). Energy status was slightly improved after EI from scheduled dormitory meals and snacks at the university cafeteria, but it could not fully compensate for excessive EE from the training sessions, resulting in negative 24 h total EB and 25.6 ± 9.8 kcal/kg FFM of 24 h total EA. A previous study on the hourly energy status of female collegiate soccer players presented negative 24 h EB (Behrens et al., 2019), which is in accordance with the results of this study. Additionally, the present study analyzed the hourly energy status to understand the reason for the severe energy deficit and to provide possible suggestions for the improvement of

energy status during training days. Previous studies on seasonal (training, match, and rest) and daily energy status of soccer players showed negative EB and decreased EA during the training and match seasons (Reed et al., 2013; Russell & Pennock, 2011), with significantly lower EA during heavy training and match days than during rest days (Moss et al., 2020). Studies on the daily distribution of EI and macronutrients in elite male soccer players have suggested the importance of collecting meal distribution data that can be related to training adaptations, performance, and health (Anderson et al., 2017; Bettonviel et al., 2016). Moreover, the importance of diet and training periodization has been widely studied, and the interaction between nutrition and exercise has been emphasized to enhance physiological adaptations and exercise capacity (Mujika et al., 2018). The present study observed hourly changes in energy status in free-living athletes; this provided the specific time and reason for the energy deficit during the day with the amount of energy required to prevent a negative energy state at the end of the day.

To our knowledge, this is the first study to analyze the WDEB variables and metabolic status in male collegiate soccer players, however, there are several limitations to the study design and methodologies. A small sample size of this study decreases the statistical power of analysis and limits the generalizability of the findings. Due to the relatively short period of the experiment, the time point of the blood analysis was restricted. Data collection of the 7-day WDEB variables relied on self-reported measurements in a free-living status, which could have resulted in under- and over-reporting of EI and the inaccuracy of activity records.

This study aimed to analyze the WDEB variables and REE_{ratio} of male athletes as the markers of energy deficiency and to investigate the relationship between energy deficiency and metabolic suppression. A significantly lower rest day EI in the

metabolically suppressed group (REE_{ratio} <0.94) and a positive association between REE_{ratio} and IGF-1 concentration were observed. Additionally, WDEB analysis of training days showed a severe energy deficit after training hours with insufficient energy consumption for daily EB.

In conclusion, metabolic suppression can be related to insufficient EI during rest days, which could not compensate for the severe energy deficit during training days and IGF-1 concentration in male soccer players. Monitoring REE_{ratio} and WDEB can help to provide practical advice and support to prevent the negative effects of energy deficiency in athletes. Future studies should consider a balance between recovery and training stress with environmental factors affecting energy deficiency and include a larger number of participants in different types of sports to obtain the data for optimal nutritional and training strategies.

Table 4.1 Descriptive characteristics of the participants

	Total (*n* = 10)	Normal (*n* = 5)	Suppressed (*n* = 5)	*p* value
Age (years)	19.1 ± 0.6	19.2 ± 0.8	19.0 ± 0.0	0.519
Height (cm)	175.8 ± 5.5	173.0 ± 1.9	178.7 ± 6.7	0.135
Weight (kg)	69.81 ± 6.14	67.66 ± 4.41	71.96 ± 7.34	0.294
BMI (kg/m^2)	22.5 ± 1.3	22.6 ± 1.4	22.5 ± 1.4	0.930
BMD (g/cm^2)	1.378 ± 0.081	1.391 ± 0.070	1.366 ± 0.097	0.655
Z-score	1.6 ± 0.8	1.8 ± 0.8	1.4 ± 0.8	0.416
Body fat (kg)	9.4 ± 2.4	9.2 ± 2.0	9.6 ± 3.0	0.831
Body fat (%)	13.3 ± 2.4	13.5 ± 2.3	13.1 ± 2.7	0.819
FFM (kg)	60.4 ± 4.3	58.5 ± 3.0	62.4 ± 4.7	0.159
FFM (%)	86.7 ± 2.4	86.5 ± 2.3	86.9 ± 2.7	0.819
VO_2 max (mL/kg/min)	55.6 ± 6.0	53.0 ± 4.6	58.2 ± 6.5	0.180

Note. BMI = body mass index, BMD = bone mineral density, FFM = fat-free mass; data are presented as mean ± SD.

Table 4.2 REE_{ratio}, EI, EE components, and WDED variables of the participants during total 7-day in total

	Total (*n* = 10)	Normal (*n* = 5)	Suppressed (*n* = 5)	*p* value
REE_{ratio} (REE_m/REE_p)	0.96 ± 0.08	1.03 ± 0.05	0.90 ± 0.04	0.002
REE_m/FFM (kcal/kg/day)	27.6 ± 2.3	29.4 ± 1.0	25.7 ± 1.4	0.001
EI (kcal/day)	3342 ± 522	3660 ± 347	3024 ± 491	0.046
DIT (kcal/day)	332 ± 52	364 ± 33	301 ± 49	0.043
Net EEE (kcal/day)	1391 ± 310	1458 ± 420	1324 ± 168	0.537
EPOC (kcal/day)	125 ± 28	132 ± 37	119 ± 17	0.513
Net NEAT (kcal/day)	456 ± 100	469 ± 70	443 ± 131	0.702
REE_h (kcal/day)	1057 ± 141	1131 ± 150	982 ± 93	0.096
SEE (kcal/day)	542 ± 42	531 ± 32	552 ± 52	0.473
TEE (kcal/day)	3903 ± 415	4085 ± 434	3721 ± 342	0.179
24 h EB (kcal)	-561 ± 529	-426 ± 621	-697 ± 444	0.450
24 h EA (kcal/kg FFM)	32.7 ± 11.0	37.8 ± 11.8	27.5 ± 8.0	0.146
WDEB <0 kcal (h/day)	20.2 ± 1.8	20.0 ± 1.9	20.4 ± 1.9	1.000

(*continued*)

Table 4.2 (*continued*).

	Total (*n* = 10)	Normal (*n* = 5)	Suppressed (*n* = 5)	*p* value
WDEB <-400 kcal (h/day)	11.9 ± 1.9	12.0 ± 1.9	11.8 ± 2.0	0.876
Largest hourly deficit (kcal)	-1509 ± 243	-1572 ± 277	-1446 ± 214	0.446

Note. REE_m = measured resting energy expenditure, REE_p = predicted resting energy expenditure, REE_m/FFM = ratio between measured resting energy expenditure and fat-free mass, EI = energy intake, DIT = diet-induced thermogenesis, EEE = exercise energy expenditure, EPOC = excess post-exercise oxygen consumption, NEAT = non-exercise activity thermogenesis, REE_h = hourly resting energy expenditure, SEE = sleeping energy expenditure, EB = energy balance, EA = energy availability, WDEB = within-day energy balance, data presented as mean ± SD.

Table 4.3. EI, EE components, and WDED variables of the participants during training days

	Total (*n* = 10)	Normal (*n* = 5)	Suppressed (*n* = 5)	*p* value
EI (kcal/day)	3365 ± 476	3615 ± 428	3116 ± 422	0.100
DIT (kcal/day)	332 ± 48	359 ± 42	304 ± 40	0.068
Net EEE (kcal/day)	1831 ± 357	1903 ± 508	1759 ± 124	0.567
EPOC (kcal/day)	165 ± 32	172 ± 45	158 ± 13	0.516
Net NEAT (kcal/day)	394 ± 114	393 ± 63	396 ± 160	0.974
REE_h (kcal/day)	1081 ± 133	1146 ± 151	1017 ± 81	0.132
SEE (kcal/day)	519 ± 47	518 ± 36	520 ± 60	0.966
TEE (kcal/day)	4323 ± 469	4491 ± 558	4154 ± 335	0.280
24 h EB (kcal)	-957 ± 530	-876 ± 701	-1038 ± 353	0.657
24 h EA (kcal/kg FFM)	25.7 ± 10.4	29.6 ± 12.8	21.9 ± 6.6	0.265
WDEB <0 kcal (h/day)	21.3 ± 1.3	21.0 ± 1.6	21.6 ± 1.1	0.511
WDEB <-400 kcal (h/day)	12.7 ± 1.6	12.8 ± 1.9	12.6 ± 1.3	0.854
Largest hourly deficit (kcal)	-1718 ± 278	-1792 ± 348	-1644 ± 197	0.434

Note. EI = energy intake, DIT = diet-induced thermogenesis, EEE = exercise energy expenditure, EPOC = excess post-exercise oxygen consumption, NEAT = non-exercise activity thermogenesis, REE_h = hourly resting energy expenditure, SEE = sleeping energy expenditure, EB = energy balance, EA = energy availability, WDEB = within-day energy balance, data presented as mean ± SD.

Table 4.4. EI, EE components, and WDED variables of the participants during rest days

	Total (*n* = 10)	Normal (*n* = 5)	Suppressed (*n* = 5)	*p* value
EI (kcal/day)	3284 ± 803	3772 ± 463	2796 ± 800	0.046
DIT (kcal/day)	335 ± 85	378 ± 47	292 ± 96	0.111
Net EEE (kcal/day)	291 ± 292	345 ± 279	238 ± 327	0.666
EPOC (kcal/day)	26 ± 26	31 ± 24	21 ± 29	0.666
Net NEAT (kcal/day)	609 ± 138	658 ± 142	560 ± 128	0.286
REE_h (kcal/day)	995 ± 172	1094 ± 156	895 ± 131	0.061
SEE (kcal/day)	598 ± 61	564 ± 46	632 ± 59	0.076
TEE (kcal/day)	2855 ± 398	3071 ± 268	2639 ± 410	0.084
24 h EB (kcal)	429 ± 693	701 ± 602	157 ± 730	0.234
24 h EA (kcal/kg FFM)	50.1 ± 16.0	58.8 ± 13.3	41.5 ± 14.5	0.075
WDEB <0 kcal (h/day)	17.8 ± 4.1	17.6 ± 3.6	18.0 ± 4.9	0.888
WDEB <-400 kcal (h/day)	9.8 ± 3.0	9.8 ± 2.8	9.8 ± 3.6	1.000
Largest hourly deficit (kcal)	-986 ± 239	-1021 ± 231	-951 ± 269	0.668

Note. EI = energy intake, DIT = diet-induced thermogenesis, EEE = exercise energy expenditure, EPOC = excess post-exercise oxygen consumption, NEAT = non-exercise activity thermogenesis, REE_h = hourly resting energy expenditure, SEE = sleeping energy expenditure, EB = energy balance, EA = energy availability, WDEB = within-day energy balance, data presented as mean ± SD.

Table 4.5 Associations of REE_{ratio} and WDEB variables with metabolic markers in the participants

	REE_{ratio} (REE_m/REE_p)		REE_m/FFM		WDEB <0 kcal (h/day)		WDEB <-400 kcal (h/day)		Largest Hourly Deficit (kcal)		24 h EB (kcal)		24 h EA (kcal/kg FFM)	
	r	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value
T_3	0.162	0.655	0.190	0.599	0.532	0.113	0.487	0.153	-0.564	0.090	-0.456	0.185	-0.234	0.514
Cortisol	0.144	0.691	0.208	0.565	-0.426	0.219	-0.498	0.143	0.206	0.568	0.188	0.602	0.208	0.564
IGF-1	0.771	0.009	0.590	0.072	0.106	0.771	0.223	0.536	-0.356	0.313	0.222	0.538	0.381	0.277
GH	0.566	0.088	0.509	0.133	0.275	0.442	0.475	0.165	-0.448	0.194	-0.117	0.748	-0.055	0.880
Insulin	0.151	0.678	0.046	0.899	0.043	0.906	0.108	0.767	-0.102	0.778	0.058	0.874	0.201	0.577
Testosterone	-0.552	0.098	-0.439	0.205	-0.029	0.938	-0.173	0.632	0.497	0.144	-0.067	0.853	-0.287	0.421
Leptin	0.308	0.387	0.293	0.410	0.197	0.585	0.238	0.508	-0.242	0.501	-0.189	0.601	-0.062	0.866

Note. REE_m = measured resting energy expenditure, REE_p = predicted resting energy expenditure, REE_m/FFM = ratio between measured resting energy expenditure and fat-free mass, T_3 = triiodothyronine, IGF-1 = insulin-like growth factor 1, GH = growth hormone.

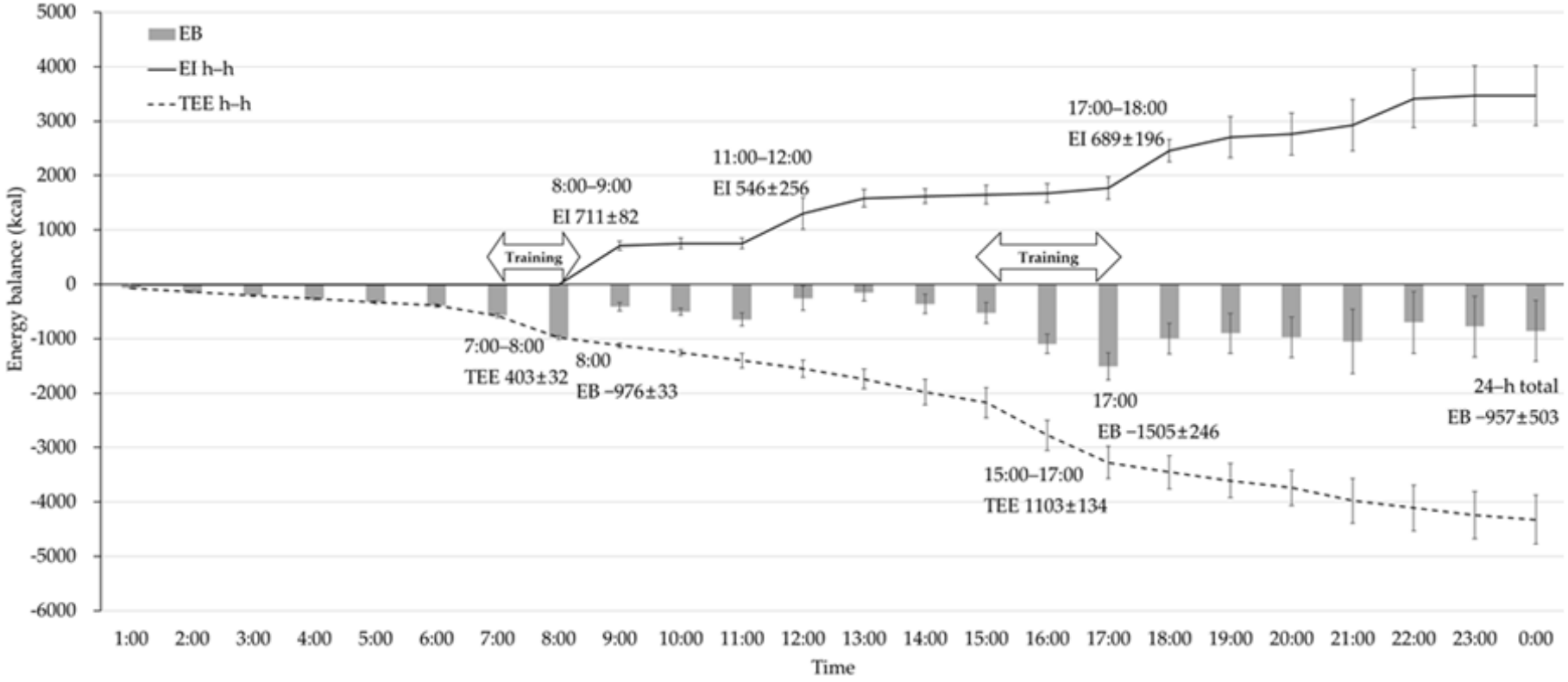

Figure 4.1 Hourly WDEB, EI, and total energy expenditure changes of the participants during the training days. EB = energy balance, EI = energy intake, TEE = total energy expenditure.

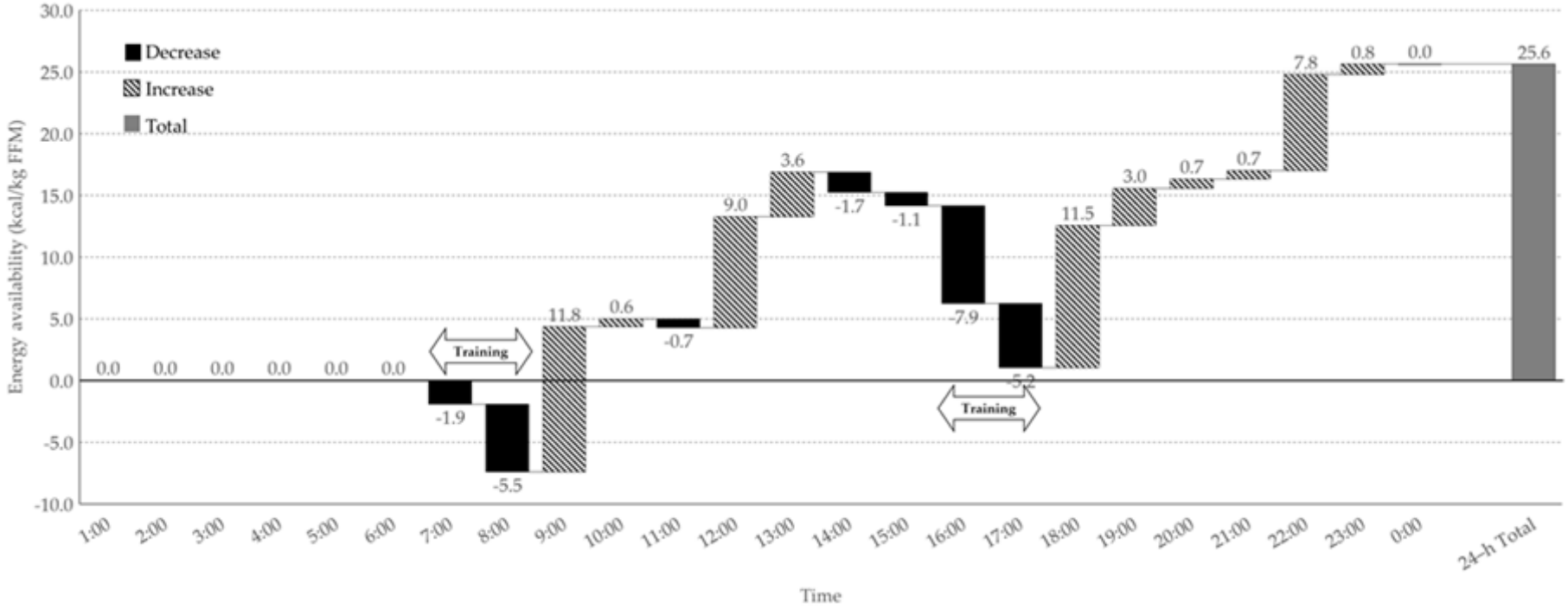

Figure 4.2 Hourly within-day energy availability change of the participants during the training days.

Chapter 5. General discussion

5.1 Main findings

The current understanding on energy deficiency in athletes has emphasized the importance of adequate EA to prevent various physiological, psychological alterations and performance decrease. Therefore, many studies conducted laboratory-controlled and observational studies on EA and energy deficiency of athletes. However, the findings are inconsistent and do not always support the previous threshold concept of EA. Importantly, no studies have elucidated the energy deficiency of Asian male athletes. This thesis aimed to provide these scientific requirements by investigating the energy status and physiological factors of Korean male collegiate athletes using the recommended methods for EA assessment and additional energy deficiency analyses methods.

In Study 1 (Chapter 3), it was aimed to evaluate the EA status, hormones, and bone status of Korean male collegiate soccer players. A cross-sectional observational study design was implemented to assess the EA status and investigate its relations with hormones and bone metabolism. The results demonstrate a high prevalence of low EA, and the group differences between the low EA and high EA in REE-related parameters suggested the effect of low EA on metabolic suppression. In addition, IGF-1 was significantly great in the high EA group, but there were no other differences in hormones and bone turnover makers.

In Study 2 (Chapter 4), it was aimed to evaluate the energy deficiency and metabolic suppression of Korean male collegiate soccer players in free-living status using the WDEB and REE_{ratio} analysis methods. There was a group difference between the suppressed REE_{ratio} and normal REE_{ratio} in rest days EI, and there was

an association between REE_{ratio} and IGF-1. These results can support that insufficient EI during recovery can result in metabolic suppression and REE_{ratio} analysis can be used as a supporting method for evaluating the energy status of athletes in free-living status. Furthermore, changes in energy status in athletes were monitored for 24 h and WDEB analysis demonstrated the insufficient EI compensation to excessive EEE during training days. Understanding the details of EI and EE of athletes can provide practical support to avoid energy deficiency.

Energy deficiency in Asian male athletes

Over the past decades, the EA of athletes has been widely studied and applied to practical strategies to prevent health problems and performance decrease. In male athletes, there are high risks of low EA and energy deficiency in specific sports, including combat sports, jockeys, distance runners, cyclists, and rowers (Burke et al., 2018a). Available cross-sectional studies of male runners supported this and showed a mean EA of 33 kcal/kg FFM/d (Heikura et al., 2018) and 27.2 kcal/kg FFM/d (Hooper et al., 2017). In addition, an observational study on EA of various sports groups presented that a low mean EA (29.0 kcal/kg FFM/d) in male athletes and a high prevalence (56%) of low EA (<30 kcal/kg FFM/d) (Koehler et al., 2013). A study from our laboratory demonstrated a severe low EA (18.9 ± 6.8 kcal/kg FFM/d) in Japanese male collegiate runners (Taguchi et al., 2020). To our knowledge, this is the only available study that evaluated the EA of Asian male athletes. Therefore, the present study assessed the EA of Korean male collegiate soccer players to provide additional scientific evidence. In Study 1 (Chapter 3), EA analysis on 12 participants presented that the most of participants (83%) showed EA lower than 45 kcal/kg FFM/d, and 40% of the participants showed low EA (<30 kcal/kg FFM/d). This study result is consistent with previous studies presenting a high risk of low EA in male athletes, but further

study on Asian male athletes and the inclusion of a large number of participants are essential to provide clear evidence of the energy deficiency in Asian male athletes.

The effects of energy deficiency on endocrine and bone

The accumulated studies on energy deficiency in athletes provided scientific backgrounds for the development of the FAT and RED-S models, and the pathophysiology of these models was based on physiological dysfunctions by energy deficiency resulting in hormonal changes and impaired bone health. Intervention studies controlling either EI or EE, or both, for inducing energy deficiency presented a decrease in IGF-1, testosterone, leptin, insulin, and P1NP and an increase in cortisol (Abedelmalek et al., 2015; Costa et al., 2005; Koehler et al., 2016; Kojima et al., 2020; Rämson et al., 2008; Stenqvist et al., 2020; Svendsen et al., 2016; Zanker & Swaine, 2000). However, findings were inconsistent, showing either no responses or opposite endocrine responses, or both, to energy deficiency (Cooper & Ackerman, 2020). Therefore, the present study obtained EA, hormones, and BTMs data and categorized the participants according to the current low EA threshold (<30 kcal/kg FFM/d) to investigate the differences in endocrine and bone status. In Study 1 (Chapter 3), the low EA group presented significantly low IGF-1 compared to the high EA group, but there were no other differences in hormones and bones. Additionally, in Study 2 (Chapter 4), REE_{ratio} had positive associations with IGF-1, and there were no associations with other hormones. These results support that low EA and energy deficiency can affect the endocrine system. More efforts are required to further elucidate the findings. A future study using prolonged monitoring to assess the energy status of athletes and more in-depth biochemical assessments is warranted.

REE_{ratio} and WDEB analysis

To identify the risk of RED-S in athletes, analysis of energy status using EA measurement is the underpinning factor of the FAT and RED-S model. Previously discussed laboratory-controlled studies have provided the effect of EA on physiological and psychological health but other cross-sectional and observational studies in athletes presented inconsistent findings. Compared to EB analysis, EA analysis has fewer variables affecting the accuracy of measurements, but the accuracy of the measurements is still limited by the components of EA. Estimation of EI is generally conducted by food record analysis, but the recording of food diary may affect the habitual intake which results in either under- or overreporting errors, or both (Burke et al., 2018b). In addition, a food diary can increase the burden on participants resulting in reduced compliance. Thus, different food record analysis methods, for example, using different software and databases, can influence the error and variability of EI analysis (Burke et al., 2018b). Estimation of EEE in free-living athletes also can increase the inaccuracy of EA analysis. In free-living athletes, it is difficult to define and classify the exercise and non-exercise activity affecting under- and overestimation of EEE (Burke et al., 2018b). Various measurement tools including HR monitors, GPS trackers, accelerometers, and activity records allow EEE analysis in different types of exercise, but with a risk of increased variability and inaccuracy of the measurements (Burke et al., 2018b). Furthermore, the current EA threshold for low EA (<30 kcal/kg FFM/d) may be used to develop strategies to prevent possible physiological dysfunctions in athletes. A recent review on the current EA threshold suggested a reconsideration of the current threshold concept which could have different susceptibility and degrees of energy deficiency by individual variability (De Souza et al., 2019a).

Given these limitations in EA measurements and their application in free-living athletes, alternative and supporting methods for the diagnosis of energy deficiency and screening of related symptoms have been suggested. For the analysis of energy deficiency in free-living athletes, longitudinal monitoring on metabolism using analysis of metabolic hormones, REE, and REE_{ratio} with EA analysis and body composition monitoring is recommended (De Souza et al., 2019a). In addition, analysis of WDEB, which assesses the EI and EE in 1 h intervals, can provide the real-time endocrine responses on energy deficiency, and it can help to determine the energy deficiency in athletes (Logue et al., 2020). Previous studies implementing REE_{ratio} evaluation presented that low EA and metabolic suppression had associations with low REE_{ratio} (Fahrenholtz et al., 2018; Melin et al., 2015; Staal et al., 2018; Taguchi et al., 2020). In addition, WDEB analysis in endurance athletes presented associations between WDED and catabolic markers (Fahrenholtz et al., 2018; Torstveit et al., 2018).

To support this, the present study analyzed the REE_{ratio} and 7-day WDEB of the participants. In Study 1 (Chapter 3), the low EA group presented lower REE_{ratio} than the high EA group, and their mean value was lower than 0.94 suggesting metabolic suppression. In Study 2 (Chapter 4), REE_{ratio} presented a relation with EI of rest days and a positive association with IGF-1 related to low EA in Study 1 (Chapter 3). WDEB analysis in Study 2 (Chapter 4), there were no associations between WDED parameters (WDEB <0, -400 kcal, and largest hourly deficit) and hormones, which was different from previous studies (Torstveit et al., 2018). However, hourly changes of EI and EE using WDEB analysis helped to understand the excessive EEE of training and insufficient EI compensation during training days. These results can provide evidence for using REE_{ratio} as an alternative method for evaluation of EA and energy status of athletes in free-living status, and WDEB analysis can help to provide efficient training

and recovery strategies to prevent severe energy deficiency in athletes.

5.2 Strength, limitations, and recommendations for future research

Strength and limitations

This study is the first to assess the EA status and investigate the energy deficiency and metabolic suppression using recognized methods and biochemical analysis in Korean male soccer players. There are some limitations in research design and methods. The research design was cross-sectional and observational, but the number of participants used in data analysis was 12 and 10, in Study 1 and 2, respectively, which is a small number that potentially lacks statistical confidence. Single time point monitoring of the endocrine system and bone metabolism in this research may also have limited the observe the effect of prolonged energy deficiency in athletes. Despite a high risk of energy deficiency in endurance and weight-categorized athletes, this research only included soccer players with different environmental factors in bone metabolism compared to those in non-weight-bearing sports. Finally, this research was the first study on the EA and energy deficiency in Korean male athletes. Therefore, there was a lack of validations of the assessment methods.

Future research

Based on the results of this research, further study is required to understand the prevalence and the effect of energy deficiency in Asian male athletes. The following questions require follow-up in future research: 1) assessment of EA and related factors in Asian male athletes for comparison between Asian countries and between Asian and Western countries, 2) observational study including a large number of participants in various sports groups and multiple-time points for biochemical analysis, 3) validity

evaluation of the EA and energy deficiency assessment methods in Korean athletes, and 4) a laboratory-controlled study on the effect of energy deficiency on the endocrine system in Asian male athletes.

5.3 Conclusion

In sports nutrition, adequate nutrition can augment the positive effect of exercise and help recovery to increase training efficacy and performance. The imbalance between EI and EEE resulting in energy deficiency in athletes has been studied for decades, but there were limited studies in Asian male athletes. This research focused on these scientific requirements, and the key findings can be summarized as follows. First, there was a high prevalence of low EA, but the effects of energy deficiency on hormones and bone metabolism in Korean male collegiate soccer players are not clear. Second, REE_{ratio} can be recommended for assessing the energy status of free-living athletes. Finally, the hourly analysis of energy status using the WDEB assessment may provide a practical strategy for preventing energy deficiency in athletes.

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Finally, I hope this research has added value to our field of study and can contribute to applied practice.

Publications & Presentations

Publications related to the dissertation

Lee, S., Moto, K., Han, S., Oh, T., & Taguchi, M. (2021). Within-day energy balance and metabolic suppression in male collegiate soccer players. *Nutrients*, 13(8), 2644. https://www.mdpi.com/2072-6643/13/8/2644

Lee, S., Moto, K., Han, S., Oh, T., & Taguchi, M. (2020). Association of low energy availability and suppressed metabolic status in Korean male collegiate soccer players: A pilot study. *American Journal of Men's Health*, 14(6), https://doi.org/10.1177/1557988320982186

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Presentations related to the dissertation

Lee, S., Moto, K., Han, S., Oh, T., & Taguchi, M. (2020, May 28). *Effects of relative energy deficiency on metabolism and biomarkers in Korean male athletes* [Conference presentation]. 67th Annual Meeting of American College of Sports Medicine, San Francisco, CA, United States. https://journals.lww.com/acsm-msse/Fulltext/2020/07001/Effects_Of_Relative_Energy_Deficiency_On.1797.aspx

Lee, S., Moto, K., Han, S., Oh, T., & Taguchi, M. (2019, October 5). *Relationship between energy status and metabolic suppression in collegiate male soccer players* [Conference presentation]. 57th Korean Alliance for Health, Physical Education,

Recreation, and Dance Conference: Korean Society for Exercise Nutrition Seminar. Seoul, Republic of Korea.

Lee, S., & Taguchi, M. (2019, March 7). *Dietary intake patterns and energy expenditure during one week in collegiate male soccer player in Korea* [Conference presentation]. Top Global University Project: KU Leuven-Waseda International Workshop, Tokorozawa, Saitama, Japan.

Lee, S., Moto, K., & Taguchi, M. (2018, May 25). *Energy availability, total bone mineral density, and serum hormone levels of male collegiate runners in Japan: A pilot study* [Conference presentation]. 39th Korean Society for Exercise Nutrition Spring Conference, Busan, Republic of Korea.

Lee, S., & Taguchi, M. (2018, March 2). *Food habits, health status, and menstrual function of female collegiate athletes in Korea* [Conference presentation]. Forum on Top Global University Project: Formulating an International Academic Network in Health and Exercise Science, Tokorozawa, Saitama, Japan.

Appendix A - Profile questionnaire

개인 프로필

작성일 년 월 일

성 명		성별	남 여
생 년 월 일	년 월 일 (만 세)		
종 목		운동 경력	년 개월
포 지 션			
체 급			
소 속			
신 장	cm	체중	kg
체 지 방 률	% (측정일: 년 월 일)(측정법:)		
현 재 상 태	□일반트레이닝기간 □시합기간 □오프시즌 □기타( )		
목 적	□몸만들기(근력향상) □체력향상(지구력향상) □감량(kg목표) □증량(kg목표) □재활 □기타( )		
주 거 환 경	□자택 □기숙사 □자취 □합숙소 □기타( )		
식 사 준 비	○아침 □자취(본인·부모님·기타) □급식(기숙사 등) □외식 □기타 ○점심 □자취(본인·부모님·기타) □급식(기숙사 등) □외식 □기타 ○저녁 □자취(본인·부모님·기타) □급식(기숙사 등) □외식 □기타		
선 호 / 기 피 음 식	선호하는 음식: 기피하는 음식:		
식 품 알 레 르 기	□없음 □있음(식품명)		
보 충 제 섭 취	□섭취하지않음 □섭취하고있음(종류:)(빈도:)		
흡 연			
음 주			

식사 또는 영양적인 부분의 질문 또는 요청

Appendix B - EAT-26

각 문장을 읽고, 당신의 식사 행동 및 태도에 가장 잘 맞는 번호를 1개 선택해 ○를 기입해주십시오.

	항상 그렇다	거의 그렇다	자주 그렇다	가끔 그렇다	거의 그렇지않다	전혀 그렇지않다
1. 살 찌는 것이 두렵다.	1	2	3	4	5	6
2. 배가 고파도 먹지 않는다.	1	2	3	4	5	6
3. 나는 음식에 집착하고 있다.	1	2	3	4	5	6
4. 멈출 수 없이 폭식을 한적이 있다.	1	2	3	4	5	6
5. 음식을 작은 조각으로 나누어 먹는다.	1	2	3	4	5	6
6. 자신이 먹는 음식의 칼로리를 알고 먹는다.	1	2	3	4	5	6
7. 탄수화물이 많은 음식(빵, 밥, 면, 파스타 등)은 특히 피한다.	1	2	3	4	5	6
8. 다른 사람들은 내가 음식을 많이 먹는 것을 좋아하는 것 같다.	1	2	3	4	5	6
9. 먹고난 뒤에 토한다.	1	2	3	4	5	6
10. 먹고난 뒤에 심한 죄책감을 느낀다.	1	2	3	4	5	6
11. 더 날씬해지고 싶다는 생각을 떨쳐버릴 수 없다.	1	2	3	4	5	6
12. 칼로리를 소모하고 있다고 생각하며 운동을 한다.	1	2	3	4	5	6
13. 다른 사람들은 내가 너무 말랐다고 생각한다.	1	2	3	4	5	6
14. 내가 살이 쪘다는 생각을 떨쳐버릴 수 없다.	1	2	3	4	5	6
15. 다른 사람들보다 식사 시간이 더 길다.	1	2	3	4	5	6
16. 설탕이 들어간 음식은 피한다.	1	2	3	4	5	6
17. 다이어트 식품을 먹는다.	1	2	3	4	5	6
18. 음식이 나의 인생의 대부분을 차지하고 있다는 생각이 든다.	1	2	3	4	5	6
19. 음식에 대해 스스로 조절 할 수 있다는 것을 과시한다.	1	2	3	4	5	6
20. 다른 사람들이 내가 더 먹도록 강요하고 있는 것 같다.	1	2	3	4	5	6
21. 음식에 대한 시간과 생각이 너무 많이 든다.	1	2	3	4	5	6
22. 단 것을 먹고난 뒤에는 마음이 불편하다.	1	2	3	4	5	6
23. 다이어트를 하고 있다.	1	2	3	4	5	6
24. 위가 텅 빈 상태가 좋다.	1	2	3	4	5	6
25. 먹어본 적이 없는 고칼로리 음식을 먹어 보는 것을 좋아한다.	1	2	3	4	5	6
26. 먹고난 다음에 토하고 싶은 충동이 생긴다.	1	2	3	4	5	6

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Appendix C - POMS-2

POMS2 성명 : 나이(만)

작성날짜 생년월일: 성별 남・여

아래의 문항들은 사람이 느끼는 다양한 기분 상태를 기술한 것입니다. 각 문항을 주의 깊게 읽고, 지난 일주일간 (오늘을 포함하여) 당신이 느껴 온 기분 상태에 해당되는 번호를 골라 ○으로 표시해주십시오.

	전혀 그렇게 느끼지 않는다	약간 그렇게 느낀다	중간 정도로 그렇게 느낀다	꽤 그렇게 느낀다	매우 그렇게 느낀다
1. 사람들과 만나는 것이 즐겁다.	0	1	2	3	4
2. 긴장된다.	0	1	2	3	4
3. 화난다.	0	1	2	3	4
4. 녹초가 된 느낌이다.	0	1	2	3	4
5. 생기 넘친다.	0	1	2	3	4
6. 혼란스럽다.	0	1	2	3	4
7. 남을 배려한다.	0	1	2	3	4
8. 슬프다.	0	1	2	3	4
9. 활동적이다.	0	1	2	3	4
10. 투덜거린다.	0	1	2	3	4
11. 활기차다.	0	1	2	3	4
12. 겁에 질려 있다.	0	1	2	3	4
13. 희망이 없다.	0	1	2	3	4
14. 안절부절못한다.	0	1	2	3	4
15. 집중이 안 된다.	0	1	2	3	4
16. 피곤하다.	0	1	2	3	4
17. 남에게 도움이 된다.	0	1	2	3	4
18. 조마조마하다.	0	1	2	3	4
19. 비참하다.	0	1	2	3	4
20. 기분이 뒤죽박죽이다.	0	1	2	3	4
21. 증오하고 있다.	0	1	2	3	4
22. 기진맥진하다.	0	1	2	3	4
23. 불안하다.	0	1	2	3	4
24. 착하고 친절하다.	0	1	2	3	4
25. 자포자기 상태이다.	0	1	2	3	4
26. 따분하다.	0	1	2	3	4
27. 당황스럽다.	0	1	2	3	4
28. 화가 나서 펄펄 뛸 지경이다.	0	1	2	3	4
29. 남을 잘 믿는다.	0	1	2	3	4
30. 쉽게 화가 난다.	0	1	2	3	4
31. 자신이 쓸모 없게 느껴진다.	0	1	2	3	4
32. 활발하고 정렬적이다.	0	1	2	3	4
33. 확신이 없다.	0	1	2	3	4
34. 지친다.	0	1	2	3	4
35. 의욕이 넘친다.	0	1	2	3	4

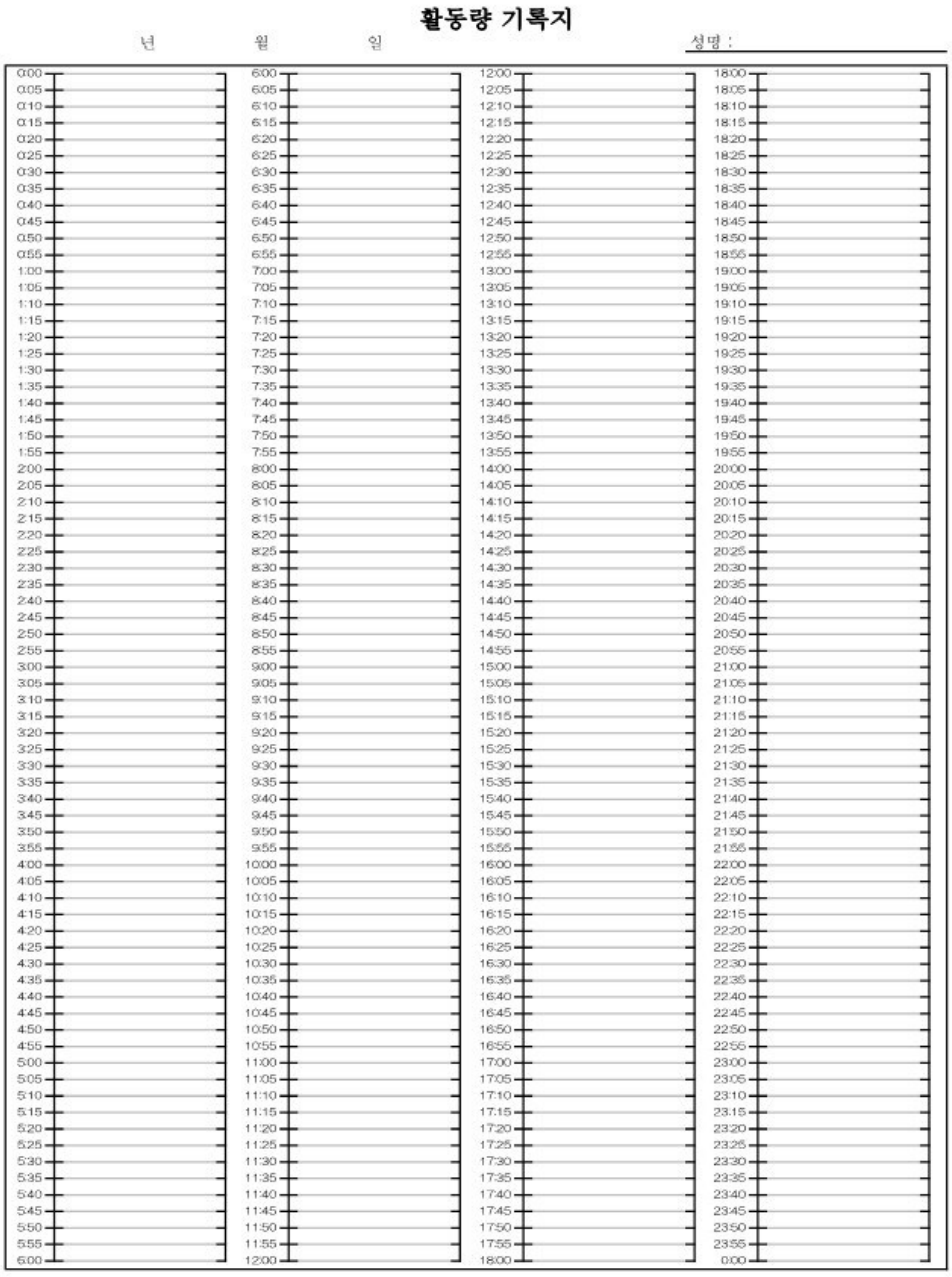
Appendix D - Food diary

식 사 일 기　　월　일 (　)

시간·장소	요리명	재료명	섭취량	비고

와세다 대학 스포츠과학 학술원 타구치 연구실

Appendix E - Activity record



활동량측정기 장착시각/분리시각	컨디션
시 분 / 시 분	
활동량측정기 분리 시간	특이사항