Within-day Energy Deficiency Before and After a Sports Nutrition Intervention in Female Endurance Athletes at Risk of Relative Energy Deficiency in Sport (REDs)

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ABSTRACT

Background: Low energy availability (LEA) occurs as a result of an insufficient energy intake (EI) which can lead to several physiological and psychological consequences that are characteristic in Relative Energy Deficiency in Sport (REDs). Energy balance (EB) is usually assessed in 24hour(h)-blocks, but this approach has been criticized for neglecting fluctuations in EB within the day. Calculating EB in one-hour intervals and assessing the hourly distribution spent in different EB-zones, also known as Within-day Energy Balance (WDEB) has therefore been advocated to better identify large deficits and Within-day Energy Deficiency (WDED). Notably, there is lack of research in this field, but the WDEB-method can potentially contribute to a better understanding of EB and the possible causes of REDs.

Objectives: The main objective of this study was to investigate the effect of a 16-week sports nutrition intervention on WDED for female endurance athletes at risk of REDs. It was also of interest to examine if the WDEB-method and the 24h-EB method generated similar interpretations of energy status, and to investigate the association between WDED and The Low Energy Availability in Females-Questionnaire (LEAF-Q) score.

Methods: The FUEL (Food and Nutrition for Endurance athletes – a Learning program) intervention included 16 online lectures in sports nutrition and eight individual athlete-centered nutrition counseling over 16 weeks. Norwegian and Swedish (n=19) female endurance athletes with LEA (a LEAF-Q score > 8) and low risk of eating disorders (EDs) completed seven-day dietary and activity records at baseline (week 0) and post-intervention (week 17) which were used to calculate WDEB, WDED and 24h-EB.

Results: There was a significant reduction in hours spent in a negative EB, and WDED was improved from baseline to post-intervention (hours in; EB< 0 kcal: 20.0h vs 5.0h, $p = 0.006$. EB< -300 kcal: 18.0h vs. 2.0h, $p = 0.005$. Largest single hour deficit (LSHD): -1686.1 kcal vs. -330.4 kcal, $p = 0.002$). 24h-EB was also improved (-327.7 kcal vs. 19.9 kcal, $p = 0.008$), and reductions in total energy expenditure (TEE) (2988 vs. 2846 kcal/day, $p = 0.021$), exercise energy expenditure (EEE) (1039 vs. 887 kcal/d) and excess post-exercise oxygen consumption (EPOC) (83 vs. 71 kcal/d) were observed from baseline to post-intervention. No significant association between WDED and the LEAF-Q score was found.

Conclusion: A 16-week sports nutrition intervention including online lectures and individual nutritional guidance can improve WDED and 24h-EB among female endurance athletes in risk of REDs. The 24h-EB method and the WDEB-method generated the same interpretation of overall energy status, but the latter provided greater insight into daily fluctuations in EB and energy deficits.

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Abbreviations

24h-EB	24hour energy balance
BF %	Body fat percentage
BMD	Bone mineral density
BMR	Basal metabolic rate
CTLEA	Computerized Time-line Energy Assessment
DE	Disordered eating
DIT	Diet induced thermogenesis.
EA	Energy availability
EB	Energy balance
ED	Eating disorder
EDE-Q	The Eating Disorder Examination-Questionnaire
EE	Energy expenditure
EEE	Exercise energy expenditure
EI	Energy intake
EPOC	Excess post-exercise oxygen consumption
FFM	Fat-free mass
GI	Gastrointestinal
HR	heart rate
IOC	The International Olympic Committee
LEA	Low Energy Availability
LEAF-Q	The Low Energy Availability in Females-Questionnaire
LSHD	Largest single hour deficit
LSHS	Largest single hour surplus
MD	Menstrual dysfunction
mRMR	Measured resting metabolic rate
NEAT	Non-exercise activity thermogenesis
NNR	Nordic Nutrition Recommendations
pRMR	Predicted resting metabolic rate
REDs-CAT	REDs Clinical Assessment Tool
REDs	Relative Energy Deficiency in Sport
RMR	Resting metabolic rate
SMR	Sleeping metabolic rate
T_3	Triiodothyronine
TT_3	Total triiodothyronine
TEE	Total energy expenditure
WDEB	Within-day Energy Balance
WDED	Within-day Energy Deficiency

Calculations and Definitions

24h-EB method	Defined as the difference between total-daily EI and total-daily EE over a 24 h period, starting from 00:00-24:00. Also referred to as “end of the day 24h-EB”. 24h-EB = EI-TEE (22).
Baseline	Defined as the “pre-intervention” assessments (week 0).
BMI	Body mass index: Body weight(kg)/height squared (m^2)
CTLEA	“Computerized Time-line Energy Assessment” established by professor Dan Benardot (7). A validated method to assess daily fluctuations in EB and to determine individual energy requirements.
DE	“Disordered Eating”, defined by Nattiv et al. as: *“various abnormal eating behaviors, including restrictive eating, fasting, frequently skipped meals, diet pills, laxatives, diuretics, enemas, overeating, binge-eating and then purging (vomiting).” (84).*
Diet-induced thermogenesis	Estimated as 10 % of kcal from the whole meal, based on the model by Reed et al.: $175.9\ X\ T\ e^{-T/1.3}$, where T is the time after ingestion and e is the base of natural logarithm (90).
EA	Defined by Loucks et al. (60) in exercise physiology as: “*the amount of dietary energy remaining after exercise for all other metabolic processes”,* and calculated as: EA [kcal/kg FFM] = EI – EEE.
ED	“Eating Disorder”, defined by Nattiv et al. (84) as: “*a clinical mental disorder defined by Diagnostic and Statistical Manual of Mental Disorders-IV (DSM- IV) and characterized by abnormal eating behaviors, an irrational fear of gaining weight, and false beliefs about eating, weight, and shape”* including anorexia nervosa, bulimia nervosa and *Eating disorder not otherwise specified (ED-NOS)* (84).
EDE-Q	A validated screening tool used to assess eating disorder symptoms, risk of LEA/REDs, DE and measure behavior and cognitive eating disorder patterns based on Fairburn et al. (33) and Rø et al. (94).
EI	Energy intake: Total kcal consumed during the day (24h-period from 00:00-24:00).
EEE	Exercise energy expenditure: 2596.05936 mL O_2/min * 0.00502332 kcal/mL * exercise-duration. (*The energy expended through planned exercise which is in addition to the energy expended through non-exercise activity during the same time period).*
EPOC	Calculated as 8 % of EEE (5 % of EEE the first hour post-exercise plus 3 % of EEE the second hour post-exercise) (32, 54, 89, 95, 108).
Eumenorrhea	Females with menstrual cycles of 28 days ± 7 days and adequate sex hormone levels (32).
Female Athlete Triad	A phenomenon observed among female athletes characterized by an interrelationship of MD, LEA (with or without an ED) and decreased BMD. Also referenced to as the “Triad” (3).

FUEL-subjects	All subjects included in the FUEL-intervention.
The FUEL-intervention	In Norwegian: Forstå Utholdenhetsidretts Ernæring – et Læringsprogram. In English: Food and Nutrition for Endurance athletes – a Learning program.
Harris Benedict -equation.	pRMR[kcal/day] = 655.0955 + 9.5634*w + 1.8495*h – 4.6756*a”, (w: body weight (kg), h: height (cm), a: age (years)) (40, 107).
MD	Menstrual dysfunction, including: “FHA (menstrual cycles > 35 days where other causes than hypothalamic suppression are ruled out), amenorrhea (either primary: no menarche after 15 years, or secondary: absence of > 3 consecutive menstrual cycles where other causes than hypothalamic suppression had been ruled out), or other MD not related to energy deficiency”, as classified by Fahrenholtz et al. (32).
LSHD	Larges single-hour deficit: the average of the lowest hourly EB observed among 24 hours within each day of the seven days of registration (kcal).
LSHS	Largest single-hour surplus: the average of the largest hourly EB observed among all hours within each day of the seven days of registration (kcal).
LEAF-Q	A validated screening tool used to identify athletes at risk of LEA. It was validated through an observational study among 45 female athletes, generating a specificity of 90 % and a reliability of 78 %, with a total LEAF-Q score ≥ 8 being indicative with “high risk of LEA" (68).
LEA-subjects	All subjects included in the LEA-study by Melin et al. (70).
Low RMR-$_{ratio}$	RMR-$_{ratio}$ < 0.90 (32, 46, 54, 57, 108).
mRMR	Measured resting metabolic rate estimated based on indirect calorimetry through a ventilated hood system.
NEAT	Energy expenditure spent from all activity not categorized as sleeping, eating, or planned physical activity.
pRMR	Predicted resting metabolic rate. Estimated based on Harris Benedict equation (40, 72, 107).
REDs	A syndrome defined in the 2014 IOC Consensus on REDs (76) as: *“impaired physiological functioning caused by relative energy deficiency and includes, but is not limited to, impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health.”*
RMR-$_{ratio}$	mRMR divided by pRMR
SMR	Sleeping metabolic rate: Calculated as 90 % of pRMR, based on NNR 2012 (32, 54, 62, 86, 108).
TEE	Total energy expenditure: RMR or SMR + DIT + EEE + EPOC + NEAT.
WDEB-method	Calculation of EB continuously in one-hour intervals, where EB = EI-TEE within each hour. The method involves an interpretation of the distribution of hours spent within different energy-zones as defined by Deutz et al. (26).

WDEB-variables	Variables used to describe WDEB as defined by Deutz et al., Fahrenholtz et al. and Torstveit et al. (26, 32, 108) including: -Hours spent in EB< 0 kcal (hours in catabolic state) -Hours spent in EB< -300 kcal (hours in energy deficit) -LSHD -Hours spent in EB between -300 kcal and +300kcal (EB[-300; +300kcal]) -Hours spent in EB> 0 kcal (hours in an anabolic state) -Hours spent in EB> 300 kcal (hours in energy surplus) -LSHS
WDED-variables	Variables used to determine Within-day Energy Deficiency, according to Deutz et al., Fahrenholtz et al. and Torstveit et al. (26, 32, 108): - Hours in EB< -300 kcal (hours in energy deficit) - Hours in EB< 0 kcal (hours in catabolic state) - LSHD

1 Background

1.1 Low Energy Availability and Relative Energy Deficiency in Sport

1.1.1 Definitions

The concept of energy availability (EA) in exercise physiology is defined as dietary energy intake (EI) minus exercise energy expenditure (EEE) relative to fat-free mass (FFM) (EA = EI – EEE/FFM), indicating the amount of energy left for all physiological systems after exercise (60, 78). EA is a widely used concept within sports medicine, and evidence have shown that optimal EA is reached when EA = 45 kcal/kg FFM/day, and that EA< 30 kcal/kg FFM/day is considered a threshold for determining low energy availability (LEA), which is a result of an insufficient EI relative to the energy demand (60, 104).

Relative Energy Deficiency in Sport (REDs) is a syndrome that is caused by overexercising or underfueling, which can occur when an athlete is subjected to chronic LEA over time (2, 76, 78). REDs can lead to a reduction in basic physiological processes in order to restore energy balance (EB) and is characterized by several physiological and psychological consequences (70, 77, 78). Moreover, REDs can occur with or without an eating disorder (ED) or disordered eating (DE), and is a revision of what was previously known as "The Female Athlete Triad" (Triad), a phenomenon that was only applicable to females and was considered an interrelationship of three components: LEA, menstrual dysfunction (MD) and decreased bone mineral density (BMD) (3, 77, 78). Evidently, it became clear that this phenomenon also affected males, and that there were several other physiological systems beyond menstrual function and bone health that was affected (77, 78). REDs was therefore developed as a broader collective term, describing an expanded concept of the Triad, as shown in **Figure 1A** (57, 77, 78),

In 2014 the International Olympic Committee (IOC) introduced REDs in a consensus statement (76), which was updated and revised in 2018 (78). Increasing evidence suggested that the consequences of REDs affected many physiological systems as shown in **figure 1A and 1B,** including metabolic health, immunity, endocrine function, hematology, gastrointestinal (GI) function, and cardiovascular and psychological health, in addition to several other factors related to athletic performance **(76, 78).** The authors claim that there are large research gaps on this topic, specifically emphasizing the need for an applicable, practical and validated screening tool to identify REDs, and validated educational programs to prevent, raise awareness and potentially treat REDs (57, 76, 78).

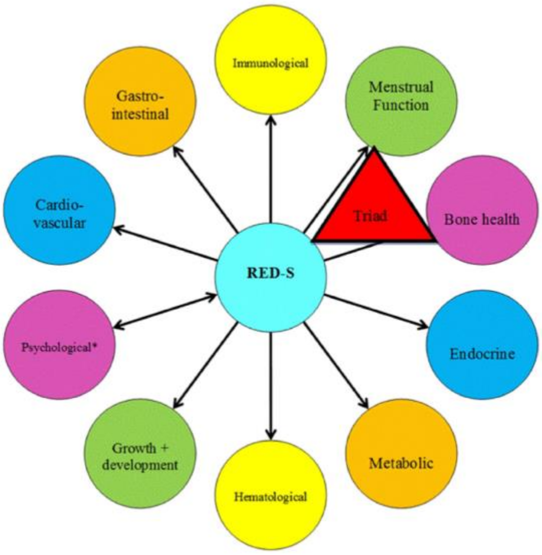


Figure 1A: Illustration of various health parameters that can be affected by REDs. The red triangle describes the interrelationship between the three parameters related to the female athlete triad ("Triad") phenomenon (3).

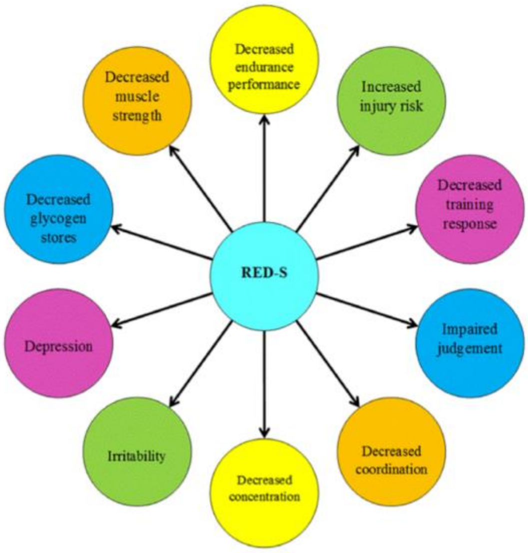


Figure 1B: Illustration of potential performance consequences of REDs.

*Note: **Figure 1A and 1B** is reprint from "IOC Consensus Statement on Relative Energy Deficiency in Sport (REDs): 2018 update" by Mountjoy et al. (78).* Abbreviations: REDs; Relative Energy Deficiency in Sport. Triad: The Female Athlete Triad.

1.1.2 Prevalence of Low Energy Availability

REDs can occur in both recreational and elite athletes, and it can affect males and females (14, 23, 70). Investigating the prevalence of REDs is challenging, especially since it is a multifactorial syndrome characterized by a variety of symptoms **(figure 1A** and **1B)** (57, 77, 78). LEA underpins the concept of REDs, and the prevalence of LEA was reported to be between 22 and 58 % in a recent review by Logue et al. (57). Moreover, signs and symptoms of REDs have been reported to be present in ~2 – 60 % of male and female athletes participating in various sports, and it has been found that particularly females endurance athletes engaging in weight-sensitive sports appears to be a group at particularly high risk of developing REDs (2, 97).

1.1.3 Screening and Identification of Low Energy Availability and Relative Energy Deficiency in Sport

Screening for REDs can be challenging since the symptomatology can be subtle and complex (77, 78). Several screening tools to identify EDs or DE exists, and The Eating Disorder Examination-Questionnaire (EDE-Q), Eating Disorder Inventory (EDI) and Drive For Thinness Score (DT-score) can possibly act as proxies for detecting energy deficiency and to identify risk of LEA/REDs (23, 33, 39, 94, 97). However, a high prevalence of energy deficiency and other symptoms of REDs in female athletes without DE or an ED has been reported, and specific screening tools to identify LEA/REDs in athletic populations have therefore been generated, including the Low Energy Availability in Females Questionnaire (LEAF-Q) and the REDs Clinical Assessment Tool (RED-S CAT) (68). RED-S CAT is based on the IOC Consensus Statement on REDs by Mountjoy et al. and was designed to evaluate athletes suspected of REDs and to manage a safe return to sport approach (76, 77).

The Low Energy Availability in Females Questionnaire

The LEAF-Q is a screening tool validated by Melin et al. particularly assessing female athletes and the risk of LEA (57, 68). The questionnaire covers three topics regarding injuries, GI-problems, and reproductive function, and a total LEAF-Q score ≥8 indicates "risk for LEA" (68). Furthermore, the LEAF-Q produces a sensitivity of 78 % and a specificity of 90 % for correctly identifying current EA and/or reproductive function and/or bone health (68). It is reported to be brief, easy to administer and alleviate some of the challenges associated with measurements of EA or EB, and is therefore appreciated by practitioners, especially when larger cohorts are assessed (15, 57). Notably, it is reported to be the most widely used questionnaire on this topic based on a recent review by Sim et al. (97).

Nevertheless, both the LEAF-Q and RED-S CAT are recognized as "brief" tools, and it is therefore proposed to not use these as diagnostic tools, but rather use them in conjunction with other more in-dept clinical examinations and assessments (57, 68, 78, 97).

1.1.4 Causes of Low Energy Availability and Relative Energy Deficiency in Sport in Female Endurance Athletes

There can be several different reasons why LEA occurs and why symptoms of REDs can arise (30). Energy deficiency can derive from intentional behaviors, such as restrictive eating patterns, DE, EDs or assumably exercise addiction (with excessive exercise) (30, 102). However, it can also occur due to unintentional manners simply due to unawareness, a busy lifestyle, or insufficient knowledge on sports nutrition (30, 64, 110).

Unintentional Causes

It is reported that the most prevalent causes for developing LEA or REDs are unintentional (30, 70, 110), and particularly periods with increased training volume without a simultaneous increase in EI have been recognized to be a common pitfall that can result in an "unintentional energy deficit" (64, 97). In addition, suppression of appetite, especially after intense workouts has been found to affect an athlete's ability to consume enough energy, and Larson-Meyer et al. found significant alterations in various appetite regulating hormones after moderate-intense exercise among female runners (53, 59). Also, Gräfnings et al. reported that female endurance athletes with symptoms of REDs struggled with meeting their recommended carbohydrate needs during hard training days compared to lighter days (38). Moreover, misunderstanding of optimal sports nutrition, restrictive dietary choices (i.e., veganism etc.) or a busy lifestyle with a lot of traveling and competition are other factors that have been reported to potentially affect the nutritional behaviors and thus energy status of an athlete (11, 14, 64).

Disordered Eating and Eating Disorders

Conversely, LEA/REDs can occur due to more apparent behaviors, such as DE or EDs (76, 78). DE occur in a continuum, ranging from EB and healthy body weight to more compulsive behaviors and clinical EDs (2, 3, 76, 78, 102). Personality traits, pressure to lose weight, frequent weight cycling and overtraining are some of the risk factors reported for DE/ED among athletes (57, 59). Many athletes believe that a lower body weight can enhance performance, and it has been reported that female athletes tend to find appearance to be one of the main reasons for dieting and conscious weight loss, and not necessarily to improve performance (47, 59, 64). Moreover, the prevalence of EDs among Norwegian female high-intensity elite athletes is reported to be 47.8 %, and a similar risk has been expected among gymnasts and runners (102). In addition, it has been reported that one third of ultra-marathon female athletes at risk of the Triad presented with DE behaviors (97).

1.1.5 Health and Performance Consequences of Relative Energy Deficiency in Sport

As mentioned, chronic LEA can cause severe consequences and can lead to REDs (56, 78). **Figure 1A and 1B** includes an overview of the possible health-and performance consequences of REDs, whereas some of the more relevant health parameters in regard to this study population are described in more detail in the following chapter (2, 57, 76, 78).

Endocrine and Menstrual Disruption

It has been reported that LEA affect endocrine function in both men and women (1, 10, 32, 57, 70, 78, 91). Moreover, research have shown that athletes with LEA have increased risk of MD, and Melin et al. found that 60 % of the female endurance athletes assessed had MD (clinically verified through gynecological assessments), altered thyroid function and altered levels of appetite regulating hormones, in addition to reduced insulin levels and elevated cortisol and growth hormone levels (70). Also, Loucks et al. found that luteinizing hormone pulsatility was decreased when EA was below 30 kcal/kg FFM for five days among healthy, menstruating young females (61).

Gastrointestinal Dysfunction

GI-disorders are reported to be a common problem among female endurance athletes, where LEA has been found to be a main contributing factor (68, 78). Melin et al. found a negative association between LEA and GI-problems (pain, diarrhea, cramps, bloating etc.) among elite Swedish and Danish female endurance athletes (68, 78). Also, high fiber intake and excessive use of artificial sweeteners may also affect these symptoms (69, 78, 98), and Ackerman et al. reported that female athletes with LEA had increased risk of GI-dysfunction compared to those with adequate EA (1).

Injuries

LEA has been reported to be one of the most significant factors associated with injury/illness among female athletes (68, 105). Also, high frequency of injuries have been reported among female endurance athletes with a high risk of exercise addiction, and Heikura et al. found that the amenorrheic female runners with LEA had 4.5times greater injury-rates compared to the eumenorrheic athletes, mainly being bone stress injuries (30, 43).

Metabolic Suppression and Resting Metabolic Rate

Several studies investigating LEA/REDs have found an association between LEA and reduced resting metabolic rate (RMR) possibly as an adaptive response to energy restriction (26, 78, 108). Using measured RMR (mRMR) when calculating EB can therefore underestimate the magnitude of an energy deficit, and predicted RMR (pRMR) has therefore been advocated to be used (4, 26, 46, 54, 108). Moreover, an RMR-ratio< 0.9 $(\frac{\text{measured RMR}}{\text{predicted RMR}})$ has been acknowledged as an indicator of energy deficiency (54, 57, 58, 68, 78, 108).

Bone Health

Prolonged LEA can lead to reduced bone health, and numerous studies have found decreased BMD and increased risk of bone stress injuries, especially in athletes participating in weight-bearing sports (1, 10, 57, 58, 70, 108). Also, it has been reported that females with MD and/or risk of REDs had decreased BMD, bone turnover markers and decreased bone strength compared to eumenorrheic-athletes or those in an adequate EB (78), and Melin et al. found that 45 % of the elite female endurance athletes had impaired bone health (70).

1.1.6 Prevention and Treatment of Relative Energy Deficiency in Sport

Increased awareness and education about REDs tailored to the athletes, coaches and support staff have been claimed to be one of the most important initiatives for preventing REDs, particularly through specific prevention/awareness-programs, similar to the FUEL-intervention (2, 78). Also, early detection and identification of REDs is considered crucial, but as previously mentioned diagnosing REDs can be challenging and a specific validated diagnostic tool to identify REDs is therefore requested (1, 2, 57). Notably, Ackerman et al. proposed several REDs-associated diagnostic factors that can be used for detecting REDs, including: chronic energy restriction, extreme diets, large and sudden alterations in body composition or body weight, drive for thinness, MD, decreased sex-drive, prolonged fatigue and two or more career bone injuries and low BMD for age (2).

Moreover, the recommended treatment of REDs is mainly to correct the relative energy deficit by either increasing EI and/or decreasing training volume, in addition to address the possible underlying causes to prevent relapse (79). Also, it has been advocated that athletes should have access to local REDs and ED-expects, registered dietitians, sports physicians and psychologists, and that an individualized treatment plan should be implemented by a multidisciplinary team together with the athlete to ensure proper recovery and overall health, and to enhance the nutritional-and training practices for the athlete (2, 78).

1.2 Energy Status in Athletes

1.2.1 Energy Availability and Low Energy Availability

As mentioned, EA is the amount of energy available in the body after subtracting the energy cost of exercise, and it is commonly determined based on different EA-zones (60, 78). These zones have primarily been established based on several studies indicating that the physiological systems affected by an energy deficit or REDs (**Figure 1A** and **1B),** particularly the female menstrual cycle, has been altered when EA exceed< 30 kcal/kg FFM/day (60, 70, 78). A procedure for calculating EA in athletes has been proposed by Loucks (59) with the following six steps: 1) FFM is measured; 2) daily EEE is measured; 3) an appropriate value of EA is determined; 4) the required daily EI is calculated (EI= FFM x (EEE + EA); 5) selection of a proper diet to match the estimated EI-level; 6) the athlete consumes the diet by discipline, regardless of appetite (59).

1.2.2 Challenges of Calculating and Interpretating Energy Availability

There are several challenges that have been addressed regarding the calculations and practical implications of using EA, and the existence of these defined thresholds are highly debated (15, 43, 104). First, recent studies indicate that the universal threshold value used to determine LEA (EA< 30) may not be applicable to all individual athletes or for all of the physiological systems affected by REDs **(Figure 1A** and **1B)** (15, 55, 59). Particularly, interindividual factors have shown to affect this and Reed et al. observed that all of the athletes assessed, regardless of menstrual function (amenorrhoeic, oligomenorrheic and eumenorrheic), had an EA> 30 kcal/kg FFM/day (91). Moreover, it has been reported that five days with an EA = 15 kcal/kg FFM/day resulted in decreased bone formation in active women compared to an EA = 45 kcal/kg FFM/day (88). These EA-zones have therefore been advocated to function as an ideological concept rather than a diagnostic tool (15).

Secondly, several methodological challenges have been reported regarding the reliability and accuracy of the different methods of data collection (i.e., dietary-records, estimation of FFM, EEE etc.) (15, 59, 78). Many of these challenges may explain the discrepancy between several of the studies that have calculated EA and used it to examine symptoms associated with REDs (15, 57, 78).

Thirdly, there is no standardized protocol for assessing EA, and both over - and underestimation can therefore occur and lead to different results depending on the method used (15). In addition, EA has been criticized as it only illustrates a single time-point estimate and do not provide information about the prolonged energy status and EA of the athlete (15, 57, 104).

1.2.3 24hour-Energy Balance

The concept of 24hour(h)-EB has been a main approach in the field of dietetics for assessing energy status, and EB is achieved when EI is equal to TEE (59). TEE is the sum of energy expended trough basal metabolic rate (BMR) or RMR, diet induced thermogenesis (DIT), EEE, excess post-exercise oxygen consumption (EPOC) and non-exercise activity thermogenesis (NEAT), and is defined as EB = EI-TEE (22, 92).

More precisely, 24h-EB is the amount of dietary energy added to or lost from the body´s energy stores after all physiological systems have completed their work for *the day*, and hence EB is an *output* from these systems (60). For a healthy young adult it is estimated that EB = 0 kcal/day when EA = 45 kcal/kg FFM/day, and that a negative EB is a result of inadequate EI relative to the total amount of energy needed (60, 102).

1.2.4 Energy Availability vs. 24hour-Energy Balance

Loucks (59) brings out important distinctions between EA and 24h-EB, emphasizing that EA is viewed as an *input* to the body rather than an *output* (59). The distinction between these two parameters can be further illustrated by the research conducted by Stubbs et al. where eight men lived in a calorimeter for one week, with a fixed EI (2770 kcal/d), EEE (840 kcal/day) and EA (1930 kcal/kg FFM/day). They found that 24h-EB was negative (-1730 kcal/day) at day 1 but increased towards 0 kcal/day during the week due to a decline in TEE (101). At this rate, they would have been defined as in "energy balance" after 3weeks, but notably this was recognized as a state of "*pathological EB"* as a result of suppressed physiological function due to a cumulative negative EB over time (59, 101). This phenomenon is one of the main reasons why RMR_{ratio} is considered a valid indicator of energy deficiency, and why pRMR is argued to be more accurate when assessing an individual´s EB, particularly if it is assumed that the subject is in an energy deficit (26, 46, 108).

Furthermore, Loucks et al. emphasize that the 24h-EB method is not suitable to assess and manage an athlete´s diet, primarily because it illustrates an output, rather than the input to the body and it does not contain reliable data about energy requirement, particularly not if the athlete already is in an energy deficit (60). Within-day Energy Balance (WDEB) on the other hand has shown to generate noticeably different values compared to the 24h-EB method, particularly when assessing athletes with clinical verified symptoms of REDs and the WDEB-method has therefore been suggested to be more appropriate to use in athletic populations (22, 28, 32, 36, 57, 62, 106, 108).

1.2.5 24hour-Energy Balance vs. Within-day Energy Balance

As mentioned, different approaches for assessing EB exist, whereas the most commonly used is the traditional 24h-EB macroeconomic view in the body (22). However, this method has been criticized to neglect large fluctuations in EB during the day, particularly the hormonal responses to real-time alterations in EB, and thus a new, more comprehensive approach for assessing EB in one-hour intervals was developed (7, 22).

1.3 The development of Within-day Energy Balance

In 1996, Professor Benardot published an article where he presented the “Computerized Time-line Energy Assessment” (CTLEA)-method, which is based on calculations of EB in sections throughout the day where TEE and EI is simultaneously calculated and assessed (7). The objective for developing this method was that he experienced several limitations with the 24h-EB method when working as a sports nutritionist, and hence requested a new approach which could clearly identify large energy fluctuations and illustrate how an individual´s EB was distributed during the day (7). Moreover, the CTLEA- method was later validated with the 3-day prospective dietary records to predict total EI and within-day surpluses and deficits, with significant correlations (7, 83). Based on the CTLEA-method, Benardot found that the largest single hour deficit (LSHD) among the female athletes was positively associated with body fat-percentage (BF %), suggesting that a restrictive eating pattern have a negative impact on body composition (7).

Within-day Energy Balance: Hourly Energy Balance Calculations

In 2000, Deutz et al. assessed EB and body composition among elite female athletes and expanded the CTLEA-method and standardized it to calculate EB in one-hour intervals cumulatively during the day, a method that is now recognized as the “Within-day Energy Balance”-method (22, 26) **(appendix 9).** Several variables were established to make it more practical to use the WDEB-method, and the following were the ones initially proposed by Deutz et al.: hours in energy surplus (EB> 0 kcal and EB> 300kcal), in energy deficit (EB< 0kcal and EB< -300 kcal), 24h-EB, and the LSHD and largest hourly energy surplus (LSHS) (26). In addition, “Within-day Energy Deficiency” (WDED) was introduced, which is now recognized as an overall term to describe the WDEB-variables associated with a catabolic state (EB <0 kcal, EB< -300 kcal and the LSHD) (32, 54, 108). Based on the WDEB-analysis, Deutz et al. found significant positive associations between WDED and BF %, and an inverse relationship between an energy surplus and BF % for the female athletes assessed (26).

Microeconomic View on Energy Balance

In 2013, Benardot introduced “the microeconomic view on EB” where he presented his version of the WDEB-method **(figure 2),** in addition to address several challenges with the traditional 24h-EB method (22).

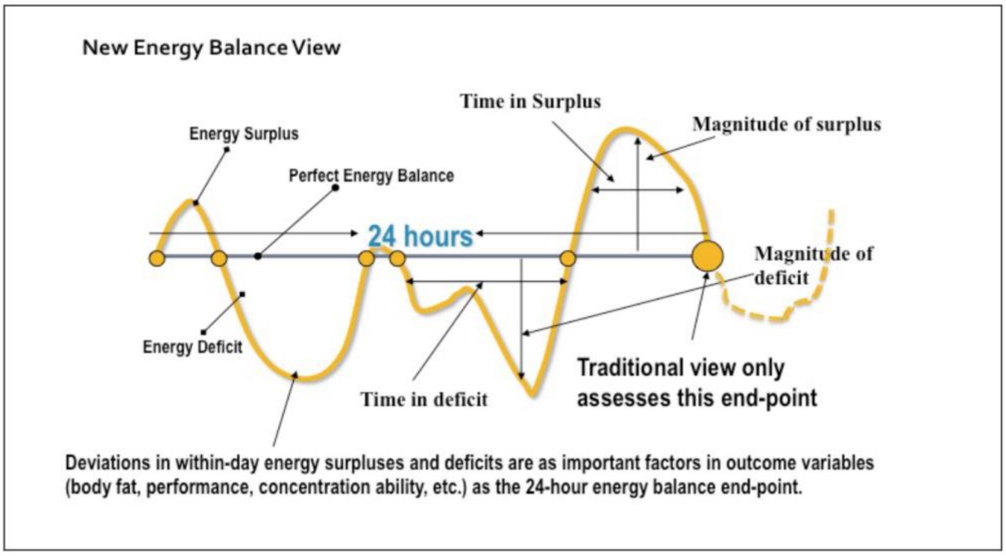


Figure 2: New Energy Balance View. Reprint from "Energy Thermodynamics Revisited: Energy Intake Strategies for Optimizing Athlete Body Composition and Performance" by Benardot 2013 (22).

One issue with the 24h-EB method claimed by Benardot was that it relies on an assumption that the EB achieved at the end of a 24h-period is the same EB that has been sustained each hour preceding it (22). However, as shown in **figure 2,** EB is not linear and several studies have found significant associations between large deviations from the optimal EB-range (EB [-300; +300 kcal]) and a higher BF %, impaired endocrine function and metabolic perturbation, even if the athlete ended the day in relatively adequate EB (8, 22, 26, 32, 108). Moreover, Benardot reported a positive association between hours in EB< -400 kcal and BF % among the female athletes, but no association between 24h-EB and BF % (22). This observation was also reported by Thivel et al. which found an association between WDEB and BF %, but no association between 24h-EB and BF % in young children (age 8-14) (106).

Another limitation with the 24h-EB method highlighted by Benardot was that it can potentially over-or underestimate EB if the timeframe of the 24hours is not properly defined (22). An example being that two dinners are included within the same 24h-EB assessment even though the meals hypothetically were consumed on two different days (22). In addition, the 24h-EB method will only represent one day, and not necessarily a typical daily intake of an individual. Lastly, Benardot emphasize that the 24h-EB method does not distinguish between a person merely consuming 2000 kcal for breakfast, 2000 kcal for dinner or 4 x 500 kcal during the day, even though these different scenarios would result in very different WDEB-profiles; in great energy surplus, energy deficit or close to EB most of the day, respectively (22).

Optimal Energy Balance Range

The EB-range considered most adequate is from EB [-300; +300 kcal], based on the predicted amount of liver glycogen, and it has been reported that spending hours below this negative threshold may initiate physiological processes and compromise brain glucose levels (6, 22, 26, 108). This threshold can be lower or greater, and several studies have for instance used EB[-400; +400 kcal] as a range, while Lundstrom et al. used EB[-300; +300 kcal] for females and EB[-400; +400kcal] for males when assessing WDEB (62).

1.4 Recent Research in Within-day Energy Balance in Athletes

Calculating hourly EB (WDEB) is a relatively new phenomenon in the scientific field of sports medicine, and based on the 2014 IOC-Consensus Statement on REDs, WDED can possibly intensify the negative effects of LEA/REDs (76). However, only a handful of studies assessing WDEB exist, and as shown above the initial studies primarily focused on its association with body composition (4, 6-8, 26, 45, 63). More recent studies however have assessed WDEB in the context of other health-and performance outcomes, particularly the ones related to the symptoms of REDs (**Figure 1A** and **1B)** (32, 46, 54, 108). A summary of the findings from recent studies assessing WDEB among athletes is described below, with additional detailed information in **appendix 9** and **appendix 10.**

In 2018, Fahrenholtz et al. compared WDEB among 25 elite female endurance athletes in regard to menstrual function (32). Athletes with clinical verified MD related to LEA spent significantly more hours in a catabolic state compared to eumenorrheic-subjects, even though they had similar 24h-EB and EA. Also, WDED was associated with lower estradiol levels and RMR_{ratio} and higher cortisol levels. Notably, a positive association between WDED and meal frequency was observed, and no significant association between WDED and BF % was found (32, 57).

The relationship between WDEB and menstrual function was also assessed in a master's thesis by Friel et al. assessing active females (36). On average, the subjects spent more hours in an energy deficit than an energy surplus, and inverse associations between MD/loss of menses exceeding three months and hours in EB> 400 kcal, meal frequency, total EI/day, LSHS and protein-intake were reported, but no association between MD and 24h-EB was found (36).

Torstveit et al. examined the association between WDED and metabolic alterations (RMR) among male endurance athletes, and found that the athletes with suppressed RMR spent more hours in EB< -400 kcal compared to subjects with normal RMR, despite similar 24h-EB and EA (108). In addition, WDED was associated with elevated cortisol levels and a lower testosterone:cortisol ratio (108). Notably, the more hours spent in an energy deficit the larger the LSHD and the lower the BF %, which is in contrasts to the findings from Beherens et al.(4) , Benardot (7), Benardot (22) and Deutz et al. (26).

In 2020, Jurov et al. assessed WDEB among female cyclists, and compared WDEB, EA and performance parameters between athletes at risk of REDs and athletes at low-risk determined based on LEAF-Q (46). They found that EA and hours in EB< -300 kcal was significantly different between the two groups, but no difference in hours in EB< 0 kcal, EI, EEE or TEE was observed, and no correlation between WDED and RMR_{ratio} or BF % was found (46). The authors claim that WDEB influence aerobic and anaerobic performance to a greater extent than EA, and emphasize that practitioners and future studies should have a greater focus on WDEB in the assessment of energy status (46).

Moreover, Behrens et al. examined EB among female soccer players and found that the subjects that spent more time in EB [400; +400kcal] and EB> 400 kcal had lower fat-mass compared to those spending time in EB< -400 kcal (4). In addition, hours spent in an energy deficit was positively associated with fat-mass, BF %, BMI and fat mass index, and these variables were inversely associated with hours spent in EB> 400 kcal and EB [400; +400 kcal] (4). Consequently, the authors conclude that nutrition counseling should focus on meal-timing and hourly food intake and not only 24h recommendations, primarily to ensure adequate energy status and body composition for the athletes (4).

Lundstrom et al. conducted a study among elite swimmers and investigated sex differences in WDEB, 24h-EB, RMR_{ratio} and alterations in total triiodothyronine (TT_3) (62). They found no significant difference in any of the WDEB-variables between athletes with suppressed and normal RMR_{ratio}, and no correlation between TT_3 and WDEB, nor 24h-EB was reported (62). Notably, a negative association between TT_3 and hours in negative EB was found for the subjects with suppressed RMR, but this association was not found for 24h-EB, indicating that the WDEB method can reveal indices of metabolic suppression not evident with the 24h-EB method alone (62).

In contrasts to the aforementioned studies, Lee et al. assessed ten Korean male soccer players and found no significant difference in any of the WDEB-variables between subjects with suppressed and normal RMR_{ratio}, and no association between the WDED-variables and various metabolic markers **(appendix 9)** (54).

As shown above and illustrated in **appendix 10**, there are conflicting findings regarding the associations between WDED on body composition, metabolic health and performance. On the other hand, most of the studies seem to correspond regarding the associations between WDED and menstrual function and endocrine function. Also, most of them found significant associations between WDEB and the physiological variable of interest, but not for 24h-EB with the same variable.

1.5 Within-day Energy Deficiency and The Low Energy Availability Questionnaire Score

As mentioned previously, the LEAF-Q is reported to be the most widely used questionnaire to identify risk of LEA (68, 94). Notably, the influence of WDED on the risk of LEA and REDs is unknown, and there are conflicting findings regarding the impact of WDED on various physiological parameters, as illustrated in **appendix 10.** While some studies have reported positive associations between WDED and different parameters, others have reported no associations or inverse associations between these same parameters (4, 7, 26, 32, 36, 46, 54, 62, 108).

Notably, Fahrenholtz et al. reported significant associations between WDED and MD, estradiol levels, cortisol levels and lower RMR_{rato} among female endurance athletes, and Friel et al. found an inverse association between loss of menses and hours spent in EB> 400 kcal, LSHS and EI/day among female endurance athletes (32, 36). These findings may therefore indicate that there is a positive association between WDED and the total LEAF-Q score.

1.6 Diet and Energy Intake

1.6.1 Dietary Characteristics

There are certain pitfalls regarding sports nutrition and EI among female athletes that have been observed, particularly inadequate EI and carbohydrate intake and a restrictive eating behavior (25, 44, 60, 67, 76). Moreover, low fat and high fiber intake, excessive use of stimulants and artificial sweeteners, low energy dense foods and inappropriate meal timing and energy distribution are other dietary characteristics that is reported to often co-exist with LEA (6, 7, 56, 69, 78).

1.6.2 Recommendations and Energy Intake

Energy Requirements

Many of the general recommendations regarding EI may not be applicable towards an athletic population, particularly due to the relatively high activity level of most athletes (6). Research among athletes has shown that EEE can be up to 40 % of their TEE, and while non-athletes need approximately 2030-2500 kcal/day, athletes often have demands two or three times greater than this (11). Manore et al. emphasize this with examples seen in female endurance athletes with extreme training volumes, where energy requirements of 4000 kcal/day is not unusual (64). In addition, research have shown that female cross-country skiers have a TEE of approximately 3611-4830 kcal/day (99).

Moreover, it is recognized that it is both time-consuming and challenging to estimate an athlete’s energy need accurately, and several sources of error can often occur (16, 19, 86, 92, 107). A more general approach is therefore often used in practice, where a minimum of 2300-2500 kcal/day is suggested for most female athletes to maintain body weight (45-50 kcal/kg) (92). However, this is considered a minimum and will often be greater when total training volume is taken into account (64, 86, 92, 108).

Energy Intake

Of concern, female endurance athletes have an average EI similar to or below the recommended values for lightly active, sedentary females (1960-2250 kcal) (28, 86).There seems to be noticeable differences in the estimated EI reported by the studies assessing EB among female endurance athletes, which may indicate that type of sport may influence EI (57). Beidleman et al. found that female cyclists had an average EI of 3005kcal/day, but the distance runners had 1950 kcal/day, while Deutz et al. reported an average EI of 1600 kcal/day for female runners (5, 26). Moreover, Melin et al. found an average EI of 2766 kcal/day among females participating in different endurance sports, where the subjects with MD had an average EI of 2677 kcal/day while the eumenorrheic-subjects had an EI of 2899 kcal/day (69, 70). Also, McCormack et al. reported that elite cross-country runners had an average EI of 1940 kcal/day (65). Notably, based on several trials, Hawley et al. reported that the typical mean EI for female endurance athletes with high energy demands is estimated to be between 2388-4060 kcal/day (41).

1.7 Meal Frequency and Meal Patterns

General Recommendations and Recent Research

In the upcoming 6th edition of Nordic Nutrition Recommendations (NNR) 2022, the authors report no consistent findings regarding meal frequency on bodyweight-or body composition in the general population (103). The importance of meal frequency and how it affects the WDEB-variables in regard to the endocrine system has been emphasized by Benardot, explaining how different appetite stimulating hormones can be altered to work in unexpected ways (22). Importantly, WDEB and meal frequency are not necessarily associated and even a positive relationship between meal frequency and WDED has been reported, indicating that energy deficient female athletes have a high meal frequency but with a low energy density (32, 69).

In contrast, Hawley et al. reported that an adequate EI was associated with a relatively high meal frequency of six-ten meals/day based on several surveys among athletes, and that female endurance athletes consume a large amount of their EI during training (41). However, it is also recognized that female endurance athletes tend to have an irregular and inappropriate meal pattern especially around training session, which may suggest that some athletes deviate from the observations reported by Hawley et al. (8, 41, 48, 69).

1.8 Knowledge on Relative Energy Deficiency in Sport and Nutritional Interventions among Athletes

1.8.1 Knowledge and Awareness of Relative Energy Deficiency in Sport

There is currently little research examining the effect of sports nutrition interventions covering REDs, and coaches and the support team around play a particular important role regarding the identification of REDs and referring athletes at risk (57). Moreover, it has been found that coaches report a lack of knowledge and education about REDs, and a survey among International Sport Federations observed that only two out of 28 Olympic Federations had completed educational courses about REDs (37, 78). Educational programs have therefore been advocated and suggested to enhance awareness and knowledge of REDs, and to possibly be an important preventive initiative (14, 51, 57, 78). Also, individual nutritional counseling has been proposed to possibly enhance several of the dietary errors observed among athletes, suggesting that interventions combining education and individual guidance can be beneficial (41, 82).

Kroshus et al. performed a survey among Athletic Trainers in the National Collegiate Athletic Association and reported that 98.5 % knew about the Triad, 33 % were aware of REDs and merely 13 % managed to identify that an energy imbalance was a part of the etiology (51, 57). Similarly, Curry et al. investigated awareness of the Triad among physicians, and found that 37 % knew about REDs, but 51 % reported feeling uncomfortable in treating or referring an athlete at risk (20). Also, Gillbanks et al. interviewed elite lightweight rowers and physiotherapists and found a significant lack of knowledge and awareness on REDs (37).

1.8.2 Nutritional Education and Individual Counseling

In 2019, Krick et al. conducted a study among female high school athletes participating in different types of sports, where one group was presented with a 10minute video created by Brown covering Triad-related topics, while the other group was not presented with the video (49, 50). Knowledge about Triad/REDs was low for both groups at baseline, but significantly increased for the video-group after the intervention (50). Moreover, Brown et al. performed a similar study among collegiate female dancers provided with the same video and found a significant increase from 58 % to 90 % correct answers in the post-intervention questionnaire covering Triad-related questions (11, 49, 68).

Interestingly, Heikkilä et al. found that nutritional knowledge was significantly improved among Finnish endurance athletes after a nutritional intervention including three educational sessions and individual dietary consultations, but no significant change in dietary behavior was observed (42). Also, Valliant et al. conducted an intervention including individualized dietary education among a group of female volleyball players over the course of four months, and found significant improvements in knowledge, total EI, carbohydrate, protein and fat-intake after the intervention (109). Interestingly, average baseline EI was 1756 kcal/day with 0 % in EB, which significantly increased to 2178 kcal/day and 18 % in EB after the intervention (109).

Together, all these findings indicate that both short and longer sports nutrition educational interventions can be effective to enhance knowledge among athletes and coaches. Notably, the association between nutritional knowledge and dietary intake in athletes is reported to be modest ($r < 0.44$), and Logue et al. emphasize that it is important to investigate whether increased knowledge is associated with behavioral change, and highlight the need for larger longitudinal studies to fully understand the possible associations between knowledge, behavior, EI and the development of LEA/REDs (42, 57).

2 Objectives

The main objective of this master´s thesis was to investigate the effect of a 16-week sports nutrition intervention on WDED for female endurance athletes at risk of REDs. A second objective was to examine if the WDEB-method and the 24h-EB method generate similar interpretations of energy status. Finally, it was of interest to investigate the association between WDED and the LEAF-Q score.

<u>The specific research questions were:</u>

1. Can a 16-week sports nutrition intervention reduce WDED among female endurance athletes at risk of REDs? More specifically, can a 16-week sports nutrition intervention decrease the number of hours spent in negative EB and/or reduce the largest hourly deficit among female endurance athletes at risk of REDs?
2. Will the WDEB-method and the 24h-EB method generate similar interpretations regarding the energy status of these athletes?
3. Is there an association between WDED and the LEAF-Q score?

3 Method

This master´s thesis was a part of the FUEL-intervention (In Norwegian: Forstå Utholdenhetsidretts Ernæring – et Læringsprogram), and the data used for the main analysis was collected beforehand by the project managers during the intervention which was shared after the project had ended in July 2022. Thus, the main task in this master´s thesis was to conduct a post-intervention analysis of the subjects dietary and activity-records, and more specifically to calculate EI and TEE (incl. DIT, EEE, RMR, EPOC and NEAT), and notably the analysis of NEAT from raw-data files (accelerometry) was a part of this process. These variables were used to estimate WDEB and 24h-EB for a group of athletes derived from the FUEL-intervention. The recruitment process and execution of the FUEL-intervention itself was therefore not a part of the main methodology carried out by the author of this master´s thesis.

3.1 Introduction to the FUEL-intervention

The FUEL-intervention was a multicenter collaboration between Norwegian, Swedish, German, and Irish research institutes, and was based at the University of Agder with PhD. student Ida L. Fahrenholtz and Prof. Monica K. Torstveit as main coordinators. Notably, the intervention was conducted during the COVID-19 pandemic, and thus several adjustments had to be made both during the planning, implementation, and execution of the intervention. The FUEL-project followed an intervention design, where the main purpose was to increase knowledge on how to optimize nutrition for female endurance athletes at risk of REDs to improve or maintain EA and athletic performance. It was also of interest to investigate the effect of this type of nutritional program on physiological and psychological health among the athletes. An overview of the intervention is presented in **figure 3,** which started with a screening phase (part 1), followed by baseline assessments (week 0, part 2), the 16-week intervention (week 1-16, part 3), and ended with post-intervention assessments (week 17, part 4). The intervention consisted of 16 sports nutrition lectures and eight individual counseling’s, which is described in more detail in **appendix 1.**

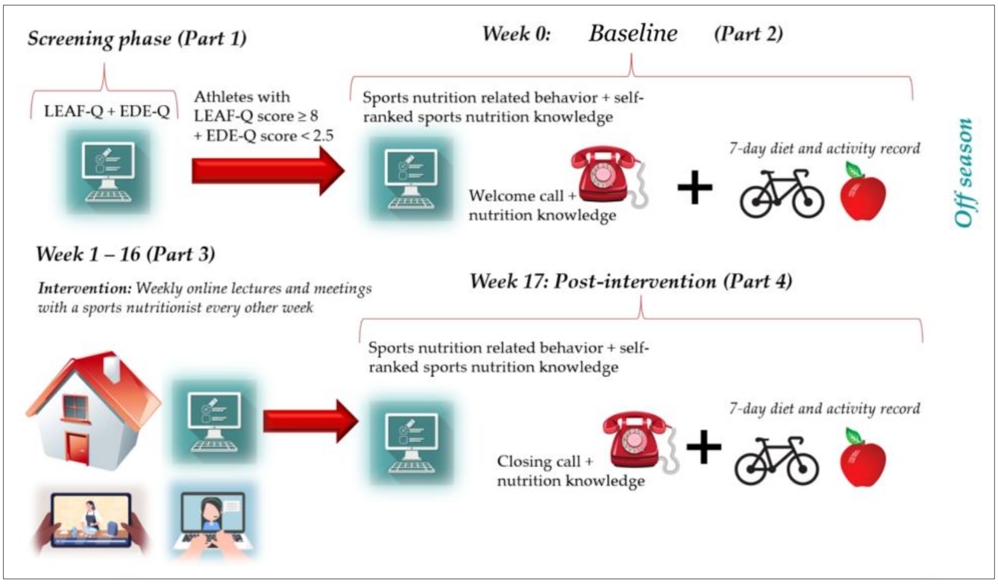


Figure 3: Overview of the recruitment process and structure of the FUEL-project. *Note: illustration is a reprint with permission from Fahrenholtz et al. (29).* Abbreviations: LEAF-Q; The Low Energy Availability in Females Questionnaire. EDE-Q; The Eating Disorder Examination-Questionnaire.

3.2 Recruitment

The subjects were recruited through social media and endurance sports clubs (either cycling, running, biathlon, orienteering, triathlon, or cross-country skiing) from Norway, Sweden, Germany and Ireland. They were included during their off-season, and therefore subjects from winter sports were recruited in May, while those from summer sports were recruited in December/January.

3.2.1 Screening Phase, Inclusion and Exclusion Criteria to The FUEL-intervention

Inclusion criteria for the intervention were that the participants had to be non-smoking, 18-35years of age, competitive female endurance athletes training a minimum of five times per week. All subjects eligible were given a written consent to approve their participation and filled out an online survey covering background information about current and past sports participation, level of competition, occupation, average training volume, age, height, and body weight. They were also asked to complete the EDE-Q and LEAF-Q validated screening tools both at baseline (week 0) and post-intervention (week 17) (**figure 3)** which are described in more detail in separate sections below.

Exclusion criteria were pregnancy, use of contraceptives or any chronic diseases (e.g., diabetes, thyroid dysfunction, Crohn’s disease). Participants who met the inclusion criteria and had an EDE-Q score< 2.5 and LEAF-Q score ≥ 8 were invited to participate in the intervention (part 2, **figure 3**). The participants were asked to complete seven-day dietary and training records at baseline (week 0) and post-intervention (week 17), and received a phone call during these weeks with questions regarding current sports nutrition knowledge (part 2 **figure 3).** The athletes completed once again the EDE-Q and LEAF-Q after the 16-week intervention (week 17, part 4 **figure 3).**

Screening for Risk of Eating Disorders

The Eating Disorder Examination Questionnaire (EDE-Q 6.0) is a validated questionnaire that was used to assess ED symptoms, risk of LEA/REDs, DE and measure behavior and cognitive eating disorder patterns (33, 39, 97). The cut-off value used to determine and distinguish between subjects with an ED and LEA/REDs in this study was an EDE-Q global score ≥ 2.5 (33, 94). Only athletes with EDE-Q global score< 2.5 were included in the FUEL-intervention.

Screening for Risk of Low Energy Availability

The Low Energy Availability in Females Questionnaire (LEAF-Q) was used to assess physiological symptoms of inadequate EI, and hence the risk for LEA/REDs. This tool has been validated and is considered highly valuable to identify female endurance athletes in risk of LEA (68). A total score ≥ 8 was classified as "risk of LEA" and used as an inclusion criterion for the FUEL-intervention (30, 68)

3.2.2 Allocation and Enrollment to the Final Within-day Energy Balance Analysis

Participants with entire days of missing data, specifically dietary, training and/or accelerometer-records were excluded. Therefore, only participants who completed the registrations for all seven days at baseline and post-intervention were included for the final analysis of this master´s thesis. Out of a total of 208 participants that volunteered, 141 were not eligible after the initial screening phase (part 1, **figure 4)** for the following reasons: male (n=2), age (n=3), sport (n=1), chronic disease (n=3), HC-user (n=55), EDE-Q >2.5 (n=23), low LEAF-Q< 8 (n=51) or no contact information (n=3). 67 subjects were invited to participate, but 14 were excluded due to illness (n=3), identified as non-REDs athlete (n=7), or withdrawment (n=4). This left a total of 33 participants eligible for the FUEL-intervention.

Out of the 33 FUEL-participants, 14 subjects from Germany or Ireland were excluded from the WDEB-analysis (part 2, **figure 4)** due to: missing days with accelerometer data (n=9), dropout (n=1), missing timepoint for dietary records (n=2) or training sessions (n=2). This resulted in a total of 19 subjects from Norway and Sweden which were eligible for the final WDEB-analysis, and no participants from Germany or Ireland due to the abovementioned causes.

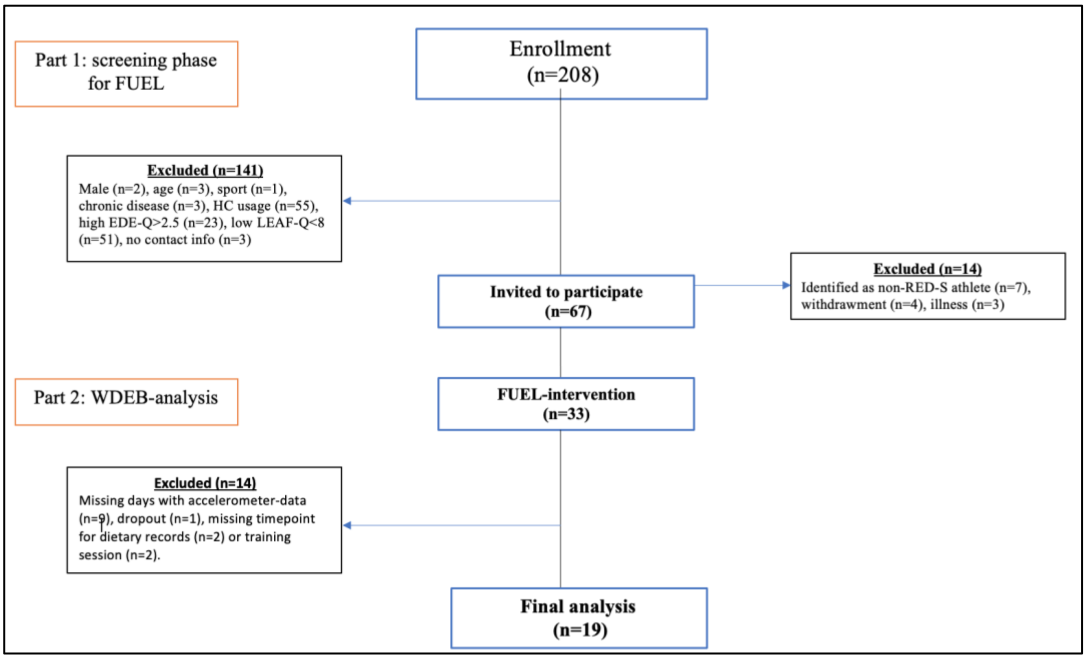


Figure 4: Flowchart of the Recruitment Process for this Study Population. An overview of the recruitment process for the FUEL-intervention (part 1) and allocation to the main analysis of this master´s thesis (part 2). Notably, several participants had more than one reason to be excluded (i.e., missing accelerometer-data + training hours). Abbreviations: HC; handicap. EDE-Q; The Eating Disorder Examination-Questionnaire. LEAF-Q; The Low Energy Availability in Females Questionnaire. WDEB; Within-day Energy Balance. FUEL; Forstå Utholdenhetsidretts Ernæring – et Læringsprogram (in Norwegian). REDs; Relative Energy Deficiency in Sport.

3.2.3 Sample Size and Power Calculation

There are currently no other intervention studies that have investigated changes in WDEB among female endurance athletes specifically, and a power estimation and determination of a minimum sample size was therefore based on a cross-sectional study by Farenholtz et al. (31) investigating differences in WDED between amenorrheic and eumenorrheic athletes. The minimum required sample size needed in this study was estimated to be 14 subjects, with 80 % power and a significance-level of 5 %.

3.3 The FUEL Intervention: Nutrition Lectures and Counseling

3.3.1 Digital Video Lectures

The participants got access to a closed online platform where the FUEL-program was initiated (week 1), and all lectures were presented via the teleconference platform Zoom (Video Communication Inc. Version 5.3.2). One lecture was presented each week from week 1 to week 16, and the athletes had the opportunity to watch these whenever it suited them the most and had the opportunity to watch it over again if necessary. The lectures, including accompanying manuscripts, were made by four experiences sports nutritionists and were based on evidence sports nutrition guidelines by the IOC, International Association of Athletics Federation (IAAF) and Olympiatoppen in Norway (12, 14, 69, 76, 78, 87, 102). Each lecture covered a specific topic relevant for female endurance athletes in risk of REDs, including sports nutrition, REDs and menstruation, and was recorded and presented by experienced sports nutrition researchers in the preferred language of the athlete. The average duration of the lectures was 25.0 ± 8.4 min, and an overview of the topics for the different lectures is described in more detail in **appendix 1.**

3.3.2 Nutritional Counseling

The participants were offered individual nutritional counseling with a registered sports nutritionist every two weeks during the 16-week intervention (a total of 8 sessions). These were held via Zoom and were scheduled at a time that suited the athlete best. The first initial consultation lasted for approximately 1.5 hours, while each following consultation was scheduled to last one hour. The FUEL-counselor team consisted of three Norwegians, four Swedes, two Irish and one German experienced sports nutritionist. Each counselor had to complete three webinars and read through a comprehensive manual before the intervention started to ensure standardization. In addition, the counselors participated in two Zoom meetings every week during the intervention, which were guided by the head of nutrition at the Norwegian Olympic and Paralympic Committee and the Confederation of Sports, and the data collection coordinator. Also, a professional psychiatrists specialized in eating disorders were included in the team to support the counselors if needed, and a flow diagram based on the RED-S CAT was available if the counselors suspected that an athlete possibly needed further assessments outside the FUEL project (i.e., gynecological examination to an undiagnosed MD).

Behavior Change Techniques and Theoretical Counseling Models

The overall structure of the FUEL-consultations were inspired by the Four Habits Model **(appendix 2),** where autonomy, competence, and relatedness were core principles primarily since a self-determination theory has been shown to be effective for behavioral change through internal motivation (34, 96). Therefore, an individualized, emphatic communication approach based on fundamental skills in motivational interviewing was applied (9, 74). The first consultation was focused on the transtheoretical model of behavior change, which was presented to the athletes to promote their awareness and readiness to change (85). New objectives and focus areas for each upcoming session was determined based on the athlete´s progress and readiness for change and were thus customized to each individual. Different behavior change techniques were used based on the athlete´s individual needs and preferences, and were designed to include the following themes according to the taxonomy (v1) by Michie et al. : “1.1 goal setting (behavior)”, “1.2 problem solving”, “1.5 review behavior goal(s)”, “1.9 commitment”, “2.2 feedback on behavior”, and “3.3 social support (emotional)”, among others (71).

Moreover, the date, time and actual duration of each session was registered, and the counselor was provided with the athlete´s LEAF-Q response in advance so they could better prepare and customize each individual consultation. In addition, they got access to the nutritional lectures in case any questions related to the educational topics were asked by the athlete. Details regarding the preparation and execution of the FUEL-counseling derive from Fahrenholtz et al. (29).

3.3.3 Dietary Records and Activity Records

Dietary Records and Estimation of Energy Intake

All participants were instructed to record their diet for seven consecutive days, both at baseline and post intervention (week 0 and 17, **figure 3**). Digital kitchen scales were shipped in the mail to each individual athlete, and they were given a manual with precise guidelines on how it should be used. “Dietist Net Matdagbok” (*Dietist Net Food-diary)* was the digital platform that the subjects used to register their diet, and they were instructed to log both the quantity, type of food and drink (incl. water), preparation method (i.e., boiled vs. fried) and exact time point of each meal. They were given detailed instructions on how to use Dietists in the app and web-version and were given practical tips and a diet-record template in paper form to write down each meal “on the go” (See example in **appendix 3**).

Non-Exercise Activity Thermogenesis and Accelerometer

To estimate NEAT the participants wore an Actigraph-accelerometer for seven days both during week 0 (baseline) and week 17 (post-intervention). They were given a written manual and a personal phone-call with information and were instructed to wear it on their hip throughout the day except during exercise, showering, and sleeping hours. Actilife Software v6.13.4 was used to analyze the data after the intervention. If a participant mistakenly wore the detector during exercise it was corrected for in the post-analysis through the "Log Diaries"-function in Actilife, matching high accelerometer-activity and training sessions simultaneously during the same time-period. Only EE from exercise was then used and NEAT was deleted during these periods, and this was done to ensure that TEE was not overestimated. An example of a template of the “Log Diaries” is illustrated in **appendix 4.**

Analysis with and without wear-time-filter was assessed in Actilife, with a wear-time-filter set to a minimum of 8h/day, as suggested from Miguele et al (73). Due to many days being classified as invalid when applying the wear-time filter, the analysis without wear-time-filter was used. This was done in order to conduct the main analysis, retain the number of days, the sample-size, and thus overall power. Notably, this was clarified by a technical support specialist from ActiGraph Support and was considered acceptable on the basis that there were minor differences in the NEAT values (kcal/d) with and without the filter applied.

3.3.4 Training Records and Heart Rate Monitors

The participants were instructed to log all their training as detailed as possible during the 16-week intervention, but particularly at baseline (week 0) and post intervention (week 17). This was done in the online training-diary platform “Bestr” (www.Bestr.no), with a description of exercise modality and time point for each session. The total duration spent in each heart rate (HR)-zone was automatically registered by the HR-monitor and registered in Bestr for each session. Notably, the participants were told to take the accelerometer off and use their own sports watch together with a HR-monitor during all bouts of training to track the intensity and duration of each session.

3.3.5 Body Mass Index

Definitions of BMI was determined based on the classification presented in NNR 2012 (86): underweight (BMI< 18.5 kg/m^2), normal weight (BMI [18.5;24.9 kg/m^2]), overweight (BMI [25.0;29.9 kg/m^2]), grade I obesity (BMI [30.0;34.9 kg/m^2]), grade II obesity (BMI [35.0;39.9 kg/m^2]) and grade III obesity (BMI> 40 kg/m^2) (86).

3.3.6 Missing Data

Whenever a participant had insufficient registration of a single meal or training session, the mean value from the other days was used as an input, and therefore missing data were handled by simple imputation based on the average. Similarly, training sessions where average HR was missing (i.e., swimming and/or strength-sessions), the mean value from the other sessions was used.

3.4 Energy Balance Calculations

3.4.1 Within-day Energy Balance

WDEB was calculated mainly based on the methods used by Fahrenholtz et al. and Torstveit et al. and was assessed in one-hour intervals (32, 108). In addition, several of the studies presented in **appendix 10** were used as a basis for determining an applicable approach to calculate WDEB in this master´s thesis (4, 7, 32, 36, 46, 54, 62, 108).

3.4.2 Calculation of Within-day Energy Balance

Hourly EB was calculated based on the following equation: EB = EI - TEE, where TEE = EEE + EPOC + NEAT + pRMR (or pSMR) (22, 54, 108). Each of the WDEB-variables are explained in more detail below. The waking hours was determined based on the timepoint from the first to the last NEAT-value since the subjects were instructed to only use the accelerometer during wakening hours. WDEB was calculated continuously from day one to day seven, and is therefore considered a cumulative value. For complete description and illustration of the WDEB-calculation see **appendix 5.**

Starting Energy Balance (day 1)

The starting EB for day one was estimated by determining the timeframe in which the participant usually consumed their last meal/snack based on the seven days of registration. The average EI and average TEE within this timeframe was then calculated and subtracted from each other, resulting in an estimated starting EB for day one for that respective week (either baseline or post-intervention week) (32, 108). See **appendix 5a** for further details and illustrations.

Largest Single-hour Deficit

The largest single-hour deficit (LSHD, magnitude of deficit) was determined by averaging all the largest single-hour deficit values observed within each day of the seven days of registration (both baseline and post intervention week) (**appendix 5)** (32, 62).

Within-day Energy Deficiency

WDED was determined according to Deutz et al., and was evaluated by calculating the frequency of hours spent in an energy deficit (EB< -300 kcal), hours spent in a catabolic state (EB< 0 kcal) and the magnitude of the LSHD (26).

3.4.3 Calculation of 24-hour Energy Balance

Average 24h-EB was defined as the end of the day EB (24h) and was determined by averaging the 24h-EB values from each day during the assessment week (both baseline and post-intervention week). 24h-EB was calculated as: total EI-TEE (**appendix 5).**

3.5 Energy Expenditure and Energy Intake

Resting Metabolic Rate

To control for problems related to a potential underestimation of energy requirements, it has previously been suggested to use pRMR instead of mRMR in the calculation of WDEB in athletes in risk of REDs (26, 32, 108) . Based on this, pRMR was used and factored in the calculation of TEE (**appendix 5**). The hourly pRMR was estimated using the Harris Benedict equation: RMR (kcal/h) = $\frac{655.0955 + 9.5634*\mathrm{w} + 1.8495*\mathrm{h} - 4.6756*\mathrm{a}}{24h}$ (w: body weight (kg), h: height (cm), a: age (years) (40, 107).

Sleeping Metabolic Rate

Sleeping metabolic rate (SMR) was calculated as 90 % of pRMR, and constituted EE during sleeping hours and therefore replaced pRMR during these time periods (**appendix 5**) (32, 54, 62, 86, 108).

Excess Post-Exercise Oxygen Consumption

Several studies assessing WDEB have used 8 % of EEE to determine EPOC (32, 54, 108), which is primarily based on previous work by Phelain et al. and Sedlock (89, 95). Therefore, EPOC was estimated by calculating 5 % of EEE the first hour post-exercise plus 3 % of EEE the second hour post-exercise (a total of 8 % of EEE) (**appendix 5**).

Exercise Energy Expenditure

A significant linear relationship between HR (beats/min) and oxygen consumption ($VO_{2)}$) (mL O_2/min) has been reported (35). Due to the pandemic, none of the physical tests needed to create individual metabolic profiles were possible to perform in the FUEL-intervention (i.e., measurement of VO_2 and carbondioxide (VCO_2) in relation to HR, and the respiratory-exchange-ratio (RER)). An average HR-oxygen consumption equation was calculated from data of a similar population (Scandinavian female endurance athletes, 18-38 years of age) previously studied by the research group (70). More details on the methodology of the LEA-study (70) is presented in **appendix 9,** and a complete overview of the calculation of EEE is explained in more detail in **appendix 6a-c.**

A mean regression curve for the relationship between VO_2 and HR for the LEA-participants was generated by Phd. Student Ida Fahrenholtz *($y = 26.97704*HR-1693.29$,* **appendix 6a***)*, and the energy equivalent was estimated to be *0.0050233kcal/mL*, based on the average RER-value, as suggested by Cavagna (17, 70) **(appendix 6b)**. The exercise duration (min) and average HR (bpm) of every training session for each of the FUEL-participants was then calculated into this formula, resulting in an estimated EE for each session *(EEE= mL O2/min*0.0050233*duration*, **appendix 6b).** For training sessions lasting beyond an hour, mean EEE following each subsequent hour was calculated and summed up (**appendix 6c).**

Diet Induced Thermogenesis

DIT was determined based on Reed et al. (90), and estimated to constitute of 10 % of EI from the meal, and calculated the following six hours after ingestion. The model used was $175.9*Te^{-T/1.3}$, where *T* is time after ingestion and *e* is the base of natural logarithm (21, 54, 90, 111) (**appendix 5).**

3.6 Energy Intake and Nutritional Analysis

Dietist Net Pro (version 1.0, 2014) was used to analyze EI and nutrient content for all subjects. To determine the time interval for each meal, the recorded starting time was rounded to the nearest full hour-interval where most of the time for that particular meal was spent. For example, a meal starting at 06:15 was estimated to be consumed in the 06:00-07:00 time-interval, whereas a meal starting at 06:30 or 06:45 was rounded up to the 07:00-08:00 time-interval.

3.7 Compliance and Attendance of Digital Lectures

Evaluation of compliance for attending the lectures was based on an evaluation form that was sent out to each participant after the intervention. It covered questions regarding their overall experience and attendance of each lecture. They were asked to what extent they found it limiting that the lectures were held digitally (via Zoom) instead of physically, based on 10-point Likert scale where 1: «not a limitation at all» and 10: «very limiting». In addition, questions regarding perceived motivation to watch the lectures, and overall satisfaction with the dietary guidance and the intervention were also evaluated.

3.8 Statistics

SPSS Statistics version 28.0.1.1 was used for all statistical analysis with a two-tailed significance level of < 0.05. All variables were tested for normality and normally distributed data were presented as mean ± standard deviation (SD), while non-normally distributed data were presented as median and interquartile range (25-75 percentiles). To test and examine differences in the average baseline and post-intervention values, Paired-Samples *t*-test was used for normally distributed data and The Wilcoxon Signed-rank test was used for non-normally distributed data.

A correlation analysis was performed to investigate associations between baseline and post-intervention WDED-variables and the corresponding baseline or post-intervention LEAF-Q score. Pearson`s correlation coefficient ($r$) was calculated for normally distributed data, while Spearman`s correlation coefficient (r_s) was used for non-normally distributed data.

3.9 Use of chatGPT or other artificial intelligence programs

ChatGPT or other artificial intelligence programs have not been used in this master´s thesis.

3.10 Ethics

The FUEL-project was approved by Regional Ethical Committee (REK) (Ref: 31640) **(appendix 11)** and the Norwegian Center for Research Data (NSD) (Ref: 968634). In addition, it followed a recommended checklist from REK, the Helsinki Declaration, and used Services for Sensitive Data (TSD) to handle and store all data records from the participants. All sensitive data were anonymized and will be deleted five years after the intervention ended (21.12.2028). It was voluntary to participate, and there were no expected side effects of the intervention, except the time the athlete needed to spend and set aside for the project.

4 Results

4.1 Subject Characteristics

Subject characteristics are presented in **Table 1.** The average BMI was 21.1 kg/m^2, where 79 % of the participants (n=15) had a normal BMI, 16 % (n=3) were underweight and 5 % (n=1) was characterized as overweight based on the NNR 2012 definitions (86). Average training hours per month was 42.6hours, and the distribution of the sports that the subjects participated in were: cycling (16 %), orienteering (32 %), triathlon (16 %), and running (37 %). There were 68 % Swedish athletes, while 32 % were from Norway.

Table 1: Subject characteristics.

Total participants (n=19)						
	Mean		**Min.**		**Max.**	**SD**
Age, years	26.1		18.0		34.0	5.0
Height, cm	170.2		158.0		186.0	7.2
Weight, kg	61.0		45.0		71.0	7.4
BMI, kg/m^2	21.1		17.9		26.3	2.2
pRMR, kcal/day	1431.6		1218.7		1553.8	89.1
Avg. training/m, (hours)	42.6		13.5		74.0	15.2
Country, n (%)						
	Norway			Sweden		
	6 (32 %)			13 (68 %)		
Sport, n (%)						
	Biathlon	Skiing	Cycling	Orienteering	Triathlon	Running
	0	0	3 (16 %)	6 (32 %)	3 (16 %)	7 (37 %)

Skiing: cross-country skiing. n: number of subjects. pRMR: predicted RMR based on Harris Benedict equation. Abbreviations; SD: standard deviation. BMI: body mass index. Avg.: average. Min: minimum. Max: maximum. m: month.

4.2 Energy Intake and Total Energy Expenditure

There was a tendency to an increase in average daily EI from baseline to post-intervention (2660 ± 553 kcal/day vs. 2865 ± 496, $p = 0.054$), and notably TEE was decreased after the intervention (2988 ± 321 kcal/day vs. 2846 ± 389 kcal/day, $p = 0.021$) (**Table 2)**. Moreover, out of the variables that constitute TEE, both EEE and EPOC were reduced from baseline to post-intervention (EEE: 1039 ± 193 kcal/day vs. 887 ± 249 kcal/day, $p = 0.009$. EPOC: 83 ± 15 kcal/day vs. 71 ± 20 kcal/day, $p = 0.012$, respectively), whereas DIT and NEAT were not significantly changed (**Table 2)**.

Table 2. Average energy intake (EI, kcal/d) and total energy expenditure (TEE, kcal/d).

	Baseline	Post-intervention	p-value
Energy intake, kcal/day^1	2660 ± 553	2865 ± 496	0.054^1
TEE (kcal/day)^1	2988 ± 321	2846 ± 389	**0.021^{1*}**

DIT, kcal/day[1]	264 ± 54	284 ± 49	0.057[1]
EEE, kcal/day[1]	1039 ± 193	887 ± 249	**0.009[1]***
EPOC, kcal/day[1]	83 ± 15	71 ± 20	**0.012[1]***
NEAT, kcal/day[2]	160 (135-300)	168 (140-233)	0.496 [2]

[1]Normally distributed data: Paired Samples T-test was used to analyze difference in individual baseline to post-intervention values. [1]Data are presented as mean ± SD for normally distributed data. [2]Non-normally distributed data: Wilcoxon Signed Rank-test (Exact Sig.2-tailed) was used, and values are presented as median and 25th-75th-percentile. *Significant difference (p <0.05). Abbreviations; TEE: total energy expenditure. DIT: diet-induced-thermogenesis. EEE: exercise energy expenditure. EPOC: excess post-exercise oxygen consumption. NEAT: non exercise activity thermogenesis. Kcal: kilocalories

4.3 Within-day Energy Balance and Within-day Energy Deficiency

Average hours/day spent in the different EB-zones together with average 24h-EB during the seven-day baseline and post-assessment is presented in **Table 3**. There was a change in all WDEB-variables during the intervention, and subjects spent more hours in a positive EB after the intervention compared to baseline (hours in: EB> 0 kcal: 19h vs. 4h, $p = 0.006$, and EB> 300 kcal: 14h vs. 2h, $p = 0.044$). Likewise, hours spent in a negative EB (marked in red) were decreased from baseline to post-intervention (EB< 0 kcal: 20h vs. 5h, p = 0.006, and EB< -300 kcal: 18h vs. 2h, $p = 0.005$). Moreover, the LSHD was decreased (-1686.1 kcal vs. -330.4 kcal, $p = 0.002$), and the LSHS was increased from a negative to a positive kcal-value after the intervention (-205.2 kcal vs. 1086 kcal, $p = 0.004$) (**Table 3**). There was also an increase in average 24h-EB from baseline to post-intervention, switching from a negative to a positive EB (-327.7 kcal vs. 19.9 kcal, $p = 0.008$). There was no significant difference in hours spent in the EB [-300; +300 kcal]-zone (3.4h vs. 3.9h, $p = 0.598$) (**Table 3**).

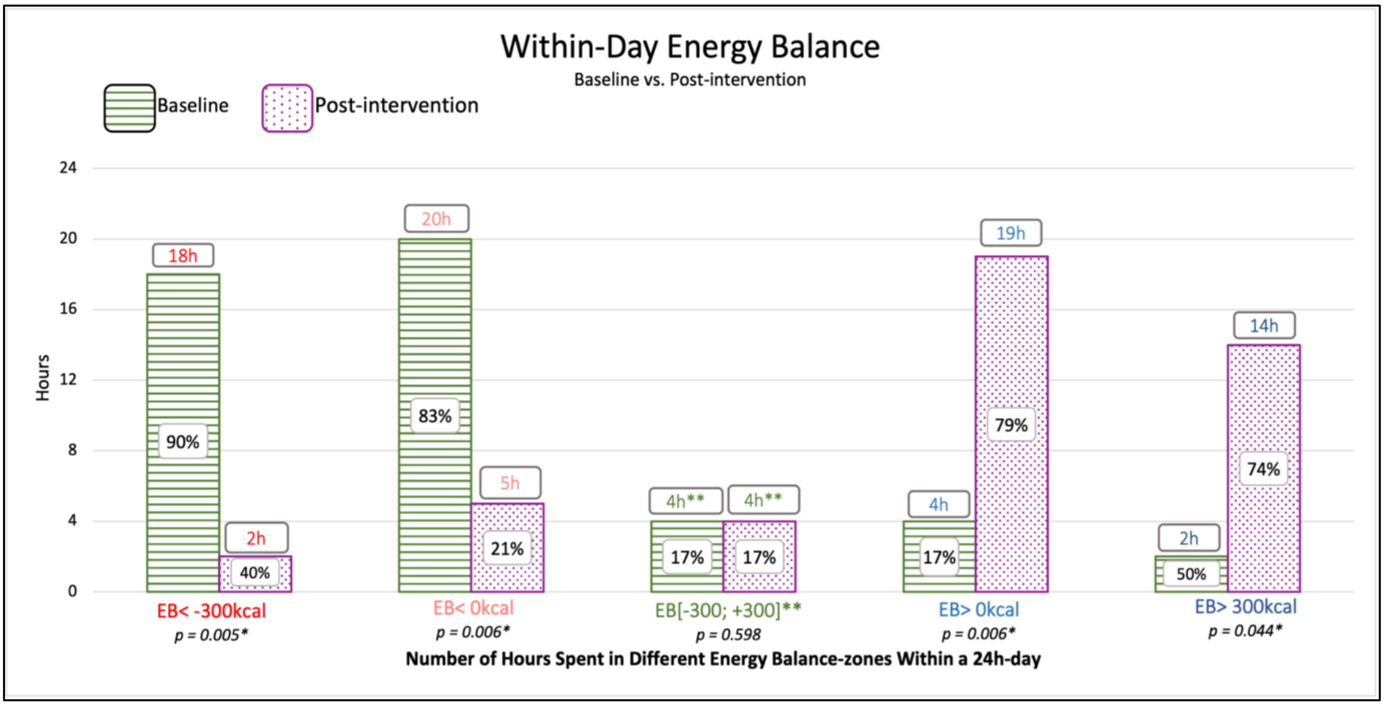
Table 3: Changes in Within-day Energy Balance and Within-day Energy Deficiency.

Hours/day spent in:	**Baseline**	**Post-intervention**	**p-value**
EB >300kcal[2]	2.0 (1 – 15)	14.0 (2 – 22)	**0.044***
EB >0kcal[2]	4.0 (1 – 20)	19.0 (7 – 24)	**0.006***
EB <0kcal[2]	20.0 (4 – 23)	5.0 (0 – 17)	**0.006***
EB <-300kcal[2]	18.0 (2 – 22)	2.0 (0 – 15)	**0.005***
Hours [-300; +300 kcal][1]	3.4 ± 2.5	3.9 ± 2.7	0.598
LSHD, kcal[1]	-1686.1 ± 1618.9	-330.4 ± 1694.0	**0.002***
LSHS, kcal[1]	-205.2 ± 1633.3	1086.4 ± 1809.0	**0.004***
Average 24h-EB, kcal[1]	-327.7 ± 432.7	19.9 ± 457.3	**0.008***

The WDED-variables are marked in red to highlight that these variables are the ones related to a negative EB. [1]Normally distributed data: values are presented as mean ± SD and Paired Samples T-test was used to analyze difference in average baseline and post-intervention values. [2]Non-normally distributed data: values are presented as median and 25th-75th-percentile and Wilcoxon Signed Rank-test was used (Exact Sig.2-tailed). *Significant difference between baseline and post-intervention values ($p < 0.05$). Abbreviations: WDEB; Within-day Energy Balance. EB: energy balance. LSHD: largest single hour deficit. LSHS: largest single hour surplus. h: hour. kcal: kilocalorie.

Figure 5 illustrate average time spent in different EB-zones during baseline (green horizontal lines) and post-intervention (purple dots). Before the intervention 83 % of the hours were spent in a catabolic state (EB< 0 kcal), and 90 % of these hours were spent below EB< -300 kcal, while 17 % of the hours were spent in EB >0 kcal, and 50 % of these hours were spent in EB >300 kcal. After the intervention, 21 % of the hours were spent in EB< 0 kcal, and 40 % of these were below EB< -300 kcal, while 79 % of the hours were spent in EB> 0 kcal, and 74 % of these were spent above EB> 300 kcal. Hours spent in EB between [-300; +300 kcal] did not change significantly during the intervention (baseline: 17 %, 4h ± 2.5 and post-intervention: 4h ± 2.7).

Figure 5: Bar charts of Within-day Energy Balance at Baseline vs. Post-intervention.



The bar chart illustrates average time spent in different EB-zones during the baseline (green horizontal lines) and post-intervention week (purple dots). Values above the bars represents median hours spent in the respective EB-zone. **: mean *: significant difference ($p < 0.05$). Values inside each bar represent the proportion of these hours as percentages. Abbreviations; h: hours, kcal: kilocalories.

4.4 Association Between Within-day Energy Deficiency and The Low Energy Availability in Females Questionnaire score

There were no significant associations between any of the WDED-variables with the corresponding LEAF-Q score (neither baseline nor post-intervention). Notably, although not significant, the association followed an inverse direction for hours in EB< 0 kcal and EB< -300 kcal for both the baseline and post-intervention values with the LEAF-Q score (**Table 4).**

Table 4. Association between Within-day Energy Deficiency variables and LEAF-Q score.

Assessment	**LEAF-Q score**	
Baseline (n=19)	**Correlation coefficient (r_s/r)**	**p-value**
Hours in EB < 0kcal	-0.15[2]	0.546[2]
Hours in EB < -300kcal	-0.18[2]	0.468[2]
LSHD, kcal	0.12[1]	0.612[1]
	LEAF-Q score	
Post-intervention (n=18)	**Correlation coefficient (r_s/r)**	**p-value**
Hours in EB < 0kcal	-0.35[2]	0.154[2]
Hours in EB < -300kcal	-0.38[2]	0.116[2]
LSHD, kcal	0.38[1]	0.124[1]

*Significant difference ($p < 0.05$). [1]Pearson`s correlation coefficient (r) was calculated for normally distributed data, and [2]Spearman`s correlation coefficient (r_s) was used for non-normally distributed data. Abbreviations; EB: energy balance. LSHD: largest single hour deficit. Kcal: kilocalories. LEAF-Q: The Low Energy Availability in Females Questionnaire. *Note: All subjects were included in the baseline correlation analysis (n=19), but not for the post-intervention analysis since one subject did not complete the post-intervention LEAF-Q questionnaire (n=18).*

4.5 Compliance of Video Lectures

Out of the 19 participants analyzed in this study, 16 subjects responded to the post-intervention evaluation form (84 % respondence rate). 15 subjects watched all the 16 video lectures, whereas one subject missed one lecture (99.6 % compliance rate). The average score for examining whether the participants found it limiting or not that the lectures were presented digitally was 2.5 ± 1.9, and 15 subjects scored < 5points (not a limitation), while 1 subject scored 10 (very limiting).

5 Discussion

The main objective of this study was to investigate the effect of a 16-week sports nutrition intervention on WDED among female endurance athletes at risk of REDs. In addition, the WDEB-method was compared to the 24h-EB method, and the association between WDED and the LEAF-Q score was investigated. Based on the analysis, three main results were found:

1) All variables of WDED were significantly reduced after the intervention, including hours spent in EB< 0 kcal, EB< -300 kcal and the LSHD. Hence, there was a significant reduction in hours spent in a negative EB after the intervention for the participating athletes.
2) The WDEB-method and the 24h-EB method generated similar interpretations of the overall energy status, as they both revealed that the subjects primarily were in a negative EB at baseline and a positive EB after the intervention.
3) There was no significant association between WDED and the LEAF-Q score.

5.1 Methodological Challenges

This study managed to generate several significant findings. However, it may be argued that causal relationships or strong conclusions cannot be drawn based on these, primarily due to several of the methodological challenges and limitations that are summarized in the following chapters.

5.1.1 Study Design and Population

This study was based on data from the FUEL-intervention where subjects were assessed at baseline and post-intervention, and one objective for choosing the FUEL-intervention as a basis for this master´s thesis was particularly the study design, as it allowed for a proper investigation of changes in EB from before and after the intervention for each subject. This study assessed female endurance athletes between 18-35 years of age from Norway and Sweden, which may limit these findings to only be applicable to this particular study population. Also, subjects were recruited through sports clubs and social media, and the risk of selection bias should therefore be addressed since other representative females might have been neglected. In addition, it might be argued that the athletes who signed up are generally more interested in REDs and thus assumably more motivated to make nutritional changes compared to other athletes, which may suggest that the changes in WDED might have been lower among other subjects. On the contrary, there might have been athletes especially concerned about their diet who alluded to participate in fear of being “exposed” and jeopardize their sport participation.

5.1.2 Missed Assessments

As mentioned, the FUEL-intervention was carried out during the COVID 19 pandemic, and many of the physical assessments that were intended to be conducted were therefore omitted. The fact that the participants were prohibited to attend any physical assessments or meet the practitioners and project managers in person might affected their accountability and personal commitment of complying to the intervention. As a result, there are several caveats to this study that should be acknowledged.

Exercise Energy Expenditure and Heart-Rate Monitors

First, as shown in **appendix 9,** previous studies examining WDEB have performed many physical tests to analyze important parameters, particularly; hormone levels, lactate- and VO_2-profiles, RMR, RER, and DEXA-scans. However, none of these assessments were possible to conduct in the FUEL-intervention, which was particularly limiting for the accuracy and calculation of EEE in this study, considering that certain data from a similar, yet another study cohort (The LEA-subjects) had to be used. Nevertheless, this solution was essential in order to carry out the WDEB-analysis, and the data from the LEA-study was considered the most applicable and available for these calculations, and thus used as a proxy in this study.

On the other hand, it may be argued that the combination of data from a similar study cohort (The LEA-subjects) in conjunction with the data from the FUEL-subjects hypothetically expand the overall study population which may make these results more generalizable and strengthen the external validity. Notably, this assumption is made on the basis that all data derive from a similar population considering that both the LEA-and the FUEL-intervention had nearly identical enrollment, and inclusion and exclusion-criteria (70).

Notably, both the training duration and HR-values used to calculate EEE for each session derive from the FUEL-participants themselves. Several practical advantages of using HR-monitors to assess exercise have been acknowledged, particularly the small instrument size and overall cost of the device (28, 35). However, various measurement errors have been reported, one being that the relationship between HR and VO_2 mainly apply when HR is between 110-150bpm, and the accuracy of EEE estimated under very light or very strenuous activity may therefore be imprecise (93). Secondly, both fatigue and hydration have been reported to affect this relationship, and factors such as high ambient temperature, humidity and emotional stress can cause an increase in HR without an actual increase in VO_2 (35).

Resting Metabolic Rate

Another important assessment that had to be omitted was individual lab measurements of RMR, which hindered the possibility to calculate RMR_{ratio}. This is especially noteworthy since an RMR-ratio < 0.9 is recognized as one of the main determinants of an energy deficit (54, 57). Also, objective measurements under controlled settings in a lab has been recognized as crucial for a proper investigation and identification of REDs (57, 70, 108). Moreover, a reduction in mRMR is reported to be one of the first identifications of an acute energy deficit, and this variable would therefore arguably been interesting and relevant to assess in this 16-week intervention, especially since it has been reported that alterations occur after only seven days in previous trials (59, 101). Moreover, a calculation of the RMR_{ratio} in this study would potentially allowed for more basis of comparison with previous publications investigating WDEB, as shown in **appendix 9** (32, 46, 54, 108).

Energy Availability

EA was another highly relevant variable that could not be calculated, primarily since FFM was not possible to measure. This was particularly limiting since investigations of changes in EA could possibly have contributed to additional interpretations of the effect of the FUEL-intervention on nutritional and training behavior among the athletes. In addition, EA could also made this study more comparable to other publications, considering that EA is one of the most relevant and frequent variables assessed in other studies on REDs and EI among athletes **(appendix 9)** (57).

Non-exercise Activity Thermogenesis

The average NEAT values were in general fairly low for most of the participants, and by comparison almost three times lower than the average NEAT-value for the LEA-subjects (LEA: 462 kcal/day vs. baseline-FUEL: 160 kcal/day **(table 2)** (70). There could be several possible explanations for this. Firstly, it may be that the participants in this study actually were less active due to the “lock down” followed by the COVID 19 pandemic. Another reason could be that the subjects were less precise and compliant with using the accelerometer.

Another explanation might be that most of the LEA-subjects were from Denmark where cycling for transportation is common, and they were therefore instructed to take off the detector during these times mainly since it known to underestimate EE (70, 73). The EE from every day-cycling was instead estimated based on HR-data and later added to the total NEAT-value. The FUEL-subjects, on the other hand, were not instructed to remove the accelerometer during cycling and the EE during these times are therefore based on the values from the accelerometer. Hypothetically, this might have led to a small underestimation of NEAT, and thus TEE for the FUEL-participants, which again could indicate that the estimated energy deficit (WDED) in this study was even more severe (70, 73). Nevertheless, the NEAT-variable is only a small portion of TEE and therefore small deviations or changes in NEAT would only affect the overall WDEB-calculation to a small extent.

Despite these methodological explanations for the discrepancy between the LEA-subjects and the FUEL-subjects, it is reasonable to believe that there is an actual difference in the daily activity level and NEAT-values between these two study groups, assuming that the cycling volume of Danes are generally much higher than for other Scandinavian populations.

Digital Lectures and Nutritional Guidance

A third challenge caused by the pandemic was that nearly all type of communication had to be web-based, which initially was thought to affect the intervention negatively particularly since all lectures had to be over video and the individual counseling had to be over the phone. However, based on the answers from the post-intervention evaluation form, all subjects attended each of the 17 digital lectures and did not find it limiting that they were over video. An exception was one subject that found it very limiting and missed one lecture. With this level of compliance, it might seem that the use of digital platforms might actually been beneficial and a strength rather than a limitation for this study, presumably as it made the intervention more available and flexible for the participants, and thus easier to adapt it to their everyday schedule.

Dietary Records

Estimations of EI are suspectable to a number of methodological errors, which unfortunately are reported to be common and inevitable in studies assessing diet and EI (41, 86, 104). Studies have found that athletes tend to either underreport or under-consume during periods of dietary records, and that those particularly aware of their body image are at highest risk of doing so to an extent that affect the estimated EI significantly (13). Under-reporting is recognized as one of the main causes for deviations observed in EB-calculations, and it is reported that the general population under-report their actual EI with approximately 20 % (13, 86). It is therefore reasonable to believe that these errors also appeared in this study, especially considering that energy restriction and DE are reported to be underlying causes of REDs (1, 76, 78). Nevertheless, a specified nutritionist provided the subjects with additional instructions on how to accurately record their diet in Dietist several times throughout the intervention to minimize these measurement errors as much as possible.

5.1.3 Summary of Methodological Challenges

As mentioned, the COVID 19 pandemic and the many limitations that followed affected the possibility to perform several relevant variables and more likely also the accuracy in some of the data that were assessed. However, it can be argued that the reliability and validity of these data are valid and sufficient considering that the same methods were used for the same subject throughout the study, which is noteworthy in this context considering that the main goal of this master´s thesis was to examine individual changes in WDED at baseline and post-intervention.

5.2 Discussion of Main Results

Within-day Energy Deficiency

The main findings of the current study were that a 16-week sports nutrition intervention can reduce WDED and thus improve WDEB among female endurance athletes at risk of REDs. All WDED-variables were significantly decreased after the intervention to an extent that the athletes changed from initially being in a catabolic state (EB< -300 kcal) at baseline over to an anabolic state (EB> 0 kcal) after the intervention, as illustrated in **table 3** and **figure 5.** This was a critical finding, especially since these particular EB-zones have been suggested to be decisive in regard to the physiological consequences of an energy deficit, but also because an anabolic state (EB> 0 kcal) has been suggested to be important for optimal endocrine and metabolic health, in addition to sports performance (**appendix 9)** (8, 22, 26, 46, 108). Notably, there was no significant change in hours spent in EB [-300; +300 kcal], illustrating a large shift from spending most hours in the lower range of WDED (EB< -300 kcal) to the higher range of an EB in surplus (EB> 300 kcal) after the intervention. Surprisingly, the change in WDED was primarily driven by a reduction in the athlete´s overall training volume during the intervention, and there was only a marginal increase EI as shown in **table 2.**

24hour-Energy Balance

Moreover, there was a significant increase in 24h-EB after the intervention, which shows that the 24h-EB method and the WDEB-method generated the same interpretations regarding the overall energy status of the athletes. Notably, this result is in contrast to the reported findings from Benardot (22), Fahrenholtz et al. (32), Friel et al. (36), Lundstrom et al. (62) and Torstveit et al. (108). This may challenge the idea that a new approach to assess EB is needed, and whether an in-depth assessment like the WDEB-method actually is necessary especially considering the different cost and benefits of these two methods (7, 22). Notably, even though the 24h-EB method was arguably easier and less time-consuming to calculate, the WDEB-method did generate an entirely different and more in-depth insight into the EB for these subjects, and thus provided details that could not be illustrated through the 24h-EB method alone (**table 3**).

As mentioned above, there was a significant decrease in TEE after the intervention, which was primarily driven by a reduction in EEE and EPOC (**table 2**). This might indicate that the positive change observed in 24h-EB was mainly due to a reduction in training volume rather than increased EI during the intervention, a theory that do correlate to the nonsignificant and marginal increase in EI observed (**table 2**). Intuitively, this might have seemed unfortunate considering that sports nutrition was one of the main focus areas of the FUEL-intervention. Nevertheless, there could be several reasons why an overall reduction in training volume was observed. Firstly, one possible theory may be that the athletes applied what they had learned about REDs during the intervention and thus intentionally chose to reduced their training to ensure an optimal EB, particularly since the recommendation for treating REDs based on the IOC consensus statement is to correct the deficit either by increasing EI and/or decreasing training volume (77, 78). However, this theory may be up for debate mainly because Fahrenholtz et al. recently reported that the control group of the FUEL-intervention, which did not receive any nutritional guidance or lectures about REDs, also had a reduction in training volume (29).

However, an important point to consider is that a reduction in EEE not necessarily mean that the athletes reduced their total training volume during the intervention. It is common among elite athletes to follow an annual training plan that is usually structured around some form of periodization according to the athlete's race-season (i.e., periods with higher intensity training and lower volume) or off-season (i.e., higher volume and lower intensity training) (81). For instance, Solli et al. compared two types of periodization modalities among Olympian female cross-country skiers which showed that their training distribution generally followed a pattern with training mostly in the lower intensity zones during their off-season, but with more training in the higher intensity zones and less low-intensity during the preparation-phase and race-season (100).

Notably, most of these athletes were classified as tier 3 athletes and thus presumably followed a pre-made, structured training plan (66). Considering that the intervention took place during the participants off-season, another possible theory may therefore be that several of the athletes coincidentally had a modification to their training plan with a greater focus on lighter training (i.e., mobility, technical sessions etc.) and thus possibly replaced some of the very energy-demanding high intensity sessions with lighter ones. On the other hand, Fahrenholtz et al. recently reported that the participants in the FUEL-intervention had a significant decrease in training volume which was due to a reduction in lighter training in zone 1-2, and not the high intensity zones (zone 3-5), which may challenge the abovementioned theory (29). It may therefore be reasonable to assume that the participants in this study did not reduce their overall training volume as a result of periodization in their off-season or intentionally to be in EB, but possibly due to other individual causes that remains unclear. Thus, a specific post-analysis similar to the one conducted by Fahrenholtz et al. assessing the frequency of training in each intensity zone for the athletes in this study-cohort in particular might therefore been appreciated to clarify these assumptions further (29).

Association Between Within-day Energy Deficiency and the Low Energy Availability in Females Questionnaire score

As shown in **table 4**, none of the WDED-variables were significantly correlated with the LEAF-Q score, which may suggest that there is no relationship between the frequency of hours spent in a negative EB and various symptoms related to of LEA/REDs, particularly being injuries, GI-problems and MD. However, both Fahrenholtz et al. (32) and Firel et al. (36) reported significant associations between hours spent in WDED and MD and endocrine function, and the initial hypothesis in this study was therefore a positive association between hours spent in a negative EB (WDED) and the LEAF-Q score, primarily since a higher

LEAF-Q score is indicative with consequences associated with LEA/REDs. However, such a relationship could not be confirmed in this study, and it may be argued that also subjects with a low LEAF-Q score < 8 should have been included in the analysis for a proper investigation, since only subjects with a LEAF-Q score ≥ 8 were assessed. Notably, if a significant association had been found it might have indicated that the LEAF-Q also could be used to screen athletes at risk of WDED, but future studies should include athletes with LEAF-Q scores < 8 to probably investigate this potential relationship.

5.3 Comparison with Recent Research

To the author´s knowledge, this is the very first study that has assessed WDEB longitudinally and compared it before and after a nutritional intervention among female endurance athletes, and the ability to compare this study to other ones are therefore limited. Notably, there are several studies that have investigated WDEB among athletes and reported significant findings, as shown in **appendix 9** and **appendix 10** (4, 7, 8, 22, 26, 32, 36, 46, 54, 62, 106, 108) . However, most of the variables investigated in these studies were not possible to assess due to the methodological challenges mentioned above (i.e., blood samples, body composition, EA, RMR_{ratio}, etc.). Still, EI and 24h-EB were assessed and are further emphasized in the upcoming sections, primarily since these two variables have been frequently examined in other studies assessing female endurance athletes at risk of REDs (4, 7, 18, 24, 26, 27, 32, 36, 43, 46, 52, 62, 65, 70, 109).

5.3.1 Energy Intake

Energy Intake and Energy Availability

Although the changes in EI in this study were not significant there was an overall trend for an increase of +205 kcal/day from baseline to post-intervention for these athletes (2660 kcal/day vs. 2865 kcal/day, $p = 0.054$) (**table 3**). As mentioned, EA was not possible to assess and a comparison of EI with similar studies that have assessed EA may therefore be appreciated. In 2018, Heikura et al. assessed female runners which all had a LEAF-Q score >8, and found that those with LEA (EA< 30 kcal/kg FFM/d) had an average EI of 2336 kcal/d, while the subjects with moderate EA (EA: 30-45) had an EI of 2767 kcal/ (43). A possible theory may therefore be that the subjects in this master´s thesis also had an EA close to or above moderate levels (EA 30-45), mainly since their EI is close to the ones reported for the moderate-EA subjects by Heikura et al. (68).

Furthermore, both the baseline and post-intervention EI (2660 and 2865 kcal/d) among the athletes in this study is substantially greater than the average EI found among the female cyclists (1802 kcal/day) assessed by Jurov et al., which notably had an average EA of 10 kcal/kg FFM/d (46). On the other hand, the female elite runners assessed by McCormack et al. had an average EA of 37 kcal/kg FFM/d with an average EI of 1940 kcal/day (65). Considering that these two studies reported almost similar EI but substantially different EA-values may emphasize that EI alone not necessarily can be used as a proxy of EA. Nevertheless, these findings may strengthen the abovementioned theory that the athletes in this master´s thesis had an average EA close to or above moderate levels, mainly since they had a greater EI than the subjects in the studies by Jurov et al. and McCormarck et al. (46, 65, 78).

Energy Intake and Menstrual Function Among Female Athletes

As mentioned, gynecological examinations were not possible to conduct in this study, but comparing the average EI with other similar studies assessing menstrual function might provide additional insight and contribution for future studies. First, both the baseline and post-intervention EI (2660 and 2865 kcal/day, respectively) among the athletes in this study is considerably greater that the average EI found among the amenorrheic endurance athletes assessed by Friel et al. (2094 kcal/d) (36). Also, the difference in EI from baseline to post-intervention for the athletes in this study (+205 kcal/d) is almost identical to the difference between the amenorrheic and eumenorrheic LEA-subjects assessed by Melin et al. (+ 222 kcal, $p = 0.475$). These findings may indicate that a nutritional intervention, such as the FUEL-intervention have great potential to affect behavior change to an extent that may be decisive for different health parameters, such as menstrual function among female endurance athletes, a theory that is in line with several other studies that are mentioned below (18, 24, 27, 52, 69, 70).

In 2014, Lagowska et al. conducted a 9-month nutritional intervention among amenorrheic female endurance athletes, which received monthly customized diets and nutritional guidance by dietitians. They found that the athletes had a gradual increase in EI (+ 234 kcal/d after three months and +449 after six months), and the average EI during the intervention was 2500-3500 kcal/day (52). Moreover, seven out the initial 31 amenorrheic athletes resumed their menses after nine months, whereas four of them resumed it after six months but no change was observed after three months. This may indicate that at least 16-weeks are needed for a nutritional intervention to have an impact on an athlete ability to make profound behavioral and dietary changes, and particularly for different physiological adaptations in the body to occur. This theory is in line with previous research among non-athletes, suggesting that nutritional educational programs lasting >5months have the best efficiency on improving dietary behaviors (80). Furthermore, this may imply that the duration of the 16-week FUEL-intervention may have been too short, which is a theory that was also recently claimed by Fahrenholtz et al. (29, 52).

Moreover, Dueck et al. reported that five out of the seven amenorrheic female athletes restored their menses after a 20-week nutritional intervention where they were given an additional of +360 kcal/day, which also resulted in a 14 % significant increase in EI (27). This is similar to the findings reported by Cialdella-Kam et al., which also included a control group of eumenorrheic athletes (18). The amenorrheic subjects were instructed to consume an additional of +360 kcal/day, and notably all of them resumed their menses after six-months and had an average increase in EI of +382 kcal/day, which were not statistically significant however (18). Interestingly, the authors claim that this might be due to large individual fluctuations in EI, possibly since they adapted their EI to changes in their training plan during the intervention. This rationale do correlate to the findings in this master´s thesis, since a significant change in EEE simultaneously with a non-significant increase in EI was observed (**table 3)**.

Furthermore, a 12-month randomized controlled trial was recently conducted by De Souza et al. which assessed female athletes with MD, mainly from endurance sports. They found that the athletes consuming 20-40 % above baseline energy needs had a significant increase in EI compared to the control group (+330 kcal/day vs. -64 kcal/day, $p < 0.05$) (24). Also, significantly more subjects in the intervention group improved their menstrual function compared to the control group (64 % vs. 19 %) (24). On the other hand, the observational study by Heikura et al. found that the eumenorrheic female endurance athletes actually had a slightly lower EI compared to the amenorrheic athletes (201 vs. 208 $kJ \cdot kg^{-1} \cdot day^{-1}$), which is an unexpected finding that may emphasize that there are several other factors than merely EI that affect menstrual function (43).

Notably, out of the studies above only the findings from De Souza et al. (24), Lagowska et al. (52) and Dueck et al. (27) were statistically significant, which reported an increase in EI ranging from +330 to 360 kcal/day and were interventions lasting from five to 12 months in duration (24, 27, 52). One may therefore speculate whether the marginal increase in EI (+205 kcal/day) observed in this study might eventually been greater if the 16-week FUEL-intervention had a longer duration (27, 52). On the other hand, Valliant et al. reported a significant increase in EI (+422 kcal/day) after a 16-week nutritional intervention among female volleyball players, which may indicate that also shorter nutritional interventions have great potential to affect an athlete's ability to make behavior and dietary changes (109). Overall, all of these studies indicate that nutritional interventions can possibly affect dietary behaviors, restoration of menstrual function and EA among female athletes (18, 24, 27, 52).

Energy Intake and Training Sessions

Previous studies examining female athletes have reported insufficient EI particularly around training sessions, and a recent study from Gräfnings et al. examining female endurance athletes with symptoms of REDs observed an insufficient EI, particularly carbohydrate, both before, during and after training sessions (12, 38, 41, 62, 78). As mentioned, Hawley et al. reported that a typical mean EI for female endurance athletes with high energy demands range from 2388-4060 kcal/day (41). Notably however, an important observation from most these studies was that the athletes had a tendency to consumed most of this energy during training sessions, which do highlight the notion that endurance athletes need to use all available opportunities in order to meet their energy needs and to possibly be in EB (6, 41). It may therefore be reasonable to speculate whether the female endurance athletes in this study also had an insufficient EI around their training sessions, and a post-analysis examining EI particularly around these times would have been valuable to clarify and investigate this theory even further.

5.3.2 24hour-Energy Balance

Several studies have also assessed 24h-EB among female endurance athletes in risk of REDs, and several of these have reported different findings which are relevant to this current study (18, 32, 52, 62). As mentioned, the athletes in this study had a significant shift in average 24h-EB from initially being in a negative EB at baseline (-327.7 ± 432.7 kcal/day) over to a positive EB after the intervention (19.9 ± 457.3 kcal/day). Notably, the relatively large dispersion and spread in these values may indicate that there were large variations among the subjects, and these values should therefore mainly be interpreted in relation to the fact that they show that the 24h-EB changed towards a positive direction **(table 3).**

The nutritional intervention conducted by Lagowska et al. found that the female endurance athletes assessed had a significant shift in 24h-EB from initially being in a negative state at baseline and after three months (EB: -288 kcal/day and -51 kcal/day, respectively), over to a positive EB after six months (EB: 59 kcal/day), which remarkably remained unchanged after nine months (EB: 59 kcal/day) (52). These findings correspond to this study, considering that a significant shift in 24h-EB also occurred after approximately four months. However, the athletes assessed by Lagowska et al. had a nearly constant TEE and EEE throughout the study, and the change in EB was therefore primarily driven by an increase in EI(52). This is in contrasts to the findings in this study, and a possible explanation for this may be that the intervention by Lagowska et al. was primarily based on customized diets with a gradual increase in EI (kcal/day), while the FUEL-intervention mainly was based on nutritional guidance and education for the subjects (52).

Similarly to Lagowska et al. (52),Valliant et al. reported that the female athletes had a significant increase in 24h-EB after the nutritional intervention which was primarily driven by an increase in EI and not TEE, which again is in contrast to the findings in this current study (109). The intervention by Valliant et al. however, was solely based on individual nutritional counseling similar to the FUEL-intervention, which may indicate that female athletes do have the ability to make profound dietary changes and improve their EI without necessarily needing a customized, fixed diet.

Furthermore, Fahrenholtz et al. reported no significant difference in 24h-EB between the female endurance athletes with MD (-659 kcal/day) and the eumenorrheic athletes (-313 kcal/day) assessed (32). This corresponds to the findings from Cialdella-Kam et al., which reported no significant difference in 24h-EB between the eumenorrheic-control group (−171 kcal/d) and the amenorrheic-intervention group, neither at baseline (-510kcal/day) or after the six-month intervention (-44 kcal/day) (18). Both of these findings may indicate that 24h-EB is not as critical and deceive for menstrual function as previously assumed, and it may be that other parameters of EB (such as WDEB or hourly EB) are more crucial (32, 57, 77-79). Also, the average 24h-EB for the eumenorrheic athletes assessed by Fahrenholtz et al. (-313 kcal/day) is almost identical to the baseline values for the participants in this study (-327.7 kcal/day), which may suggest that most of the athletes in this study had a 24h-EB sufficient enough for a normal menstrual function (32).

Moreover, Lundstrom et al. found that the female elite swimmers assessed had a positive 24h-EB of 52 ± 505 kcal/day, but interestingly had a negative hourly EB of -107 ± 225 kcal/hour. This is yet another finding that emphasizes the importance of assessing EB through different approaches (i.e., hourly, WDEB, "end of the day-EB"), mainly since it generated different interpretations of the energy status of the athletes, which corresponds to the findings from Benardot (22), Fahrenholtz et al. (32), Torstveit et al. (108) and Firel et al. (36) (**appendix 9** and **appendix 10)**. Furthermore, this misinterpretation of a positive 24h-EB while most of the hours were in a negative EB was also frequently observed among several of the individual athletes in this study, since a positive 24h-EB often occurred even on days where most of the hours actually were spent in WDED (EB< 0 kcal and EB< -300 kcal) **(appendix 8).**

5.3.3 The 24hour-Energy Balance Method vs. The Within-day Energy Balance Method

This study compared the WDEB-method to the traditional 24h-EB method and found that the two methods generated similar interpretations regarding energy status, and both reveled a significant change from baseline to post-intervention (**table 3**). This might question some of criticism and statements that have been previously raised, claiming that the 24h-EB method is an oversimplified approach for assessing EB in athletes (7, 26, 59). Actually, the findings in this study might emphasize that the 24h-EB method is highly valuable and important, particularly in a practical setting where the main objective is to primarily get an overview of the energy status. This is mainly because the 24h-EB method is arguably less time consuming, both for the practitioner and the athlete compared to the WDEB-method.

Furthermore, this finding might seem contrary to the findings from most of the other studies highlighted in **appendix 9** and **appendix 10** like Benardot (22), Friel et al. (36), Fahrenholtz et al. (32), Lundstrom et al. (62) and Torstveit et al. (108), which found no associations between 24h-EB on either body composition, menstrual function or RMR, but found significant associations with one or all of the WDEB-variables.

5.3.4 Practical Considerations and Limitations of the Within-day Energy Balance Method

Assessment Days and Comparing Within-day Energy Balance-values.

One limitation with the WDEB-method is that one cannot principally compare WDEB-values if a different number of days are assessed, as it can lead to incorrect and bias interpretations due to the cumulative effect of the WDEB-calculations. This applies both when comparing values between individuals or studies, but also when comparing values for the same subjects between two different periods. As an example, a subject with four days registered mostly in WDED will appear to be “less deficient” compared to a person with an identical WDEB-profile with seven days registered. This phenomenon was one of the main reasons for the fairly strict inclusion criteria in this study, as only participants with a complete dataset for the whole week (7/7 days) was included, which arguably was one of the main reasons for the resulting small sample size (**figure 3**).

Out of the studies presents in **appendix 9,** only Fahrenholtz. et al. and Lee et al. assessed WDEB over a seven day period, whereas the other ranged from merely one day up to four days of registration (4, 7, 26, 32, 36, 46, 54, 108). The significance of this is illustrated in **appendix 7**, with an example of the WDEB-values for one subject which shows notable differences in the WDEB-values, particularly the LSHD and LSHS-variable, depending on whether the registration was based on one day or seven-days. This observation may explain the large difference between the average LSHD reported by Deutz et al. (LSHD: -358 ± 557 kcal) based on one day registration, compared to the baseline LSHD found in this study (LSHD: -1686.1 ± 1618.9 kcal) based on seven days (26). This difference was also evident and reported by Fahrenholtz et al. when comparing their results to the values presented by Deutz et al. (26, 28). Notably, this is not to say that it is incorrect to compare WDEB-values between studies or subjects with different numbers of days registered, but when doing so one needs to take this possible methodological bias into consideration when interpreting the results, as shown in **appendix 7**.

Energy Balance Assessments and Misinterpretations of Energy Deficits

As mentioned, it can be argued that the 24h-EB method has the advantage of being less time-consuming and easier to calculate in a practical setting compared to the WDEB-method. However, one important pitfall of the 24h-EB method that was frequently observed in this study was that it often could mask an energy deficit that had evolved over days, with a possible explanation being that the 24h-EB calculations are zeroed out every day at midnight. As mentioned earlier, this approach has been criticized to be misleading considering that such a “reset” does not occur physiologically in the human body (22, 28). The WDEB-method on the other hand is a cumulative calculation, and hence the EB from the previous day is integrated into the next including significant energy fluctuations and deviations. Moreover, this observation corresponds to the findings by Stubbs et al. regarding the occurrence of a *“pathological EB”* as an adaptive response of a cumulative negative EB over time (59, 101).

Even though the EB-values from both of these methods changed significantly during the intervention, there were many cases where these two methods generated distinctive different results when evaluating EB in single days, with the most evident instance being a positive 24h-EB, even though all of the hours were spent in a negative EB (i.e., EB< 0 kcal or EB< -300 kcal), which is illustrated with an example in **appendix 8.** This was particularly apparent on days with little or no activity, which may indicate that low-training days have a compensatory effect on 24h-EB, an observation and theory that has also been reported in previous studies (28). In addition, this was also evident on days later in the week, probably due to the cumulative effect of WDEB as more days were registered by the participant.

Throughout this study there has been a lot of emphasis on the difference between these two methods, evaluating their advantages and disadvantages. However, it is needless to say that the WDEB-method should not be considered a substitute of the 24h-EB method, or vice versa, especially since both of these are highly appreciated and do complement each other on different areas. While the 24h-EB method can be appreciated as a more simple and applicable method to get an overall picture of the energy status of the athletes, the WDEB-method can serve as an additional in-depth analysis especially in particular circumstances, for instance when screening for REDs or LEA, to investigate dietary behaviors or patterns, or before important competitions or events.

5.4 Perspectives and Future Research

As mentioned, both REDs and WDEB are fairly new phenomena, which makes the demand for more research on these topics particularly high. There are several other associations and hypothesis that can be investigated based on a WDEB-assessment, particularly since it involves many variables and thus includes a lot of data. It would have been interesting to investigate the energy distribution among these subjects, and to examine whether they had a meal pattern similar to what has been previously reported to be characteristic among female athletes (3, 69, 78). In addition, a calculation of meal-frequency and an investigation of its relationship to overall EI, WDED and body composition would have been interesting to assess, and to further compare this to the reported findings from the other studies assessing EB among athletes **(appendix 9** and **appendix 10)** (22, 32, 41, 103).

Moreover, it is evident that the prevalence of REDs is higher among females than males, but it is likely that more males will be identified in the future since specific male-screening tools recently has been developed (57, 63, 78). In addition, REDs among other athletic populations (i.e., ball games, martial arts, climbing) will probably be more recognized in the future especially since there is an increased focus on REDs globally in the sporting arena (2). Also, a high prevalence of LEA/REDs was recently reported among elite rock climbers, and symptoms related to LEA have been assessed in para-athlete populations (4, 10, 75)

5.4.1 Strengths and Summary of Limitations

This is, to the author's knowledge, currently the only study that have examined WDEB among female endurance athletes and evaluated the effect of a sports nutrition education program on WDED, which is one of the largest strengths in this master´s thesis. Moreover, the fact that the managers of the FUEL-intervention managed to recruit participants and conduct a relatively comprehensive intervention study during the COVID 19 pandemic was also a great accomplishment which should not be neglected. Another strength of this study was that subjects with EDs, use of hormonal contraceptives and those previously diagnosed with MD not related to REDs was excluded, which minimized the chance of including false positive cases of REDs and possible biases.

On the other hand, an important limitation of this study was the absence of a control group, which limited the possibility to identify any causal relationships between the sport nutritional intervention and changes in WDED. Also, there is a possibility that various confounding factors might have influenced these results, mainly since the participating athletes were not randomized. Moreover, since no other similar intervention studies on this topic existed, the power analysis and estimation of sample size was based on a cross-sectional study and the accuracy of this estimate may therefore be debated. Furthermore, there were relatively large dispersion in most of the calculated variables in **table 2** and **table 3,** and this might indicate that there were large interindividual variations between the subjects and that the intervention effected each individual differently. Also, this dispersion might indicate that there were large day-to-day variations within each subject.

Despite these limitations however, these results may be appreciated since they can contribute and inspire future studies and provide inspiration for possible hypothesis that can be investigated in the future. This is especially important in this field of research considering that REDs, and particularly WDEB, are both fairly new phenomenon and a lot is still unclear and unknown regarding these concepts (2, 57).

6 Conclusion

In this study, the effect of a 16-week sports nutrition intervention for female endurance athletes at risk of REDs was investigated by examining changes in WDED. Also, the WDEB-method was compared to the 24h-EB method, and the association between WDED and the LEAF-Q score was examined. The conclusions for the specific research questions from this study are:

- There was a significant reduction in hours spent in a negative EB, and all variables of WDED were significantly reduced (EB< 0 kcal, EB< -300 kcal and LSHD) from baseline to post-intervention for the participating athletes.
- Both the 24h-EB method and the WDEB-method generated similar interpretations of energy status, and they both revealed that the athletes went from primarily being in a negative EB at baseline to a positive EB after the intervention.
- No significant association between WDED and the LEAF-Q score was found.

Notably, 24h-EB was significantly increased after the intervention, which was primarily driven by a reduction in overall training volume rather than increased EI for most of the athletes. Also, it was recognized that the 24h-EB method had a tendency to mask an energy deficit which had evolved over time, however this misinterpretation did not appear with the WDEB-method since it is a cumulative value that take EB from the previous day, including large energy fluctuations and deviations, into consideration.

WDED was significantly improved for the athletes, and they had a significant shift from initially spending most of their hours in a negative EB, over to a positive EB after the sports nutrition intervention. This can indicate that a sports nutrition intervention like the FUEL-project is of great value and has potential to enhance nutritional behavior among female endurance athletes at risk of REDs. However, since this study did not include a control group, in addition to several methodological limitations particularly related to data collection and the restrictions followed by the COVID 19-pandemic, these findings cannot be used to determine any causal relationships but rather be used to generate future hypothesis and inspiration for upcoming studies that will be conducted in the future.

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8 Appendices

Appendix 1: Overview of the Weekly Program and Content of the FUEL-intervention.

Appendix 2: The Four Habits of Change.

Appendix 3: Example of a Diet Registration Form.

Appendix 4: Illustration of the “Log Diaries-function” for NEAT-analysis in Actilife Software.

Appendix 5: Example of Within-day Energy Balance calculations.

  Appendix 5a: Example of how “starting EB” was determined.

Appendix 6: Example of Exercise energy expenditure calculations.

  Appendix 6a: Relationship between heart rate and VO_2 (mL O_2/min).

  Appendix 6b: Example of calculating total EEE.

  Appendix 6c: Example of calculating EEE for each individual training hour.

Appendix 7: Example of WDEB for 1-day vs. 7-days of registration.

Appendix 8: Comparison of 24h-EB and WDEB.

Appendix 9: Detailed Description of Studies Assessing WDEB Among Athletes.

Appendix 10: Overview and Comparison of Studies Assessing EB and WDEB in Relation to Different Health-and Performance Outcomes.

Appendix 11: REK approval form.

Appendix 1 1: Overview of The Weekly Program and Content of the FUEL-intervention

The table illustrate the structure and an overview of the weekly schedule of the FUEL-intervention, including details of the different events that occurred during the 16-week intervention and the themes for the different lectures.

Project week	**Weekly Program**
0	Questionnaire + welcoming conversation 7-day nutrition registration 7-day activity registration (accelerometer + HR-sensor during exercise and registration in BESTR)
1	Teaching video: Introduction to sports nutrition Individual conversation with nutritionist on Zoom
2	Teaching video: REDs part 1
3	Teaching video: REDs part 2 Individual conversation with nutritionist on Zoom
4	Teaching video: REDs part 3
5	Teaching video: Myths in sports nutrition Individual conversation with nutritionist on Zoom
6	Teaching video: Carbohydrate
7	Teaching video: Protein Individual conversation with nutritionist on Zoom
8	Teaching video: Fats
9	Teaching video: Meal timing and food choices Individual conversation with nutritionist on Zoom
10	Teaching video: Performance nutrition – fuel prior to, during, and after training and competition
11	Teaching video: Periodization Individual conversation with nutritionist on Zoom
12	Teaching video: Micronutrients
13	Teaching video: Supplements Individual conversation with nutritionist on Zoom
14	Teaching video: Weight, body composition, health, and performance
15	Teaching video: Injuries and nutritional rehabilitation Individual conversation with nutritionist on Zoom
16	Teaching video: Menstruation and performance
17	Questionnaire + concluding conversation. 7-day nutrition registration 7-day activity registration (accelerometer + HR-sensor during exercise and registration in BESTR)

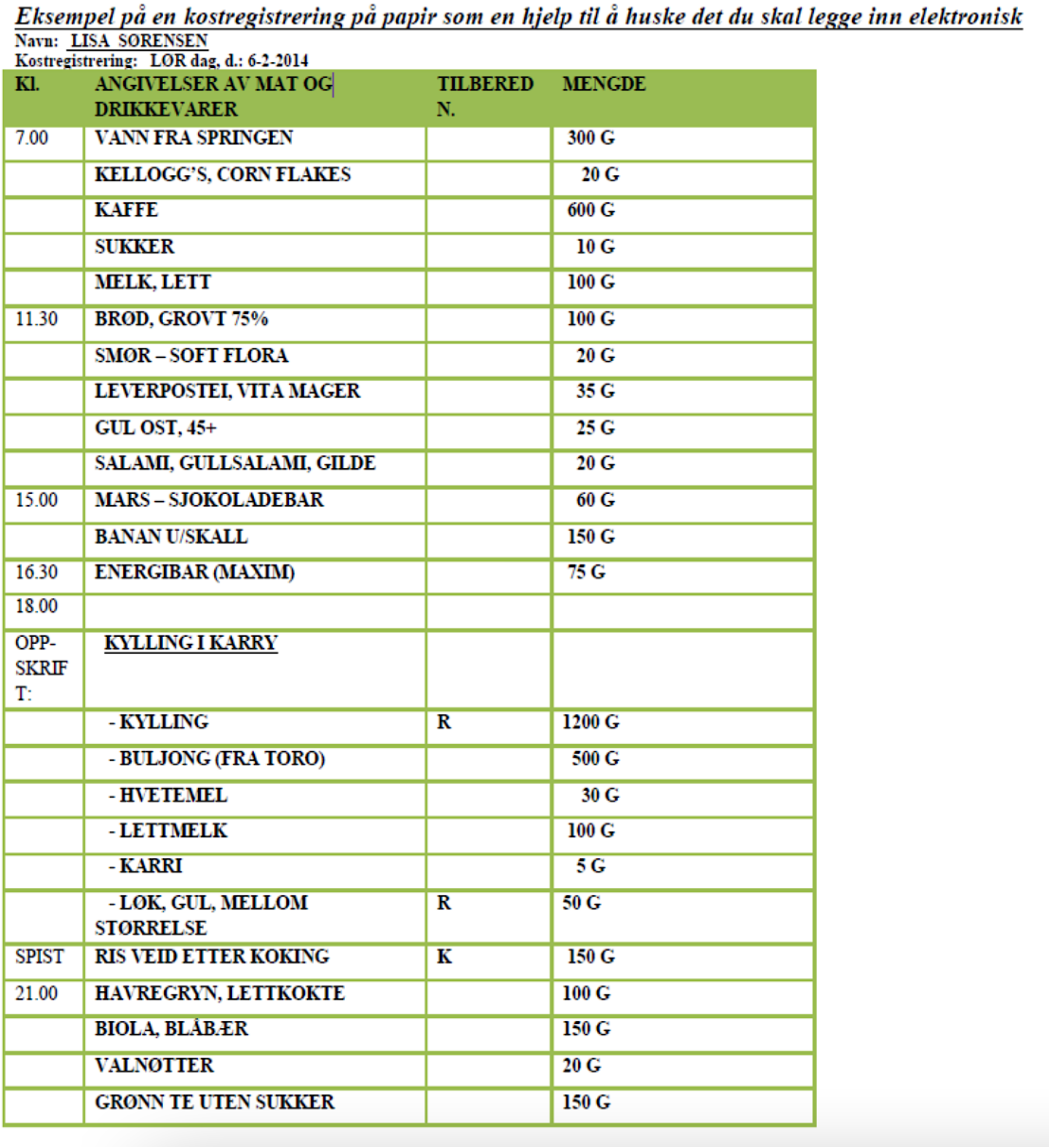
Appendix 2. Structure of the FUEL-intervention Consultations Based on The Four Habits Model.

The table illustrate the structure of the FUEL-nutritional consultations inspired by the Four Habits Model by Frankel (34).
Note: table is inspired by Fahrenholtz et al. (2023) (29).

The Four Habits Model (34)				
Preparation	**Beginning**	**Work Part**	**Conclusion**	**Reflection**
• Athlete takes notes and summarizes what is important to bring up during the lecture. • The counselor prepares the conversation based on the manual and template for conversations and previous conversations with the athlete, as well as online lectures viewed by the athlete.	• Establish contact (especially for the first meeting; establish trust, identify motivation for change, inform about further course progression and planning). • Engage • Focus • Set point: What does the athlete experience as important (today and in the future)?	• Explore the athlete´s perspective (thoughts, feelings) of their personal situation related to REDs, e.g., potential symptoms, dietary restrictions. • Wish and intention to make changes. • Goal, action plans and approaches. • Support mastery, loyalty and trust in one´s own ability to conduct change/mastery (including exploring previous experiences). • Inform	• Explore the athlete´s experience of the consultation. • Ask for additional questions. • Summarize and conclude.	• The athlete writes personal notes. • The counselor writes a journal.
⇐Maintain alliance · Express empathy · Support autonomy and mastery · Give information ⇒				

Appendix 3: Example of a Diet Registration Form.

An example of a diet registration form in paper format. This was provided for the participants to show how to log their meals “on the go”, enabling them to later enter it digitally in Dietist if they did not have a phone/computer available. The example is for the Norwegian participants and is a reprint with permission from the project managers.



Eksempel på en kostregistrering på papir som en hjelp til å huske det du skal legge inn elektronisk

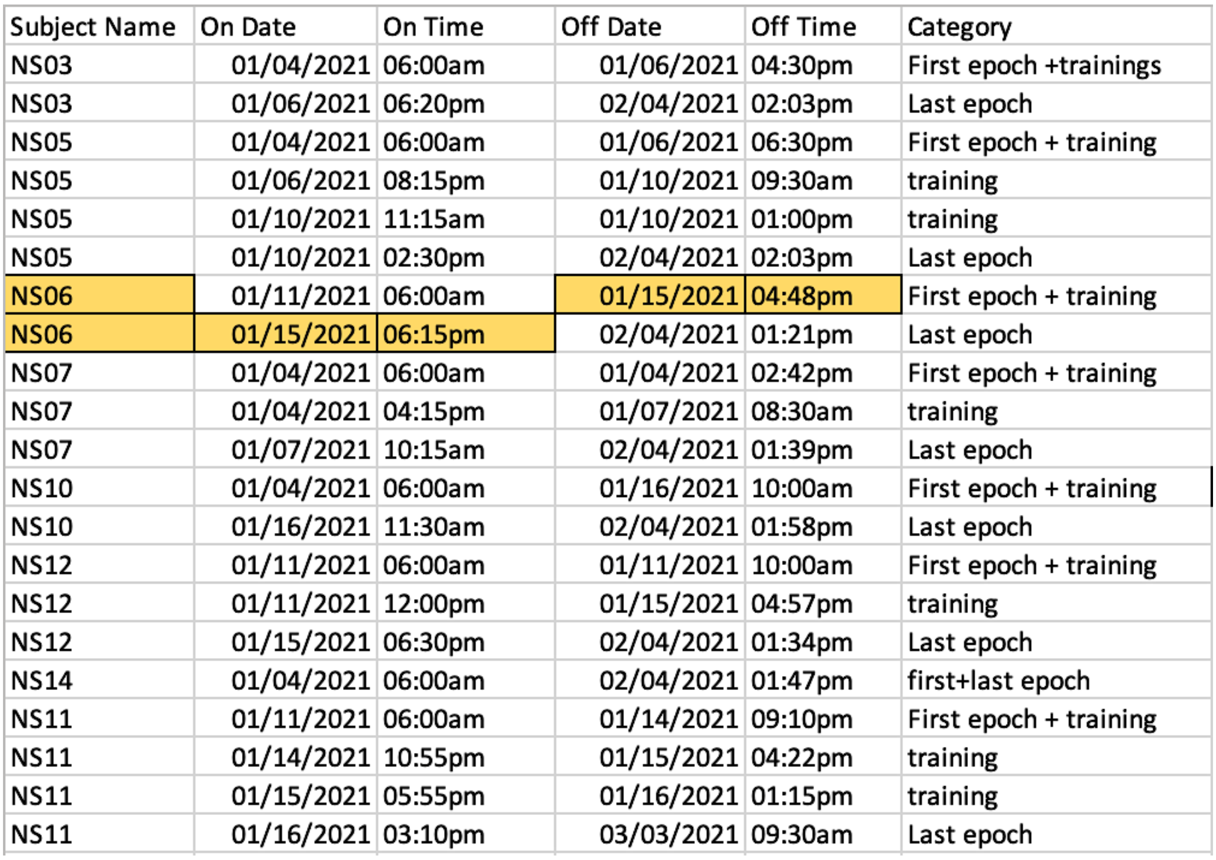
Navn: LISA SØRENSEN

Kostregistrering: LØR dag, d.: 6-2-2014

Kl.	ANGIVELSER AV MAT OG DRIKKEVARER	TILBEREDN.	MENGDE
7.00	VANN FRA SPRINGEN		300 G
	KELLOGG’S, CORN FLAKES		20 G
	KAFFE		600 G
	SUKKER		10 G
	MELK, LETT		100 G
11.30	BRØD, GROVT 75%		100 G
	SMØR – SOFT FLORA		20 G
	LEVERPOSTEI, VITA MAGER		35 G
	GUL OST, 45+		25 G
	SALAMI, GULLSALAMI, GILDE		20 G
15.00	MARS – SJOKOLADEBAR		60 G
	BANAN U/SKALL		150 G
16.30	ENERGIBAR (MAXIM)		75 G
18.00			
OPP-SKRIFT:	KYLLING I KARRY		
	- KYLLING	R	1200 G
	- BULJONG (FRA TORO)		500 G
	- HVETEMEL		30 G
	- LETTMELK		100 G
	- KARRI		5 G
	- LØK, GUL, MELLOM STØRRELSE	R	50 G
SPIST	RIS VEID ETTER KOKING	K	150 G
21.00	HAVREGRYN, LETTKOKTE		100 G
	BIOLA, BLÅBÆR		150 G
	VALNØTTER		20 G
	GRØNN TE UTEN SUKKER		150 G

Appendix 4: Example of The “Log-diaries”-Function in Actilife.

Illustration of a Log Diary-template from excel, which was later uploaded to the Actilife Software. It included subject name and time interval of which the subject wore the accelerometer during training. During these times, only EE from exercise was used in the calculation of WDEB, and the accelerometer-data was deleted to ensure that TEE was not overestimated. Example: Subject NS06 had a training session 15.01.2021 from 04:48pm-06:15pm but wore the accelerometer at the session. This time-interval was therefore categorized in the “off-date, off-time” column and hence, accelerometer data was deleted (highlighted in yellow).

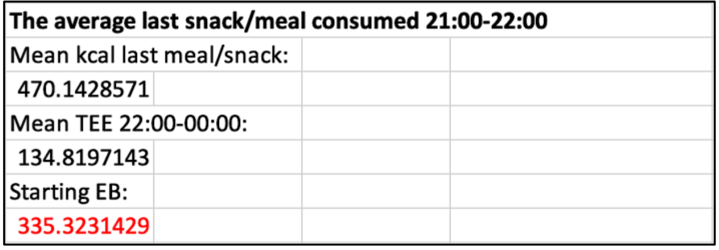


Subject Name	On Date	On Time	Off Date	Off Time	Category
NS03	01/04/2021	06:00am	01/06/2021	04:30pm	First epoch +trainings
NS03	01/06/2021	06:20pm	02/04/2021	02:03pm	Last epoch
NS05	01/04/2021	06:00am	01/06/2021	06:30pm	First epoch + training
NS05	01/06/2021	08:15pm	01/10/2021	09:30am	training
NS05	01/10/2021	11:15am	01/10/2021	01:00pm	training
NS05	01/10/2021	02:30pm	02/04/2021	02:03pm	Last epoch
NS06	01/11/2021	06:00am	01/15/2021	04:48pm	First epoch + training
NS06	01/15/2021	06:15pm	02/04/2021	01:21pm	Last epoch
NS07	01/04/2021	06:00am	01/04/2021	02:42pm	First epoch + training
NS07	01/04/2021	04:15pm	01/07/2021	08:30am	training
NS07	01/07/2021	10:15am	02/04/2021	01:39pm	Last epoch
NS10	01/04/2021	06:00am	01/16/2021	10:00am	First epoch + training
NS10	01/16/2021	11:30am	02/04/2021	01:58pm	Last epoch
NS12	01/11/2021	06:00am	01/11/2021	10:00am	First epoch + training
NS12	01/11/2021	12:00pm	01/15/2021	04:57pm	training
NS12	01/15/2021	06:30pm	02/04/2021	01:34pm	Last epoch
NS14	01/04/2021	06:00am	02/04/2021	01:47pm	first+last epoch
NS11	01/11/2021	06:00am	01/14/2021	09:10pm	First epoch + training
NS11	01/14/2021	10:55pm	01/15/2021	04:22pm	training
NS11	01/15/2021	05:55pm	01/16/2021	01:15pm	training
NS11	01/16/2021	03:10pm	03/03/2021	09:30am	Last epoch

Appendix 5: Example of Within-day Energy Balance Calculation

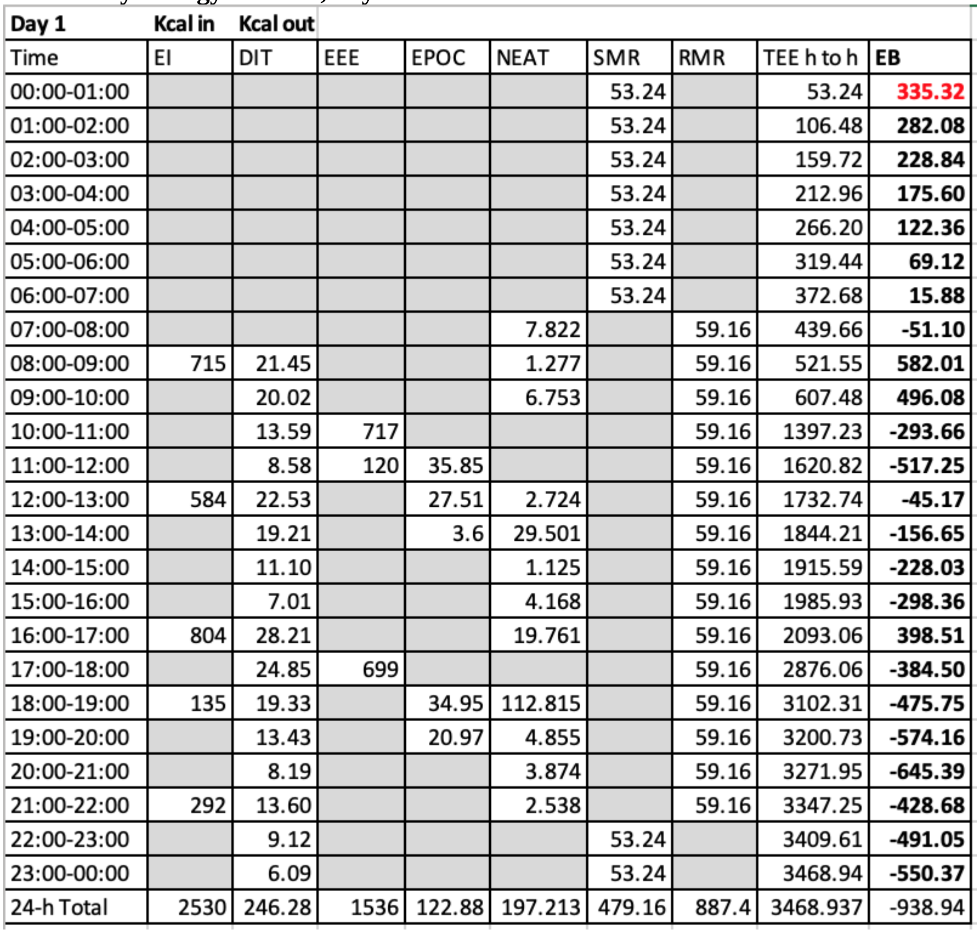
Below is an example of 7day-WDEB calculations for one subject during the baseline registration week. *Note: Largest-single hour surplus (LSHS) is referred to as "Greatest surplus" and Largest-single hour deficit (LSHD) is referred to as "Greatest deficit".*

Appendix 5a: Illustration on how "starting EB" was determined using an example where a subject consumed most of her evening meals between 21:00-22:00. Average EI was 470 kcal and average TEE was 135 kcal in this time interval (21:00-22:00) for all seven days. Starting EB (day 1): 479 kcal – 135 kcal = 335kcal (marked in red).



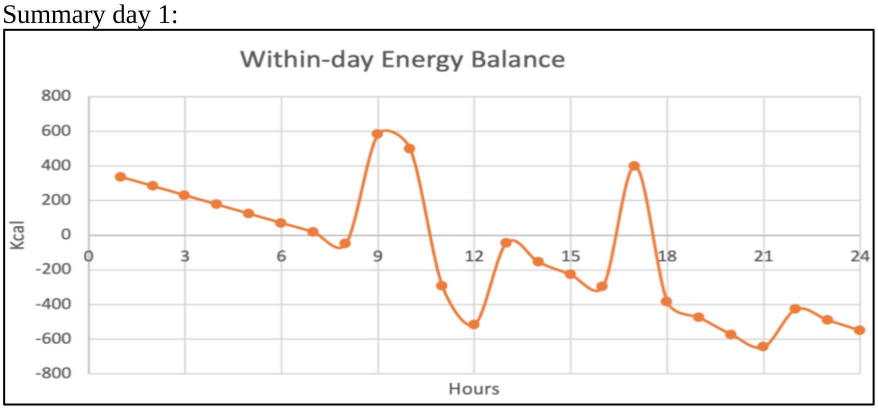
The average last snack/meal consumed 21:00-22:00		
Mean kcal last meal/snack:		
470.1428571		
Mean TEE 22:00-00:00:		
134.8197143		
Starting EB:		
335.3231429		

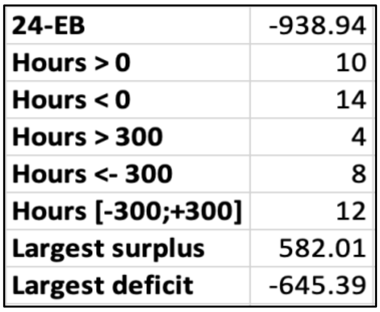
Within-day Energy Balance, day 1:



Day 1	**Kcal in**	**Kcal out**							
Time	EI	DIT	EEE	EPOC	NEAT	SMR	RMR	TEE h to h	**EB**
00:00-01:00						53.24		53.24	**335.32**
01:00-02:00						53.24		106.48	**282.08**
02:00-03:00						53.24		159.72	**228.84**
03:00-04:00						53.24		212.96	**175.60**
04:00-05:00						53.24		266.20	**122.36**
05:00-06:00						53.24		319.44	**69.12**
06:00-07:00						53.24		372.68	**15.88**
07:00-08:00					7.822		59.16	439.66	**-51.10**
08:00-09:00	715	21.45			1.277		59.16	521.55	**582.01**
09:00-10:00		20.02			6.753		59.16	607.48	**496.08**
10:00-11:00		13.59	717				59.16	1397.23	**-293.66**
11:00-12:00		8.58	120	35.85			59.16	1620.82	**-517.25**
12:00-13:00	584	22.53		27.51	2.724		59.16	1732.74	**-45.17**
13:00-14:00		19.21		3.6	29.501		59.16	1844.21	**-156.65**
14:00-15:00		11.10			1.125		59.16	1915.59	**-228.03**
15:00-16:00		7.01			4.168		59.16	1985.93	**-298.36**
16:00-17:00	804	28.21			19.761		59.16	2093.06	**398.51**
17:00-18:00		24.85	699				59.16	2876.06	**-384.50**
18:00-19:00	135	19.33		34.95	112.815		59.16	3102.31	**-475.75**
19:00-20:00		13.43		20.97	4.855		59.16	3200.73	**-574.16**
20:00-21:00		8.19			3.874		59.16	3271.95	**-645.39**
21:00-22:00	292	13.60			2.538		59.16	3347.25	**-428.68**
22:00-23:00		9.12				53.24		3409.61	**-491.05**
23:00-00:00		6.09				53.24		3468.94	**-550.37**
24-h Total	2530	246.28	1536	122.88	197.213	479.16	887.4	3468.937	-938.94

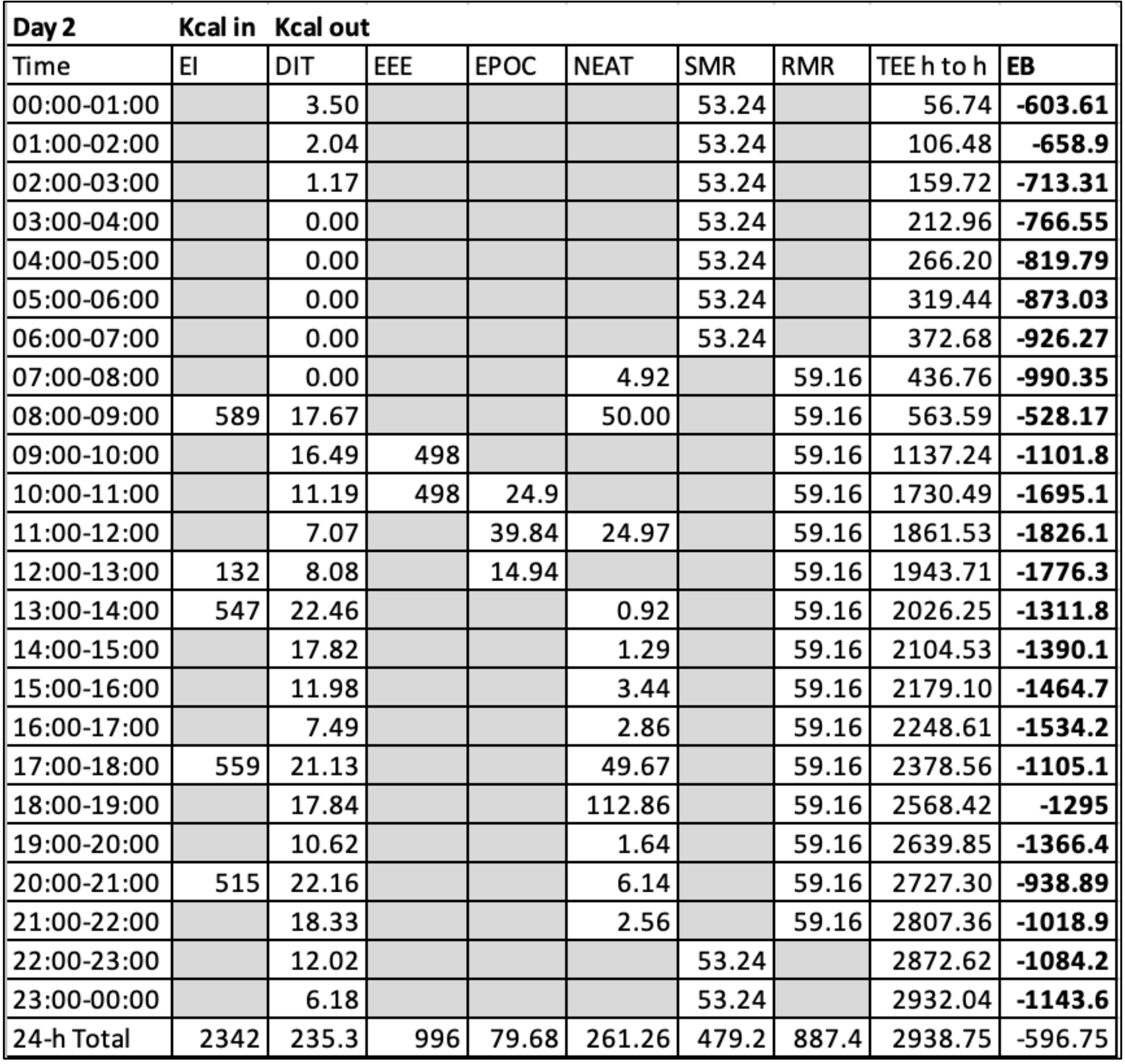
Summary day 1:





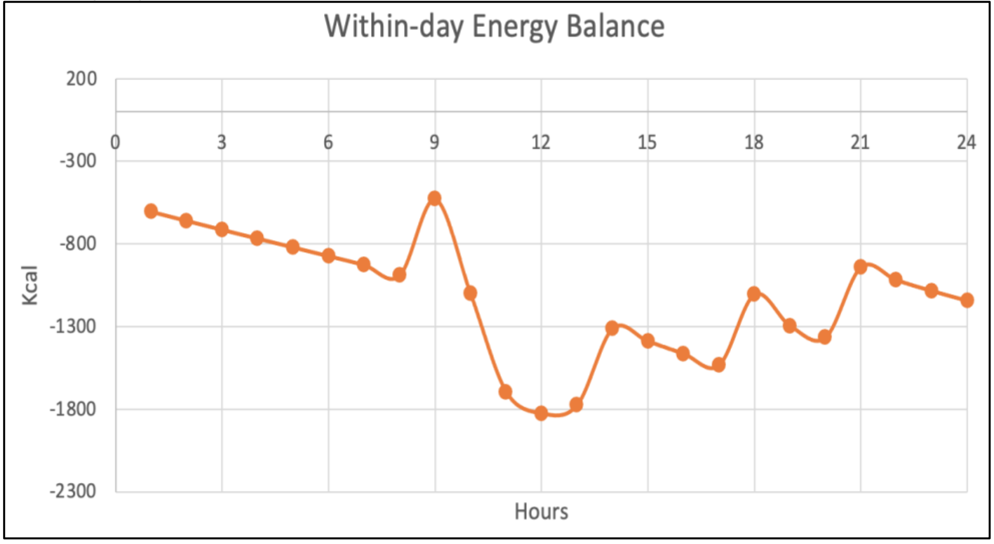
24-EB	-938.94
Hours > 0	10
Hours < 0	14
Hours > 300	4
Hours <- 300	8
Hours [-300;+300]	12
Largest surplus	582.01
Largest deficit	-645.39

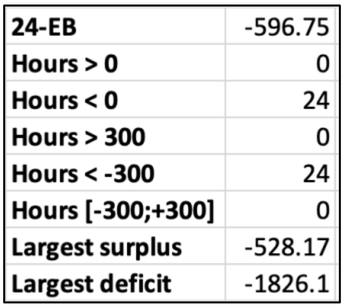
Within-day Energy Balance, day 2:



Day 2	**Kcal in**	**Kcal out**							
Time	EI	DIT	EEE	EPOC	NEAT	SMR	RMR	TEE h to h	**EB**
00:00-01:00		3.50				53.24		56.74	**-603.61**
01:00-02:00		2.04				53.24		106.48	**-658.9**
02:00-03:00		1.17				53.24		159.72	**-713.31**
03:00-04:00		0.00				53.24		212.96	**-766.55**
04:00-05:00		0.00				53.24		266.20	**-819.79**
05:00-06:00		0.00				53.24		319.44	**-873.03**
06:00-07:00		0.00				53.24		372.68	**-926.27**
07:00-08:00		0.00			4.92		59.16	436.76	**-990.35**
08:00-09:00	589	17.67			50.00		59.16	563.59	**-528.17**
09:00-10:00		16.49	498				59.16	1137.24	**-1101.8**
10:00-11:00		11.19	498	24.9			59.16	1730.49	**-1695.1**
11:00-12:00		7.07		39.84	24.97		59.16	1861.53	**-1826.1**
12:00-13:00	132	8.08		14.94			59.16	1943.71	**-1776.3**
13:00-14:00	547	22.46			0.92		59.16	2026.25	**-1311.8**
14:00-15:00		17.82			1.29		59.16	2104.53	**-1390.1**
15:00-16:00		11.98			3.44		59.16	2179.10	**-1464.7**
16:00-17:00		7.49			2.86		59.16	2248.61	**-1534.2**
17:00-18:00	559	21.13			49.67		59.16	2378.56	**-1105.1**
18:00-19:00		17.84			112.86		59.16	2568.42	**-1295**
19:00-20:00		10.62			1.64		59.16	2639.85	**-1366.4**
20:00-21:00	515	22.16			6.14		59.16	2727.30	**-938.89**
21:00-22:00		18.33			2.56		59.16	2807.36	**-1018.9**
22:00-23:00		12.02				53.24		2872.62	**-1084.2**
23:00-00:00		6.18				53.24		2932.04	**-1143.6**
24-h Total	2342	235.3	996	79.68	261.26	479.2	887.4	2938.75	-596.75

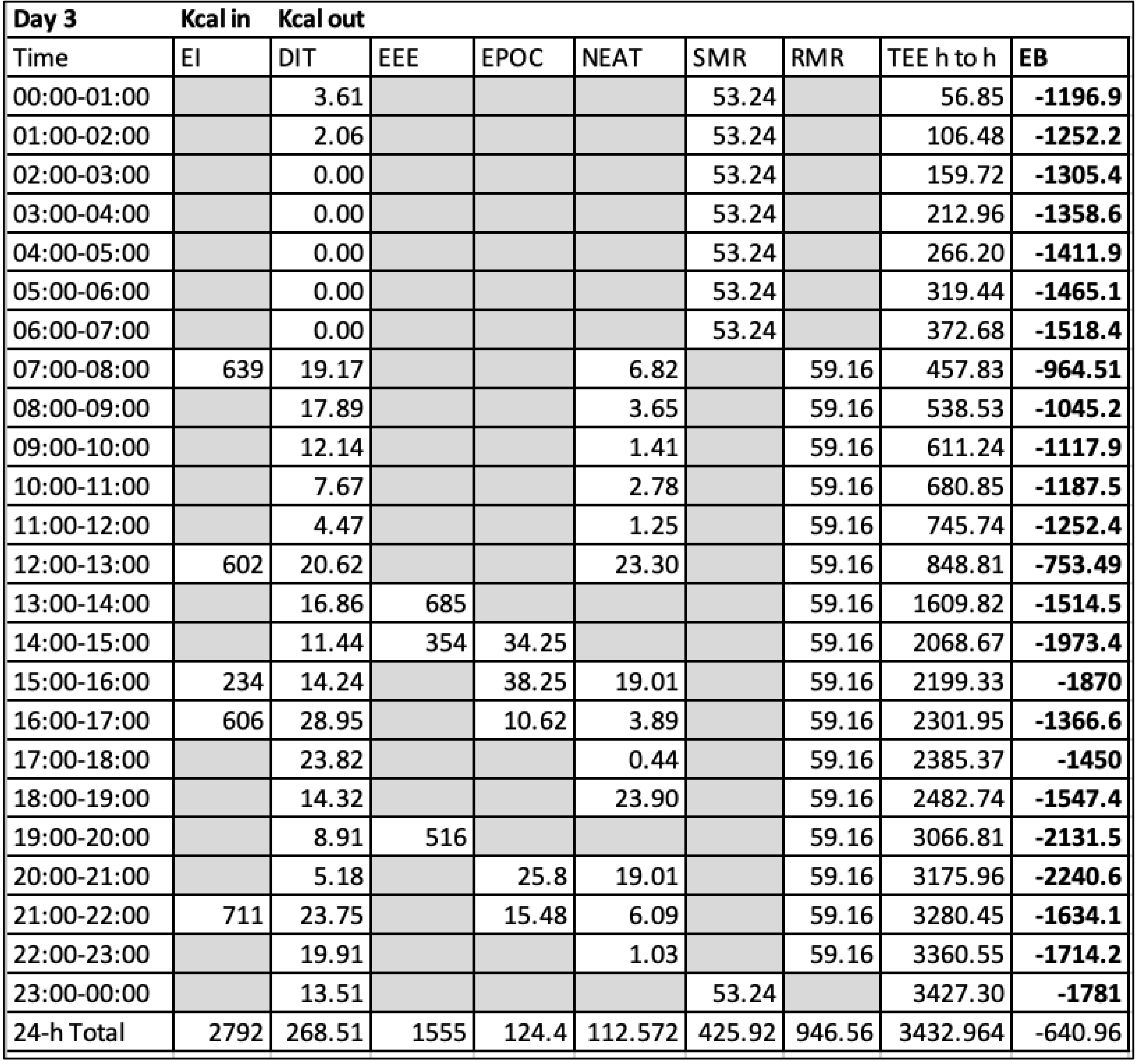
Summary day 2:





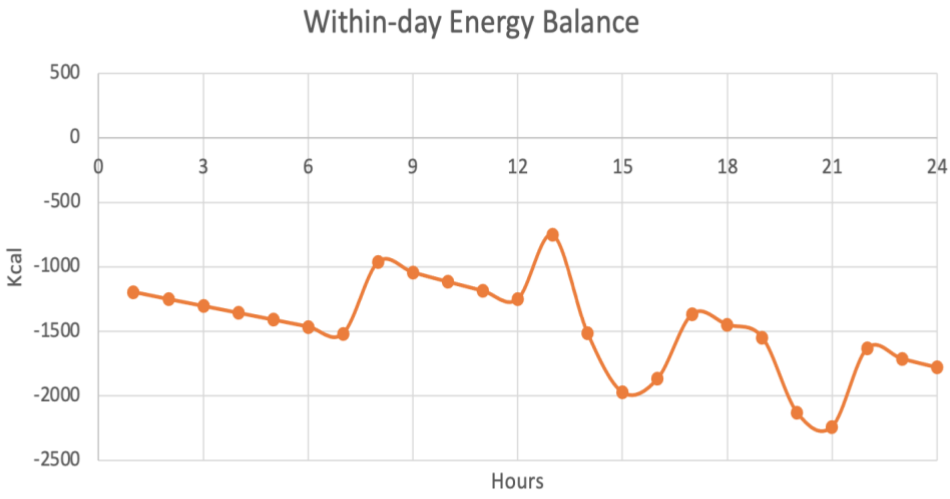
24-EB	-596.75
Hours > 0	0
Hours < 0	24
Hours > 300	0
Hours < -300	24
Hours [-300;+300]	0
Largest surplus	-528.17
Largest deficit	-1826.1

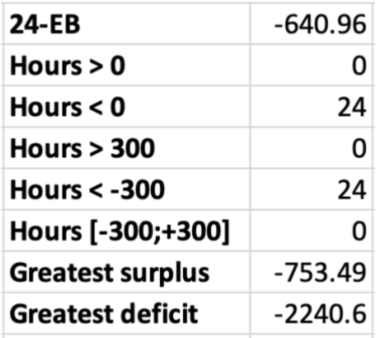
Within-day Energy Balance, day 3:



Day 3	**Kcal in**	**Kcal out**							
Time	EI	DIT	EEE	EPOC	NEAT	SMR	RMR	TEE h to h	**EB**
00:00-01:00		3.61				53.24		56.85	**-1196.9**
01:00-02:00		2.06				53.24		106.48	**-1252.2**
02:00-03:00		0.00				53.24		159.72	**-1305.4**
03:00-04:00		0.00				53.24		212.96	**-1358.6**
04:00-05:00		0.00				53.24		266.20	**-1411.9**
05:00-06:00		0.00				53.24		319.44	**-1465.1**
06:00-07:00		0.00				53.24		372.68	**-1518.4**
07:00-08:00	639	19.17			6.82		59.16	457.83	**-964.51**
08:00-09:00		17.89			3.65		59.16	538.53	**-1045.2**
09:00-10:00		12.14			1.41		59.16	611.24	**-1117.9**
10:00-11:00		7.67			2.78		59.16	680.85	**-1187.5**
11:00-12:00		4.47			1.25		59.16	745.74	**-1252.4**
12:00-13:00	602	20.62			23.30		59.16	848.81	**-753.49**
13:00-14:00		16.86	685				59.16	1609.82	**-1514.5**
14:00-15:00		11.44	354	34.25			59.16	2068.67	**-1973.4**
15:00-16:00	234	14.24		38.25	19.01		59.16	2199.33	**-1870**
16:00-17:00	606	28.95		10.62	3.89		59.16	2301.95	**-1366.6**
17:00-18:00		23.82			0.44		59.16	2385.37	**-1450**
18:00-19:00		14.32			23.90		59.16	2482.74	**-1547.4**
19:00-20:00		8.91	516				59.16	3066.81	**-2131.5**
20:00-21:00		5.18		25.8	19.01		59.16	3175.96	**-2240.6**
21:00-22:00	711	23.75		15.48	6.09		59.16	3280.45	**-1634.1**
22:00-23:00		19.91			1.03		59.16	3360.55	**-1714.2**
23:00-00:00		13.51				53.24		3427.30	**-1781**
24-h Total	2792	268.51	1555	124.4	112.572	425.92	946.56	3432.964	-640.96

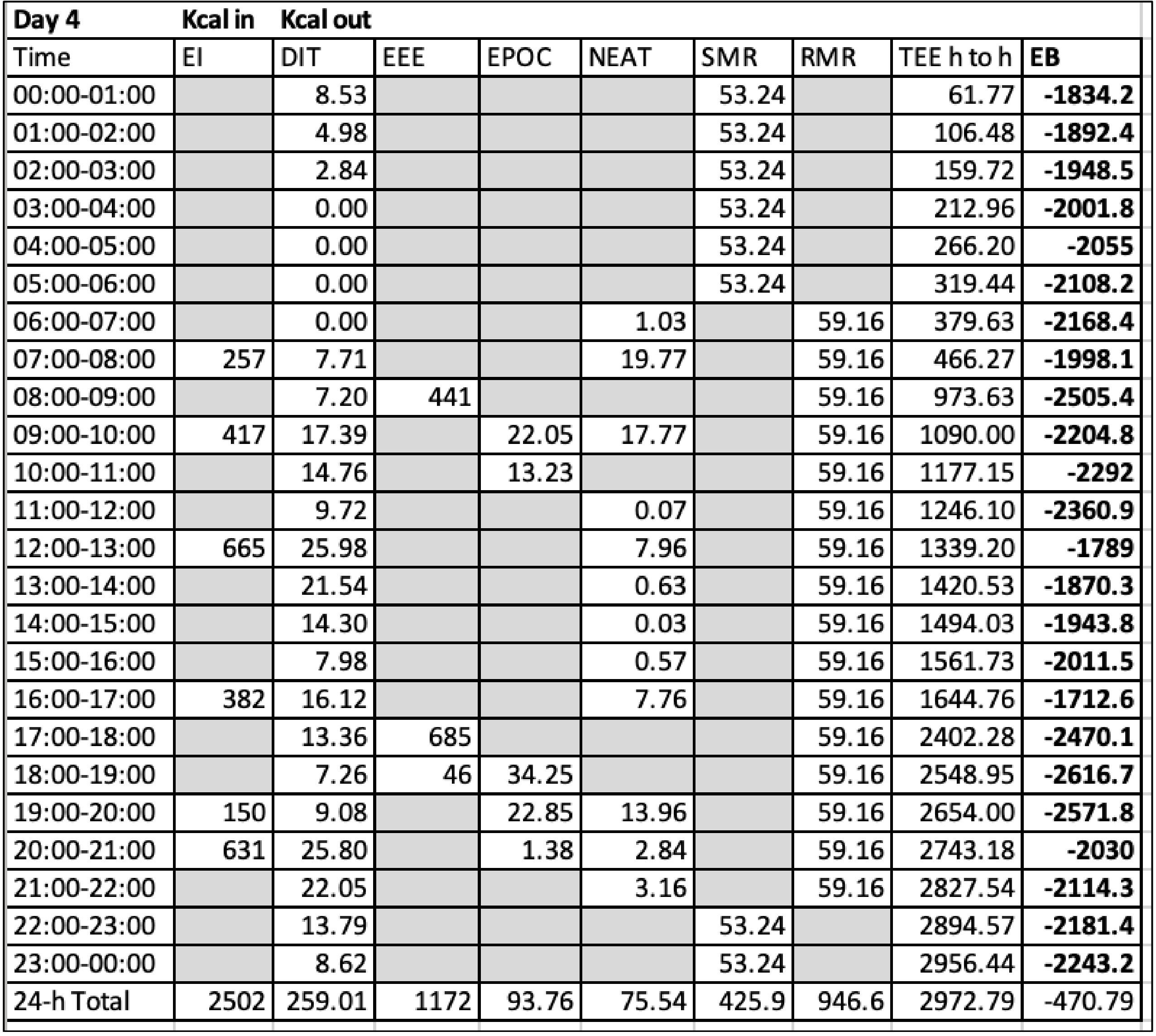
Summary, day 3:





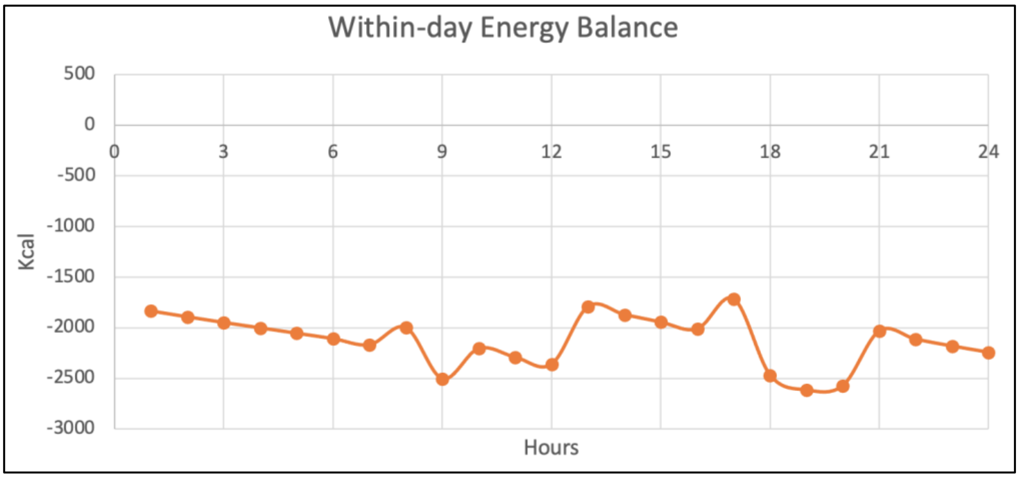
24-EB	-640.96
Hours > 0	0
Hours < 0	24
Hours > 300	0
Hours < -300	24
Hours [-300;+300]	0
Greatest surplus	-753.49
Greatest deficit	-2240.6

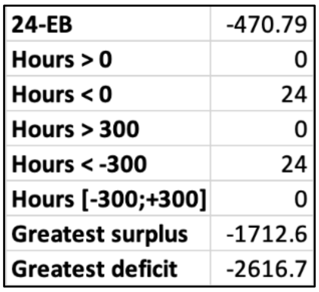
Within-day Energy Balance, day 4:



Day 4	**Kcal in**	**Kcal out**							
Time	EI	DIT	EEE	EPOC	NEAT	SMR	RMR	TEE h to h	**EB**
00:00-01:00		8.53				53.24		61.77	**-1834.2**
01:00-02:00		4.98				53.24		106.48	**-1892.4**
02:00-03:00		2.84				53.24		159.72	**-1948.5**
03:00-04:00		0.00				53.24		212.96	**-2001.8**
04:00-05:00		0.00				53.24		266.20	**-2055**
05:00-06:00		0.00				53.24		319.44	**-2108.2**
06:00-07:00		0.00			1.03		59.16	379.63	**-2168.4**
07:00-08:00	257	7.71			19.77		59.16	466.27	**-1998.1**
08:00-09:00		7.20	441				59.16	973.63	**-2505.4**
09:00-10:00	417	17.39		22.05	17.77		59.16	1090.00	**-2204.8**
10:00-11:00		14.76		13.23			59.16	1177.15	**-2292**
11:00-12:00		9.72			0.07		59.16	1246.10	**-2360.9**
12:00-13:00	665	25.98			7.96		59.16	1339.20	**-1789**
13:00-14:00		21.54			0.63		59.16	1420.53	**-1870.3**
14:00-15:00		14.30			0.03		59.16	1494.03	**-1943.8**
15:00-16:00		7.98			0.57		59.16	1561.73	**-2011.5**
16:00-17:00	382	16.12			7.76		59.16	1644.76	**-1712.6**
17:00-18:00		13.36	685				59.16	2402.28	**-2470.1**
18:00-19:00		7.26	46	34.25			59.16	2548.95	**-2616.7**
19:00-20:00	150	9.08		22.85	13.96		59.16	2654.00	**-2571.8**
20:00-21:00	631	25.80		1.38	2.84		59.16	2743.18	**-2030**
21:00-22:00		22.05			3.16		59.16	2827.54	**-2114.3**
22:00-23:00		13.79				53.24		2894.57	**-2181.4**
23:00-00:00		8.62				53.24		2956.44	**-2243.2**
24-h Total	2502	259.01	1172	93.76	75.54	425.9	946.6	2972.79	-470.79

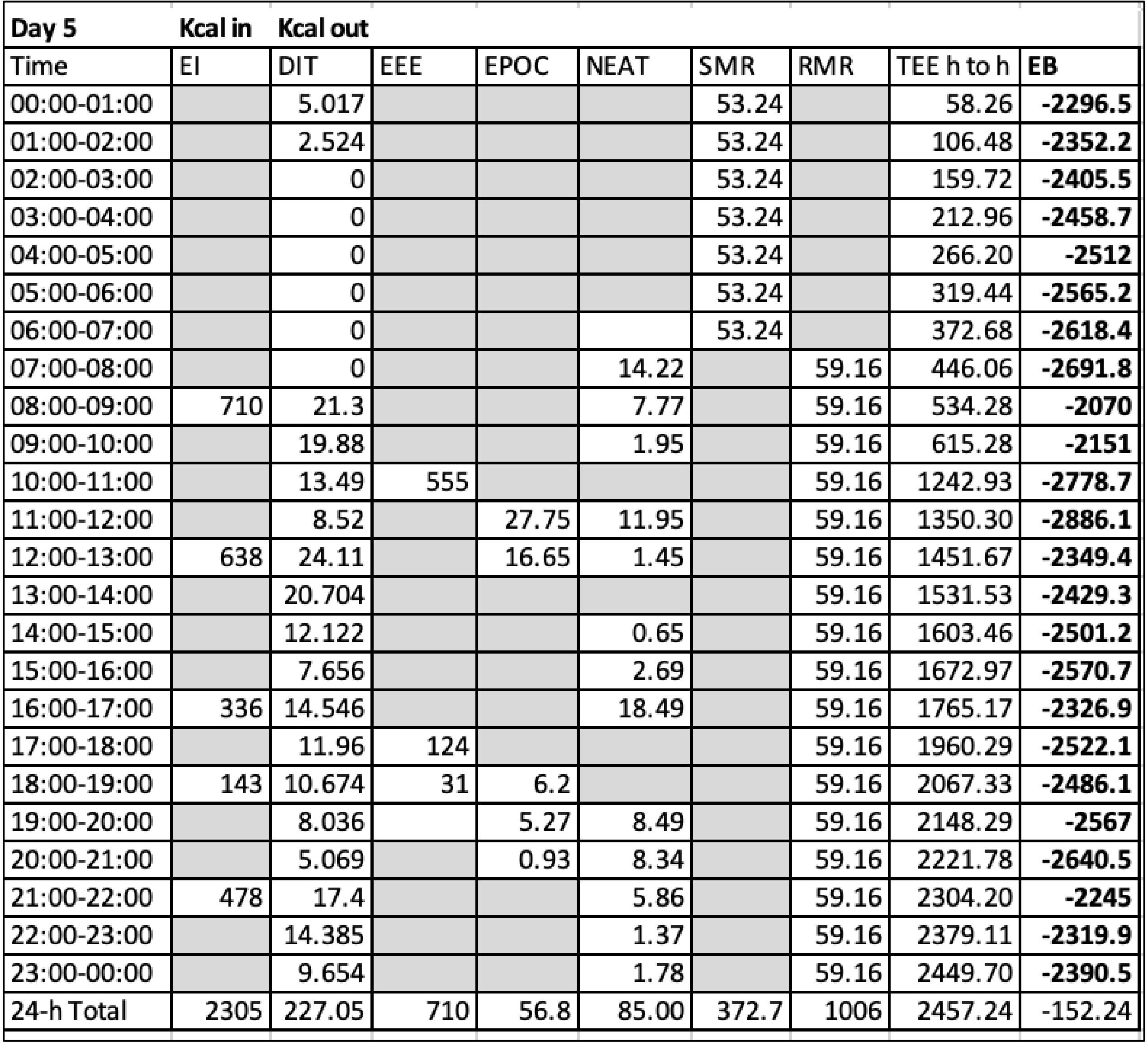
Summary, day 4:





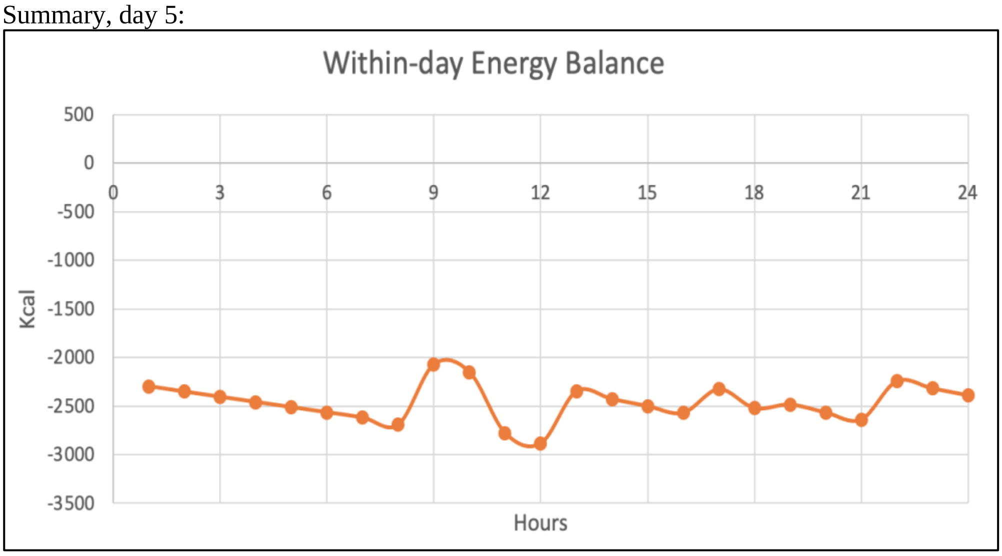
24-EB	-470.79
Hours > 0	0
Hours < 0	24
Hours > 300	0
Hours < -300	24
Hours [-300;+300]	0
Greatest surplus	-1712.6
Greatest deficit	-2616.7

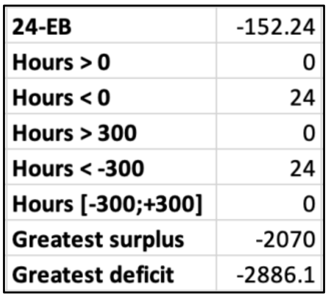
Within-day Energy Balance, day 5:



Day 5	**Kcal in**	**Kcal out**							
Time	EI	DIT	EEE	EPOC	NEAT	SMR	RMR	TEE h to h	**EB**
00:00-01:00		5.017				53.24		58.26	**-2296.5**
01:00-02:00		2.524				53.24		106.48	**-2352.2**
02:00-03:00		0				53.24		159.72	**-2405.5**
03:00-04:00		0				53.24		212.96	**-2458.7**
04:00-05:00		0				53.24		266.20	**-2512**
05:00-06:00		0				53.24		319.44	**-2565.2**
06:00-07:00		0				53.24		372.68	**-2618.4**
07:00-08:00		0			14.22		59.16	446.06	**-2691.8**
08:00-09:00	710	21.3			7.77		59.16	534.28	**-2070**
09:00-10:00		19.88			1.95		59.16	615.28	**-2151**
10:00-11:00		13.49	555				59.16	1242.93	**-2778.7**
11:00-12:00		8.52		27.75	11.95		59.16	1350.30	**-2886.1**
12:00-13:00	638	24.11		16.65	1.45		59.16	1451.67	**-2349.4**
13:00-14:00		20.704					59.16	1531.53	**-2429.3**
14:00-15:00		12.122			0.65		59.16	1603.46	**-2501.2**
15:00-16:00		7.656			2.69		59.16	1672.97	**-2570.7**
16:00-17:00	336	14.546			18.49		59.16	1765.17	**-2326.9**
17:00-18:00		11.96	124				59.16	1960.29	**-2522.1**
18:00-19:00	143	10.674	31	6.2			59.16	2067.33	**-2486.1**
19:00-20:00		8.036		5.27	8.49		59.16	2148.29	**-2567**
20:00-21:00		5.069		0.93	8.34		59.16	2221.78	**-2640.5**
21:00-22:00	478	17.4			5.86		59.16	2304.20	**-2245**
22:00-23:00		14.385			1.37		59.16	2379.11	**-2319.9**
23:00-00:00		9.654			1.78		59.16	2449.70	**-2390.5**
24-h Total	2305	227.05	710	56.8	85.00	372.7	1006	2457.24	-152.24

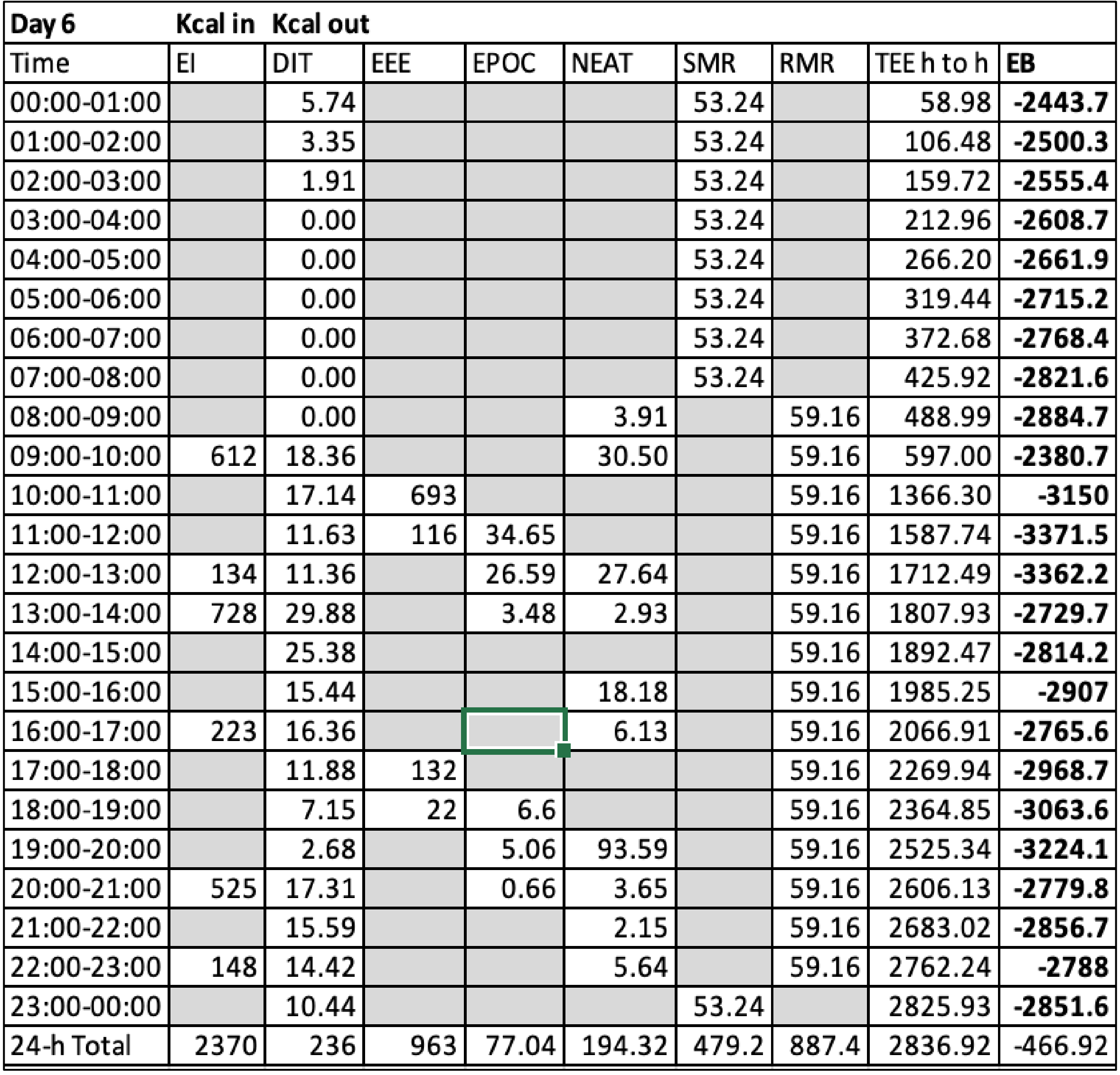
Summary, day 5:





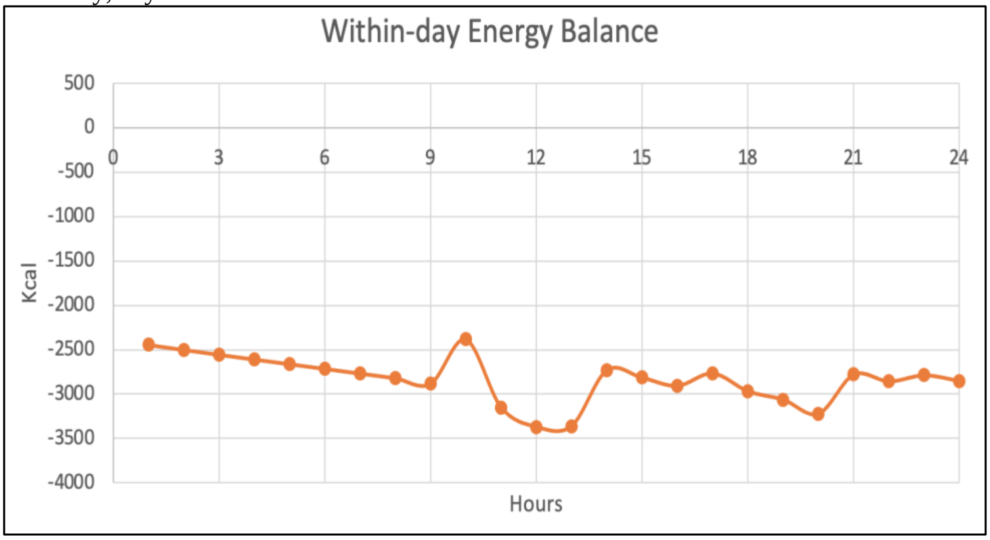
24-EB	-152.24
Hours > 0	0
Hours < 0	24
Hours > 300	0
Hours < -300	24
Hours [-300;+300]	0
Greatest surplus	-2070
Greatest deficit	-2886.1

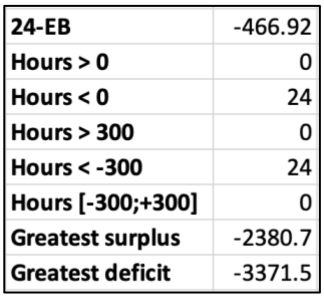
Within-day Energy Balance, day 6:



Day 6	Kcal in	Kcal out							
Time	EI	DIT	EEE	EPOC	NEAT	SMR	RMR	TEE h to h	**EB**
00:00-01:00		5.74				53.24		58.98	**-2443.7**
01:00-02:00		3.35				53.24		106.48	**-2500.3**
02:00-03:00		1.91				53.24		159.72	**-2555.4**
03:00-04:00		0.00				53.24		212.96	**-2608.7**
04:00-05:00		0.00				53.24		266.20	**-2661.9**
05:00-06:00		0.00				53.24		319.44	**-2715.2**
06:00-07:00		0.00				53.24		372.68	**-2768.4**
07:00-08:00		0.00				53.24		425.92	**-2821.6**
08:00-09:00		0.00			3.91		59.16	488.99	**-2884.7**
09:00-10:00	612	18.36			30.50		59.16	597.00	**-2380.7**
10:00-11:00		17.14	693				59.16	1366.30	**-3150**
11:00-12:00		11.63	116	34.65			59.16	1587.74	**-3371.5**
12:00-13:00	134	11.36		26.59	27.64		59.16	1712.49	**-3362.2**
13:00-14:00	728	29.88		3.48	2.93		59.16	1807.93	**-2729.7**
14:00-15:00		25.38					59.16	1892.47	**-2814.2**
15:00-16:00		15.44			18.18		59.16	1985.25	**-2907**
16:00-17:00	223	16.36			6.13		59.16	2066.91	**-2765.6**
17:00-18:00		11.88	132				59.16	2269.94	**-2968.7**
18:00-19:00		7.15	22	6.6			59.16	2364.85	**-3063.6**
19:00-20:00		2.68		5.06	93.59		59.16	2525.34	**-3224.1**
20:00-21:00	525	17.31		0.66	3.65		59.16	2606.13	**-2779.8**
21:00-22:00		15.59			2.15		59.16	2683.02	**-2856.7**
22:00-23:00	148	14.42			5.64		59.16	2762.24	**-2788**
23:00-00:00		10.44				53.24		2825.93	**-2851.6**
24-h Total	2370	236	963	77.04	194.32	479.2	887.4	2836.92	-466.92

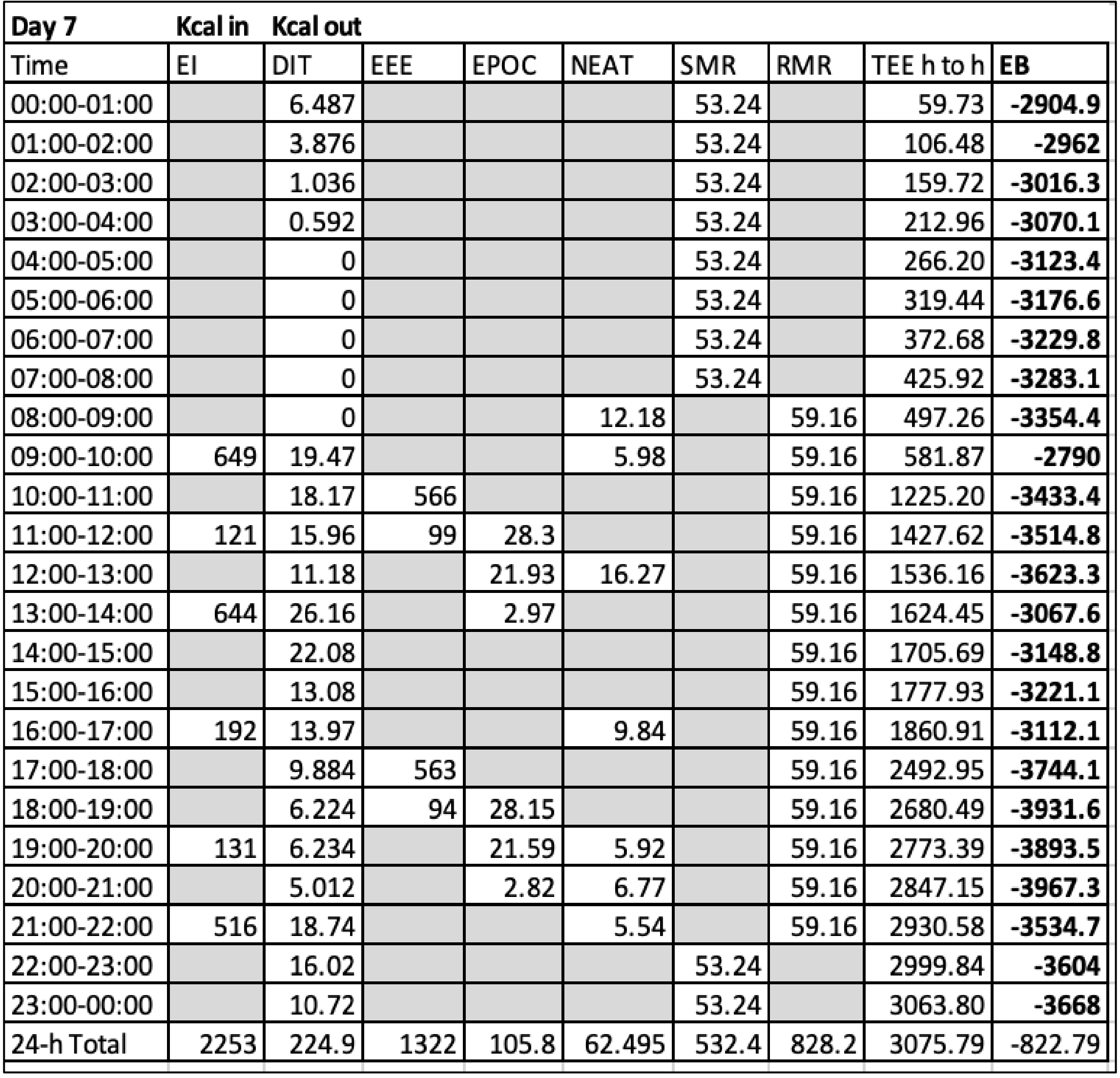
Summary, day 6:





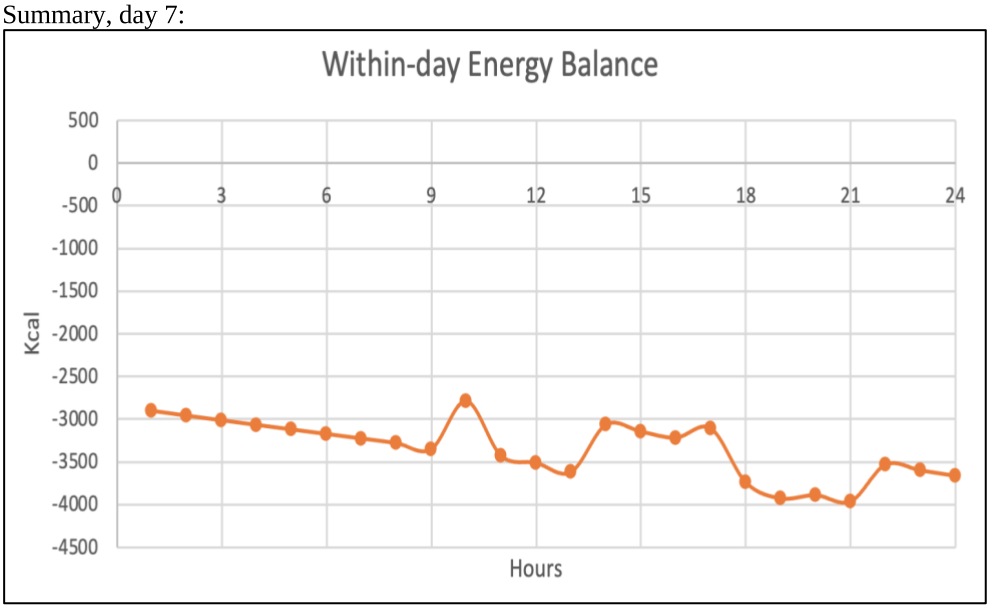
24-EB	-466.92
Hours > 0	0
Hours < 0	24
Hours > 300	0
Hours < -300	24
Hours [-300;+300]	0
Greatest surplus	-2380.7
Greatest deficit	-3371.5

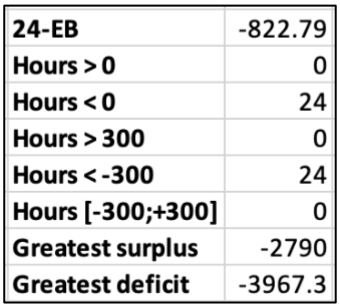
Within-day Energy Balance, day 7:



Day 7	**Kcal in**	**Kcal out**							
Time	EI	DIT	EEE	EPOC	NEAT	SMR	RMR	TEE h to h	**EB**
00:00-01:00		6.487				53.24		59.73	**-2904.9**
01:00-02:00		3.876				53.24		106.48	**-2962**
02:00-03:00		1.036				53.24		159.72	**-3016.3**
03:00-04:00		0.592				53.24		212.96	**-3070.1**
04:00-05:00		0				53.24		266.20	**-3123.4**
05:00-06:00		0				53.24		319.44	**-3176.6**
06:00-07:00		0				53.24		372.68	**-3229.8**
07:00-08:00		0				53.24		425.92	**-3283.1**
08:00-09:00		0			12.18		59.16	497.26	**-3354.4**
09:00-10:00	649	19.47			5.98		59.16	581.87	**-2790**
10:00-11:00		18.17	566				59.16	1225.20	**-3433.4**
11:00-12:00	121	15.96	99	28.3			59.16	1427.62	**-3514.8**
12:00-13:00		11.18		21.93	16.27		59.16	1536.16	**-3623.3**
13:00-14:00	644	26.16		2.97			59.16	1624.45	**-3067.6**
14:00-15:00		22.08					59.16	1705.69	**-3148.8**
15:00-16:00		13.08					59.16	1777.93	**-3221.1**
16:00-17:00	192	13.97			9.84		59.16	1860.91	**-3112.1**
17:00-18:00		9.884	563				59.16	2492.95	**-3744.1**
18:00-19:00		6.224	94	28.15			59.16	2680.49	**-3931.6**
19:00-20:00	131	6.234		21.59	5.92		59.16	2773.39	**-3893.5**
20:00-21:00		5.012		2.82	6.77		59.16	2847.15	**-3967.3**
21:00-22:00	516	18.74			5.54		59.16	2930.58	**-3534.7**
22:00-23:00		16.02				53.24		2999.84	**-3604**
23:00-00:00		10.72				53.24		3063.80	**-3668**
24-h Total	2253	224.9	1322	105.8	62.495	532.4	828.2	3075.79	-822.79

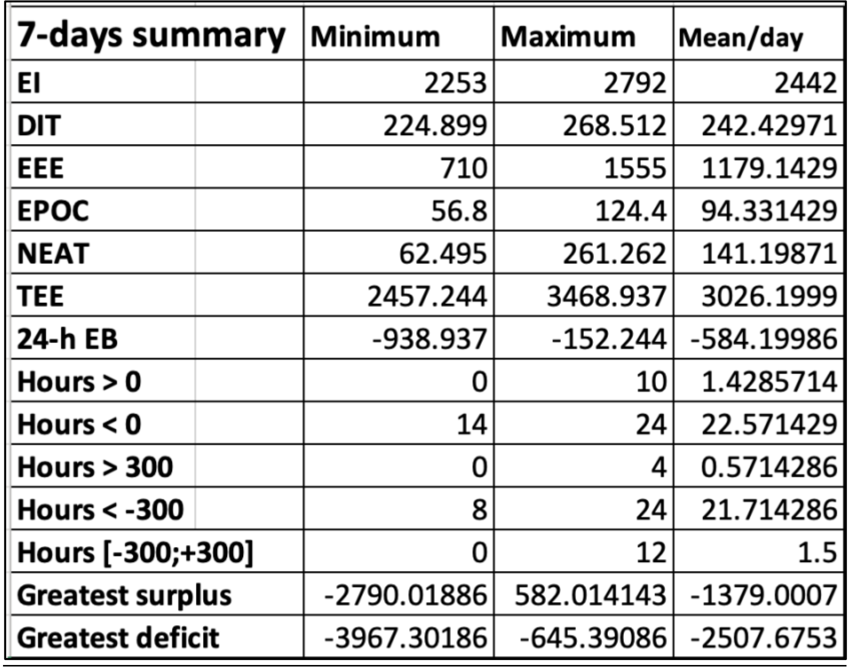
Summary, day 7:





24-EB	-822.79
Hours > 0	0
Hours < 0	24
Hours > 300	0
Hours < -300	24
Hours [-300;+300]	0
Greatest surplus	-2790
Greatest deficit	-3967.3

Within-day Energy Balance: 7-day weekly summary

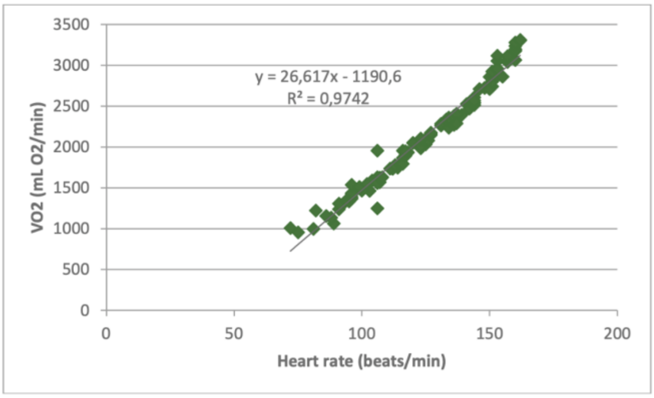


7-days summary	Minimum	Maximum	Mean/day
EI	2253	2792	2442
DIT	224.899	268.512	242.42971
EEE	710	1555	1179.1429
EPOC	56.8	124.4	94.331429
NEAT	62.495	261.262	141.19871
TEE	2457.244	3468.937	3026.1999
24-h EB	-938.937	-152.244	-584.19986
Hours > 0	0	10	1.4285714
Hours < 0	14	24	22.571429
Hours > 300	0	4	0.5714286
Hours < -300	8	24	21.714286
Hours [-300;+300]	0	12	1.5
Greatest surplus	-2790.01886	582.014143	-1379.0007
Greatest deficit	-3967.30186	-645.39086	-2507.6753

Appendix 6: Calculation of Exercise Energy Expenditure

Appendix 6a:

An example of the relationship between HR and VO_2 (mL O_2/min) for one of the LEA-subjects, generated by Phd. Student Ida Fahrenholtz. Reprint with approval from Fahrenholtz (28, 70).



Calculation example for the LEA-subject:

- VO_2 = (26.617 * 146) – 1190.6 = 2695.482 mL O_2/min
- EEE = 2695.482 mL O_2/min * 0.004998 kcal/mL * 41 min = 552.35 kcal

Appendix 6b: Example of calculating total EEE for the subjects assessed in this master´s thesis.

Mean regression equation based on the average regression curve from the LEA-subjects:

- VO_2 (mL O_2/min) = 26.97704*HR – 1693.29 =______ mL O_2/min

Average RER-value from the LEA-subjects used to determine the energy equivalent (kcal/L) was based on the table from Cavagna (2019) (17):

- RER = 0.98 = 5.02332 * 1000 = 0.00502332 kcal/mL

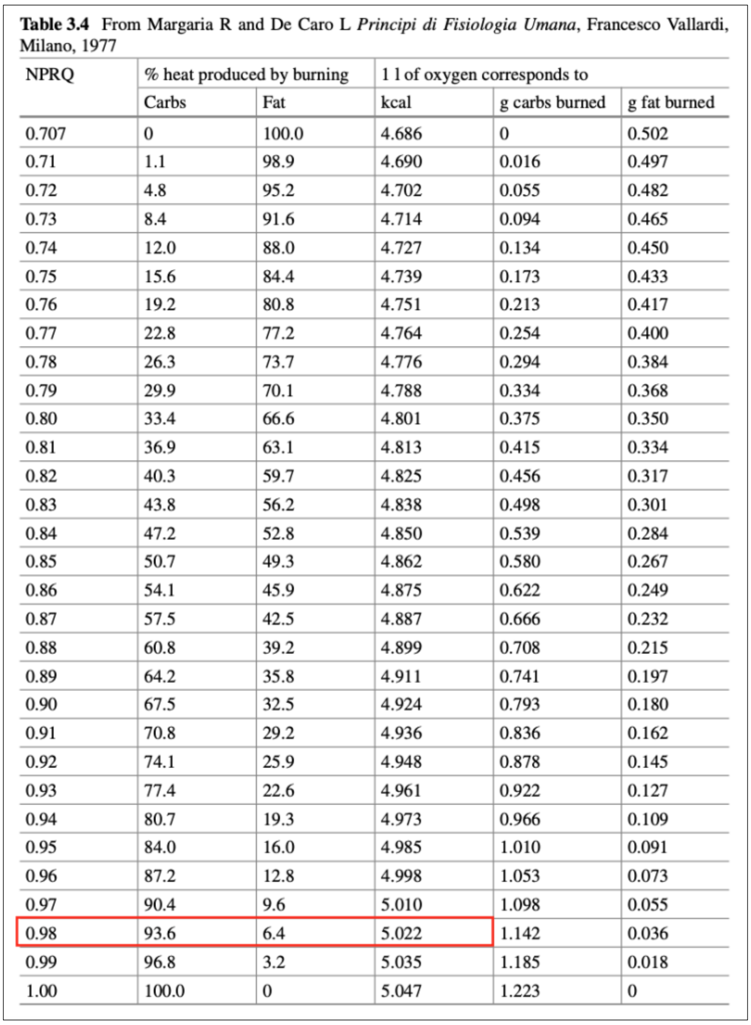


Table 3.4 From Margaria R and De Caro L *Principi di Fisiologia Umana*, Francesco Vallardi, Milano, 1977

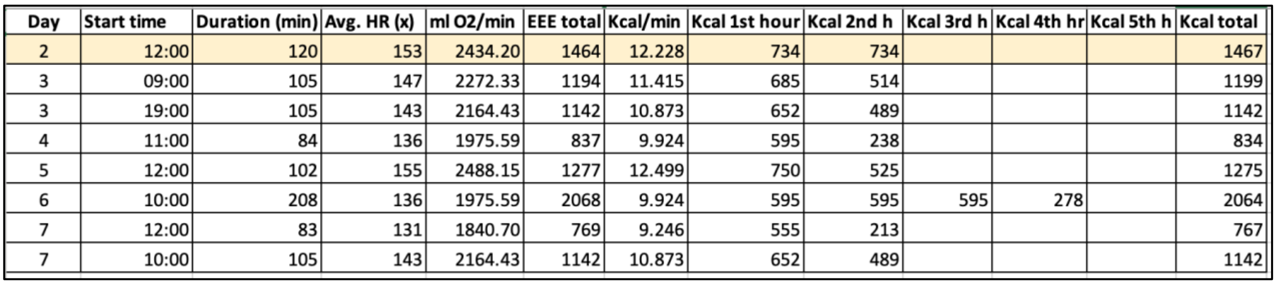
NPRQ	% heat produced by burning		1 l of oxygen corresponds to		
	Carbs	Fat	kcal	g carbs burned	g fat burned
0.707	0	100.0	4.686	0	0.502
0.71	1.1	98.9	4.690	0.016	0.497
0.72	4.8	95.2	4.702	0.055	0.482
0.73	8.4	91.6	4.714	0.094	0.465
0.74	12.0	88.0	4.727	0.134	0.450
0.75	15.6	84.4	4.739	0.173	0.433
0.76	19.2	80.8	4.751	0.213	0.417
0.77	22.8	77.2	4.764	0.254	0.400
0.78	26.3	73.7	4.776	0.294	0.384
0.79	29.9	70.1	4.788	0.334	0.368
0.80	33.4	66.6	4.801	0.375	0.350
0.81	36.9	63.1	4.813	0.415	0.334
0.82	40.3	59.7	4.825	0.456	0.317
0.83	43.8	56.2	4.838	0.498	0.301
0.84	47.2	52.8	4.850	0.539	0.284
0.85	50.7	49.3	4.862	0.580	0.267
0.86	54.1	45.9	4.875	0.622	0.249
0.87	57.5	42.5	4.887	0.666	0.232
0.88	60.8	39.2	4.899	0.708	0.215
0.89	64.2	35.8	4.911	0.741	0.197
0.90	67.5	32.5	4.924	0.793	0.180
0.91	70.8	29.2	4.936	0.836	0.162
0.92	74.1	25.9	4.948	0.878	0.145
0.93	77.4	22.6	4.961	0.922	0.127
0.94	80.7	19.3	4.973	0.966	0.109
0.95	84.0	16.0	4.985	1.010	0.091
0.96	87.2	12.8	4.998	1.053	0.073
0.97	90.4	9.6	5.010	1.098	0.055
0.98	93.6	6.4	5.022	1.142	0.036
0.99	96.8	3.2	5.035	1.185	0.018
1.00	100.0	0	5.047	1.223	0

Note: NPRQ (non proteic respiratory quotient) corresponds to RER.

Appendix 6c: Example of calculating EEE for each individual training hour.

An example showing how the formulas were used to estimate EEE for a training session, highlighted in yellow: data from a subject who had a training session lasting 120minutes with an average HR of 153bpm at day 2 of the registration week.

- VO_2 (mL O_2/min) = 26.97704 * 153 – 1693.29 = 2434.19712 mL O_2/min
- EEE = 2434.19712 mL O_2/min * 0.00502332 kcal/mL * 120min = 1467.33 kcal



Day	Start time	Duration (min)	Avg. HR (x)	ml O2/min	EEE total	Kcal/min	Kcal 1st hour	Kcal 2nd h	Kcal 3rd h	Kcal 4th hr	Kcal 5th h	Kcal total
2	12:00	120	153	2434.20	1464	12.228	734	734				1467
3	09:00	105	147	2272.33	1194	11.415	685	514				1199
3	19:00	105	143	2164.43	1142	10.873	652	489				1142
4	11:00	84	136	1975.59	837	9.924	595	238				834
5	12:00	102	155	2488.15	1277	12.499	750	525				1275
6	10:00	208	136	1975.59	2068	9.924	595	595	595	278		2064
7	12:00	83	131	1840.70	769	9.246	555	213				767
7	10:00	105	143	2164.43	1142	10.873	652	489				1142

Appendix 7: WDEB for 1 day vs. 7-day registration.

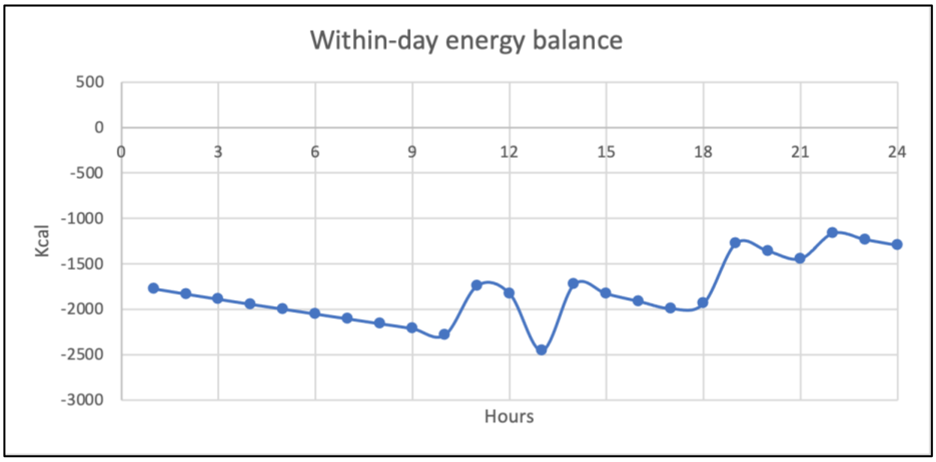
Table showing the difference in average time spent in different EB-zones depending on number of registration days (1-day vs. 7-days registration). For simplicity reasons values from one subject was used as an example. The first day of the week for both baseline or post-intervention-assessment (day 1, week 0 or week 17) was used for the values presented in the "1-day column".

	Baseline		Post-intervention	
Hours in:	1-day	7-day	1-day	7-day
EB >300 kcal^2	4	1	20	20
EB >0 kcal^2	10	1	23	23
EB <0 kcal^2	14	23	1	1
EB <-300 kcal^2	8	22	0	0
EB [-300; +300kcal]^1	12	1	4	4
LSHD, kcal^1	-645.4	-2507.7	-92.7	358.7
LSHS, kcal^1	582.0	-1379.0	1372.1	1806.4
Average 24h-EB, kcal^1	-938.9	-584.2	-111.4	244.03

Appendix 8: 24h-EB vs. Whitin-day Energy Balance

Illustration of day 6 at baseline for one subject showing the difference between 24h-EB and the cumulative WDEB-values on a relatively light training day. As shown, 24h-EB is positive (416 kcal), even though all hours were spent in a catabolic state (EB < -300 kcal).

Day 6	**Kcal in**	**Kcal out**							
Time	EI	DIT	EEE	EPOC	NEAT	SMR	RMR	TEE h to h	**EB**
00:00-01:00		8.93				53.48		62.41	**-1772.9523**
01:00-02:00		5.64				53.48		106.96	**-1832.0723**
02:00-03:00		3.29				53.48		160.44	**-1888.8423**
03:00-04:00		1.88				53.48		213.92	**-1944.2023**
04:00-05:00		0				53.48		267.4	**-1997.6823**
05:00-06:00		0				53.48		320.88	**-2051.1623**
06:00-07:00		0				53.48		374.36	**-2104.6423**
07:00-08:00		0				53.48		427.84	**-2158.1223**
08:00-09:00		0				53.48		481.32	**-2211.6023**
09:00-10:00		0			10.021		59.42	550.761	**-2281.0433**
10:00-11:00	626	18.78			4.858		59.42	633.819	**-1738.1013**
11:00-12:00		17.528			11.012		59.42	721.779	**-1826.0613**
12:00-13:00		11.894	556				59.42	1349.093	**-2453.3753**
13:00-14:00	858	33.252		27.8	6.719		59.42	1476.284	**-1722.5663**
14:00-15:00		28.406		16.68	1.741		59.42	1582.531	**-1828.8133**
15:00-16:00		18.806			4.236		59.42	1664.993	**-1911.2753**
16:00-17:00		10.296			11.241		59.42	1745.95	**-1992.2323**
17:00-18:00	138	10.146			10.376		59.42	1825.892	**-1934.1743**
18:00-19:00	755	29.946			3.499		59.42	1918.757	**-1272.0393**
19:00-20:00		23.762			4.017		59.42	2005.956	**-1359.2383**
20:00-21:00		16.001			6.749		59.42	2088.126	**-1441.4083**
21:00-22:00	374	21.246			12.97		59.42	2181.762	**-1161.0443**
22:00-23:00		16.309				53.48		2251.551	**-1230.8333**
23:00-00:00		10.126				53.48		2315.157	**-1294.4393**
24-h Total	2751	286.238	556	44.48	87.439	588.28	772.46	2334.897	416.103



24h-EB	**416.103**
Hours > 0	0
Hours < 0	24
Hours > 300	0
Hours < -300	24
Hours [-300; +300kcal]	0
Greatest surplus (LSHS)	-1161.044
Greatest deficit (LSHD)	-2453.375

Appendix 9: Detailed Description of Studies Assessing WDEB Among Athletes.

The table contains detailed information about the studies that have examined WDEB among athletes. These studies are the main ones used for background information and comparison regarding WDEB in this master's thesis. The details are mainly focused on the variables and information that are most related to WDEB and EB.

Reference, year	Study Design, Country, Population and Aim	Method and Energy Balance-analysis	Results/summary
Behrens et al. 2020	<u>Design:</u> Cross-sectional study <u>Country:</u> United States <u>Study population:</u> 20 collegiate female soccer players from a university in Southeast United States NCAA division I students Age: 18-21y <u>Aim:</u> 1. Test whether time spent in EB is inversely associated with FM 2. Investigate whether athletes in WDED have lower FM compared to those in surplus/balance	<u>Method:</u> <u>Assessments:</u> -3days WDEB-registration -3-day hourly dietary and training records, inc. details: time of meals, food preparation, quantity, type and intensity of training -Body composition: BIA -Questionnaire of medical history, demographics, injuries, and health goals <u>WDEB</u> WDEB-calculated through *NutriTiming® -software (NutriTiming® LLC, Atlanta, GA, USA),* <u>Output:</u> total EI (kcal), 24h-ending EB, 24-h net EB <u>WDEB-variables, hours in:</u> -deficit (EB < -400 kcal) -EB (between ± 400 kcal) -surplus (EB > +400 kcal) -relative amount of time spent in deficit and surplus (%)	<u>Results:</u> - <u>Average distribution of hours/24h day for the participants:</u> 7h in energy deficit (WDEB<-400kcal), 14h in EB [-400; +400kcal]) and 3h in surplus (EB>400kcal). -Subjects that spent more time in EB [-400; +400kcal]) and surplus (EB > 400kcal) had lower FM compared to those in an energy deficit (EB <-400kcal). In addition, hours spent in EB< -400kcal was positively associated with FM, BF %, BMI and FMI, but inversely associated for hours spent in surplus (EB > 400kcal) and EB [-400; +400kcal]. -TEE: 2240.2 ± 271.3 kcal/day. -EI: 2112.4 ± 504.7 kcal/day. -24h-EB: -127.8 ± 504.6 kcal/day. -<u>Conclusion:</u> Female soccer players who manage to maintain an adequate EB during the day (EB [-400; +400kcal]), and spent minimal time in an energy deficit have lower FM.
Benardot 1996	<u>Design:</u> Article <u>Country:</u> United States <u>Population:</u> 22 female elite gymnasts from USA national team 1994 <u>Aim:</u> 1.Introduce a new approach to determine energy requirements (microeconomic view on energy balance) **(figure 2)** 2. Provide insight to his personal experience working as the nutritionist for the US national team	<u>Validation of CTLEA with 3-day food diary:</u> -3days WDEB-registration -14 female gymnasts - 3-day food diary and CTLEA-assessment <u>CTLEA:</u> CTLEA-method: assessment of 24h day; meals, snacks, sleep, daily activity/NEAT and exercise. Assesses EE and EI simultaneously. Cumulative EB calculated in the timeframe of which a new activity occurred (i.e., travel, school, showering, workout). TEE was estimated based on the RDA (54, 55). EI assessed in the timeframe of the activity. RMR:	<u>CTLEA-validation:</u> -CTLEA and 3d food diary corresponded significantly (r = 0.7561, *p=0.002*) for total EI, but not distribution of macronutrients. -That the largest energy deficit estimated from CTLEA-analysis were significantly associated with BF % (p = *0.0035*) and BMI (p *=0.0063*) -Majority of athletes consumed most of their EI in the evening. <u>Benardot´s experiences (CTLEA):</u> 1) The athletes were pleased with the analysis because it directly reflected their individual energy status and needs at the time. 2) simplified the workload for practitioner. 3) CTLEA can be a valuable tool for counseling with the athletes, especially to change eating behavior and habits. -EI (3day food diary): 1222kcal.9kcal/day

	3.Introduce CTLEA-method and validation with 3d food diary	preferably through indirect calorimetry, or predictive formulas (54).	-EI (CTLEA): 1495.5kcal/day
Deutz et al. 2000	Design: Observational study (3years) Country: United States Population: 42 female elite gymnasts and 20 runners (N=62) from United States National Team Mean age:15.5y Aim: 1.Investigate how WDEB affects body composition 2.Evaluate and compare WDEB between female gymnasts and runners	Method: -1-day assessment - 62 female athletes: 42gymnasts + 20 runners -Assessments: DEXA, SFT, body weight, height WDEB/CTLEA: 1day EB-assessment: • EI: 24h-recall of a typical day • TEE: prediction from NRC (83) + training sessions	Observations: -A tendency for all athletes to be in EB (±100kcal) for the first 10h, but by hour 13-14 they had a tendency to stay at an avrg. energy deficit exceeds -300kcal. -The LSHD occurred for all athletes immediately following their afternoon session Results: -Gymnasts vs. runners: runners had higher EI/less energy deficit than gymnasts -Avg. EI: 1600kcal in 1day (24h) -Avg. TEE: 2384 kcal/day EB and body composition - Sig. association between hours in energy deficits (EB<-300kcal) and BF %. -Positive association between LSHD and BF % -Sig. relationship between total hours in deficit (EB <0kcal) and BF %, negative association between hours in surplus (EB >0kcal) and BF %. -Sig. inverse association between LSHS and BF %. -Positive association with BF % and age, height, and weight.
Fahrenholtz et al. 2018	Design: Observational study, cross-sectional design. Reanalysis from participants in the LEA-study by Melin et al. (70). Country: Sweden and Denmark Population: 25 female endurance athletes from Denmark and Sweden Mean age: 26.6y Aim: 1.Estimate and compare WDEB, 24h-EB and EA	Method: -7day WDEB-registration -25 participants collected from the LEA-study (40 in total, but included only data from eligible 25subjects) -Participants: athletes on elite level or training >5times/week. Assessments: Day 1: DEXA, reproductive function/gynecological examination + LEAF-Q score. Day2: -Aerobic capacity and EEE: an incremental exercise test on an Monark bicycle-ergometer with an increased workload, with a mask covering the mouth and nose to measure VO_2, VCO_2 and RER. This was the basis for individual regression lines and calculations of EEE. -Assessment of Disordered eating: EDI-Q	Results: 1.Subjects with menstrual dysfunction (MD) spent more time in negative EB compared to EUM-athletes: *WDEB < 0 kcal: 23.0 hour (20.8-23.4) vs 21.1 hour (4.7-22.3), P = .048; WDEB < −300 kcal: 21.8 hour (17.8- 22.4) vs 17.6 hour (3.9-20.9), P = .043, although similar 24-hour EA: 35.6 (11.6) vs 41.3 (12.7) kcal/kg FFM/d, (p = .269), and 24h-EB: −659 (551) vs −313 (596) kcal/d, (P = .160).* 2. Similar 24h-EB and 24h-EA between EUM-subjects and subjects with MD. 3. Hours in WDED (EB <0 kcal and <−300 kcal) were inversely associated with RMR_{ratio} (r = −.487, *p = .013*, r = −.472, *p = .018*) and estradiol-levels (r = −.433, *p = .034*, r = −.516, *p = .009*), and positively associated with cortisol-levels (r = .442, *p = .027*, r = .463, *p = .019*).

	between female athlete with MD and EUM. 2.Investigate the association between WDED and suppressed RMR, body composition and endocrine function (cortisol, estradiol, T3, blood glucose)	-mRMR: ventilated hood system after overnight fast -Blood samples: fasted, between 08:30-08:50am. 7-day records: -EI: weighted food-diary (Diestist) -EEE: heart rate monitors and training diaries -NEAT: Actigraph-accelerometers on the hip (except when sleeping, showering, swimming, biking, training) -Meal frequency: daily number of meals and snacks were counted Data collection and calculations: -EA: (EI/day) – (EEE/kg FFM/day) -WDEB: EI-TEE (DIT + NEAT + pRMR/SEE + EEE + EPOC) in 1h-intervals continuously for 7days. -pRMR: Cunningham equation -WDED: defined as the variables according to Deutz et al. 2000 (hours in EB <0kcal, EB<-300kcal and LSHD)	4. Positive association between WDED and meal frequency, suggesting that a high meal frequency does not improve WDEB in athletes mainly eating low energy dense foods. 5.No association with WDED and body composition (BF %).
Friel & Benardot 2011	Design: Master thesis via College of Health and Human Sciences, Atlanta, Georgia 2010 Country: United States Population: 20 subjects (10 non-competitive, 2 triathletes, 1elite runner, 7collegiate XC-runners) Mean age: 23.4y Aim: 1. Determine whether WDEB is related to menstrual function in active female adults.	WDEB: -3 days WDEB-registration (one highly active, moderate, and rest day). Hourly EI and TEE registered in *NutriTiming® -software (NutriTiming® LLC, Atlanta, GA, USA)* to analyze WDEB and 24h-EB. PAL was determined by the athlete. Nutritiming: -EI: records in Nutritiming -WDEB-variables: -LSHD, LSHS, EB < -400kcal, EB >400kcal, EB >0kcal, EB <0kcal	Results: -Mean EI: 2094kcal -Mean predicted EEE: 2698kcal -Mean 24h-EB: -504kcal WDEB: Athletes spent more hours in EB <-400kcal than EB >400kcal (15.2h vs. 1.7hrs), and more hours EB <0kcal than EB>0kcal (20.5h vs. 3.5h). The average LSHD was -1034kcal, and the LSHS was 187kcal, while mean 24h-EB was -703kcal. Menstrual function: An inverse relationship between loss of menses (>3months) and hours spent in energy surplus (EB >400kcal), meal frequency, total EI/day, LSHS and protein-intake, but no relationship between 24h-EB was found. Participants averaged more hours in a catabolic state than an anabolic state (hour in: EB<0kcal; 20.5h vs. EB >0kcal; 3.5h).
Jurov et al. 2020	Design: Cross-sectional study Country: Slovenia Population: 8 elite female cyclists	Method: -5 days registration -Baseline assessments: LEAF-Q, BIA, mRMR (indirect calorimetry). pRMR (Harris Benedict formula)	Results: Mean values (for all subjects): -EI: 1802.52 kcal/day -24h-EB: 61.68 kcal/day

	Mean age: 17.5y Aim: 1. Investigate the effects of LEA on athletic performance. 2. Examine the correlation between RMR and performance	-Day 1-5: dietary records, HR during training, accelerometer (NEAT, 24h except during training) -Last day (day 5): incremental exercise test on ergometer -Allocated subjects in two groups: LEAF+ (risk of LEA group, >1 score on LEAF-Q) and LEAF– (no risk group, 0 score on LEAF-Q) -Compared EA, WDED, performance and other metabolic variables between LEAF-Q+ and LEAF-Q group WDEB: WDEB = EI-(EEE + EPOC + NEAT + DIT + mRMR) in 1h intervals. WDED-variables: -WDEB-neg: EB < 0kcal -WDEB < -300kcal -EA=EI-EE/FFM	-EA: 10 kcal/kg FFM/day -EEE: 1357.81 kcal/day LEAF+ vs. LEAF-: -EA was significantly different between the LEAF+ and. LEAF– group. -No difference in WDEBneg, EI, EEE and TEE between the groups. -Sig. difference in WDEB < -300kcal between LEAF- compared to LEAF+ Performance: -No difference in performance parameters, body composition (BF %, FFM) or energy metabolism (TEE, EEE, EI and mRMR) between the groups. -Significant association between WDEB< -300kcal and LAmax (r=-0.859,) but not for the other performance parameters. WDEB and EA: -Sig. correlation between WDEB < -300kcal and EA, and WDEB < -300kcal and 24h EB -No correlation between WDEB < -300kcal and BF %, mRMR or RMR-ratio. Conclusion: -No association between WDEB < 0kcal and performance, but WDEB < -300kcal was negatively associated with lactate values in the incremental test, suggesting that the severity of energy deficit have a greater impact in performance rather than merely time spent in an energy deficit (WDEB <0kcal).
Lee et al. 2021	Design: 1-month follow-up observational study. Country: Korea Population: 10 Korean male collegiate soccer players. Mean age: 19.1y Aim: Evaluate WDEB and its effect on RMR$_{\text{-ratio}}$ and other markers of metabolic suppression.	Method: -7-days registration -10 male collegiate soccer players from Korea (18-21y) - Baseline assessments: -DEXA for body composition. VO_2max: incremental test on bicycle ergometer. -Blood sampling for hormonal analysis (T3, cortisol, insulin, IGF-1, GH) -NEAT: accelerometer during waking hours except sleep, shower, swimming, training -Training: HR-monitor -mRMR: The Douglas bag method	Results: -No difference in WDEB-variables between normal group and suppressed-RMR group -Sig. difference: Normal group had a higher RMR-ratio, mRMR/FFM-ratio, EI and DIT compared to suppressed group. -RMR-ratio positively associated with IGF-1-levels. -No other sig. associations between WDED and metabolic markers (leptin, testosterone, insulin, GH, IGF-1, cortisol and T3). -Average values (all subjects): -24-h EB: 429 ± 693kcal -Hours in EB < 0kcal: 17.8 ± 4.1 hours

		-pRMR: values predicted from DEXA Allocation: 1. normal group (RMR-ratio > 0.94), n=5 2. metabolic suppressed group (RMR-ratio < 0.94), n=5 7day food and training diary: EI: Weighted 7day food diary (5training days + 2rest days) TEE: -mRMR (adapted instead of unadapted) -SMR (90 % av RMR) -DIT (10 % of kcal from meal) -EEE: Polar HR-watch, regression lines generated by HR-data, HR-VO2 and HR-CO2 from incremental test (Wier equation). -EPOC: 5 % + 3 % two hours post exercise -NEAT: accelerometer WDEB-calculations: - WDEB: EI − TEE; mRMR (or SMR) + DIT + EEE + EPOC + NEAT) -24h-EB: Total EI – TEE within a 24h period WDED: -Defined as: total hours of EB < 0kcal, EB < -400kcal and LSHD EA: -hourly EA: net EEE minus EI /FFM for every hour -> sum -> average EA.	-Hours in EB < -400kcal: 9.8 ± 3.0hours -LSHD: -986 ± 239kcal -24h-EA: 25.6 ± 9.8kcal/kg FFM/day Conclusion: -Hourly changes in WDEB during training days showed a severe energy deficit after training with insufficient compensation of EI which resulted in a negative EB at the end of the day.
Lundstrom et al. 2022	Design: Cross-sectional study conducted over 6week period. Country: United States. Population: 25 elite swimmers (10 males + 15 females) from United States Mean age: 19.6years Aim: Determine whether an imbalance between EI and EE across the day may be related to	Energy-assessments/WDEB: - 6week total data collection - WDED and 24h-EB determined based on 3-day measurements of TEE (mRMR/SEE + EEE + NEAT + EPOC + DIT) and 3-day dietary records. EB assessments: - Starting WDEB: determined midnight first day of starting food records. - WDEB-variables: -LSHD, LSHS, EB < -400/300kcal, EB > 400/300kcal, EB > 0kcal, EB < 0kcal	Results: -84 % (21/25) enrolled in the study achieved positive 24h-EB or energy surplus, while 16 % remained in a negative 24h-EB. -All males (100 %) were in positive 24h-EB, while 11/16 (69 %) of the females were in a positive 24h-EB or surplus. -Male swimmers had significantly more positive overall EB (incl. hourly EB and 24h-EB) compared to females, which were primarily driven by a greater hourly EI. WDEB: - Avg. hourly EB was negative (-3 ± 270 kcal per h) - LSHD: -1090 ± 460kcal for all participants

	metabolic compensation (RMR) and other health outcomes and associated sex differences.	-Consecutive hours in each EB-category (hourly blocks ≥ 3 h in each EB-state). -hourly EB: average hourly EB determined as EIhourX −EEhourXEB. Data collection: -EI: 3-day food diary recorded in mobile application (*MyFitnessPal)* -TEE (incl.: EEE, NEAT, sleep quantity): based on data from wrist worn multi-optical sensor (*WHOOP).* - Training: subjective training load based on weekly average of RPE (Borg CR10 scale) -mRMR: indirect calorimetry through ventilated hood and metabolic chart following a 12h fast. -pRMR: based on DEXA-derived equation. -TT_3: collected from antecubital vein -Body composition: DEXA, BMI, height, and weight -RMR-ratio: subjects grouped into suppressed group (<0.94) and non-suppressed group (>0.94) <u>Meal distribution examination</u> -The subjects in positive total-daily EB (24h-EB > 0kcal) were allocated into 1) “back loaders”: consumed >50 % of EI after 17:00, or “non-back loaders”: more evenly distributed meal pattern	- Hours in EB < 0kcal: 15 ± 5h - Hours in EB < -300/-400kcal: 5 ± 4h - Hours in EB < 0kcal (>3h blocks): 14 ± 6 consecutive hours - Hours in EB < -300/-400kcal (>3h blocks): 4 ± 4 consecutive hours) <u>Sex differences and WDEB:</u> -Males had a greater positive hourly EB compared to females who displayed a lower (negative) average hourly EB -LSHD was significantly more negative in males compared to females -Females spent more hours in EB < 0kcal and EB < 0kcal (>3h blocks) than males, but there was no difference between the sexes for hours in EB <-300/400kcal or EB <-300/400kcal (>3h blocks) -Positive WDEB: Hours in EB > 0kcal were significantly greater for males, but no difference in EB > +300/+400kcal between the sexes. <u>Metabolic compensation and WDEB:</u> - TT_3 was not correlated to 24h-EB or any WDEB-variables (either males or females), but TT_3 was negatively correlated to hours in EB <0kcal (>3h blocks) and positively correlated to EB [−300/400 to + 300/400kcal] but only for the subjects with suppressed-RMR (n=12). -Metabolic compensation was not associated with variations in hourly EB (kcal) -RMR-ratio: no significant difference in 24h-EB, hourly EB nor any of the WDEB-variables between suppressed and non-suppressed-RMR group. <u>Meal distribution and WDEB</u> -No significant difference in any of the WDEB-variables (p > 0.05) nor RMR-ratio between “backloaders” (n = 7f, 5m) and “non backloaders” (n= 4f, 5m). - “Backloaders” had a significant lower TT_3 compared to. “Non backloaders”
Torstveit et al. 2018	<u>Design:</u> Cross-sectional study. <u>Country:</u> Norway and Denmark <u>Population:</u> 31 male endurance athletes.	<u>Method:</u> -4-days registration -31 male endurance athletes (triathletes, cyclists, long distance runners), age 18-50years, VO_2-max > 55,	<u>Results:</u> -65 % of the subjects had suppressed RMR (<0.9) -No sig. difference in 24-h EB or 24h-EA between the groups

	Mean age: 34.7y Aim: -To assess WDEB in male athletes and compare WDEB between those with suppressed RMR and normal RMR. -Explore whether WDED is associated with endocrine markers of energy deficiency.	training frequency > 4sessions/week, competing at regional or national level. -Allocated into two groups; 1) normal (RMR-ratio >0.9), 2) suppressed group (RMR-ratio < 0.9). Protocol: -mRMR using a ventilated hood, DEXA, blood plasma (T3, cortisol, testosterone, glucose) -DIT (10 % of kcal from meal), NEAT (accelerometer), EI (weighted 4-day food diary in Dietist), EPOC (8 % of EEE, 5 % of EEE first hour + 3 % of EEE second hour post exercise). pRMR (Cunningham equation), SEE (90 % of pRMR), -EEE: (HR-monitor, EEE (kcal/kg/min) = (5.95*HRaS) + (0.23·age) + (84·1)-134)/4186.8. -VO_2-max: incremental test to exhaustion (running on treadmill or cycling on ergometer) WDEB: -WDEB in 1h intervals consecutively for 4days -WDEB: EB=EI-TEE (DIT + EEE + EPOC + NEAT + pRMR). WDED-variables: -Total hours in EB < 0kcal -Total hours in EB < -400kcal -LSHD	-Subjects with suppressed RMR spent more time in EB < -400kcal (20.9h vs. 10.8h) and had a greater LSHD comparted to the normal group (3265kcal vs. -1340kcal). -A greater LSHD was associated with higher cortisol levels and lower testosterone:cortisol-ratio. -No difference in protein intake between the groups. -No association between WDED and glucose-levels nor T_3-levels. -The more time spent in EB < 0kcal and the greater LSHD the lower the BF %. -No association between protein intake and body composition, but a tendency towards a lower BF % for those with lower protein intake. -No sig. difference in 24-h EB or 24-h EA between the groups. Summary: - It was evident that WDEB was associated with suppressed RMR and endocrine alterations (LSHD was associated with lower testosterone:cortisol-ratio and higher cortisol levels). -Inverse relationship between WDED and BF % (the greater the deficit, the lower the BF %).

Country: main country for the execution of the study and study population. Abbreviations: Avg: average. BF %: body-fat percentage. BIA: bioelectrical impedance analysis. BMI: body mass index. CTLEA: Computerized Time-line Energy Assessment”. DEXA: dual-energy X-ray absorptiometry. DIT: diet-induced thermogenesis. EA: energy availability. EA: energy availability. EB: energy balance. EE: energy expenditure. EEE: exercise energy expenditure. EI: energy intake. EPOC: Excess post-exercise oxygen consumption. EUM: eumenorrhea. f: females. FFM: fat-free mass. FM: fat-mass. FMI: fat-mass index. GH: growth hormone. h: hours. HR: heart rate. HRaS: HR above sleeping HR (beats/min). IGF-1: Insulin-like growth factor 1. Incl: including. kcal: kilocalorie. LAmax: lactate max (maximum production rate of lactate). LEA: low energy availability. LEAF-Q: The Low Energy Availability in Females Questionnaire. LHED: largest hourly energy deficit. LHES: larges hourly energy surplus. m: males. MD: menstrual dysfunction. RMR: resting metabolic rate. mRMR: measured resting metabolic rate. pRMR: predicted resting metabolic rate. NCAA: National Collegiate Athletic Association. NEAT: Non-exercise activity thermogenesis. NRC: Current National Research Council NRC: National Research Council US. PAL: physical activity level. pRMR: predicted RMR. RDA: Recommended Dietary Allowance. RER: respiratory exchange ratio RMR: resting metabolic rate. RPE: ratings of perceived exertion. SEE: sleeping energy expenditure. SFT: Skinfold thickness. Sig.: significant. STF; skinfold thickness. TEE: total energy expenditure. TT3: total triiodothyronine. WDEB: Within-day energy balance. WDED: within-day energy deficiency. XC: cross-country. y: years.

Appendix 10: Overview and Comparison of Studies Assessing EB and WDEB in Relation to Different Health-and Performance Outcomes.

The table below (B-G) includes various studies that have investigated the relationship between WDEB, WDED or meal frequency on various health-and performance parameters. Also, Table A illustrate the discrepancy between the WDEB-method and 24h-EB method, showing studies that have found significant findings with the WDEB-method but not the 24h-EB method.

A. WDEB vs. 24h-EB

Reference, year	.	
Benardot 2013 (22)	Significant positive association between hours in EB < -400kcal and BF %, and the more hours spent in EB [-400kcal; +400kcal] the lower the BF %. No significant relationship between 24h-EB and BF % was found.	
Fahrenholtz et al. 2018 (32)	Amenorrheic subjects spent more hours in WDED compared to eumenorrheic subjects, despite similar 24h-EB and EA between these subjects.	
Friel et al. 2011	An inverse relationship was found between loss of menses (>3months) and: hours spent in energy surplus (EB > 400kcal), meal frequency, total EI/day, LSHS and protein-intake, but no relationship between 24h-EB with these variables were found.	
Lundstrom et al. 2022 (62)	For subjects with suppressed RMR there was a negative correlation between TT_3 and WDEB > 0kcal (in >3hour consecutive blocks), while no correlation was found between TT_3 and 24h-EB for the same subjects.	
Thivel et al. 2013 (106)	Significant association between WDEB and BF %, but no association between 24h-EB and BF % (among young children aged 8-14) was found.	
Torstveit et al. 2018 (108)	Similar 24h-EB between the groups with suppressed RMR and normal RMR, despite significant difference in hours in EB < -400kcal.	

B. WDEB/WDED/Meal Frequency and Body Composition

Reference	Positive association: WDED and altered body composition	Additional information/comments
Beherens et al. 2020	Positive associations between WDEB < -400kcal and FM, BF %, BMI and FMI were found.	Inverse association between WDEB >400kcal and WDEB [-400; +400kcal] with FM, BF %, BMI and FMI.
Benardot 1996	Positive association between LSHD and BF % was found.	The greater the magnitude of LSHD (the more negative the LSHD), the greater the BF %.
Benardot 2005	Additional 250kcal between meals (total of 750kcal/day) was associated with reduced BF % and increased LBM.	
Benardot 2013	Positive association between WDEB < -400kcal and BF % was found.	The more hours spent in EB< -400kcal, the greater the BF %.
Deutz et al. 2000	Positive association between all WDED-variables (EB <-300kcal, EB <0kcal, LSHD) and BF % was found.	Inverse association between all WDEB-surpluses (EB >300kcal, EB >0kcal and LSHS) and BF %.

Iwao et al. 1996	Two groups of boxers (2meals vs 6meals/day-group): 2meals/day-group had greater muscle loss and decreased LBM compared to 6meals/day-group.	Inverse association between meal frequency and muscle loss.
Thivel et al. 2013	Positive association between WDED and BF % was found among young children aged 8-14.	
	No association: WDED and body composition	
Fahrenholtz et al. 2018	No association between WDED (EB <-300kcal, EB <0kcal, LSHD) and BF % was found.	
Jurov et al. 2020	No association between WDED and BF % was found.	
	Inverse association: WDED and body composition	Additional info/comments
Torstveit et al. 2018	The greater the magnitude of LSHD and the more time spent in EB <0kcal, the lower the BF %.	The greater the magnitude of LSHD (the more negative the LSHD), the lower the BF %.

C. WDEB/WDED and Metabolic Health

Reference	Association: WDED and metabolic suppression (RMR)	
Fahrenholtz et al. 2018	WDEB <0kcal and WDEB <-300kcal were associated with lower RMR-ratio.	
Torstveit et al. 2018	An association between suppressed RMR and WDEB < -400kcal was found.	
	No association: WDED and metabolic suppression	Additional info/comments
Jurov et al. 2020	No association between WDED and RMR-ratio was found.	
Lee et al. 2021	No association/difference in any of the WDEB-variables between suppressed RMR-group and normal-RMR group.	
Lundstrom et al. 2022	No significant difference in WDEB-variables between suppressed group (RMR-ratio < 0.94) and non-suppressed group (RMR-ratio > 0.94)	No significant difference in RMR-ratio between subjects eating >50 % of EI after 17:00 and those with a more evenly meal distribution throughout the day.

D. WDEB/WDED and Menstrual Function

Reference	Association: WDED, EI, meal frequency and menstrual dysfunction	Additional info/comments
Fahrenholtz et al. 2018	WDED (EB <0kcal, EB <-300kcal) was associated with MD.	MD including functional hypothalamic oligomenorrhea, amenorrhea (either primary or secondary) or other MD not related to energy deficiency.
Friel et al. 2011	Inverse relationship between loss of menses exceeding three months and hours spent in energy surplus (EB >400kcal), meal frequency, total EI/day, LSHS and protein-intake.	

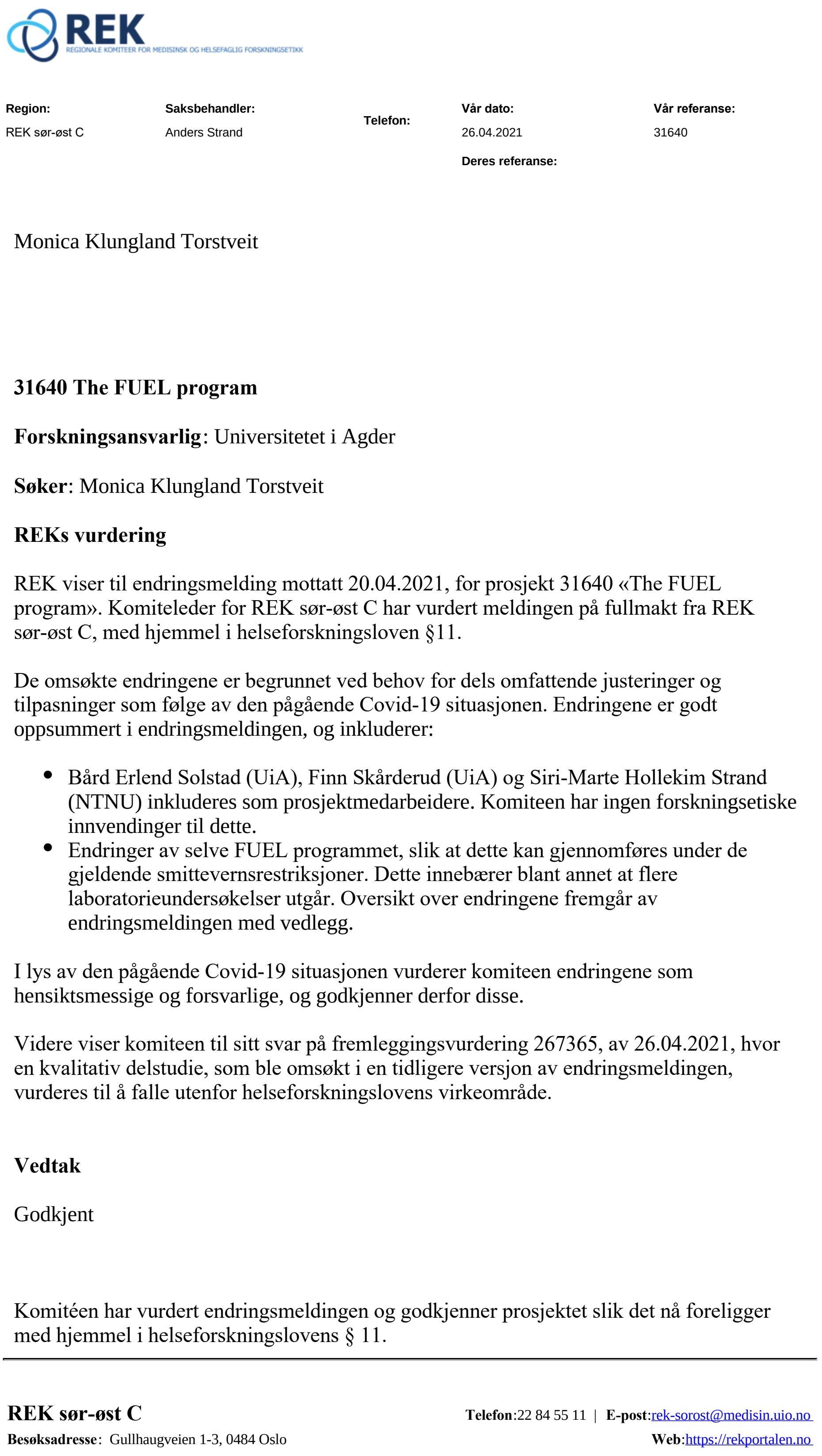
E. WDED/WDED/Meal Frequency and Endocrine Function

Reference	Association: WDED and endocrine function	
Fahrenholtz et al. 2018 (32)	WDEB <0kcal and WDEB <-300kcal were associated with lower estradiol levels and elevated cortisol levels.	

Torstveit et al. 2018	WDED was associated with elevated cortisol and lower testosterone:cortisol-ratio.	
Lundstrom et al. 2022	Subjects consuming >50 % of EI after 17:00 had significantly lower TT$_3$ than subjects with a more evenly meal distribution.	
	No association: WDED and endocrine function	
Lee et al. 2021	No association between WDED and metabolic markers (T$_3$, cortisol, insulin, IGF-1 and GH) was found.	
Lundstrom et al. 2022	No correlation between TT$_3$ and any of the WDEB-variables nor 24h-EB (either for male or females) was found.	
	F. WDEB/WDED and Athletic Performance	
Reference	**Association: WDED and performance**	
Jurov et al. 2020	WDED <-300kcal associated with reduced anaerobic capacity (but not for WDEB <0kcal).	
Benardot 2005	An additional of 250kcal between meals (total of 750kcal per day) was associated with increased anaerobic power and energy output.	
	G. WDEB/WDED and Meal Frequency	
Reference	**Association: WDED and meal frequency**	**Additional info/comments**
Fahrenholtz et al. 2018	WDED was positively association with increased meal frequency.	The greater the energy deficit, the more frequent meal frequency.
	No association: WDEB/WDED and meal frequency	
Lundstrom et al. 2022	No significant difference in WDEB between subjects consuming >50 % of EI after 17:00 and those who had a more evenly meal distribution throughout the day.	
Svensen et al. 2022 (NNR 2022)		No consistent findings regarding the association between meal frequency and body composition.

WDEB< >: hours spent in the respective "EB-zone". WDED-variables: EB <-300kcal, EB <0kcal. Abbreviations: BF %: Body fat percentage. BMI: Body mass index. EB: Energy balance. EI: Energy intake. FM: Fat mass. FMI: Fat mass index.GH: Growth hormone. h: hour.IGF-1: Insulin like growth factor-1. Kcal: Kilocalorie. LBM: Lean body mass. LSHD: Largest single hour deficit. LSHS: Largest single hour surplus. MD: Menstrual dysfunction. RMR: Resting metabolic rate. T3: Triiodothyronine. TT3: Total triiodothyronine. WDEB: Within-day energy balance. WDED: Within-day energy deficiency.

Appendix 11: REK-approval sheet. Latest version of the REK approval sheet after an updated revision of the study protocol as a result of the COVID 19 pandemic



Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst C	Anders Strand		26.04.2021	31640
			Deres referanse:	

Monica Klungland Torstveit

31640 The FUEL program

Forskningsansvarlig: Universitetet i Agder

Søker: Monica Klungland Torstveit

REKs vurdering

REK viser til endringsmelding mottatt 20.04.2021, for prosjekt 31640 «The FUEL program». Komiteleder for REK sør-øst C har vurdert meldingen på fullmakt fra REK sør-øst C, med hjemmel i helseforskningsloven §11.

De omsøkte endringene er begrunnet ved behov for dels omfattende justeringer og tilpasninger som følge av den pågående Covid-19 situasjonen. Endringene er godt oppsummert i endringsmeldingen, og inkluderer:

- Bård Erlend Solstad (UiA), Finn Skårderud (UiA) og Siri-Marte Hollekim Strand (NTNU) inkluderes som prosjektmedarbeidere. Komiteen har ingen forskningsetiske innvendinger til dette.
- Endringer av selve FUEL programmet, slik at dette kan gjennomføres under de gjeldende smittevernsrestriksjoner. Dette innebærer blant annet at flere laboratorieundersøkelser utgår. Oversikt over endringene fremgår av endringsmeldingen med vedlegg.

I lys av den pågående Covid-19 situasjonen vurderer komiteen endringene som hensiktsmessige og forsvarlige, og godkjenner derfor disse.

Videre viser komiteen til sitt svar på fremleggingsvurdering 267365, av 26.04.2021, hvor en kvalitativ delstudie, som ble omsøkt i en tidligere versjon av endringsmeldingen, vurderes til å falle utenfor helseforskningslovens virkeområde.

Vedtak

Godkjent

Komitéen har vurdert endringsmeldingen og godkjenner prosjektet slik det nå foreligger med hjemmel i helseforskningslovens § 11.

REK sør-øst C **Telefon**:22 84 55 11 | **E-post**:rek-sorost@medisin.uio.no
Besøksadresse: Gullhaugveien 1-3, 0484 Oslo **Web**:https://rekportalen.no