UNIVERSITY OF AGDER

Prevalence of Relative Energy Deficiency in Sport among well-trained male Norwegian cyclists and long-distance runners

Thomas Birkedal Stenqvist

Supervisors

Monica Klungland Torstveit, Associate Professor
Øystein Sylta, PhD student

This master's thesis is carried out as a part of the education at the University of Agder and is therefore approved as a part of this education. However, this does not imply that the University answers for the methods that are used or the conclusions that are drawn.

University of Agder, 2016
Faculty of Health and Sport Science
Institute of Public Health, Sport & Nutrition

Abstract

Introduction

Relative Energy Deficiency in Sport (RED-S) links low and reduced energy availability (EA) with negative health and performance consequences, though not well investigated in male endurance athletes. The aim of this study was to investigate the prevalence of RED-S and associated health consequences in well-trained male endurance athletes.

Methods

Forty-one subjects, cyclists (n=21) and runners (n=20) [age: 40 (31-45) years; BMI: 23.5 (21.4-24.0) kg/m^2; body-fat: 14.0% (10.0-16.5%); training volume: 12 (9-16) h/week presented as median + interquartile range] were recruited. Protocol included assessment of bone health, body composition, resting metabolic rate (RMR), blood pressure, energy intake, energy expenditure, hormonal biomarkers, blood glucose and lipids. 27 subjects were included in the final analysis.

Results

Eighteen subjects had reduced EA (<40kcal/kgFFM/day) and showed a trend of lower RMR ratio compared to the optimal EA group (0.83 vs. 0.86, *P*=0.026). Six subjects had low bone mineral density (BMD), but not related to EA status. The reduced EA group showed a trend of higher BMD in femur (*P*=0.037), hip (*P*=0.057), lumbar spine (*P*=0.01) and total body (*P*=0.035). No associations between groups were observed in hormonal biomarkers, blood glucose or blood lipids.

Conclusion

We found high prevalence of reduced EA accompanied by metabolic alterations in this group of well-trained athletes. However, no differences were observed between EA groups in either anthropometric, hormonal biomarkers, blood glucose, blood lipids or BMD. This may indicate that well-trained male endurance athletes are better protected against associations to negative health consequences in combination with reduced EA, compared to female endurance athletes.

Keywords

Athlete health, bone health, energy availability, hormonal biomarkers, male endurance athletes, resting metabolic rate

Due to word limitations in the master thesis, the following will only be present in the article (part 2); results, discussion regarding results and conclusion.

Sammendrag

Introduksjon

Relativ energimangel innen idrett (RED-S) knytter lav- og redusert energitilgjengelighet (EA) med negative helse- og prestasjonskonsekvenser, men dette har ikke vært undersøkt i tilstrekkelig grad blant mannlige utholdenhetsutøvere. Formålet med denne studien var å undersøke forekomsten av RED-S og de assosierte helsekonsekvenser blant godt trente mannlige utholdenhetsutøvere.

Metode

41 subjekter, syklister (n=21) og løpere (n=20) [alder: 40 (31-45) år; kroppsmasse indeks (KMI): 23.5 (21.4-24.0) kg/m^2; kroppsfett: 14.0% (10.0-16.5%); trener: 12 (9-16) timer/uke presentert som median med interkvartil bredde] ble rekruttert. Protokoll inkluderte måling av beinhelse, kroppssammensetning, hvilemetabolisme (RMR), blodtrykk, energiinntak, energiforbruk, hormonelle biomarkører, blodglukose og blodlipider. Total ble 27 subjekter inkludert i den endelige analysen.

Resultater

18 subjekter hadde redusert EA (<40kcal/kgFFM/dag). Gruppen med redusert EA tenderte til lavere RMR ratio sammenlignet med gruppe med optimal EA (0.83 vs. 0.86, *P*=0.026). Seks subjekter hadde lav benmineraltetthet (BMD), men dette var ikke relatert til EA. Videre, gruppen med redusert EA viste en trend til høyere BMD i lårhals (*P*=0.037), hofte (*P*=0.057), lumbalcolumna (*P*=0.01) og helkropp (*P*=0.035). Det ble ikke observert noen forskjeller mellom antropometriske data eller hormonelle biomarkører.

Konklusjon

En høy forekomst av redusert EA ble observert, inkludert en trend til en metabolsk forskjell. Det ble ikke observert noen forskjeller mellom gruppene på hverken antropometriske data eller hormonelle biomarkører i relasjon til EA. Videre, redusert EA var ikke relatert til lav BMD. Slike resultater kan indikere at mannlige utøvere kanskje er bedre beskyttet mot negative helsekonsekvenser som kommer fra redusert EA, sammenlignet med kvinnelige utholdenhetsutøvere.

Nøkkelord

Beinhelse, energitilgjengelighet, hormonelle biomarkører, hvilemetabolisme, mannlige utholdenhetsutøvere, utøverhelse

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Abbreviations

BMC	Bone mineral content
BMD	Bone mineral density (g/cm^2)
BMI	Body mass index [weight (kg) divided by height squared (m^2)]
BP	Blood pressure
DEE	Daily energy expenditure
DXA	Dual-energy x-ray absorptiometry
EA	Energy availability
EEE	Exercise energy expenditure
EI	Energy intake
FFM	Fat-free mass
GH	Growth hormone
HDL	High density lipoprotein
IGF-1	Insulin-like growth factor-1
IOC	International Olympic Committee
Kcal	Kilocalorie
LDL	Low density lipoprotein
MET	Metabolic equivalents
NEAT	Non-exercise activity thermogenesis
RED-S	Relative Energy Deficiency in Sport
RMR	Resting metabolic rate
RMR$_m$	Resting metabolic rate measured
RMR$_p$	Resting metabolic rate predicted
RMR ratio	The ratio between measured and predicted RMR
T$_3$	Triiodothyronine
TC	Total cholesterol
Triad	Female athlete triad
$\dot{V}O_{2max}$	Maximal oxygen uptake (L·min^{-1} or mL·kg^{-1}·min^{-1})
$\dot{V}O_{2peak}$	Peak oxygen uptake (L·min^{-1} or mL·kg^{-1}·min^{-1})

Part 1

Theoretical background and methods

Thomas Birkedal Stenqvist
University of Agder

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1. Introduction

In all types of sports, body weight and body composition are important to both athletic performance and athlete health (Ackland et al., 2012). Athletes' ways of maintaining body composition vary from both healthy methods to pathological and extreme methods in order to reduce body mass quickly and/or to gain competitive advantages (Donnelly et al., 2009; Sundgot-Borgen et al., 2013). The consequences of extreme dieting or very low body weight in female athletes in particular are associated with unhealthy hormonal alterations, degeneration of bones and alterations of reproductive function, and in a worst case scenario, can be fatal (Ackland et al., 2012; Mountjoy et al., 2014; Nattiv et al., 2007). Such states of extreme dieting and low body weight are now present in more and more sport disciplines, where especially athletes in endurance sport, aesthetic sport and weight-class sports seem to be more at risk compared to athletes from other sports (Sundgot-Borgen et al., 2013). Alterations in energy intake (EI) to change body composition do not always alter body weight however, and it has been observed, that athletes with low dietary intake in some cases maintain their body weight, but alter metabolic functions such as their resting metabolic rate (RMR) (Redman et al., 2009).

Research on possible negative associations between reproductive function and body composition originally began in the mid 1980s when Barbara Drinkwater found a relationship between menstrual dysfunction and low bone mineral density (BMD) in female athletes (Drinkwater et al., 1984; Drinkwater, Nilson, Ott, & Chesnut, 1986). Research developed, but for a long period focused on females where several position stands on this topic were released in the 1990s up until 2014 (Drinkwater, Loucks, Sherman, Sundgot-Borgen, & Thompson, 2005; Nattiv et al., 2007; Otis, Drinkwater, Johnson, Loucks, & Wilmore, 1997). During this period it was observed that females, especially in endurance sports and sport emphasizing leanness, had a prevalence of one or more of the discovered components of what is defined as the female athlete triad (Triad) (Nattiv et al., 2007). The components of the Triad are energy availability (EA), menstrual function and BMD (see theoretical background for more information), and move on a continuum from health to disease and the prevalence has been reported to be relatively high amongst elite female athletes (Torstveit & Sundgot-Borgen, 2005). Studies of females have shown that reducing EA by <30 kilocalories (kcal)/kg fat-free mass(FFM)/day results in different hormonal changes, such as a reduction in blood glucose, triiodothyronine (T_3), luteinizing hormone, insulin and insulin-like growth factor-1 (IGF-1) (Loucks & Thuma, 2003; Loucks, Verdun, & Heath, 1998).

Scientific research has subsequently classified EA that is less than 30kcal/kgFFM/day as low EA, 30-44 kcal/kgFFM/day as reduced EA and ≥45 kcal/kgFFM/day as optimal EA, but these classifications are limited to female athletes (Mountjoy et al., 2014).

Only defined for females until recently, the interpretation of the Triad underwent a major revision in an International Olympic Committee (IOC) position stand from 2014, where the IOC stated that the Triad was no longer limited to female athletes but also included male athletes (Mountjoy et al., 2014). The IOC incorporated the Triad into a more comprehensive description named "Relative Energy Deficiency in Sport" (RED-S), where EA is still the main essence of the RED-S model (Mountjoy et al., 2014). There further exists an uncertainty on how to classify cut-off points for the different EA categories (low, reduced and optimal) in male athletes, where different studies have used different cut-off for the categories (Koehler et al., 2016; Viner, Harris, Berning, & Meyer, 2015). Based on assumptions that the energy costs of the male reproductive function costs less compared to females (Bronson, 1985), the cut-off point for reduced and optimal EA in males have been proposed to be set at 40kcal/kgFFM/day, but the cut-off point for low EA remains uncertain due to lack of scientific research targeting males (Koehler et al., 2016). Research into RED-S in male athletes, however, is not as extensive as research involving females and the Triad, and studies investigating the prevalence of EA among male athletes and how this impacts both performance and health variables are generally lacking (Mountjoy et al., 2014). A study of male soldiers subjected to severe energy deficiency found lower levels of testosterone, T_3 and IGF-1 compared to controls with much lesser energy deficit (Friedl et al., 2000). Another study by Koehler et al. (2016) found that male athletes, who exercised more than three hours/week, subjected to an EA of <15 kcal/kgFFM/day reduced their leptin and insulin levels, but not IGF-1, testosterone or T_3. Despite these findings, the research involving male athletes in general is very limited.

The main aim of this master's thesis is to investigate the prevalence of reduced energy availability and associations between reduced energy availability (<40kcal/kgFFM/day) and selected health variables among well-trained male Norwegian cyclists and long-distance runners.

1.1. Research question and null-hypothesis

Research question

What is the prevalence of reduced energy availability and what are the associations between reduced energy availability and selected health variables among well-trained male Norwegian cyclists and long-distance runners?

Null-hypothesis

This study's null-hypotheses are the following:

- Well-trained male cyclists and long-distance runners do not show signs of reduced energy availability.
- Reduced energy availability in well-trained male cyclists and long-distance runners is not associated with low BMD.
- There are no differences in metabolic function between well-trained male cyclists and long-distance runners with reduced energy availability and optimal energy availability.
- There are no differences in hormonal biomarkers between well-trained male cyclists and long-distance runners with reduced energy availability and optimal energy availability.

1.2. Delimitation of the thesis

This thesis will look only at the prevalence of reduced EA and possible associations with selected health variables, but recognizes that EA is a more complex phenomena and the elements are more interrelated than the theoretical section emphasizes. Due to limitations in this thesis, other components of the concepts of RED-S than described in the theoretical section will therefore not be in focus (such as, but not limited to, gastrointestinal, hematological and immunological factors). It is recognized, however, that all factors can and will in some way affect each other in combination with EA. This master's thesis will therefore focus only on bone health, hormonal biomarkers and metabolic factors and describe the available knowledge on this theme in combination with EA. There is no room for detailing the different effects of every hormone tested, and this can be found in textbooks such as McArdle, Katch, and Katch (2015) and papers such as Loucks (2014).

Researchers have also focused on female athletes and defined three groups of EA; Optimal EA, reduced EA and low EA (Nattiv et al., 2007). Due to limited research on male athletes, this thesis will only focus on reduced EA in males, defined as <40kcal/kgFFM/day based on Koehler et al. (2016). The term "reduced EA" in this thesis in male athletes is therefore adequate in terms of both low and reduced EA used in female athletes.

2. Theoretical background

2.1. Cycling

Cycling is a type of exercise where mechanical energy is generated in order to overcome external resistance (riding position, body mass, rolling resistance, air resistance and gradient) (Jeukendrup, Craig, & Hawley, 2000). One method of going faster in cycling refers to decreasing the various sources of resistance, where one key element is to reduce the mass (i.e. the weight of the cycle or the rider), which is crucial for changing the power demands (Jeukendrup et al., 2000). Furthermore, there are three ways in which bodyweight slows a rider down: hindering acceleration, adding mass to be carried uphill and adding rolling resistance (Jeukendrup et al., 2000). Professional riders who participate in Tour de France are estimated to have a daily energy expenditure (DEE) of between 5,700–9,500 kcal per day during the race (Saris, van Erp-Baart, Brouns, Westerterp, & ten Hoor, 1989). Although well-trained cyclists never reach the same energy demands as professionals, they still have high energy demands during training (Jeukendrup et al., 2000). Cycling is defined as a non-weight-bearing activity due to the absence of ground reaction force during cycling and therefore has little osteogenic effect on the skeletal system (Nichols, Palmer, & Levy, 2003; Warner, Shaw, & Dalsky, 2002).

2.2. Running

In running, the aim is to move the centre of mass of the body forward, using muscles to exert force on the ground and thereby creating a forward movement (Kaneko, 1990). Some of the factors which influence running performance are maximum oxygen uptake ($\dot{V}O_{2max}$), running economy, stride length and stride rate (Foster & Lucia, 2007; Hall, 2007). It usually takes three to six hours for recreational athletes to complete a marathon and requires a great expenditure of energy (Loftin et al., 2007). Energy expenditure for completing the Boston marathon was estimated by Costill and Fox (1969) to be 2410 kcal for elite runners. Loftin et al. (2007) estimated the energy expenditure of recreational runners to be approximately 2792kcal in a marathon, but this depended on factors such as body size. Running is defined as a weight-bearing activity and research has shown that runners, compared to sedate control groups, have equal or slightly better bone health (Scofield & Hecht, 2012). Compared to other types of high-impact sports, running has consistently shown lower BMD, especially within endurance running where EA can be critical for bone health (Scofield & Hecht, 2012).

2.3. Historical perspectives on the Triad and Relative Energy Deficiency in Sport

Research into the Triad syndrome had a breakthrough in the early 1980s, when it was observed in two studies that female athletes with menstrual dysfunction had low BMD (Cann, Martin, Genant, & Jaffe, 1984; Drinkwater et al., 1984). Furthermore Drinkwater et al. (1986) found that females with menstrual dysfunction did not significantly improve their BMD even after they had returned to a normal menstruating cycle pattern (eumenorrhea) and normal body weight. These new findings led to the assumption that females with a menstrual dysfunction would be at a greater risk of losing bone mass and subsequently developing osteoporosis. Research furthermore linked female athletes with an eating disorder and a menstrual dysfunction to low BMD and in 1992, the American College of Sports Medicine (ACSM) defined this phenomenon as "the female athlete Triad" (Triad) (Yeager, Agostini, Nattiv, & Drinkwater, 1993). The Triad was later revised by the ACSM in 1997 (Otis et al., 1997), and in 2005 the IOC released its position stand relating to the Triad. They outlined that girls and women should participate in sport, but that health professionals played an important part in the well-being of these athletes and in understanding the influence of nutritional factors on both the reproductive function and skeletal health (Drinkwater et al., 2005). The ACSM redefined its position stand on the Triad again in 2007 (see Figure 1) as follows: "*The female athlete triad (Triad) refers to the interrelationships among energy availability, menstrual function, and bone mineral density, which may have clinical manifestations including eating disorders, functional hypothalamic amenorrhea, and osteoporosis*" (Nattiv et al., 2007, p. 1867).
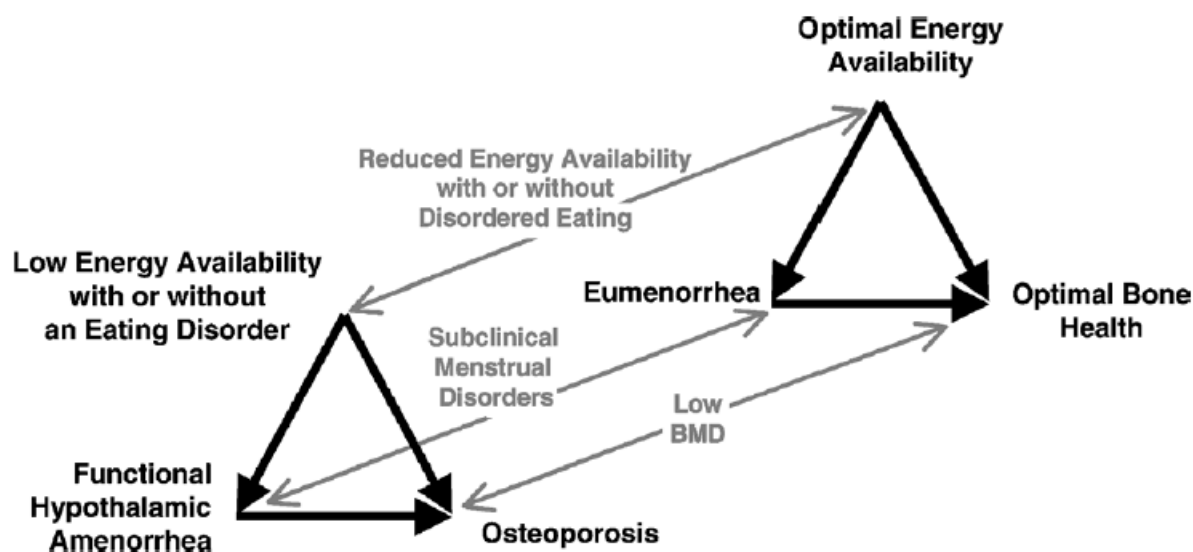
Figure 1. The female athlete Triad includes a continuum from optimal energy availability, optimal bone health and normal menstrual function to low energy availability, osteoporosis and menstrual dysfunction, which impairs the health of athletes (Nattiv et al., 2007).

Almost all research on the Triad up until 2014 was done on female athletes, and the Triad seemed to be limited to women only. This changed, however, when the interpretation of the Triad underwent a major revision in an IOC position stand from 2014 (Mountjoy et al., 2014). In this statement the IOC declared that the Triad is no longer confined to female athletes but also includes male athletes (Mountjoy et al., 2014). The IOC reformed and renamed the syndrome to Relative Energy Deficiency in Sport (RED-S). This new definition states that; "*The syndrome of RED-S refers to impaired physiological function including, but not limited to, metabolic rate, menstrual function, bone health, immunity, protein synthesis, cardiovascular health caused by relative energy deficiency*" (Mountjoy et al., 2014, p. 1). Insufficient energy or EA is the essential component of RED-S which supports a range of functions in the organism involved in optimal health and performance (Mountjoy et al., 2014). RED-S includes not only menstrual function and bone health as variables, but also immunological factors, endocrine factors (hormonal biomarkers), metabolic factors, haematological factors, growth/development, physiological factors, cardiovascular factors and gastrointestinal factors (see Figure 2) (Mountjoy et al., 2014). Another new element of the RED-S syndrome is that it now also includes and recognizes different negative aspects on performance variables (see Figure 3) such as decreased glycogen stores, decreased muscle strength, decreased endurance performance (anaerobic and aerobic), increased injury risk, decreased training response, impaired judgment, decreased coordination and decreased concentration as well as irritability and depression (Mountjoy et al., 2014).
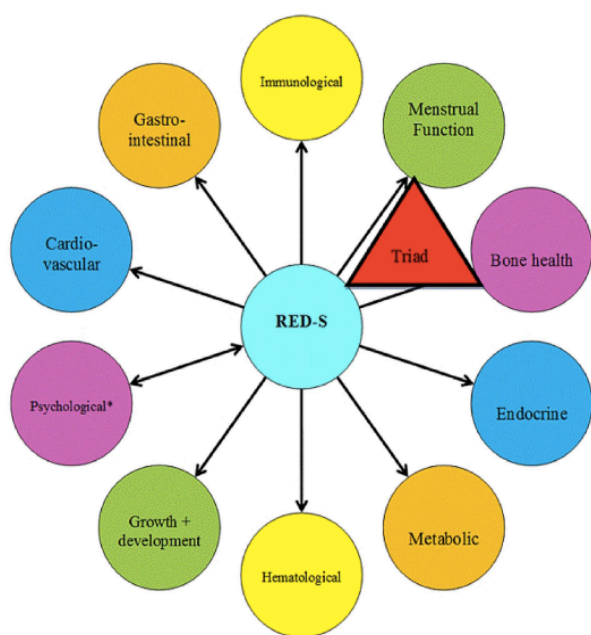
Figure 2. Health consequences from Relative Energy Deficiency in Sports (RED-S), from Mountjoy et al., (2014). The red area is what is known as the Triad for female athletes.
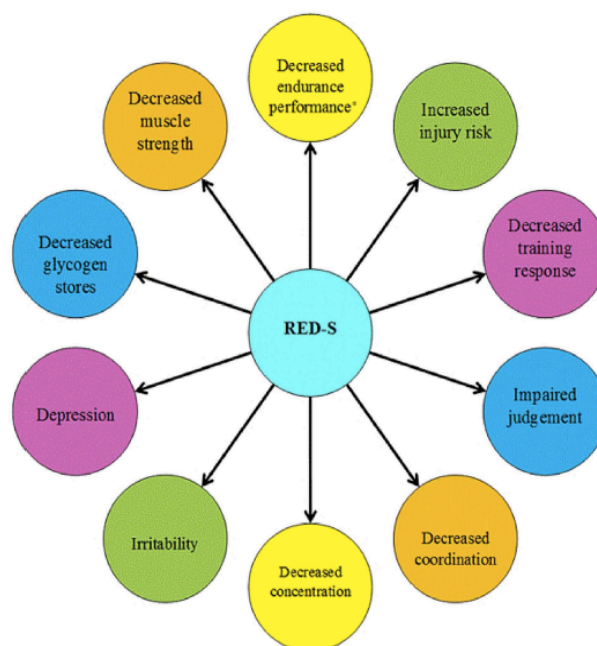
Figure 3. Performance consequences from Relative Energy Deficiency in Sports (RED-S), from Mountjoy et al., (2014).

2.4. Energy availability

The main problem underlying RED-S is a lack of energy to support a range of different body functions in order to achieve optimal health and performance for all athletes (Mountjoy et al., 2014). As a way of quantifying EI in relation to energy spent in training and daily life, scientists use EA in relation to fat-free mass as a way of assessing athletes and their potential risk of jeopardizing their health (Mountjoy et al., 2014; Nattiv et al., 2007).

2.4.1. Definition

EA is often referred to as a continuum from optimal EA to low EA with or without an eating disorder (Nattiv et al., 2007). Nattiv et al. (2007, p. 1868) define EA as "*dietary energy intake minus exercise energy expenditure, energy availability is the amount of dietary energy remaining for other body functions after exercise training.*" Mathematically, EA can be calculated as follows: EA=(EI - EEE)/FFM, where EI is energy intake (kcal/day or kJ/day), EEE is exercise energy expenditure and FFM is fat-free mass (Loucks, 2014). Melin (2015) has defined EEE more precisely in her equation and subtracts both resting metabolic rate and non-exercise activity thermogenesis (NEAT) from the duration of exercise. The formula used by Melin (2015) is as follows: EA=(EI - (EEE - (DEE - EEE))/(FFM).

Different studies and position stands have defined EA, but almost all use female athletes as their point of reference (Loucks, 2014; Melin et al., 2015; Mountjoy et al., 2014; Muia, Wright, Onywera, & Kuria, 2015; Sundgot-Borgen et al., 2013). Traditionally, EA has been grouped into different categories; low EA (<30 kcal/kgFFM/day); reduced EA (30-44 kcal/kgFFM/day); and optimal EA (≥45 kcal/kgFFM/day) (Gibbs, Williams, & De Souza, 2013; Loucks, 2004). Only a few studies have investigated EA in male athletes and defined the categories of EA, and no agreement exists about these categories. Two studies have used the same definitions for both male and female athletes (Loucks, Kiens, & Wright, 2011; Viner et al., 2015), and one recent study used a slightly lower cut-off (<40kcal/kgFFM/day) between optimal and reduced EA in male athletes, but proposed no new cut-off point for low EA in male athletes (Koehler et al., 2016).

2.4.2. Prevalence

Studies of low EA have focused on female athletes, but low EA has also been reported in male athletes (Sundgot-Borgen et al., 2013). Although studies of low EA in male athletes are few, it seems that low EA is found in some of the same risk sports as for female athletes (Mountjoy et al., 2014). These sports include weight-sensitive sports, where leanness and/or weight are important factors for performance (long-distance running, road cycling, etc.) and weight-class sports disciplines such as boxing and wrestling (Sundgot-Borgen et al., 2013). Low EA with as little as 8 kcal/kgFFM/day has

been reported in elite male cyclists in a cross-sectional study by Vogt et al. (2005). Furthermore, a high prevalence of underweight was reported in a longitudinal study including world-class male ski jumpers (Muller, Groschl, Muller, & Sudi, 2006).

Low EA causes the amount of energy used for thermoregulation, growth, cellular maintenance and reproduction to be reduced by physiological mechanisms (Wade, Schneider, & Li, 1996). However, this compensation seems to reinstate energy balance, which helps survival, but can damage the health of the athlete (Nattiv et al., 2007). Factors especially influencing EA seem to be various eating disorders and/or excessive exercise (Mountjoy et al., 2014). Both eating disorders and disordered eating are serious mental disturbances with huge effects on the body and, in a worst case scenario, are lethal (Smink, van Hoeken, & Hoek, 2012). These types of mental disturbances are highly prevalent among athletes, both adolescents and adults participating in weight sensitive sports such as aesthetic sports, cycling, running, cross-country skiing, and horse-racing (Baum, 2006; Torstveit, Rosenvinge, & Sundgot-Borgen, 2008). In one of the largest epidemiological studies to date, Sundgot-Borgen and Torstveit (2004) assessed the prevalence of eating disorders in 687 Norwegian male and female athletes compared to 629 non-active control persons. The study showed an 8% prevalence of eating disorders among the male athletes and 0,5% prevalence among the male controls. The researchers also found a higher prevalence (12.9%) of eating disorders among males participating in sports where leanness is important (aesthetics, weight-class, anti-gravitation and endurance sports) compared to sports where leanness is not as important (4.6%) (power, technical, ball-game and motorsports) (Sundgot-Borgen & Torstveit, 2004). The prevalence of eating disorders and/or disordered eating in male elite athletes has been found to be as high as 50% in cycling (Ferrand & Brunet, 2004) but also in contact and combat sports such as wrestling and boxing (Schaal et al., 2011; Sundgot-Borgen & Torstveit, 2004).

2.4.3. Risk-factors and possible associations between reduced energy availability and selected health variables

2.4.3.1. *Bone health*

Movement, exercise and training are all important factors for the development of bone health and for increasing BMD, and are especially important during development and growth (Khan, 2001). To optimize bone strength and thereby decrease the risk of fracture, both males and females must accumulate and maintain peak bone mass throughout the lifespan (Tenforde, Barrack, Nattiv, & Fredericson, 2015). Bone strength is dependent on bone mass, bone size, shape, microarchitecture or a combination of all elements (Khan, 2001). The density, including internal structures of the bone mineral and the quality of bone protein are important factors for bone strength and the risk of

fracture, and may explain why some people suffer from fractures while others do not, even though their BMD is equal (Nattiv et al., 2007).

BMD is often referred to on a continuum from optimal bone health to osteoporosis, whereas osteoporosis is defined as ''*a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture*'' (Kanis, 2002, p. 1929) (see also Figure 1).

Low BMD is defined by the International Society of Clinical Densitometry in children and adolescents as a Z-score of -2 or less, and osteoporosis is defined as a Z-score of -2 or less including a fracture, and therefore cannot be classified by Z-score alone (Lewiecki et al., 2008). Athletes participating in high-impact sports are often observed having a 5–15% higher BMD than non-athletes (Tenforde & Fredericson, 2011). Athletes are therefore expected to have a higher BMD compared to the normal population (Tenforde, Barrack, et al., 2015). This is not always found in endurance athletes, where a "below average" or even "low BMD" is often observed (Tenforde, Barrack, et al., 2015). Low BMD for athletes is defined by ACSM as a Z-score between -1 and -2 and can include a stress fracture, nutritional deficiencies or other secondary clinical risk factors for fracture. Osteoporosis is defined by a Z-score of -2 or lower in athletes (Nattiv et al., 2007).

Several other elements play a role in the acquisition and maintenance of peak bone mass, where genetics is the strongest (Nattiv et al., 2007). The timing of impact also seems to play a role in how strong a gain in BMD a person will acquire, where the second decade of life seems especially important and here BMD may almost double (Tenforde, Barrack, et al., 2015). Research has indicated that most males achieve their peak accumulation of BMD between 13 to 15 years of age, and that they acquire their maximum bone mass when they reach the age of 20 years (Heaney et al., 2000). The loss of BMD in adulthood is not always an accelerator of osteoporosis, but may be linked to not acquiring optimal BMD as a child or adolescent (Borer, 2005; Nattiv et al., 2007). A recent 39-year prospective cohort study by Tveit, Rosengren, Nilsson, Ahlborg, and Karlsson (2012) showed no serious decline in BMD from an already high baseline (active athletes) to follow-up (retired athletes) in male runners, soccer players, swimmers and weight-lifters, indicating that peak bone mass is an important factor for BMD later in life. Research suggests that people who continue to participate in sports will maintain the full benefits of a peak bone mass and even maintain some benefits if they stop participating (Tenforde & Fredericson, 2011). Results of under-stimulation of the bones is clearly seen during prolonged bed rest and in space-flight where weightlessness eliminates the mechanical stress on the bones resulting in a decline in bone strength (Bikle & Halloran, 1999).

Other significant factors for optimal BMD are diet (especially EA, calcium and vitamin D) and exercise (type, intensity, frequency and duration), where high-impact and multidirectional exercise, such as soccer, volleyball and martial arts seems to promote the strongest gains in BMD (Fredericson et al., 2007; Kohrt et al., 2004). For this reason, bone strength and bone density seem to be determined by the kind of strain exerted by the bones. Wolff´s law explains how various physiological stress factors results in the adaptation of the bone structure, thereby ensuring that material proportion, geometry and bone mass are equal to and appropriate for the applied load of the bones (Khan, 2001; Wolf, 1995). Endurance activities such as cycling, long distance running and swimming do not seem to promote a gain in BMD due to the low impact loads and repetitive movements (Guillaume, Chappard, & Audran, 2012; Tenforde, Barrack, et al., 2015; Tenforde, Fredericson, Sayres, Cutti, & Sainani, 2015). A systematic review by Olmedillas, Gonzalez-Aguero, Moreno, Casajus, and Vicente-Rodriguez (2012) found that cycling did not have osteogenic effects on bone, most likely because these athletes spend many hours in a weight-supported position on the bike and also due to the long recovery time of sitting or lying, especially in competitive road cycling. Research has found that the prevalence of osteopenia and osteoporosis is quite high (up to 70%) among male road cyclists (Nichols et al., 2003). Cross-sectional studies of male athletes have reported low BMD or osteoporosis in sports that emphasize leanness, such as horce-racing (jockeys), running and cycling (Dolan, Crabtree, et al., 2012; Rector, Rogers, Ruebel, & Hinton, 2008).

Degeneration of the bones also seems to be related to weight loss and in general low body weight amongst athletes. Low body weight or weight reduction reduces BMD which seems to be caused by reduction in mechanical loading on the bones and changes in hormones (Ihle & Loucks, 2004). Research also indicates that low EA at all deficiency levels is an independent factor of poor bone health, due to a decrease in bone formation markers and IGF-1 levels (Mountjoy et al., 2014). Furthermore, an increase in hormones such as stress-hormones and cortisol in relation to low EA seems to have a negative effect on BMD (Fuqua & Rogol, 2013), whereas Hind, Truscott, and Evans (2006) have proposed that low levels of testosterone might be related to low BMD in male athletes. It has also been shown that, in the absence of disordered eating there is still a high risk of low BMD in endurance athletes in non-weight-bearing activities (Guillaume et al., 2012; Rector et al., 2008; Smathers, Bemben, & Bemben, 2009). Finally, bone loss appears to be irreversible (Keen & Drinkwater, 1997).

2.4.3.2. *Hormonal biomarkers*

Research on female athletes has shown that there is a risk of developing functional hypothalamic amenorrhea in a state of low EA (Nattiv et al., 2007). Menstrual dysfunction is relatively simple to detect (Tenforde, Barrack, et al., 2015), but the long term-effect of low EA on the reproductive functions in females is still unknown (Mountjoy et al., 2014).

Studies involving females have also focused on other hormonal biomarkers. An experimental study found that sedentary women with an EA of <30kcal/kgFFM/day for more than five days reduced insulin, leptin, IGF-1 and glucose availability, and increased growth hormone (GH) (Loucks et al., 1998). Leptin, which is mostly produced in fat cells, plays an important role in regulating and suppressing the appetite in healthy adults (Fuqua & Rogol, 2013). Low EA seem to reduce the levels of leptin, which then enhances the release of neuropeptide Y and agouti-related peptide which ultimately leads to an increase in appetite and a risk of binge eating (Loucks & Thuma, 2003; Torstveit et al., 2008). Furthermore, in a review article, Warren (2010) reported lowered levels of leptin in patients with eating disorders, and the author highlights that in females, low blood glucose, low T_3, elevated cortisol and total cholesterol are often observed in patients with eating disorders such as anorexia nervosa. One randomized, repeated-measure experiment in sedentary women found that five days of EA of < 30kcal/kgFFM/day reduced blood glucose and biomarkers of bone formation, elevated cortisol and suppressed hypothalamic-pituitary-axis hormones such as T_3 and luteinizing hormone (Loucks & Thuma, 2003).

Loucks (2014) has further proposed that biomarkers for the investigation of low availability of energy and glucose in athletes could be the ratio between GH and insulin. GH along with insulin is a regulator of the cycling of fatty acids in adipose tissue, whereas GH stimulates lipolysis, insulin inhibits lipolysis (Loucks, 2014). Therefore, the ratio between GH and insulin may lead to elevation in ketones in the urine, which is a key sign of accelerated lipolysis. Glucose deficiency also lowers levels of T_3, which is a key stimulant in mitochondrial biogenesis and ATP production, and could reduce skeletal muscles' ability to produce mechanical work and power, thereby influencing muscular endurance, strength and power (Loucks, 2014). It has also been found that lowered levels of oestrogen in females as a result of low EA can negatively adjust the lipid profile and vascular function (Rickenlund, Eriksson, Schenck-Gustafsson, & Hirschberg, 2005). Furthermore it has been found that athletes with menstrual dysfunction can have higher levels of low-density lipoprotein (LDL) compared to normal menstruating athletes (Rickenlund et al., 2005).

Similar conditions in male athletes, such as the evaluation of the reproductive function, require advanced techniques and may therefore obscure the connection between low EA and reduced

reproductive function. In trying to clarify and find such conditions, the clinical symptoms are few and such evaluation may require fertility and sperm analysis (De Souza & Miller, 1997; Tenforde, Barrack, et al., 2015). Studies including sports such as cycling, running or other sports that contribute to leanness or weight-sensitiveness have found lower levels of reproductive hormones in male athletes including but not limited to testosterone (Tenforde, Barrack, et al., 2015). In relation to testosterone only, a study by Griffith, Dressendorfer, Fullbright, and Wade (1990) found that testosterone levels were reduced by 12% in well-trained endurance athletes training more than 1 - 2 hours a day, 6-7 days a week. A prospective study by Wheeler, Singh, Pierce, Epling, and Cumming (1991) reported a decrease in testosterone levels in 15 previously sedentary males, who started running up to 56 km a week over a period of six months. A cross-sectional study by Hackney, Fahrner, and Gulledge (1998) showed significantly lower serum testosterone levels in well-trained male endurance athletes, compared to a sedate control group. However, two other studies of runners did not find lower levels of testosterone compared with a sedentary control group (Bagatell & Bremner, 1990; McColl, Wheeler, Gomes, Bhambhani, & Cumming, 1989). Another study from Safarinejad, Azma, and Kolahi (2009) found that testosterone decreased, but sex hormone-binding globulin, which is a major transport protein for testosterone in males, increased as a result of 12 weeks of moderate and high-intensity training.

In relation to alterations in hormone production, combined with reduced EA, two prospective studies of male wrestlers found that GH increased during the season, while testosterone and IGF-1 decreased in conjunction with reduced EA, which lead to reduced body weight, muscle strength and fat mass (Roemmich & Sinning, 1997a, 1997b). Field studies of male soldiers have identified a reduction in T_3, testosterone and IGF-1 when the soldiers were exposed to various levels of energy deficiency during long military exercises (Friedl et al., 2000; Kyrolainen et al., 2008). In a study by Friedl et al. (2000), soldiers participating in an 8-week US Army Ranger course were exposed to four repeated cycles of energy restriction of either 1200 or 1000 kcal/day. During these energy restrictions, a decline to "below normal reference" in T_3 was observed, testosterone plummeted to near-castration-levels, levels of IGF-1 were halved and cholesterol increased. When refeeding the soldiers between the cycles, an immediate normalization was observed in T_3, testosterone and IGF-1, leading the researchers to suggest that T_3, testosterone and IGF-1 are reliable markers of energy deficiency in males (Friedl et al., 2000). In the study by Kyrolainen et al. (2008) male soldiers participating in a three-week (20-days) field exercise, with a weekly energy deficit of 4000, 450 and 1000kcal/day, reported an increase in cortisol and GH combined with a decrease in insulin and testosterone levels in week one. During the second week, cortisol and GH returned to base levels. It was further observed that testosterone and insulin also recovered to base levels after week three, suggesting that

energy deficiency of <1000 kcal/day allows for recovery of hormonal changes in males over time (Kyrolainen et al., 2008). Another recent study on male jockeys by Dolan, McGoldrick, et al. (2012) reported that elevated IGF-1 and sex hormone-binding globulin concentrations was related to low BMD in relation to the low body weight of the jockeys.

2.4.3.3.   Metabolic factors

RMR, which usually represents 55 - 65% of the DEE in normal sedentary people (Speakman & Selman, 2003), can be described as a combination of different metabolic processes that includes the energy cost of basic physiologic functions (growth, reproduction, thermoregulation, immunity and cellular maintenance) (Melin, 2015). FFM is the largest determinant of RMR, but energy balance also seems to play a key role (Speakman & Selman, 2003). A review by Wade and Jones (2004) found that when humans do not ingest enough energy to maintain basic physiological processes, the body prioritizes the processes that are crucial for survival, such as cell maintenance, circulation and neural activity. A RCT-study by Redman et al. (2009) and an experimental study by Goldsmith et al. (2010) found that humans who were subjected to long-term low EA were in some cases preserving their body tissue as a result of metabolic adaptations such as increased work efficiency or reduced RMR.

Thyroid hormones such as thyroxine (T_4) and T_3 are often referred to as the major metabolic hormones, involved in the adjustment of RMR (McArdle et al., 2015). Thyroid hormones are also involved in the regulation of growth and development, the skeletal and nervous system and reproduction (McArdle et al., 2015). Research on trained endurance athletes has shown that they have an elevated RMR after exercise, which can be maintained for a minimum of 36 hours immediately after the end of the training session (Sjodin et al., 1996). A cross-sectional study of female athletes with a menstrual dysfunction, has shown a lower RMR compared to eumenorrheic athletes (Lebenstedt, Platte, & Pirke, 1999). In order to identify low RMR, the term "RMR ratio" is widely used in the literature in relation to female athletes. The RMR ratio is described as the ratio between measured RMR (RMR_m) and predicted RMR (RMR_p) and is defined as "normal" when the RMR-ratio is >0.90 in female athletes (De Souza et al., 2008; Melin, 2015). In some studies involving patients with an eating disorder, the RMR ratio has been reported to be between 0.60 - 0.80 (Marra et al., 2002; Platte, Lebenstedt, Ruddel, & Pirke, 2000).

Few studies have focused on metabolic alterations in relation to reduced EA in male athletes, but one recent randomized cross-over study by Papageorgiou, Elliott-Sale, Greeves, Fraser, and Sale (2015) evaluated changes in metabolic markers, including IGF-1 and T_3 hormones in 11 males. The subjects completed two experimental five-day trial periods; a restricted EA trial (<15kcal/kgFFM/day) and a

controlled EA trial (45kcal/kgFFM/day). The researchers found no differences in either metabolism or hormonal biomarkers between the periods, which may suggest that more restricted levels of EA are necessary for seeing changes in this group of males (Papageorgiou et al., 2015; Tenforde, Barrack, et al., 2015).

2.4.4. Prevention and treatment of low energy availability

In order to prevent low EA, the IOC has established recommendations to address RED-S (Mountjoy et al., 2014). Several important factors are highlighted, including but not limited to educational programmes on RED-S (including healthy eating, nutrition, EA, risks etc.) and to reduce the emphasis on weight (and emphasize nutrition) (Mountjoy et al., 2014). To develop realistic goals in relation to body composition and weight, coaches should avoid negative and critical comments regarding the weight of an athlete, use well-established scientific information and, finally, promote an awareness that good performance does not always equal good health (Mountjoy et al., 2014).

To treat low and reduced EA, athletes should either increase their EI, decrease their energy output or persue a combination of both (Mountjoy et al., 2014). Adding energy-rich supplements to athletes' daily EI combined with a small reduction in energy output or a weekly resting day is currently the only established scientific method of successfully increasing EA among athletes (Mountjoy et al., 2014). Despite the fact that these studies are few and their sample sizes are small, the researchers' interventions successfully restored energy balance and restored hormones to normal levels (Dueck, Matt, Manore, & Skinner, 1996; Kopp-Woodroffe, Manore, Dueck, Skinner, & Matt, 1999). Not all studies following the same strategy have produced similar results, perhaps due to the many underlying and physiological factors of RED-S (Guebels, Kam, Maddalozzo, & Manore, 2014; Mountjoy et al., 2014). The consensus statement from the IOC suggests a practical treatment of implementing an eating plan to increase EI by 300-600 kcal/day combined with practices in relation to the time of energy ingestion around training sessions and dietary composition (Mountjoy et al., 2014).

In general, there are limited evidence-based guidelines to assist both the athlete and the coach in relation to RED-S and sport participation (Mountjoy et al., 2014). Guidelines from the Norwegian Olympic Training Centre and expertise from the IOC Consensus group have been used to develop a new model to help athletes and coaches assess the health-risk of sport participation (Mountjoy et al., 2014). This model is named Return-to-Play, and is currently used in Norway (Mountjoy et al., 2014). The model consists of three categories (high risk: red light, moderate risk: yellow light and low risk: green light). Athletes in the red light group are not allowed to participate in competitions, since it could seriously jeopardize their health. Athletes in the yellow light group can participate in

competition, but with supervised participation and a medical treatment plan. Athletes in the green light group can participate in competition without restraint (Mountjoy et al., 2014).

3. Materials and methods

3.1. Design and recruitment

The study design in this master thesis is cross-sectional, designed to measure the prevalence of reduced EA in 41 well-trained male endurance athletes. In this study, subjects were recruited in two phases. Twenty one cyclists were recruited in phase one during the spring of 2015 in addition to 20 runners in phase two during the autumn of 2015 (see Figure 4). The ethical part is presented in section 5.

3.2. Subjects

The cyclists were recruited through local cycling clubs, as part of an on-going training intervention study (Sylta et al., 2016, unpublished). The runners recruited were all active at regional level, competing and training for various distances such as 10 km, half-marathon, marathon, ultra-running and orienteering. Some of the runners also competed at a national level at distances of 10km, marathon and orienteering. At the group level, subjects were categorized as well-trained according to Jeukendrup et al. (2000).

3.2.1. Criteria for inclusion/exclusion of subjects

This study used inclusion criteria based on Jeukendrup et al. (2000) defining the difference between trained, well-trained, elite and world-class cyclists. Due to the anthropometry and the difference between cyclists and runners, both inclusion criteria from the trained and well-trained groups were used to assess whether the subjects should be included.

The inclusion criteria were:

- male.
- absence of disease or injuries that prohibit participation
- ≥ 18 but ≤ 50 years
- $\dot{V}O_{2max} \geq 60 mL \cdot kg^{-1} \cdot min^{-1}$ or $\geq 4.0\ L \cdot min^{-1}$
- training volume last year: ≥ 5 sessions per week
- history of training: Active bicycle/running for more than 1 year

The exclusion criteria were:

- disease/injuries preventing participation in the study

3.2.2. Dropouts and exclusion

One cyclist dropped out for personal reasons. Three other subjects (out of the 41) were excluded from the final analysis due to the provision of invalid activity data. One activity tracker had faulty measurements and two subjects had only worn the activity tracker during training. Two subjects were excluded from the analysis due to complications with the registration, saving and sending of EI data for analysis (e.g. one subject only sent two out of four days of food registration). Eight subjects were excluded for providing food-registration data of poor validity. Therefore, this study includes 27 subjects (66% of the subjects recruited) in the statistical analysis (see Figure 4).
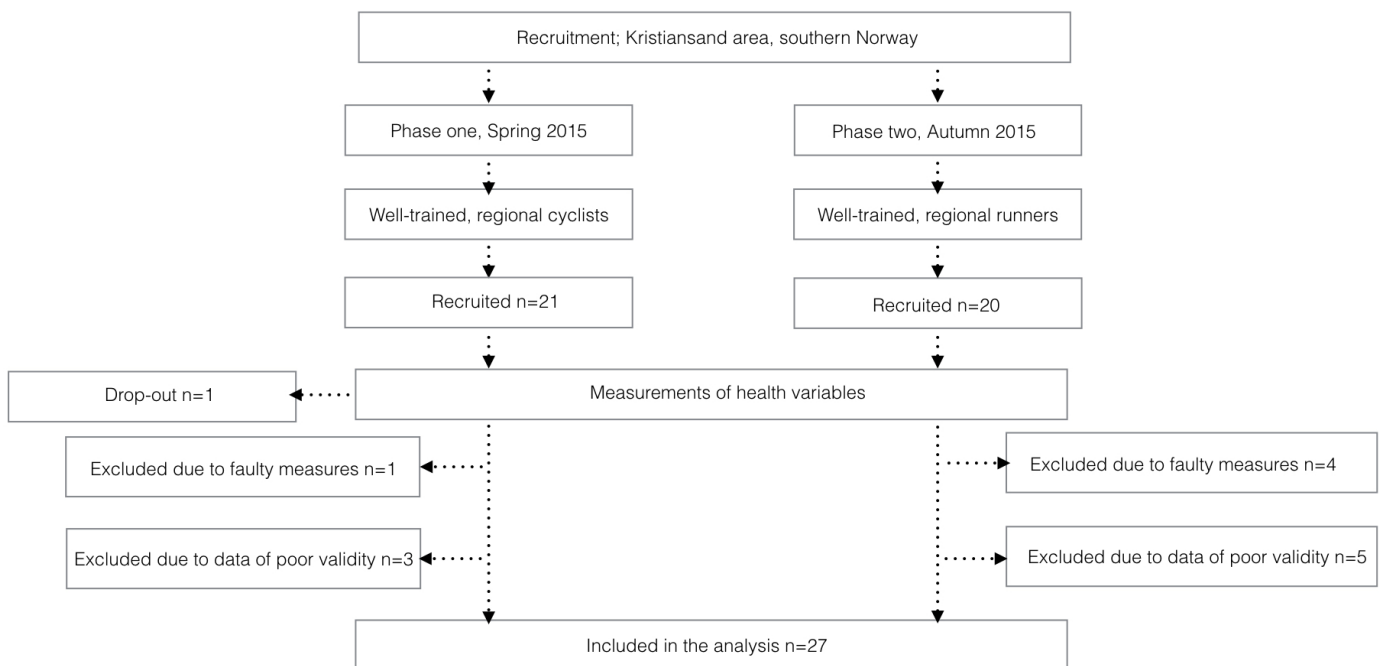

Figure 4. Timeline of recruitment of subjects for phases one and two, dropouts, and exclusion.

3.3. Testing procedure and measurements

The subjects in this study were tested on four non-consecutive days. Day 1 measured the performance variable, where as day 2-4 measured health variables (see Figure 5).

Day 1: 10.00 a.m. - 09.00 p.m.

Height/weight measurement	30 min warm-up	VO2max

Day 2: 06.00 a.m. - 08.00 a.m.

Weight measurement	15 min. rest	30 min. RMR-test	Blood Pressure	Food/watch Registration	Questionnaire

Day 3: 07.00 a.m. - 09.00 a.m.

Blood sampling

Day 4: 07.00 a.m. - 09.00 a.m.

DXA scan

*Day «5-8» : 00.00 a.m. - 00.00 p.m.

Recording of energy intake (EI), daily energy expenditure (DEE) and exercise energy expenditure (EEE)

*Cyclist recorded 3 days of EI, DEE & EEE. Runners recorded 4 days.

Figure 5. The study protocol. Testing on day 1, 2 and 3 was performed on the University campus. Testing on day 4 was performed at Sørlandets Hospital, Kristiansand, Norway. The subjects met in a state of fasting on day 2, 3 and 4. Recording of parameters on days 5-8 was performed in their home-environment. RMR; resting metabolic rate, measured using a ventilated hood; DXA, dual x-ray absorptiometry.

Day 1

3.3.1. Anthropometry

The height of the subjects was measured using a wall-affixed centimetre scale (Seca Optima, Seca, UK). The test was performed without shoes, and was recorded to the nearest cm. The weight of the subjects was obtained using an Inbody 720 body composition analyser (InBody 720, Biospace, Seoul, Korea), and was performed without shoes and only light clothing.

3.3.2. Performance measurement

The subjects went through a standardized warm-up protocol on the treadmill/bicycle, before an incremental test to exhaustion was performed to determine the subjects $\dot{V}O_{2max}$. The cyclists were tested on a Velotron cycling ergometer (Velotron Dynafit Pro, Racermate, Inc., Seattle, WA, USA) whereas the test started with one minute of cycling at a power output corresponding to 3 W/kg (rounded down to the nearest 50 W), and increased by 25 W/min. until voluntary exhaustion or

failure to maintain a cadence ≥ 70 RPM. The runners were tested on a Lode Katana Sport treadmill (Lode B.V., Groningen, The Netherlands). In the protocol used, the runners started at 12 km/h with a constant positive incline of 3 degrees (corresponding to 5.3% incline). The speed was increased by 1 km/h/min until voluntary exhaustion.

$\dot{V}O_2$ was measured using Oxycon Pro™ with mixing chamber and 30 seconds sampling time (Oxycon, Jaeger GmbH, Hoechberg, Germany), using a two-way T-shape non-rebreathing valve and a reusable nose clip series 9015 (Hans Rudolph, Kansas, MO, USA). Gas sensors were calibrated using an automated process using certified calibration gasses of known concentrations before every test. The flow turbine (Triple V, Erich Jaeger) was calibrated using a 3L calibration syringe (Hans Rudolph, Kansas, MO, USA). Heart rate (HR) was measured using Polar V800 (Polar Elektro Oy, Kempele, Finland). Capillary blood samples were analyzed for whole blood using a stationary lactate analyzer (EKF BIOSEN, EKF diagnostic, Cardiff, UK). $\dot{V}O_{2max}$ was calculated as the average of the two highest 30-sec consecutive $\dot{V}O_2$ measurements. Plateau of $\dot{V}O_2$ curve and/or HR ≥95% of known HRmax, RER ≥1.10 and [la-] ≥8.0 mMol.L^{-1} were used as criteria for the attainment of $\dot{V}O_{2max}$. If the subject did not have a $\dot{V}O_2$ plateau, the test was classified as a $\dot{V}O_{2peak}$ -test, showing the highest possibly $\dot{V}O_2$ the subject could attain on that day, and not the true maximal $\dot{V}O_2$ level.

Day 2

3.3.3. Resting metabolic rate

Subjects arrived at the laboratory between 6 and 8 a.m. and the testing was estimated to last approximately 1 - 1.5 hours. All subjects met in a fasting state according to protocol (Compher, Frankenfield, Keim, Roth-Yousey, & Evidence Analysis Working, 2006) and no use of alcohol or tobacco was allowed for a minimum of 12 hours prior to the test. Furthermore, the subjects were instructed to travel to the lab using only motorized transportation, and under no circumstances were allowed to walk or ride a bicycle. Training was restricted to a maximum of 60 minutes of low intensity endurance training the day before the test, and at least 12 hours before the test (strength training was not allowed).

RMR was measured via indirect calorimetry using a canopy hood (Oxycon Pro, Jeager, Germany). and calibrated using the same standards as described in section 3.3.2. The subjects were instructed to lie on a bed for a 15-minute rest, in order to minimize errors in measuring RMR before the test began (Compher et al., 2006). The measuring of RMR lasted a total of 30 minutes, bringing the total resting time to 45 minutes. During the RMR test, the subjects was not allowed to move, talk or fall asleep and was checked up on several times by lab personnel. A heart rate monitor from Polar

(V800/M400) was used to record the lowest resting HR during the test. An RMR test was declared successful if the coefficient of variation for $\dot{V}O_2$ and $\dot{V}CO_2$ for the last 20 minutes of the test was ≤ 10% (Compher et al., 2006).

3.3.4. Blood pressure

The resting blood pressure ($_rBP$) and orthostatic blood pressure ($_oBP$) of the subjects were measured using an electronic sphygmomanometer (Microlife BP A100, Widnau, Switzerland). Before the measurement the cuff was carefully placed 2 cm above the elbow with the tube on the inside of the arm pointing downwards. The cuff was firmly closed to fit the arm and at the same level as the heart, according to the manufacturer's instructions (Microlife, 2015). The $_rBP$ was first measured three times in a lying position. The $_oBP$ was then measured three times in a standing position. The first result of both $_rBP$ and $_oBP$ was excluded from the analysis; hence the average of the two last measures was used.

In addition, the subjects were lectured on how to weigh and register food and, plot this in on the computer and how to use the Polar heart rate monitor. The runners were further instructed in how to use and wear an additional activity tracker (Sensewear armband, BodyMedia, Inc., Pittsburgh, PA, USA) for comparison between Polar and Sensewear (see section 4.3.3).

Day 3

3.3.5. Blood sampling

On day three, the subjects arrived in a fasting state at the lab between 7 and 9 a.m. for a blood sample. A bioengineer took the sample using a tourniquet and the Safety blood collection set (Greiner Bio-One, GmbH, Kremsmünster, Austria). A 10-mL BD Vacutainer CAT (BD, Plymouth, United Kingdom) was filled and left to stand for at least 30 minutes. After 30 minutes the blood sample was centrifuged for 10 minutes at 3000 rpm (StatSpin Express 4, Beckman Coulter, USA) and two 1.8-mL Cryotube Vials (Termo Fischer Science, Roskilde, Denmark) were filled with the serum using a pipette. The Cryotubes with the serum were immediately placed in freezer at a temperature of -18 degrees (Electrolux CF100, Stockholm, Sweden), where they were stored until transportation to the analysis institute. Cyclists blood samples was analysed at Hormonlabor C831, Bern, Switzerland and the runners blood samples was analysed at Sørlandets Hospital, Arendal and at Aker Hormonlab Norway. The serum was analysed for the following hormones: cortisol, total testosterone, IGF-1, insulin, glucose, T_3, total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides.

Day 4

3.3.6. Body composition, including bone health

The body composition and BMD was measured by the same experienced observer and was obtained using dual-energy X-ray absorptiometry (DXA; GE-Lunar Prodigy, Madison, WI, USA) at Sørlandets Hospital, Kristiansand, Norway. DXA is an objective method that is non-invasive, very accurate and widely used for assessing BMD and risk of osteoporosis. It is considered the "gold standard" for measuring bone mass (Kleerekoper, 1998; Marshall, Johnell, & Wedel, 1996). The DXA delivers a two-dimensional picture of the site scanned, rather than volumetric density, and therefore DXA determines the bone mineral content (BMC) of the scanned area, and divides this by area. It is therefore areal density (g/cm^2), and not volumetric density (mg/cm^3) as a quantitative computed tomography would show, that is used in the analysis.

The test was performed in a fasting state between 7 and 9 a.m. on the fourth test day. The subjects were instructed to arrive by motorized transport to the hospital. Before the test both weight and height were measured, and no jewellery or other ornaments were allowed to be worn during the DXA test. Subjects were instructed to lie down, with both hands slightly away from but alongside the body and legs were to be kept straight. The DXA scanned the subjects in a supine position from head to toe (see Picture 1).

Picture 1. Body composition, including bone health assed by a DXA-scan ("DXA-scan "Picture"," 2014)

DXA determines BMC, FFM and BMD at femur neck, total hip, lumbar spine (L1-L4), and total body. BMD was classified in the subjects as recommended in previous position statements of athletes (Mountjoy et al., 2014; Nattiv et al., 2007):

- normal BMD: Z-score of higher than -1 in the measured areas
- low BMD: Z-score between -1 to -2 in at least one area
- osteoporosis: Z-score of -2 or lower in at least one area

Day 5-8

3.4. Estimations and calculations of energy intake and energy availability

3.4.1. Estimating energy intake

A researcher carefully guided the subjects in how to weigh and register the intake of food and beverages. All subjects borrowed a kitchen scale from either Exido (Exido 246030 Kitchen Scale, Gothenburg, Sweden) or OBH (OBH Nordica 9843 Kitchen Scale Color, Taastrup, Denmark) and were instructed in how to weigh their daily intake of food and beverages. The kitchen scales weighed to the nearest 1 gram. The subjects logged their food and beverage intake with the food registration software Dietist Net (Dietist Net, Kost och Näringsdata, Bromma, Sweden). Dietist Net has access to the Norwegian food table (Matvaretabellen 2014), an open Norwegian nutritional information database (MILLUM PDB) and the U.S national nutrient database (US Department of Agriculture).

The cyclists were instructed in how to weigh their food and beverages for three days, two weekdays and one weekend day and the runners were instructed in how to weigh their food and beverages for four days, two weekdays and two weekend days. The days for registering food intake were chosen by the researchers, in cooperation with the subjects. The days for registering should be representative of their normal days of eating habits, and not contain days that were not representative (e.g. partying, travelling). A manual for the programme was distributed to the subjects, which also included a paper form where they could record their intake of food and beverages (see Appendix 6). Data on food registration was controlled by researchers for under-reporting and poor validity using the Goldberg cut-off as described by Black (2000). Subjects who delivered records of poor validity were excluded from the analysis (see Figure 4).

3.4.2. Calculating energy availability

Few studies have examined male endurance athletes and defined the categories of EA. Those found up until 2015 have used the same definitions of both male and female endurance athletes (Loucks et al., 2011; Viner et al., 2015). Based on a study of male and female mammals by Bronson (1985), it has been proposed that the female reproductive system requires more energy compared to the male reproductive system. An experimental study by Koehler et al. (2016) therefore proposes that the cut-off point for optimal EA in males should be set at 40kcal/kgFFM/day based on the assumption described above. Koehler et al. (2016) proposes no new cut-off for low EA in males, but in their experimental design participants experienced EA both at 15kcal/kgFFM/day and 40kcal/kgFFM/day. This thesis will therefore use the 40kcal/kgFFM/day as the cut-off point for optimal and reduced EA. EA was calculated as EA=(EI - (EEE - (DEE - EEE))/(FFM) as outlined in section 2.4.1. When calculating DEE and EEE, we used predefined MET values in different activity and intensity zones as defined by Polar (see Appendix 1).

3.5. Statistics

All data were analyzed using SPSS for Macintosh (v. 22; SPSS Inc., Chicago, IL, USA). Figures and tables were made using Microsoft Excel 2016 for Macintosh (Microsoft Corporation, Redmond, Washington, USA) and GraphPad Prism 7 for Macintosh (GraphPad Software, Inc., 7825 Fay Avenue, La Jolla, CA 92037, USA). The subjects were divided into two groups based on their current EA status (reduced and optimal EA) and were controlled for missing data and sign of non-normality using histograms. Due to the small numbers in each group, it was difficult to identify normal distribution. All data are therefore presented as non-normally distributed data; median plus interquartile range (25 - 75). Due to the non-normality, non-parametric tests (Mann-Whitney U-test) was used to search for statistical differences between the groups on all variables. When checking for correlation between variables we used Spearman´s rho (ρ) due to non-normal distributed data. When assessing for associations between reduced EA and selected health variables, a logistical regression was performed on one variable at a time. Odds-ratio (with 95% confidence interval), *P*-value and Nagelkerke R Square was used to assess wheter there were an association between the variable and reduced EA. In order not to draw false conclusions based on false statistical significant levels for all the different statistical tests performed, an α-level of 0.01 was used. Effect size (ES) was calculated in order to interpret the meaningfulness of the results and to identify trends. ES in non-parametric data was calculated as $r = \dfrac{Z}{\sqrt{N}}$, where N is the total number of cases. We used the criteria from Cohen (1988) for ES: 0.1= small effect, 0.3=medium effect, 0.5 = large effect. When medium or large ES was observed, results are discussed as trends if comparisons are non-significant.

4. Method discussion

4.1. Design

A cross-sectional design tries to describe a phenomenon or different phenomena at a fixed point in time, and it is one of the most frequently used study designs for examining relationships between exercise and health variables (Polit & Beck, 2014; Thomas, Nelson, & Silverman, 2011). Accordingly, this type of study design seems to be a suitable method for investigating the aim of this thesis (see section 1.1).

A cross-sectional design is limited in that it only measures outcome and exposure at the given time of the study. As a result, it is not possible for this study to identify whether EI, EA or physical activity is responsible for the observed effects. This type of design is therefore not suited for drawing any conclusions about cause-and-effect since the timing of the relationship between outcome and exposure is unknown (Thomas et al., 2011). Another limitation using this type of study design is not knowing the longitudinal effects of diet and exercise on the variables assessed in this study. For example, we may find that reduced EA correlates with low BMD over a given time point, but the longitudinal effects are unknown.

4.2. Study sample

This study recruited 41 well-trained male endurance athletes from the regional area, active within either cycling or running. The subjects were a homogeneous group (see Table 1 in the article), and represented well-trained athletes as defined by Jeukendrup et al. (2000). Despite having to eliminate 14 of our study participants from the final analysis, we still have a relatively high study sample compared to other studies of EA among male athletes, where the numbers of participants often is 10 or less (Koehler et al., 2016; Viner et al., 2015). However, despite the relative high number of participants compared to other studies, the different numbers in each group (reduced EA, n=18 and optimal EA, n=9), make it difficult to gain statistical power and detect true differences (Thomas et al., 2011). As a result, one question is whether our results can be generalized. All subjects were in good shape and health, and the inclusion criteria ruled out participants with injuries. The subjects in our study could therefore be a representative sample of other well-trained cyclists and runners at the same age, and could be generalized as other studies have done (Melin et al., 2015). However, due to the low numbers of studies published, different sub-group sizes in our study and the fact that we have no control group, we find it difficult to generalize our findings. More studies are needed and a higher N is probably required to find true statistical differences.

4.3. Measurements

It can be asserted that testing in sport science is an essential tool for assessing different effects of both exercise and lifestyle interventions or for measuring the prevalence of a phenomenon. Testing is an "objective" way to assess such variables, but can be difficult. Several factors are important for testing and essential to verify; 1) the equipment measurement must be accurate; 2) it is essential to control the lab conditions; 3) it is essential to have the same standard protocols for all testing and the most important 4) the test must be both reliable and valid (Thomas et al., 2011).

In this study, every effort was made to increase both the reliability and validity of all measurements such as using gold-standard methods wherever possible (food weighing and DXA), and to use the same procedure and protocol on both cyclists and runners. When not using gold-standard methods, calibration, measurements and methods were carried out according to both instructions from manufacturers and best-practice scientific papers. Furthermore, we used the same test leader in both phases of testing. We also set up the procedure of measurements in an attempt to eliminate any possible influencing elements that could potentially affect the tested variables. Clear instructions prior to tests, supervision of subjects, well-established contacts between subjects and researchers and best-practice methods were used to try to control such elements. In the following, a more in-depth discussion of the different measurements is presented.

4.3.1. Resting metabolic rate

Due to the complicated structure of RED-S, energy expenditure is an important factor for the determination of EA in athletes. Energy expenditure can be both measured and estimated using mathematical formulas. However, measurements should be more accurate than estimations (based on mathematics), but only if the measurements are performed correctly (Compher et al., 2006). In order to measure RMR we chose indirect calorimetry using a canopy hood, due to the proven validity if calibrated correctly and low costs of measurements (Westerterp, 2015). To minimize error when measuring RMR in our subjects, we carefully followed the instructions from both the manufacturer of the Oxycon and the systematic review of best practice methods for RMR by Compher et al. (2006). We further extended the total time of active measurement from 10 minutes to 30 minutes, and used the last 25 minutes to calculate RMR, instead of 5 minutes as proposed by Compher et al. (2006). The Oxycon was calibrated before each measurement according to instructions. Subjects were furthermore instructed in how to behave (eating habits and exercise) on the days before and during the test, all according to the best-practice method (Compher et al., 2006). In order to calculate RMR based on the measurements, we used the Weir (1990) equation, since this equation is widely used in studies assessing EA in athletes (De Souza, Hontscharuk, Olmsted, Kerr, & Williams, 2007; Melin et al., 2015).

When assessing the predicted RMR for use in the calculations of the RMR ratio, several methods exist. In the literature both the Harris-Benedict equation (Harris & Benedict, 1918) and the Cunningham equation (Cunningham, 1980) are widely used for studying athletes (De Souza et al., 2007; Kim, Kim, Kim, Park, & Kim, 2015; Melin et al., 2015). Since this study looks at well-trained endurance athletes, we used the Cunningham equation, as it is assumed to be the most accurate for predicting RMR in athletes (Kim et al., 2015).

4.3.2. Energy intake

In general, a reliable and valid assessment of EI is an important factor in sport science and for athletes striving to perform (Hill & Davies, 2001). Different forms of food registration, spanning from a recall of food eaten, retrospective questionnaire, and diet records exist. Diet records where subjects weigh and register their food over a given period are largely considered the most precise and accurate method of food registration compared to the cost (Barrett-Connor, 1991). However, weighing and registration of food intake over several days has proven to be time consuming and boring, and is associated with poor agreement and/or changes of eating habits during the registration periods (Barrett-Connor, 1991). The doubly labelled water (DLW) method has proven that subjects in many different groups under-report their dietary intake, but the reason is to some extent still unknown (Hill & Davies, 2001). The DLW method is furthermore a difficult and expensive method for assessing EI, and was not available for use in this present study. Researchers have proposed that elements such as poor body-image and weight consciousness may play a significant role in under-reporting of dietary intake (Lafay et al., 1997). However, despite such challenges in diet recording, it is still one of the most widely used methods in studies on both males and females athletes (Melin et al., 2015; Muia et al., 2015; Viner et al., 2015; Vogt et al., 2005). To try to overcome the problem of under-reporting and to avoid drawing false conclusions, we identified data of poor validity using the Goldberg cut-off described by Black (2000) and removed a total of eight subjects from the analysis. When reviewing the literature, few studies eliminate under-reporters (Melin et al., 2015), and it is therefore believed that the validity of EI in this study is higher compared to other studies. The consequences of not eliminating under-reporters could be an over-reporting of the prevalence of reduced EA. To this author's knowledge, however, no formal, well-recognized standard for how to evaluate and exclude possible under-reporters exists.

4.3.3. Physical activity and exercise tracking

To assess physical activity, the use of accelerometers has become a standard method when using field-based research (Welk, Schaben, & Morrow, 2004). Furthermore, activity trackers have become more readily available through different manufacturers and provide the consumer with different options for self-monitoring of physical activity. In order to use such activity trackers in research they

should be reliable and valid. Some validation studies on different types of activity trackers compared to both whole-room indirect calorimeter and portable metabolic analysers, which analyses oxygen consumption under free-living conditions exists (Adam Noah, Spierer, Gu, & Bronner, 2013; Dannecker, Sazonova, Melanson, Sazonov, & Browning, 2013). Studies of validity show that activity trackers are useful for estimating both activity and energy expenditure, although all of them underestimate physical activity levels and energy expenditure to some extend (Adam Noah et al., 2013; Lee, Kim, & Welk, 2014). Activities such as cycling and upper body movement are also harder to detect for activity trackers due to the motion pattern of the exercise (Lee et al., 2014). Furthermore, most commercial activity trackers do not allow researchers direct access to raw data or minute-by-minute data, so estimations and calculations must be obtained directly from the software provided by the manufacturers (Lee et al., 2014).

However, despite the underestimation by the commercially available activity trackers, they are inexpensive, easy to wear (incorporated into a watch), easy to use, non-invasive and small and they are objective indicators of physical activity during the period of use (Lee et al., 2014). Our subjects were also already very familiar with the use of heart rate monitor. The fact that the Polar V800 and M400 were easy to use and accessible at a low cost played an important role in the selection of activity tracker to be used in the present study, although this exact type of activity tracker is not yet validated. To address this lack of validation, we fitted the runners in phase 2 with a Sensewear armband (BodyMedia, Inc., Pittsburgh, PA, USA) paralleling their use of the M400. This was done to get an indication of how well Polar and Sensewear correlated at different intensities and activities. Our unpublished data show some of the same patterns as other activity trackers do, highlighted by Lee et al. (2014), where low-intense activities seem to be the hardest to detect by the Polar V800/M400, but overall, the performance of the Polar V800/M400 compared to Sensewear is good. Another reason for choosing Polar accelerometer was its ability to get direct access to heart-rate monitoring during exercise, which is especially important during activities such as cycling (where activity trackers perform poorly). By quantifying heart-rate data during exercise, we were able to calculate EEE more directly compared to Sensewear which estimates based on the accelerometer, sweat and heat-flux. Due to lack of access to raw data or minute-by-minute data by Polar, we chose a metabolic equivalent (MET) value for both sitting, low and medium intensity categories, and we chose to base this on the median of each category defined by Polar (Virtanen, 2014). This is difficult to interpret, and difficult to control for correctness, but we deemed it the best-suited method of assessment. It should be noted, however, that there is a risk of both underestimating and overestimating of energy expenditure in these categories.

Different methods for quantifying DEE and EEE exist, where heart-rate data, oxygen consumption, DLW, accelerometers and self-reported exercise are useful, but all methods have both strengths and limitations (Lamonte & Ainsworth, 2001). The DLW method is a very precise method of assessing energy expenditure, but is expensive and not readily available (Lamonte & Ainsworth, 2001). Assessing EEE in cycling based on accelerometers has proven difficult due to the limited movement of the body's centre of gravity (Lee et al., 2014; Virtanen, 2014). Measuring oxygen consumption during exercise in free living conditions has demonstrated good accuracy, but has limitations due to cost issues, obtrusive instrumentations, changes in exercise patterns and being resources-intensive (Lamonte & Ainsworth, 2001). Although energy expenditure in general has a strong linear relation between heart-rate and VO_2, there are issues concerning energy expenditure during low and very high intensities (Lamonte & Ainsworth, 2001). There are also different factors affecting heart-rate, such as stress, body temperature and medication (Lamonte & Ainsworth, 2001). On the other hand, heart-rate monitoring is a low-cost method of quantifying the time spent in different intensity zones, and all our subjects were experienced in the use of heart-rate monitors during training. During the incremental test to exhaustion we determined the HR_{peak} and the different intensity zones as mentioned in section 3.3. As we quantified the time spent in these zones and compared the different activity levels based on Ainsworth et al. (2000) and Ainsworth et al. (2011), we believe we have a well-reasoned method for estimating EEE in relation to the cost and accessibility of methods.

4.3.4. Body composition including bone health

DXA is currently reported to be the gold standard for assessing bone health and diagnosing osteoporosis (Kleerekoper, 1998) and is widely used at different hospitals in Norway. To minimize the possibility of error, the same technician performed the scans among the groups of cyclists and runners on the same DXA machine. Looking at the validity of DXA, we should observe at the difference in the estimation between lean and fat-mass measured by DXA compared to true lean and fat mass in dead animals (Clarys et al., 2010). However, studies of humans concerning the validity and reliability of DXA have only been done using the four-compartment model, which is currently regarded as the gold standard to assess body composition (Toombs, Ducher, Shepherd, & De Souza, 2012). The four-compartment model is a time consuming and expensive method, however, and was not available for this study. Several studies have also reported reliable and valid assessments of body composition using DXA (Brodowicz, Mansfield, McClung, & Althoff, 1994; Prior et al., 1997), and this method was used in the present study instead of bio-impedance (Inbody 720) available at the university. Different studies have looked at the precision of DXA, where the aim is to see results with little variation, when tests are repeatedly performed under identical conditions. In order to quantify the precision, coefficient of variation and correlation coefficients have been used in the studies. The CV has been reported to be between 1.7 - 3.6%, depending on the site of measurement,

and correlation coefficients have been reported up to 1.0 (Lohman, Tallroth, Kettunen, & Marttinen, 2009; Phillipov, Seaborn, & Phillips, 2001). We also instructed the participants to follow the guidelines prescribed by the hospital and used best-practice articles for subjects in order to obtain as precise results as possible (Nana, Slater, Hopkins, & Burke, 2012, 2013).

4.4. Calculations

The predicted RMR (RMR_p) can be calculated using several methods such as the Cunningham equation or the Harris Benedict equation (see Appendix 1). Studies have shown that the Harris Benedict equation in both lean athletes and recreationally active females might underestimate RMR_p and using it leads to a risk of overestimating the RMR ratio (Kim et al., 2015). Studies have reported the Cunningham equation to be the most precise (Gibbs et al., 2013; Kim et al., 2015). Low RMR is often defined as an RMR ratio below 0.90, but this definition is mostly used for female athletes (De Souza et al., 2008). Since the reproductive function of males probably has lower energy costs as described earlier (see section 3.4.2), we chose therefore not to use the 0.90 cut-off point as a reference for low RMR, since this may not be the correct reference for males.

When calculating DEE and EEE, we used predefined MET values in different activity and intensity zones as described earlier (see Appendix 1). The MET values for normal activity were already defined by Polar. Since we had no access to any raw data or minute-by-minute data, we chose to use the median MET of each predefined activity zone. When estimating EEE in their daily training, we used the time spent in each heart-rate zone (defined by their maximal achieved HR during the incremental test), and calculated energy expenditure in each zone by average MET values at different intensities for each group of subjects, as defined by Ainsworth et al. (2000). This method has some limitations, however. By using the median MET of all activity zones, it is difficult to assess the precision of the calculation, due to choosing and locking our calculations based on one specific value instead of a range of values when analysing minute-by-minute data. When defining EEE, a study by Tomten and Hostmark (2006) used a more extensive protocol, where running economy and heart-rate at different intensities were analysed and used to calculate EEE during training. This method was not used by us, due to a lack of time and funding. This method is deemed more precise, however, and is preferable for future studies. On the other hand, to strengthen our calculation for EA, we used the same formula as described by Melin (2015), where we subtracted both RMR and NEAT from the EEE, thereby giving a more precise estimate of true EEE and thereby eliminating a risk of over-reporting reduced EA.

4.5. Strengths and limitations

The main strengths of the present study are the fact that it involves research into a new and not well-studied area, where in general, a limited number of studies of male athletes with few participants exist. This study is a comprehensive study performed with a high number of participants compared to other studies investigating the same subjects (Koehler et al., 2016; Viner et al., 2015). We further increased the strength of this study by using the same equipment, test leader and technician in both periods of data collection. The study used several well-established and objective measuring methods, where DXA is the gold standard in assessing bone health. Although self-reported EI has some challenges, the method is currently best suited for assessing EI (Hill & Davies, 2001). Furthermore using the Goldberg cut-off is a strength in trying to exclude under-reporters from analysis, which may otherwise lead to false conclusions.

There are however several limitations in this study. For instance, the design precludes the establishment of a causal relationship between the various variables measured in relation to EA, and only examines the prevalence of reduced EA. Also, this study has a duration time, which only looks at EA over a few days and may therefore not be able to identify subjects with a genuinely reduced EA over time. The absence of a control group in this study makes it hard to compare our findings to other populations of athletes or non-athletes. When divided into groups and analysed, this study has a lopsided sample size (9 vs. 18), with perhaps not enough statistical power to detect differences between groups.

As a result of such a lopsided sample size, lack of statistical power and exclusion of subjects in the analysis, a potential of making a type II error exists, where researchers wrongly accepts the null hypothesis based on false assumptions and premises. However, due to the large numbers of statistical tests performed, we chose to set the α-level at 0.01 to avoid making a type I error, which is to reject the null-hypothesis even if it is true, and thereby stating a false negative, but this could further lead to an increased risk of a type II error (Thomas et al., 2011). Lastly, due to a lack of funding, we did not investigate hormonal biomarkers such as ghrelin and leptin, which are involved in regulating hunger and body weight (Melin, 2015), and these could have provided interesting results. A limitation in this study was that other markers such as illness, injuries, gastrointestinal function, disordered eating and performance effects were not analysed due to the vast method of this study combined with limited time to analyse, interpret and write the thesis.

5. Ethics

This project was performed on healthy individuals and examined variables such as body composition, nutrition, exercise, hormonal biomarkers and BMD. Both the University of Agder Faculty's Ethical Committee (FEK) and the Norwegian Centre for Research Data (NSD) approved this study. The participants in this study received written information before the study began, explaining that the study involved testing to exhaustion, fasting before testing, measurement of body composition, BP and blood sampling, all of which could cause some discomfort. Furthermore, all participants were told that they could withdraw from the study without giving any reason. All participants submitted a written consent form. All information about the participants was anonymised using a person-specific code, and the key paper that linked the participants name to the code was stored in a safe-deposit box. All the available information about the participants (including test results) was stored both as hard copy and digitally (USB flash drive). These data were stored in a safe-deposit box. All subjects received individual, formal and detailed feedback on variables measured, to minimize any possible misinterpretation of the results by the subjects themselves. If some of the findings were either lower or higher than population-based reference values, the subjects were advised to contact their physician for a follow up. No adverse events of the testing/data collections were reported.

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Part 2:

Paper

Prevalence of Relative Energy Deficiency in Sport among well-trained male cyclists and long-distance runners

This paper is written according to the standards of the following journal:

Thomas Birkedal Stenqvist

University of Agder

May 2016

Prevalence of Relative Energy Deficiency in Sport among well-trained male cyclists and long-distance runners

Thomas Birkedal Stenqvist [1]

[1]Department of Public Health, Sport and Nutrition, Faculty of Health and Sport Science, University of Agder, Kristiansand, Norway

Running head: Prevalence of RED-S among male endurance athletes

Corresponding author:
Thomas Birkedal Stenqvist
University of Agder
Faculty of Health and Sport Science
PO. Box 422
4604 Kristiansand
Norway
E-mail: thoms14@uia.no

Conflicts of interest: the author has no conflicts of interest in this study

Abstract

Relative Energy Deficiency in Sport (RED-S) links low and reduced energy availability (EA) with negative health and performance consequences. However, this is not well investigated in male athletes. The aim of this study was to investigate the prevalence of RED-S and associated health consequences in well-trained male endurance athletes.

Forty-one subjects, cyclists (n=21) and runners (n=20) [age: 40 (31-45) years; BMI: 23.5 (21.4-24.0) kg/m^2; body-fat: 14.0% (10.0-16.5%); exercise: 12 (9-16) h/week presented as median + interquartile range] were recruited. Protocol included assessment of bone health, body composition, resting metabolic rate (RMR), blood pressure, energy intake, energy expenditure, hormonal biomarkers, blood glucose and lipids. 27 subjects were included in the final analysis.

Eighteen subjects had reduced EA (<40kcal/kgFFM/day) and showed a trend of lower RMR ratio compared to the optimal EA group (0.83 vs. 0.86, $P=0.026$). Six subjects had low bone mineral density (BMD), however, this was not related to EA status. The reduced EA group showed a trend of higher BMD in femur ($P=0.037$), hip ($P=0.057$), lumbar spine ($P=0.01$) and total body ($P=0.035$). No associations between groups were observed in hormonal biomarkers, blood glucose or blood lipids.

We found high prevalence of reduced EA accompanied by metabolic alterations in this group of well-trained athletes. However, no differences were observed between EA groups in either anthropometric, hormonal biomarkers, blood glucose, blood lipids or BMD. This may indicate that well-trained male endurance athletes are better protected against associations to negative health consequences in combination with reduced EA, compared to female endurance athletes.

Introduction

Controlling body weight and body composition are crucial elements of performance in different sport disciplines and athletes' ways of maintaining body composition vary from healthy methods to extreme methods in order to reduce body mass quickly and/or to gain competitive advantages (Sundgot-Borgen et al., 2013). Athletes experiencing an energy deficiency in order to loose weight is not a new phenomenon, but a controlled energy deficit is recommended to safely reduce weight as an athlete if needed (Donnelly et al., 2009).

Female athletes with low energy availability (EA) have been observed with a normal body weight, and body weight seems to be preserved during long-term low EA. EA is defined as "*dietary energy intake minus exercise energy expenditure, energy availability is the amount of dietary energy remaining for other body functions after exercise training.*" (Nattiv et al., 2007, p. 1868), and has traditionally been categorized in females as follows: low EA (<30 kcal/kg fat-free mass (FFM)/day), reduced EA (30-44kcal/kgFFM/day) and optimal EA (≥45 kcal/kgFFM/day) (Loucks, 2004). Low EA and reduced EA can be sustained either by lowering energy intake or exercising excessively or a combination of both (Koehler et al., 2016). Studies of female athletes undergoing EA of <30kcal/kgFFM/day have shown a reduction in blood glucose, triiodothyronine (T_3), luteinizing hormone, insulin, leptin and insulin-like growth factor-1 (IGF-1) (Loucks & Thuma, 2003; Loucks et al., 1998). Other factors observed are metabolic alterations, such as a reduction in resting metabolic rate (RMR) and non-exercise activity thermogenesis (NEAT) (Redman et al., 2009). The prevalence of low EA in females has not been investigated properly, however, but it is widely recognized that the prevalence of one or more of the severe components (low/reduced EA, menstrual dysfunctions or low bone mineral density (BMD)) of what is known as the female athlete triad (Triad) is quite high (Nattiv et al., 2007; Torstveit & Sundgot-Borgen, 2005). Furthermore, females participating in endurance sports, aesthetic sports, and weight-class sports seem to have the greatest risk of developing some of the Triad components compared to athletes competing in other types of sport (Nattiv et al., 2007).

Until 2014, the Triad was only related to female athletes, despite increasing support that male athletes could also be at risk (Mountjoy et al., 2014). This changed, however, when the International Olympic Committee (IOC) released a revised and expanded version, including the Triad, named "Relative Energy Deficiency in Sport" (RED-S), where EA is still the main and essential part, but now includes male athletes (Mountjoy et al., 2014). It seems that low EA occurs in some of the same sports for male athletes as for females, such as weight-sensitive sports, where leanness and/or a low

weight are important factors for performance (gymnastics, long-distance running, road cycling, etc.) and weight-category sports disciplines like boxing and wrestling (Sundgot-Borgen et al., 2013). Only a few studies have investigated male endurance athletes and defined categories of EA, in comparison to females. Viner et al. (2015) used the same EA criteria for males as females, whereas Koehler et al. (2016) used 40 kcal/kgFFM/day as a cut-off for males between optimal and reduced EA. Severe energy deficit has been observed in elite male world-class cyclists at a training camp, where EA as low as 8 kcal/kgFFM/day has been reported (Vogt et al., 2005). Furthermore, studies of male soldiers undergoing extreme energy deficiency during military exercises have found a severe reduction in testosterone, T_3 and IGF-1 during four weeks of exercise (Friedl et al., 2000). Another study of male soldiers by Kyrolainen et al. (2008) found an increase in cortisol and growth hormone, whereas a reduction in insulin and testosterone was observed during energy restriction. A recent controlled experimental study of male athletes with EA of 15kcal/kgFFM/day for four days led to a suppression of leptin and insulin, but no alterations in IGF-1, T_3 or testosterone (Koehler et al., 2016).

The prevalence of low and reduced EA and possible associations with selected health variables in well-trained male endurance athletes has not been well investigated. Therefore, we performed an observational study aimed at investigating the prevalence of reduced EA and possible associated health variables such as bone health and hormonal- and metabolic variables in a group of well-trained male endurance athletes.

Materials and methods

Subjects

Twenty-one well-trained male cyclists and 20 well-trained male long-distance runners were recruited to the study through local clubs in two phases (Figure 1). All subjects competed at a regional level, and a few of the runners also competed at national level as well. Inclusion criteria were: 18-50 years old, absence of disease or injury, maximal oxygen uptake ($\dot{V}O_{2max}$) $\geq$60 mL$\cdot$kg$^{-1}\cdot$min^{-1} or $\geq$4.0 L$\cdot$min^{-1}, training frequency last year $\geq$5 sessions/week and active at regional level. For exclusion and drop-outs, see Figure 1. A total of 27 subjects (out of 41) were included in the final analysis in this study. All subjects received information regarding the background of the study and test-procedures and signed an informed consent document. Permission to undertake the study was granted by the University Faculty Ethics Committee (FEK) and the Norwegian Centre for Research Data (NSD – project no.: 46706).

Insert Figure 1 here

Methods

Data collection was performed over four non-consecutive days, followed by three or four consecutive days of food and exercise registration (Figure 2). On day one subjects performed a test to determine $\dot{V}O_{2max}$ and measurements of anthropometry. On day two, the subjects was assessed for RMR, blood pressure (BP) and resting heart rate (HR), in addition to instructions in how to record their energy intake and energy expenditure and filled out a questionnaire. On day three, blood samples were drawn and during day four, bone health was assessed. All subjects were told to arrive in a fasted state on days two, three and four and refrain from using products containing tobacco and caffeine and not to engage in more than one hour of mild exercise the day before. Cyclists registered their consumption of food and beverage and their activity and exercise sessions for three consecutive days (two weekdays and one weekend day) and runners registered the same for four consecutive days (two weekdays and two weekend days). All registration was done in the subjects normal environment.

Insert Figure 2 here

Anthropometry

Height was measured to the nearest 0.1 cm using a centimetre scale affixed to the wall (Seca Optima, Seca, UK), and was done without shoes. Body weight was measured in light clothing to the nearest 0.01 kg using an Inbody 720 bioelectrical impedance analyzer (InBody 720, Biospace, Seoul, Korea). Body mass index (BMI) was calculated as weight in kilos divided by height squared in m^2.

Maximal oxygen uptake

$\dot{V}O_{2max}$ was determined performing an incremental test to exhaustion: cyclists on a stationary bike (Velotron Dynafit Pro) and runners on a treadmill (Lode Katana Sport). Cyclists began with one minute of cycling at a power output corresponding to 3 W/kg, and increased by 25 W/min until voluntary exhaustion or failure to maintain a cadence $\geq$ 70 RPM. Runners began at 12 km/h on a constant positive incline of 3 degrees. Speed was increased by 1 km/h/min until voluntary exhaustion. $\dot{V}O_2$ was measured using Oxycon Pro™ with mixing chamber and 30 seconds sampling time (Oxycon, Jaeger GmbH, Hoechberg, Germany), using a two-way T-shape non-rebreathing valve and a reusable nose clip series 9015 (Hans Rudolph, Kansas, MO, USA). All systems were calibrated according to standards.

Resting metabolic rate, blood pressure and resting heart rate.

Subjects arrived at the lab by motorized transport, in a fasted state between six and nine a.m. to assess RMR. Indirect calorimetry using a canopy hood system was used (Oxycon Pro, Eric Jeager, Germany), and systems were calibrated before each test according to standards. Subjects rested lying down for 15 minutes before the measurements began. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were assessed over a 30-minute period. The last 20 minutes of measurement were used to assess RMR according to protocol (Compher et al., 2006). Measured RMR was assessed using the Weir (1990) equation: $(3.94\,(\dot{V}O_2) + 1.1\,(\dot{V}CO_2)) \times 1.44$. To calculate the ratio between measured RMR and predicted RMR we used the Cunningham (1980) equation to predict what the subjects individual RMR should be: $500 + (22 \times$ FFM [kg]). The lowest obtained heart rate during the measurement of RMR was registered using a Polar V800 heart rate monitor.

BP was obtained using an electronic sphygmomanometer (Microlife BP A100, Widnau, Switzerland) immediately after measuring RMR. Systolic BP was measured three times in a resting supine position and orthostatic BP was measured three times in an upright position (the mean of the last two measures was used). Hypertension was defined as a systolic BP of >140mmHG and/or a diastolic BP of >90mmHG (Legemiddelhåndbok, 2013).

Blood sampling

After the subjects had fasted overnight, blood samples were drawn by a qualified nurse. Blood was drawn from a cephalic vein of the subjects in a sitting position, between seven and nine a.m. One 10 mL BD Vacutainer CAT (BD, Plymouth, United Kingdom) was filled and centrifuged after 30 minutes. Two 1.8 mL Cryotube Vials (Termo Fischer Science, Roskilde, Denmark) were filled with serum and frozen to -18 degrees Celsius. Blood from the cyclists was analysed at Hormonlabor

C831, Bern, Switzerland and blood from the runners was analysed at Sørlandets Hospital Arendal and Aker Hormonlab in Oslo. The blood was analysed for its content of glucose, cortisol, testosterone, T_3, IGF-1, total cholesterol (TC), high density-lipoprotein (HDL), low density-lipoprotein (LDL), triglycerides and insulin. Reference values of hormones and blood glucose was defined as follows (Laboratorium, 2016; Universitetssykehus, 2016): cortisol (138-690mmol/L); testosterone (18-40 y, 7.2-24 nmol/L; >41 y, 4.6-24 nmol/L); T_3 (1.2-2.7 nmol/L); IGF-1 (19-30 y, 17-63 nmol/L; 31-54 y, 11-40 nmol/L); TC (<5.0 mmol/L); HDL (0.8-2.1 mmol/L); LDL (<3.0 mmol/L); LDL/HCL ratio (<3.0); Triglycerides (<2.6 mmol/L); insulin (<160 pmol/L); glucose (4-6 mmol/L).

Bone health and body composition

BMD and body composition were obtained using Dual-energy X-ray absorptiometry (DXA) (GE-Lunar Prodigy, Madison, WI, USA) at Sørlandet Hospital. BMD was assessed in femur neck, total hip, lumbar spine (L1-L4) and total body, and the assessment was performed between 7 and 9 a.m. on fasting subjects who were in a resting position. The same technician performed all tests on the same scanner on all subjects. Normal BMD was classified as a z-score of higher than -1, low BMD was classified as a z-score of -1 to -2 in at least one of the measured sites and osteoporosis was classified as a z-score of -2 or lower (Nattiv et al., 2007).

Energy availability, energy intake and energy expenditure

Subjects registered energy intake (EI) during a three to four days period mirroring their typical food patterns and training regime. EI, daily energy expenditure (DEE) and exercise energy expenditure (EEE) were recorded and calculated to assess EA. Subjects weighed their food intake using a digital kitchen scale (Exido 246030 Kitchen Scale, Gothenburg, Sweden; OBH Nordica 9843 Kitchen Scale Color, Taastrup, Denmark). Subjects further logged their food records using software from Dietist Net (Dietist Net, Kost och Näringsdata, Bromma, Sweden) with access to the Norwegian food table and an open Norwegian nutritional information database. In-depth oral and written instructions were given to the subjects explaining how to weigh and register consumed food and beverages, and they were told to maintain a normal eating pattern during the registration period. The Goldberg cut-off (Black, 2000) was used to identify subjects who delivered food records of poor validity and to identify who would be excluded in the analysis.

EA was calculated by subtracting EEE from DEE and further calculated relative to FFM (Nattiv et al., 2007). In order not to overestimate EA, EEE only represented the energy attributable to training. RMR and NEAT were therefore subtracted from EEE before being used in the equation for EA.

To record DEE and EEE, subjects were told to use a combined HR monitor and activity tracker from Polar (V800/M400) during their registration period. The subjects wore the activity tracker from 00.00 a.m. on the first day to 00.00 p.m. on the last day in order to record activities such as sleeping, sitting and low, medium and high-intense activity as defined by Polar. To record EEE, the subjects were told to record all training using the HR sensor in combination with the HR monitor. EEE was calculated as the sum of the time spent in each HR zone described by Seiler and Tønnessen (2009) multiplied by metabolic equivalent (MET) value for the subjects' main type of activity based on Ainsworth et al. (2000). This was verified against their electronic training diary of Polar. DEE was calculated as the sum of the time spent in each classification zone defined by their activity monitor from Polar (resting, sitting, and low, medium and high activity). Resting was defined as 1 MET, sitting as 1.5 MET, low activity as 2.7 MET, medium activity as 4.7 MET and high activity as 6 MET. The values are the median of each zone defined by Polar. NEAT was calculated by the data from the activity tracker using the formula described by Levine (2004).

Statistics

All data were analysed using Statistical Package for the Social Sciences (SPSS) for Macintosh (v. 22; SPSS Inc., Chicago, IL, USA) and graphs using GraphPad Prism 7 for Macintosh (Graph Pad Software, Inc., 7825 Fay Avenue, La Jolla, CA 92037, USA). The dataset was controlled for missing data and signs of non-normality using histograms as reference. All data were non-normal distributed and are presented as median and interquartile range (25-75). Subjects were divided into two groups based on their current EA status: reduced EA <40 kcal/kgFFM/day and optimal EA $\geq$ 40 kcal/kgFFM/day based on Koehler et al. (2016). Mann-Whitney U-test was used to compare groups and correlation between variables was calculated using Spearman's rho (ρ). To identify possible associations, not predict, we used logistic regression between EA groups and selected health variables. Odds-ratio (with 95% confidence interval), *P*-value and Nagelkerke R Square were used to assess whether there was an association between reduced EA and the variable tested. An alpha-level of 0.01 was used, due to high numbers of tests performed. Effect size (ES) was calculated to interpret the meaningfulness of results and to identify trends, defined as a medium or large ES if comparisons are non-significant. Cohen (1988) criteria for ES were used (0.1 = small effect, 0.3 = medium effect, 0.5 = large effect).

Results

Overall, the participants in this study were a well-trained homogeneous group, with all anthropometric data within reference values, but three subjects had a fat-percentage in the lower reference value range (6-7%).

Energy availability

A total of nine subjects (33%) had optimal EA and 18 subjects (67%) had reduced EA. One subject with reduced EA had hypertension. Descriptive statistics for all subjects and classified according to current EA-status, are presented in Table 1. No differences between the groups were observed in terms of age, anthropometric data or aerobic capacity ($\dot{V}O_{2max}$, $L \cdot min^{-1}$ or $mL \cdot kg^{-1} \cdot min^{-1}$). Subjects with optimal EA tended to have competed in their sport for a longer period compared to subjects with reduced EA, but this was not statistically significant (P=0.07, ES=0.35). The reduced EA group had lower EI (16%) compared with the optimal EA group (P<0.001). Subjects with reduced EA had 28% lower EA compared to subjects with optimal EA (P<0.001). No difference was observed between the reduced EA group and the optimal EA group in DEE, NEAT or EEE (see Table 2).

Insert Table 1 here

Insert Table 2 here

Resting metabolic rate

There was no difference in mean RMR (kcal/day, kcal/kgFFM/day) between the groups, but we found a positive correlation between EA and RMR ratio (ρ=0.426, P=0.027). Subjects with reduced EA showed a trend of having a lower RMR ratio (P=0.026, ES=0.43) compared to subjects with optimal EA (see Figure 3).

A subgroup analysis show that seven subjects (26%), six with reduced EA and one with optimal EA, had a RMR ratio of ≤0.80. An additional six subjects (22%), three with reduced EA and three with optimal EA had a RMR ratio of ≥0.87. An analysis of subjects with a RMR ratio of ≤0.80 and a RMR ratio of ≥0.87 revealed no differences in hormonal biomarkers or anthropometric data between the groups. Subjects with a RMR ratio of ≥0.87 showed a trend of higher EI [3787 kcal (3414-4627) vs. 2734 kcal (2356-3000), P=0.015, ES=0.67] and DEE [4067kcal (3902-4338) vs. 3333 kcal (3077-3790), P=0.015, ES=0.67] compared to subjects with a RMR ratio of ≤0.80.

Insert Figure 3 here

Bone health and body composition

A total of six subjects (22%) had low BMD, where two of the subjects were close to being diagnosed with osteoporosis. The reduced EA group had higher BMD compared to the optimal EA group in femur neck (P=0.037, ES=0.40), total hip (P=0.057 ES=0.37), L1-L4 (P=0.10, ES=0.49) and total body (P=0.035, ES=0.41), but none of these was statistically significant (see Figure 4). Only BMD in L1-L4 was close to being statistically significant. In addition, we found a negative correlation between EA and BMD in L1-L4 (ρ=-0.553, *P*=0.003) and in total body (ρ=-0.405, *P*=0.036). No differences in body composition such as body fat or FFM between the EA-groups were found, nor any associations between reduced EA and body composition.

Subgroup analysis showed three subjects with optimal EA had low BMD, two subjects in L1-L4 and one subject in both L1-L4 and femur neck. Three subjects with reduced EA had low BMD, one subject in L1-L4, one subject in total hip and one subject in femur neck. No associations was found between reduced EA and bone health.

Insert Figure 4 here

Biomarkers for energy deficit

There were no statistically significant differences in hormonal biomarkers between subjects with reduced EA and subjects with optimal EA (see Table 3). Overall, subjects with reduced EA had 14% non-significant (*P*=0.094, ES=0.32) higher cortisol levels compared to subjects with optimal EA. Two subjects with reduced EA had elevated cortisol, where one approached levels of 900mmol/L. Subjects with reduced EA had a non-significant 16% higher IGF-1 levels compared to optimal EA, (*P*=0.076, ES=0.34), and all levels were within normal range. Nine subjects (33%), three with optimal EA and six with reduced EA had elevated TC levels. Twelve subjects (44%), three with optimal EA and nine with reduced EA, had elevated levels of LDL. There were no statistically significant associations between reduced EA and hormonal biomarkers (see Table 4, supporting information)

Insert Table 3 here

Discussion

To the best of the author's knowledge, this study is one of the first studies to examine the prevalence of reduced EA in well-trained male endurance athletes and its associations between reduced EA and selected health variables during a single week. The main findings of this study were that 67% of our subjects had reduced EA. This group showed signs of metabolic alterations such as lower RMR ratio compared to the optimal EA group, where six of the subjects in the reduced group had an RMR ratio of ≤0.80. Independent of EA status we observed elevated TC (n=9) and elevated LDL (n=12), despite subjects having a normal BMI, body composition and a generally active lifestyle with lots of exercise and movement on a daily basis (exercising on average 12 hours/week). We further found that 22% of the subjects (n=6) had low BMD, where two subjects were close to a state of osteoporosis. Finally no differences or associations between EA groups were observed in relation to biomarkers for energy deficit.

Energy availability

Methodological challenges in assessing EA exists. There are currently no recommended cut-off points for optimal, reduced or low EA among male endurance athletes, compared to female athletes for whom some recommendations exist (Mountjoy et al., 2014; Nattiv et al., 2007). For male athletes competing in team sports and ball games, however, research proposes that an EA of 40-60 kcal/kgFFM/day is adequate to maintain the health of the athletes (Tenforde et al., 2015). Only a few studies have looked at male athletes competing in endurance sports, where the use of different cut-off points regarding EA classifications occurs (Koehler et al., 2016; Viner et al., 2015). In this study, we chose to use a cut-off point of <40 kcal/kgFFM/day for reduced EA, based on the clinical study by Koehler et al. (2016), who reported that participants felt pressured to eat, had weight concerns and experienced bloating when controlling them for EA of ≥45 kcal/kgFFM/day. This was our rationale for using this cut-off, in combination with research suggesting that male reproductive costs are less compared to females (Bronson, 1985; Koehler et al., 2016) which, when combined with data from Loucks (2007) indicates that a habitual EA of exercising males is closer to 40 than 45 kcal/kgFFM/day. However, we did not chose a third category of low EA, as others have (Koehler et al., 2016; Viner et al., 2015), due to lack of scientific support for such cut-offs. We are aware, however, that we present data with only one cut-off, and are thereby putting all participants with an EA of <40kcal/kgFFM/day in the same group (ranging from 15 to 40 kcal/kgFFM/day) and have a low number of subjects participating in the study which may result in not being able to observe differences and associations between the tested variables and the groups. We have, however,

analysed subgroups in order to describe such and, to look for differences between high/low values in different variables.

One of the biggest challenges is the reporting of EI, where a change of eating habits, disordered eating and under-reporting of EI are vital and important issues to detect and address. Eight subjects in our study were identified reporting EI with low validity (under-reporting), and were therefore removed from the analysis. Few studies have tried to monitor under-reporting, but the study by Melin et al. (2015), showed some of the same trends in relation to under-reporting, but chose not to remove under-reporters from the analysis due to physiological symptoms of energy deficit. Other methodological factors are the assessment of DEE and EEE using a commercial product with no access to minute-by-minute data and are not validated. Unpublished data from our research group indicates, however, that the accelerometers used in this study are generally as good as other commercially available accelerometers.

The prevalence of reduced EA among male endurance athletes in our study seem to be high (67%). Few studies have investigated the prevalence of reduced EA in male athletes, but our findings are supported by the study of Viner et al. (2015), who also found a high prevalence (>70%) of low EA among male cyclists and Vogt et al. (2005), who found that male cyclists reported an EA as low as 8kcal/kgFFM/day. However, these studies reported much lower EA status (from 8 kcal/kgFFM/day to 27 kcal/kgFFM/day), compared to our study, which may be due to subjects being recruited came from different performance levels or data collection was performed during different periods of the competition season. Our findings also match other studies of female athletes, were Melin et al. (2015) found a prevalence of low and reduced EA of 63% in female endurance athletes and Hoch et al. (2009) found a prevalence of low EA of 36% in female athletes and 39% in sedentary controls. These findings indicate that reduced and low EA are present in both male and female athletes, but, it is still not known to the full extension how high and especially not the consequences of such in male endurance athletes. We found no difference between EA-groups in BMI or body composition, which may be caused by us measuring EA over a relative short period, thereby not reflecting a true long-term energy deficit. It could, however, also reflect energy preserving alterations, such as a reduced RMR: six subjects from the reduced EA group had an RMR ratio of ≤0.80, despite normal BMI and body composition (see Table 1). Other studies of female athletes have reported some of the same findings regarding RMR ratio, normal BMI and body composition (De Souza et al., 2008; Melin et al., 2015), which could indicate that our subjects are able to adapt to negative changes in energy status, thereby enhancing survival (Nattiv et al., 2007).

It is also debatable whether athletes or coaches have the right knowledge for understanding, dealing with and treating the potentially serious consequences of reduced EA. In our study we experienced a lack of such knowledge when several subjects (of which some had reduced EA) expressed that they believed having a normal and stable body weight equalized their energy balance, which explained why they were not worried about their energy status or possible health and performance consequences.

Resting metabolic rate

The optimal EA group had as expected a non-significant higher RMR compared to the reduced EA group, however, since RMR (kcal/day) are affected of both body size and body composition, such a value is difficult to compare between subjects. RMR ratio, however, which is the ratio between predicted and measured RMR is a better way of assessing how RMR potentially have changed in each subject (De Souza et al., 2008). RMR ratio is based on a theoretically calculated RMR, which is well investigated in athletes (Kim et al., 2015), and an objective measure of true RMR. In general, we found a positive correlation between EA and RMR ratio (ρ=0.426, P=0.027). Between EA groups a trend was observed (P=0.026, ES=0.43) of RMR ratio being lower in subjects with reduced EA compared to subjects with optimal EA, which could indicate that male endurance athletes are at risk of alterations in their RMR when exposed to reduced EA.

Uncertainty exist, at which cut-off low RMR ratio should be classified in male athletes, but the literature indicates that there is a probability of it being lower than the <0.90 cut-off for females (Bronson, 1985; Koehler et al., 2016). Our trend could therefore also be linked to the assumption that males have less reproductive costs compared to females, thereby requiring less energy (Bronson, 1985), and indicates that males theoretically should have a lower cut-off for RMR ratio compared to females. This however, would require more extensive research. An agreement on cut-off values could, on the other hand, offer a more practical and easy way of assessing EA and compare studies, due to the accuracy of RMR ratio compared to the problems of subjects providing data on poor validity on EI. RMR ratio could therefore potentially help researchers identify athletes with reduced or low EA. This will, however, require more and extensive research. Our findings might therefore indicate that energy deficit potentially can affect the RMR ratio of athletes, thereby to some extend indicating that metabolic alterations also occur in male athletes.

Bone health and body composition

In general, studies investigating the prevalence of low BMD in endurance athletes have reported much higher numbers than we found. Hind et al. (2006) found that 39% of their tested endurance runners had low BMD in L1-L4. Nichols and Rauh (2011) reported an alarmingly high prevalence of osteopenia or osteoporosis, by up to 90% of master cyclists, and Barry and Kohrt (2008) found that BMD decreased during a cycling season, and it was not possible to recover the lost BMD after the end of the season.

In our study, 22% of the subjects had low BMD. However this did not seem to be affected by their EA status, as other studies have shown (Ihle & Loucks, 2004; Loucks, 2007; Tenforde et al., 2015). Subjects in our study were cyclists and runners, sports that have shown no and even in some cases negative osteogenic effects on bones (Olmedillas et al., 2012). Athletes in this type of sport therefore cannot be expected to experience the same osteogenic effects as in other sports, such as gymnastics and soccer (Tenforde & Fredericson, 2011). This may be due to weight-bearing exercise benefitting from and accumulating and maintaining higher BMD of up to 15% and in addition higher peak bone mass (Tenforde & Fredericson, 2011). Studies of adolescent female runners (Barrack et al., 2010), male runners (Hind et al., 2006) and cyclists (Smathers et al., 2009) have implied that such athletes might have a greater risk of low BMD compared to untrained controls. Another study by Rector et al. (2008) has proposed that cyclists are more exposed to low BMD, particularly in L1-L4 than other low-impact sports such as long-distance running. In our study, we did not, on the other hand, find that cyclists were more exposed to low BMD in the lumbar spine compared to runners (two cyclists and two runners had low BMD in L1–L4).

Confounding factors that will affect bone health of athletes exist. Such factors are genetics, malnutrition and youth activities (Khan, 2001). For instance, Tveit et al. (2012) have shown in a 39-year prospective controlled study that males who are active in sports that emphasize a high BMD as a child will have higher BMD decades later, showing the importance of maintaining peak bone mass at an early stage. Our results on BMD seems therefore to be more affected of cycling being a non-weight bearing activity and running a low-impact activity than by the effect of EA status, and we can only speculate if our subjects with higher BMD most likely had a high peak bone mass as a child. Our subjects with optimal EA, who generally had lower BMD compared with the reduced EA group (see Figure 3), had all been active for a longer period (8.0 years vs. 2.8 years, P=0.07, ES=0.35) in a low-impact and non weight-bearing sport. In this short-term observational study, we did not find, that male athletes with reduced EA therefore are in a greater risk of deteriorating bone health such as other studies on females have shown (Melin et al., 2015; Tenforde & Fredericson, 2011).

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Biomarkers for energy deficit

It has been proposed earlier that validated biomarkers, e.g. hormonal status obtained from a blood sample, could be used as an easier and less time-consuming way of assessing energy deficiency compared to weighing, register and analysing energy intake (Melin et al., 2015; Mountjoy et al., 2014). Such biomarkers proposed for being linked to both the Triad in females and RED-S are for example leptin, T_3, growth-hormone, IGF-1 and cortisol (De Souza et al., 2008; Mountjoy et al., 2014) It should be noted, however, that most of this research is currently based on studies on females.

Almost all subjects in both groups had within reference values of hormonal biomarkers and we found no associations between reduced EA and hormonal biomarkers. Two of the subjects with reduced EA had, however, elevated levels of cortisol (<690 mmol/L) which can be an indication of more stress (McArdle et al., 2015). We found no difference between the EA-groups in levels of testosterone, T_3 and IGF-1. Such findings are supported by a recent study by Koehler et al. (2016), who investigated changes in metabolic hormones when male athletes were exposed to an EA status of 15 kcal/kgFFM/day and found no change in testosterone, T_3 or IGF-1. On the other hand, our findings are not directly supported in a study of male soldiers undergoing extreme energy deficit over a period of four weeks (Friedl et al., 2000). The researchers instead found a dramatic reduction in testosterone, T_3 and IGF-1, but almost all levels returned to normal after refeeding the soldiers. Another study of male soldiers by Kyrolainen et al. (2008) found that extreme energy deficit reduced insulin and testosterone levels in the short term, but these slowly recovered to normal levels when a deficit of <1000kcal/day was sustained. The soldiers' EA status was not reported in either study, however. In terms of biomarkers such as insulin and fasting glucose, we found no difference between our groups, which could indicate no serious disruption of energy and substrate homeostasis as otherwise reported (Koehler et al., 2016; Loucks & Thuma, 2003).

A further 33% of our subjects (n=9) had elevated TC (>5mmol/L), where 66% of the affected subjects had reduced EA. Several factors can affect high cholesterol, where genetics is the strongest but also food containing saturated fat and high levels of cholesterol influences TC (Mensink et al., 2003), such data, however, were unavailable for this project. An additional 44% of our subjects (n=12), where the majority (75%) had reduced EA, also had elevated levels of LDL (>3mmol/L), yet all subjects had within reference values of LDL/HDL ratio, due to the concurrently high HDL levels. No subjects reported having high levels of triglycerides. Despite such prevalence of both elevated TC and elevated LDL, we did not find associations between the groups on these two variables. It has been reported, however, that patients with anorexia nervosa can have high levels of TC, and negative

lipid profiles have been found in females with menstrual dysfunction (Rickenlund et al., 2005), however, such data was not available to this study. Causes of such negative lipid profile are reportedly due to an increase in inflammatory markers combined with menstrual dysfunction and oestrogen deficiency, but studies of the lipid profile of male athletes are few (Rickenlund et al., 2005). It is noteworthy, however, that such a high number of our subjects, who all lead a physically active lifestyle, which normally decreases the risk of cardiovascular disease, have such numbers of elevated TC and LDL.

Our findings could point in the direction, that a habitual EA of 15-40kcal/kgFFM/day is not sufficient to experience unhealthy changes in either the reproductive function or to experience changes in hormonal biomarkers over a short period. It could also point in the direction that the reproductive function or hormonal biomarkers may recover when a mild energy deficiency are sustained over time, such as Kyrolainen et al. (2008) found. This will require further research.

In conclusion we found a high prevalence of reduced EA accompanied by signs of metabolic alterations in this group of well-trained male endurance athletes. Contrary to our expectations we did not find that subjects with reduced EA had low BMD or any significant associations in hormonal biomarkers compared to subjects with optimal EA.

This could possibly be explained by the fact that 40 kcal/kgFFM/day is too high a cut-off for reduced EA, which may point in the direction that male athletes are more protected against alterations and negative associations in our tested health variable compared to females, such as Papageorgiou et al. (2015) also found. The fact however, that we found a high prevalence of reduced EA with signs of alterations in RMR, but otherwise no associations with hormonal biomarkers and bone health emphasizes the need for more research to fully understand EA implications on both health and performance variables in male endurance athletes. Longitudinal studies following different subjects at different levels (untrained, well-trained and elite) over a longer period than just three or four days are wanted to understand the long-term effect of reduced or low EA. Other variables such as ghrelin and leptin should also be included in future analysis, due to their role in regulating and suppressing appetite and body weight.

Strengths and limitations

This study involves research in a new area, where few studies have examined the prevalence of reduced EA and consequences for health variables. A strength of this study is, that we used objective measurements of bone health, resting metabolic rate, blood pressure, daily physical activity, exercise expenditure, energy intake and biomarkers for energy deficiency. This study is also one of the few to address the problem of under-reporting energy intake and eliminating this from the analysis, thus reducing the risk of a type-I error. A limitation of this study is its cross-sectional design, which is not able to detect or describe the outcome of reduced EA in male athletes in our tested health variables. When testing for such a large number of variables as we did, we are at risk of making a type I error. However we chose to set an alpha level at 0.01 to avoid such an error. By excluding as many participants as we did, our sample size, and the lopsided group size, make it difficult to gain enough statistical power, thereby increasing the risk of a type II error. In addition, we had no control group, thereby making it difficult to assess whether the prevalence of reduced EA is greater among well-trained endurance male cyclists and runners in Norway compared to a reference population.

Perspectives

This study is one of the first to assess the prevalence of reduced EA in well-trained male endurance cyclists and runners. The high numbers of athletes with reduced EA and that no alterations were observed in anthropometrical data, bone health and biomarkers for energy deficiency, emphasizes the need to conduct more research among this group of athletes, and to do further and more in-depth testing. The research should also include larger sample sizes along with the testing of health and performance variables, as outlined by Mountjoy et al. (2014). More research is also needed on the cut-offs for both reduced EA and low EA in order to assess whether males have the same risk as females or are at a lower risk of developing negative health and performance consequences as a result of their EA status. The findings in this study further emphasize and confirm the complexity of RED-S.

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Tables

Table 1. Descriptive statistics of whole group and by groups according to energy availability (EA) status.

	All (n=27)	Optimal EA (n=9)	Reduced EA (n=18)	*P*-value	Effect Size
Age (year)	40.0 (31.0-45.0)	45.0 (34.5-46.0)	37.0 (29.8-43.3)	0.135	0.29
Height (cm)	182.0 (175.0-185.0)	180.0 (178.5-182.8)	182.5 (175.0-188.0)	0.624	0.09
Weight (kg)	†76.5 (73.1-82.7)	†77.5 (75.2-82.0)	†76.5 (71.3-82.8)	0.700	0.07
BMI (kg/m^2)	23.5 (21.4-24.0)	23.5 (21.3-24.4)	23.4 (21.4-24.1)	0.738	0.06
Body fat (kg)	†10.7 (7.5-12.0)	†9.4 (7.5-12.2)	†10.8 (6.6-11.6)	1.000	0
Body fat (%)	†14.0 (10.0-16.5)	†11.8 (9.9-17.9)	†14.0 (9.8-16.6)	1.000	0
FFM (kg)	†64.7 (60.9-68.8)	†65.6 (60.9-69.9)	†63.4 (60.9-68.4)	0.440	0.15
Resting heart rate (beats/min)	43.0 (37.0-47.0)	45.0 (40.0-49.0)	42.0 (36.0-46.5)	0.303	0.20
BP systolic lying (mmHG)	118.5 (114.0-128.0)	118.5 (116.0-126.8)	119.3 (113.5-129.8)	0.719	0.07
BP diastolic lying (mmHG)	70.0 (65.0-77.0)	70.5 (65.8-78.8)	69.8 (64.8-77.8)	0.797	0.05
BP systolic standing (mmHG)	120.5 (116.5-132.0)	117.0 (113.0-131.0)	121.8 (118.9-133.6)	0.328	0.19
BP diastolic standing (mmHG)	80.5 (73.5-84.5)	80.5 (77.0-86.3)	80.8 (73.4-85.0)	0.699	0.07
Exercise (h/week)	12.0 (9.0-16.0)	11.0 (7.0-19.3)	13.5 (9.8-18.3)	0.246	0.22
Active in sport (years)	4.0 (2.0-10.0)	8.0 (4.5-10.0)	2.8 (1.8-6.3)	0.070	0.35
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	64.3 (60.2-67.6)	62.6 (60.1-66.0)	65.1 (60.2-68.6)	0.437	0.15
$\dot{V}O_{2max}$ (L·min^{-1})	4.95 (4.54-5.14)	4.89 (4.49-5.37)	4.98 (4.54-5.14)	0.849	0.04

Data are presented as median and interquartile range (25-75).

†=measured by DXA

BMI, body mass index; FFM, fat free mass; BP, blood pressure; $\dot{V}O_{2max}$, maximal oxygen uptake

Table 2. Energy intake, energy availability, energy expenditure and resting metabolic rate results of the whole group and by groups according to energy availability (EA) status.

	All (n=27)	Optimal EA (n=9)	Reduced EA (n=18)	*P*-value	Effect Size
Energy intake (kcal/day)	3252 (3000-3599)	3665 (3471-4328)	3066 (2726-3263)**	<0.001	0.75
EA (kcal/kgFFM/day)	37.2 (32.6-42.6)	48.6 (42.5-49.4)	35.0 (31.0-37.3)**	<0.001	0.80
Energy balance (%)	88.6 (82.1-97.5)	99.1 (96.2-111.1)	84.7 (74.8-89.2)**	<0.001	0.80
Daily EE (kcal/day)	3807 (3456-4021)	3813 (3374-4090)	3799 (3425-4024)	0.797	0.05
NEAT (kcal/day)	1419 (1143-1582)	1415 (1234-1641)	1423 (1113-1541)	0.643	0.09
EEE (kcal/day)	990 (781-1226)	990 (312-1380)	1011 (823-1144)	0.758	0.06
RMR (kcal/day)	1681 (1597-1740)	1695 (1608-1821)	1647 (1522-1725)	0.237	0.23
RMR (kcal/kgFFM/day)	25.9 (25.2-26.6)	26.2 (25.8-26.8)	25.7 (24.5-26.6)	0.217	0.24
RMRratio	0.84 (0.80-0.86)	0.86 (0.84-0.90)	0.83 (0.80-0.86)	0.026	0.43

Data are presented median and interquartile range (25-75).
EA, energy availability; energy balance, (energy intake/daily energy expenditure × 100); daily EE, daily energy expenditure; NEAT, non-exercise activity thermogenesis; EEE, exercise energy expenditure; RMR, resting metabolic rate; RMRratio, the ration between the predicted and measured RMR; FFM, fat-free mass.
**$P<0.001$

Table 3. Blood sample results of whole group and by groups according to energy availability (EA) status.

	All (n=27)	Optimal EA (n=9)	Reduced EA (n=18)	Normal range	*P*-value	Effect Size
Cortisol (mmol/L)	514 (440-587)	475 (375-574)	542 (462-610)	138-690	0.094	0.32
Testosterone (nmol/L)	22.0 (17.0-30.0)	22.0 (16.7-29.6)	22.6 (17.8-31.4)	4.6-24	0.625	0.09
T_3 (nmol/L)	1.77 (1.61-1.95)	1.75 (1.59-1.96)	1.78 (1.65-1.96)	1.2-2.7	0.746	0.06
IGF-1 (nmol/L)	21.2 (18.5-24.7)	18.6 (16.1-21.8)	21.6 (19.5-25.6)	11-63	0.076	0.34
TC (mmol/L)	4.71 (4.35-5.24)	4.57 (4.32-5.25)	4.85 (4.37-5.39)	<5.0	0.487	0.15
HDL (mmol/L)	1.69 (1.46-1.87)	1.67 (1.47-1.83)	1.71 (1.46-1.96)	0.8-2.1	0.877	0.03
LDL (mmol/L)	2.68 (2.51-3.35)	2.64 (2.48-3.36)	3.00 (2.57-3.39)	<3.0	0.537	0.12
LDL/HDL cholesterol ratio	1.81 (1.31-2.07)	1.71 (1.35-2.02)	1.83 (1.30-2.17)	<3.0	0.837	0.04
Triglyceride (mmol/L)	0.78 (0.62-1.07)	0.73 (0.59-1.06)	0.81 (0.61-1.11)	<2.6	0.520	0.12
Insulin (pmol/L)	30.9 (23.7-41.6)	30.9 (21.6-40.6)	29.5 (22.8-42.3)	<160	1.000	0
Fasting glucose (mmol/L)	4.95 (4.74-5.29)	4.86 (4.66-5.12)	4.97 (4.80-5.36)	4-6	0.607	0.10

Data are presented as median and interquartile range (25-75).

T_3, triiodothyronine; IGF-1, insulin like growth factor-1; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein. Normal range provided by analytic laboratory.

Figures
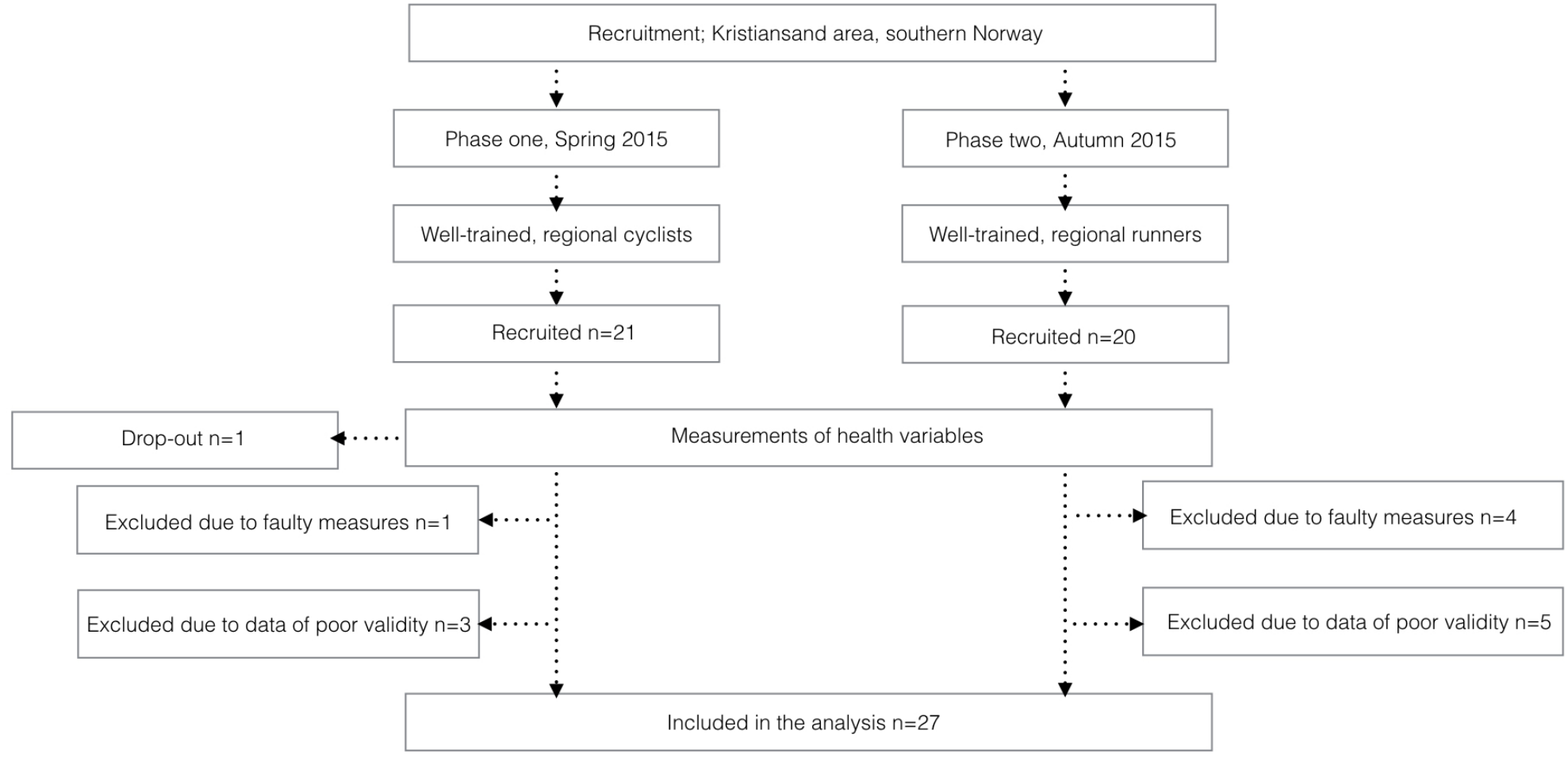
Figure 1. Flowchart recruitment and inclusion. The figure illustrate the recruiting process in two phases. 41 subjects participated. 1 cyclist dropped out for personal reasons. 5 subjects were excluded due to faulty measures (activity or energy intake issues) and 8 subjects were excluded for submitting poor-validity data on energy intake. A total of 27 subjects were included in the analysis.

Day 1: 10.00 a.m. - 09.00 p.m.

Height/weight measurement	30 min warm-up	VO2max

Day 2: 06.00 a.m. - 08.00 a.m.

Weight measurement	15 min. rest	30 min. RMR-test	Blood Pressure	Food/watch Registration	Questionnaire

Day 3: 07.00 a.m. - 09.00 a.m.

Blood sampling

Day 4: 07.00 a.m. - 09.00 a.m.

DXA scan

*Day «5-8» : 00.00 a.m. - 00.00 p.m.

Recording of energy intake (EI), daily energy expenditure (DEE) and exercise energy expenditure (EEE)

*Cyclist recorded 3 days of EI, DEE & EEE. Runners recorded 4 days.

Figure 2. Protocol. This figure illustrates the protocol used during data collection. Collection on days 1–3 took place at the university campus, on day 4 at Sørlandets Hospital and on days 5–8 in their home environment. The subjects arrived in fasting state on days 2–4. RMR; resting metabolic rate, measured using a ventilated hood; DXA, dual x-ray absorptiometry.
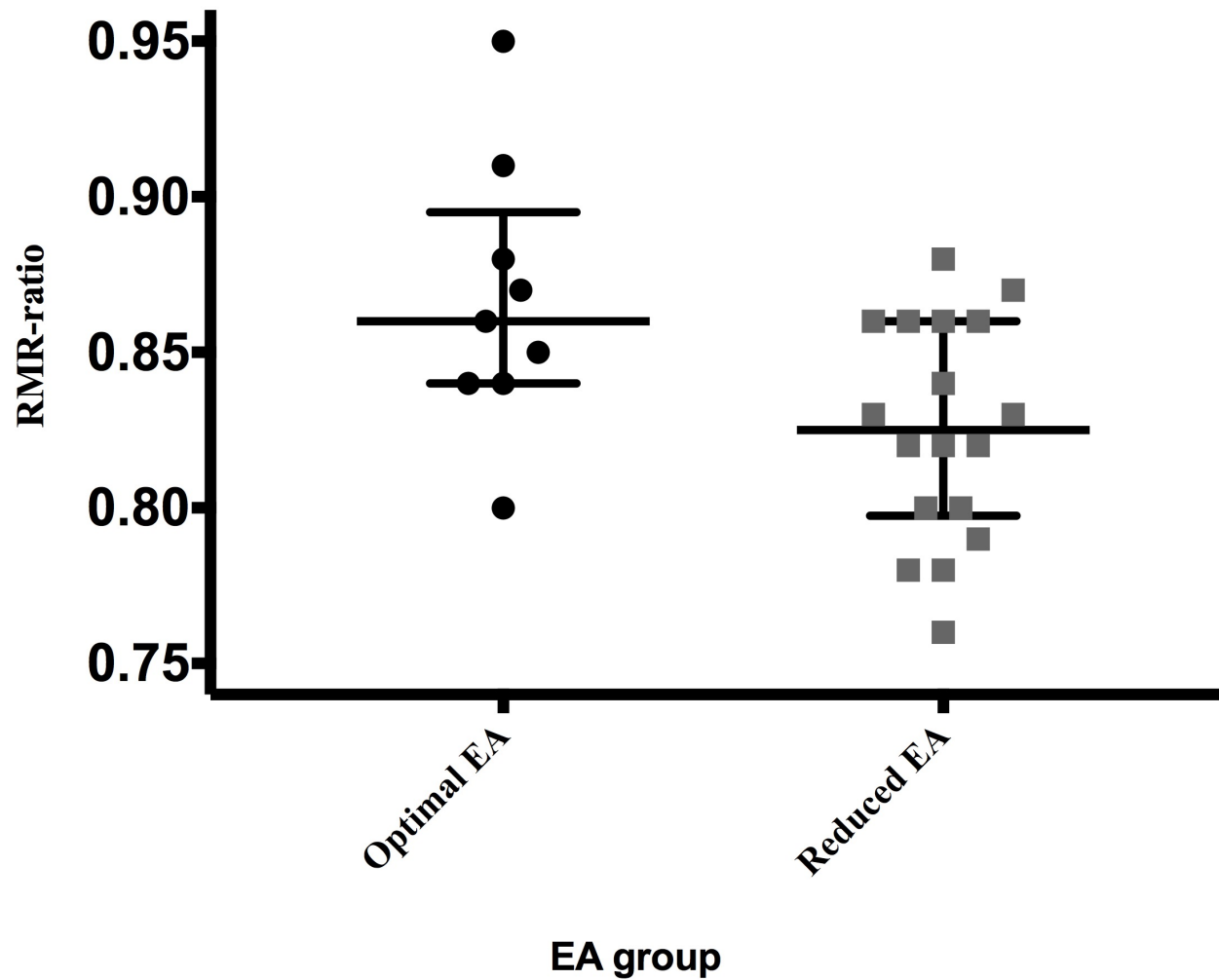
Figure 3. RMR ratio between groups. Data are presented as median and interquartile range (25-75). The reduced EA group shows a trend (P=0.026, ES=0.43) of a lower RMR ratio compared to the optimal EA group.
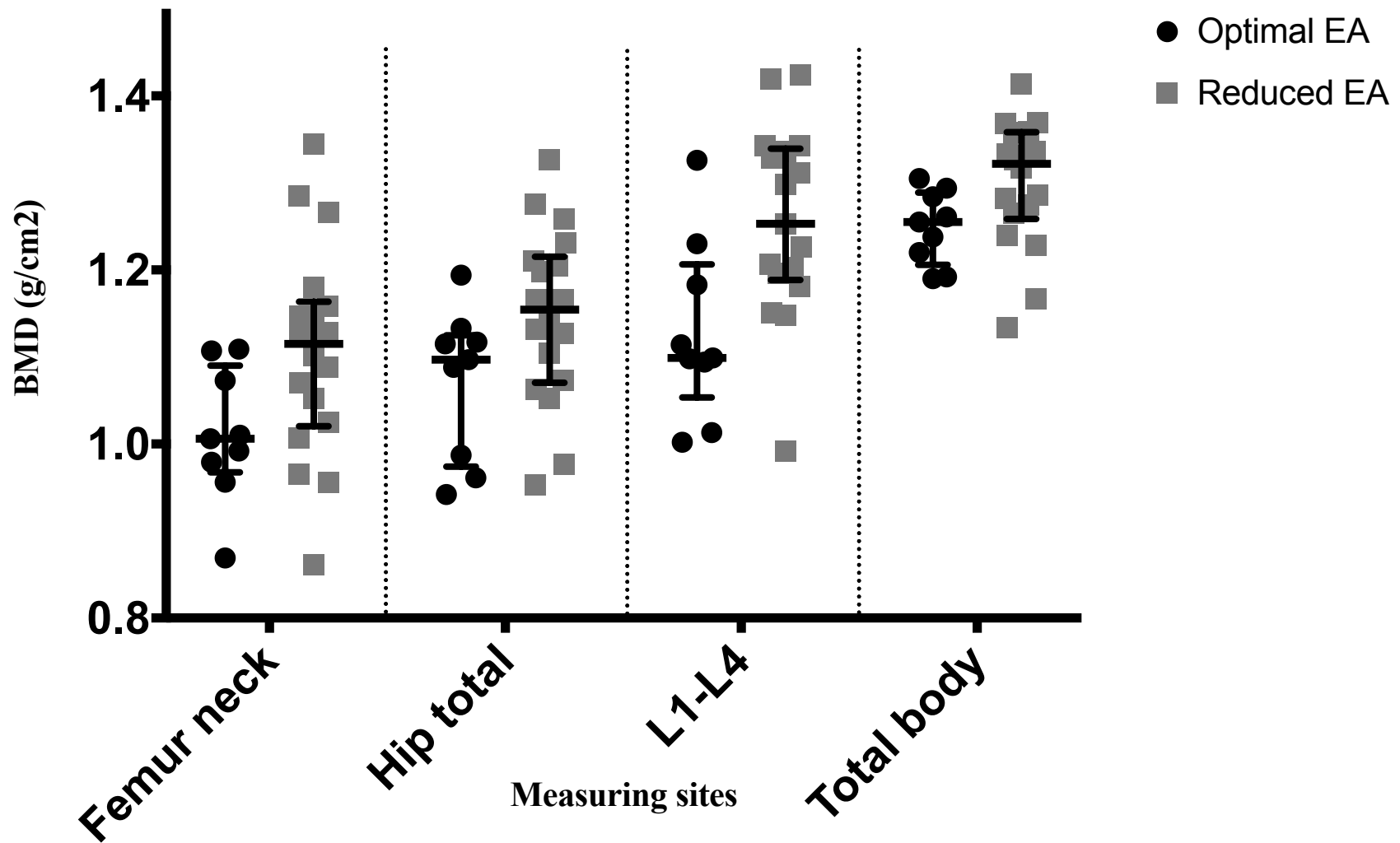
Figure 4. Measurement of BMD at different sites using dual x-ray absorptiometry. Data are presented as median and interquartile range (25-75). The reduced EA group show a trend of higher BMD in femur neck (*P*=0.037, ES=0.40), total hip (*P*=0.057, ES=0.37), L1-L4 (*P*=0.01, ES=0.49) and total body (*P*=0.035, ES=0.41)

Supporting information

Table 4. Possible associations between reduced EA and selected health-variables using logistic regression

	Variable	Odds ratio (95% CI)	P-value	Nagelkerke R square
Performance data	Exercise (h/week)	1.05 (0.93-1.18)	0.426	0.04
	Active in sport (years)	0.92 (0.79-1.07)	0.282	0.06
	$\dot{V}O_{2max}$ (L/min)	1.14 (0.18-7.14)	0.892	0.00
Biomarkers for energy deficit	Cortisol (mmol/L)	1.01 (1.00-1.02)	0.118	0.15
	Testosterone (nmol/L)	1.01 (0.90-1.14)	0.848	0.00
	T3 (nmol/L)	0.98 (0.03-34.89)	0.991	0.00
	IGF-1 (nmol/L)	1.20 (0.98-1.48)	0.084	0.18
	HDL (mmol/L)	1.33 (0.13-13.69)	0.812	0.00
	LDL (mmol/L)	1.24 (0.35-4.32)	0.740	0.01
	LDL/HDL cholesterol ratio	1.18 (0.24-5.74)	0.842	0.02
	Triglyceride (mmol/L)	2.32 (0.11-48.73)	0.589	0.02
	Insulin (pmol/L)	1.00 (0.94-1.01)	0.884	0.00
	Fasting glucose (mmol/L)	1.78 (0.19-16.61)	0.615	0.01
Energy expenditure	Daily EE (kcal/day)	1.00 (0.99-1.00)	0.784	0.00
	NEAT (kcal/day)	1.00 (0.99-1.00)	0.680	0.01
	EEE (kcal/day)	1.00 (0.99-1.00)	0.336	0.05
	RMR (kcal/day)	1.00 (0.99-1.00)	0.354	0.05
Bone health (Age-related Z-scores)	Femur neck mean	2.75 (0.81-9.36)	0.107	0.16
	Hip total mean	2.37 (0.71-7.88)	0.159	0.11
	Lumbar spine L1-L4	2.47 (0.97-6.29)	0.058	0.21
	Total body	2.84 (0.87-9.28)	0.084	0.17

Data are presented as an Odds ratio with a 95% confidence interval (CI), significance level and the Nagelkerke R squared model (indicating the amount of variation in the dependent variable)

Part 3:

Appendix

Contents:

Thomas Birkedal Stenqvist
University of Agder
May, 2016

Appendix 1

Estimating energy output

Subjects were equipped with a combined heart rate monitor and activity tracker, Polar V800/M400 (Polar Electro Oy, Finland) in order to record and analyse daily activity including training sessions and normal everyday activity and movement. The V800/M400 was at the time, Polar´s newest and most advanced training and activity trackers and uses a heart rate soft strap and a heart rate sensor (Polar Bluetooth Smart H7) to detect the heart rate during training. The sensor is strapped around the chest of the athlete, just below the chest muscles (Polar-Electro, 2015). One of the features of the V800/M400 is measurement of acceleration, where acceleration signals are filtered and classified to varies activity modes, calculated to metabolic equivalent (MET), kilocalories (Kcal) and steps (Virtanen, 2014). The V800/M400 uses a 3D digital acceleration sensor, register acceleration at 50 Hertz frequencies, and reported to be accurately calibrated to measure acceleration. The polar method uses frequency, intensity and regularity of movements as a key element (Virtanen, 2014). The V800/M400 uses MET to assess daily activity level. Polar expresses MET as an expression of energy expenditure and it´s multiples of resting metabolic rate (1 MET = BMR (app. 1kcal/kg/h)). When transforming this into energy expenditure the V800/M400 uses parameters as age, gender and weight. The V800/M400 analyses the MET data in 30 seconds periods using 1-5 minute's time window. The activity detected by the V800/M400 is divided into 6 categories; low activity, medium activity, high activity, sitting, rest/sleep and non-wear (Virtanen, 2014). The METs for acquiring activity is as following; Low: 1.8-3.5 MET, Medium: 3.5-6 MET, High: >6 MET (also see Figure 1), but the limits within these categories will vary according to personal data as mentioned above (Virtanen, 2014).

Polar
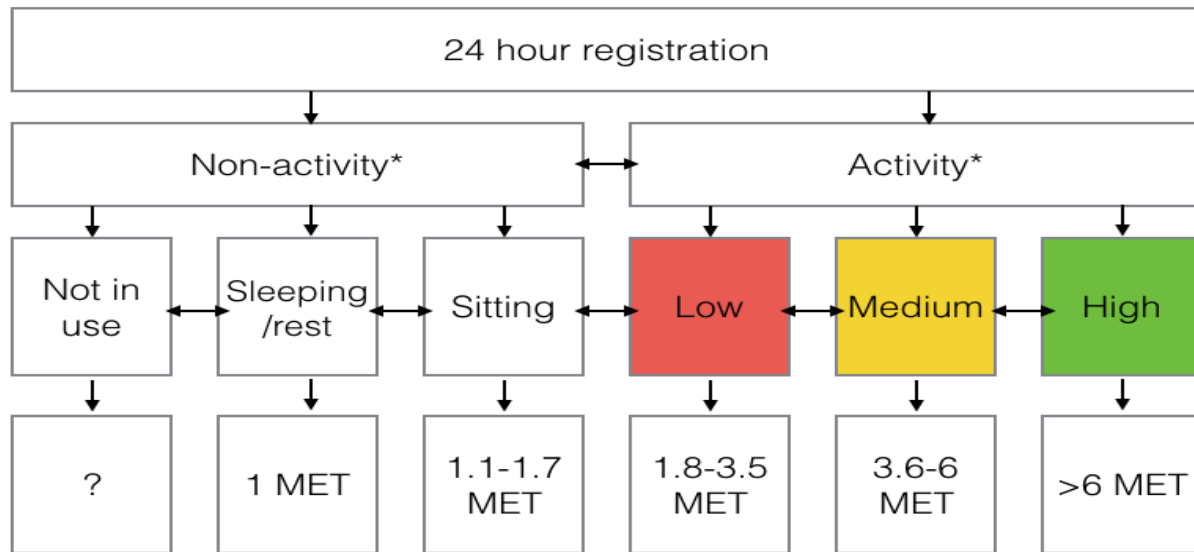

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┌─────────────────────────────────────────────────────────────┐
│                    24 hour registration                      │
└─────────────────────────────────────────────────────────────┘
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Figure 1. Polar V800/M400 method of categorizing and calculating energy expenditure within each sub-classification of activity. Due to lack of minute-by-minute data, it is not known to the researchers how and when the activity tracker classifies different activities and at which MET.

To calculate one MET, Polar uses the Schofield algorithm, including age, height and weight, which allow the V800/M400 to adjust the resting metabolic rate (RMR) according to the age of the person, wearing it. Polar furthermore developed their calorie calculation using indirect calorimetric (cardiopulmonary gas exchange analysis) in short performances less than two hours. For longer performances Polar used the doubly labeled water (DLW) method for the measurement of energy expenditure as a reference for measuring. Polar refers to a correlation coefficient of 0.86-0.89 regarding daily energy expenditure (DEE) (Virtanen, 2014).

Our calculations of daily energy expenditure using Polars activity tracker

Based on the measurements by the activity tracker, the daily activity and exercise of the athletes were divided into three main categories: "Daily activity", "exercise" and "not in use". The daily activity was furthermore divided into five predefined sub classifications as described earlier by Polar (see Table 1). We defined "Rest" as 1 MET based on Ainsworth et al. (2000) and Virtanen (2014). The 1.5-MET definition for "sitting" was chosen by us based on Mansoubi et al. (2015). When the activity tracker for some reason was "not in use", we defined this as 1.5 MET, in order not to overestimate DEE if for some reason the athletes did anything else than sitting while not wearing the activity tracker. The MET in the predefined classifications ("low" and "medium) were furthermore

defined by us as the median of the predefined MET range by Polar (low=1.8-3.5 MET´s (median=2.7); Medium 3.5-6 MET (median=4.7); high >6 MET (6). This estimate was done since there is no access to the raw data file from the activity tracker, and it is therefore difficult to quantify the activity into various MET classifications. Since Polar defines "high" activity as activity >6 MET, this study uses 6 MET as a reference to this activity level, and furthermore calculated exercise at various intensity zones into this activity level and subtracting the exercise time from the "high" time. By recording all exercise using the heart rate monitor, the exact time spent in the different intensity zones during exercise was obtained (see Table 2 for complete calculation of DEE). The values chosen for the exercise levels at the different intensity zones was defined with the use of Ainsworth et al. (2000) for both cycling and running.

Table 1. Different classifications and sub-classifications of activity and corresponding MET values

Activity classification	Activity type	MET used to calculate energy expenditure
Daily activity	Rest	1
	Sitting	1.5
	Low	2.7
	Medium	4.7
	High	6
Exercise	< Intensity zone 1	6
	Intensity zone 1	7
	Intensity zone 2	10.5
	> Intensity zone 3	14
Activity tracker not in use		1.5

The athletes were told to wear their activity tracker 24 hours/day for either three or four days

In order to calculate individual intensity zones and thereby estimating the exercise energy expenditures (EEE), the calculations of intensity zones were based on the HR_{peak} the athletes achieved during the incremental maximal oxygen uptake $\dot{V}O_{2max}$ test in the lab. The zones where based on the "five zone intensity scale" used to assess training of well-trained endurance athletes (Seiler & Tønnessen, 2009) developed by the Norwegian Olympic Federation.

Based on these HR-zones and intensity zones, the MET´s at different intensity zones where defined using Ainsworth et al. (2000) as reference for both runners and cyclists. The bodyweight in kg (BW) of the athlete was used to calculate the DEE in each sub-category using the formula:

$$DEE = (\frac{BW * MET}{60}) * \min$$

Where "MET" is the selected MET value for intensity and "min" is the time (in minutes) in each zone. We further evaluated the difference in the measured RMR and estimated RMR and adjusted the energy expenditure according to this difference using the Schofield algorithm (Kim, Kim, Kim, Park, & Kim, 2015)

Table 2. Example of calculation of daily energy expenditure

Day 1					
Bodyweight: 76.2		MET	Minutes	Minutes correction	Kcal
Daily act	Rest	1	440		558,8
	Sitting	1,5	506		963,9
Training	<Z1	6	32,68		249
	Zone 1	7	90,25		802,3
	Zone 2	10,5	46,53		620,5
	>Zone 3	14	1,53		27,3
	Total		171		
Daily act	Low	2,7	262		898,4
	Medium	4,7	69	60.00 (69-9)	358,1
	High	6	162	-9.00 (162-171)	
Not in use		1,5	1		1,9
RMR adjustment; measured RMR minus Schofield					-148,5
Daily EE					**4331,8**
V800 EE					4039
Difference					-292,8

The table outlines how daily energy expenditure is calculated in each category, added up and adjusted for the measured resting metabolic rate. This person has a DEE of 4331.8 kcal, after we adjusted the RMR values based on the measured RMR. In this case the V800 underestimated DEE by 293kcal.

The DEE for this day (see Table 2) was estimated to be 4431.8 kcal, which was 292.8 kcal higher than the activity tracker estimated. The activity tracker in this case is therefor underestimating with about 7% compared to our calculations.

Calculation of RMR and RMR-ratio

Measured RMR in the laboratory was calculated as a mean of the last 20 minutes of the measurement, using VO_2 and VCO_2 minute by minute. These values and the Weir (1990) equation:

$RMR = 3.94 * (\dot{V}O_2) + 1.1 * (\dot{V}CO_2) * 1.44$ was used to calculate the measured RMR of the athletes. The Cunningham (1980) equation calculates the predicted RMR as following: RMR=500+22(LBM) where as RMR-ratio is calculated as following:

$$RMR_{ratio} = \frac{RMR_{measured}}{RMR_{predicted}}$$

Calculation of Goldberg cut-off for underreporting energy intake

The Goldberg-cut-off for underreporting of energy intake was calculates as described by Black (2000). Our calculation in short term is listed below, and was further confirmed by a mathematician.

Lower bound: $\qquad EI_{rep}: BMR > 1{,}87 \times e^{\left(-2 \times \frac{\left(\frac{22{,}75}{100}\right)}{\sqrt{35}}\right)} = 1{,}73$ PAL

Upper bound: $\qquad EI_{rep}: BMR > 1{,}87 \times e^{\left(2 \times \frac{\left(\frac{22{,}75}{100}\right)}{\sqrt{35}}\right)} = 2{,}01$ PAL

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Appendix 2

Vi søker deltakere til et spennende forskningsprosjekt på prestasjonsutvikling!

Vi er interesserte i Sørlandets 20 beste mannlige langdistanseløpere – er du en av dem?

Vi gjennomførte våren 2015 testing av 20 mannlige godt trente syklister og er nå på jakt etter langdistanseløpere på samme nivå. Forskningsprosjektet heter *Energitilgjengelighet, helse og prestasjon* hvor hensikten er å kartlegge energitilgjengelighet, helsevariabler og prestasjonsvariabler blant godt trente langdistanseløpere. Som deltaker vil du bli bedt om å gjennomføre en maksimal oksygenopptakstest, en spensttest og en helsekartlegging, samt registrere dine kostholds- og aktivitetsvaner. Du vil selvsagt få alle dine resultater inkludert måling av kroppssammensetning kostnadsfritt. Vi ønsker å inkludere mannlige langdistanseløpere i alderen 18-50 år som har drevet aktivt med løping i minst 3 år og som per i dag trener 5 økter per uke eller mer.

Er du interessert? Send en mail, sms eller ring snarest og senest innen 15. oktober til testansvarlig Thomas Stenqvist; thoms14@uia.no tlf: xxx xx xxx. Aktuelle deltakere vil få tilbud om en maksimal oksygenopptakstest på arbeidsfysiologisk laboratorium på UIA i uke 44. Deretter vil det gjøres en vurdering hvem som får bli med videre i prosjektet. Vi ser frem til å høre fra deg!!

Appendix 3

Norsk samfunnsvitenskapelig datatjeneste AS
NORWEGIAN SOCIAL SCIENCE DATA SERVICES

Monica Klungland Torstveit
Institutt for folkehelse, idrett og ernæring Universitetet i Agder
Serviceboks 422
4604 KRISTIANSAND S

Vår dato: 10.02.2016   Vår ref: 46706 / 3 / MSS   Deres dato: Deres ref:

TILBAKEMELDING PÅ MELDING OM BEHANDLING AV PERSONOPPLYSNINGER

Vi viser til melding om behandling av personopplysninger, mottatt 19.01.2016. Meldingen gjelder prosjektet:

46706	*Energitilgjengelighet, helse og prestasjon*
	Hovedhensikt: *Kartlegging av energitilgjengelighet blant godt trente mannlige langdistanseløpere og undersøke om det er en sammenheng med ulike helse- og prestasjonsvariabler.*
Behandlingsansvarlig	*Universitetet i Agder, ved institusjonens øverste leder*
Daglig ansvarlig	*Monica Klungland Torstveit*
Student	*Thomas Stenqvist*

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, ombudets kommentarer samt personopplysningsloven og helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema, http://www.nsd.uib.no/personvern/meldeplikt/skjema.html. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database, http://pvo.nsd.no/prosjekt.

Personvernombudet vil ved prosjektets avslutning, 01.10.2019, rette en henvendelse angående status for behandlingen av personopplysninger.

Vennlig hilsen

Katrine Utaaker Segadal

Marie Strand Schildmann

Dokumentet er elektronisk produsert og godkjent ved NSDs rutiner for elektronisk godkjenning.

Appendix 4

Informasjon og forespørsel om deltakelse i forskningsstudien

«Energitilgjengelighet, helse og prestasjon»

Informasjon til forsøkspersoner

Vi vil med dette skriv informere og spørre om du vil delta i et forskningsprosjekt kalt *Energitilgjengelighet, helse og prestasjon* hvor hensikten er å kartlegge energitilgjengelighet, helsevariabler og beinmasse blant godt trente langdistanseløpere. Som deltaker vil du bli bedt om å gjennomføre noen prestasjonstester og en helsekartlegging, samt registrere dine kostholdsvaner. I det følgende gis mer detaljert informasjon.

Bakgrunn og hensikt

Tilstrekkelig energitilgjengelighet (samsvar mellom energiinntak og energiforbruk) er viktig både for god helse og god prestasjon for idrettsutøvere. Lav energitilgjengelighet (for lite energiinntak/mat i forhold til energiforbruket/trening) innebærer at den mengde energi som er tilgjengelig for kroppens fysiologiske funksjoner blir utilstrekkelig. Dette er vist å ha sammenheng med lavt energistoffskifte, lav kroppstemperatur, mage- og tarm problemer, reduserte nivåer av kjønnshormoner, økt risiko for skader og sykdom samt redusert beinmasse i skjelettet blant kvinnelige utøvere. Det er noen funn som tyder på at liknende sammenhenger også er tilstede blant mannlige utøvere, men dette er mindre undersøkt sammenlignet med kvinnelige utøvere. Utøvere i krevende utholdenhetsidretter (som langdistanseløpere, triatleter og syklister) er vist å være i ekstra risiko for lav energitilgjengelighet, lave testosteronverdier og lav beinmasse. Våren 2015 undersøkte vi de nevnte variabler blant mannlige godt trente syklister og i høst ønsker vi å undersøke tilsvarende blant mannlige godt trente langdistanseløpere. Hovedhensikten er å undersøke grad av energitilgjengelighet og faktorer som kan assosieres med dette.

Målinger

Ved å takke ja til deltakelse i denne studien vil du bli bedt om å møte i laboratoriet for å måle maksimalt oksygenopptak og spenst, kroppssammensetning (fordeling av fettvev og muskelvev), hvilestoffskiftet, blodtrykk, utvalgte blodparametre (som stresshormoner, kjønnshormoner, lipidprofil), samt besvare et spørreskjema. Du vil også bli bedt om å måle kroppssammensetningen din med en gullstandard målemetode på sykehuset (DXA) hvor detaljer knyttet til beinhelsen din blir målt (styrken på ulike deler av skjelettet ditt) (se detaljer om målingene i vedlegget). Videre vil du bli bedt om å registrere kostholdet og aktivitetsnivået ditt i 4 dager. All kostregistrering gjøres elektronisk via PC eller Mac med et kostholdsprogram som også benyttes av Olympiatoppen. Du vil få låne en vekt hvor du skal veie all mat du inntar disse 4 dagene. Oppsummert består testbatteriet av følgende:

1. Møte på laboratoriet for måling av maksimalt oksygenopptak og spenst (ca. 45 minutter) (gjerne kveldstid)
2. Møte fastende på laboratoriet for måling av hvilestoffskifte og helseparametre (ca. 75 min) (tidlig morgen)
3. Møte fastende på sykehuset for DXA måling (ca. 15 min) (tidlig morgen)

4. Registrere kostholdet ditt i 4 dager og bruke puls/aktivitetsmåler samme 4 dager

Fordeler og ulemper ved å delta

Som deltaker i studien bidrar du til å skaffe kunnskap for å utvikle toppidretten i samarbeid med Olympiatoppen. Du vil få kartlagt din energitilgjengelighet med muligheter for tilbakemelding på kostholdet ditt og utvalgte helsevariabler. Du vil også få målt ditt hvilestoffskifte som sier noe om din forbrenning i hvile. Videre vil du få måle din kroppssammensetning med gullstandard målemetode (DXA) med detaljerte opplysninger om din fett-, muskel- og beinmasse. Alle målinger er kostnadsfrie for deg. Eventuelle ulemper er at du må kartlegge kostholdet ditt i 4 dager og ha på aktivitetsmåler (pulsklokke) samme 4 dager, møte på laboratoriet til testing av maksimalt oksygenopptak/spenst en gang og til helseprofilvurdering en gang, samt måle kroppssammensetningen din på Sørlandet sykehus 1 gang (tar ca. 15 min).

Hva skjer med testresultater, prøver og informasjonen om deg?

Alle testresultater, prøver og informasjon som registreres på deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet anonymt. En kode knytter deg til dine opplysninger og testresultater gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse

Det er frivillig å delta i denne forskningsstudien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige deltakelse. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte ansvarlige for studien (se info nederst på arket).

Prosjektleder for studien er Monica Klungland Torstveit, førsteamanuensis i idrettsvitenskap ved fakultet for helse- og idrettsvitenskap, Universitetet i Agder. Testleder i laboratoriet er Thomas Stenqvist, vitenskapelig assistent og masterstudent i idrettsvitenskap. *Du samtykker til deltakelse ved å fylle ut samtykkeskjemaet på siste siden i dette informasjonsbrevet. Dette leveres til en av testlederne når du ankommer til første test.*

VEDLEGG: Ytterligere informasjon om målemetodene

Dag 1 i laboratoriet: Måling av maksimalt oksygenopptak og spenst (ca 40 minutter totalt)

Det vil gjennomføres en standardisert test av maksimalt oksygenopptak ($\dot{V}O_{2max}$ test) på tredemølle i laboratoriet. Etter en kontrollert oppvarming på ca 10 minutter vil nødvendig målingsutstyr ble tatt på og selve testen til utmattelse bli gjennomført. Arbeidets varighet ligger normalt i området 4 til 10 minutter. Oksygenopptak og hjertefrekvens samt andre variabler måles kontinuerlig gjennom hele testen.

Du vil også bli bedt om å gjennomføre en enkel spensttest på kraftplatform (counter movement jump og squat jump).

Retningslinjer før måling

- *De siste 48 timer før testdagen må du ikke utføre intensiv eller utmattende trening/konkurranser eller drikke alkohol.*
- *De siste tre timer før testene må du ikke drikke te, kaffe eller annen koffeinholdig drikke.*
- *Du har ikke tillatelse til å spise den siste time før testene.*

Dag 2 i laboratoriet: Måling av kroppssammensetning, hvilestoffskiftet, blodtrykk og blodparametere (fastende) (ca. 75 minutter totalt):

Ved ankomst i laboratoriet ønsker vi aller først å måle kroppssammensetningen ved en Inbody test (estimerer mengden kroppsfett, fettfri masse og beinvev, se bilde). Vi måler høyden deres og selve testen tar omtrent 1 minutt. Målingen medfører ingen smerte eller ubehag.
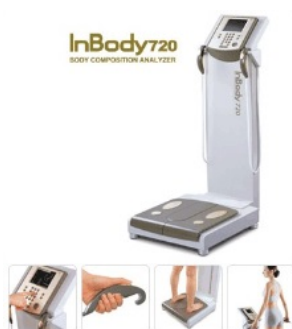
Energitilgjengelighet er den mengden av energi som er igjen til alle andre funksjoner i kroppen etter at energikostnaden ved trening er trukket fra. For å kunne måle energitilgjengelighet må vi estimere energiforbruk ved trening og fysisk aktivitet (som dere gjør ved hjelp av pulsklokkene og treningsdagbøkene), energiinntak (som dere estimerer via kostholdsregistreringen), fettfri masse (som vi måler ved hjelp av Inbody 720) og til slutt måling av hvilestoffskiftet. Vi vet at det kan være store forskjeller i hvilestoffskiftet mellom individer og de aller færreste vet hvor mye energi de bruker i hvile da målemetodene sjelden er tilgjengelig.

Som forsøksperson skal du ligge på en benk i ca 30 minutter med en «hette» (som vist på bildet). Målingen medfører ingen smerte eller ubehag. Hvilepulsen vil bli registrert og vi vil se til at du ikke sovner underveis i målingen.

Mens dere ligger på benken vil vi også måle blodtrykket liggende og deretter i stående posisjon. Dere vil så bli bedt om å ta en blodprøve for å analysere enkelte hormoner og kostfaktorer. Dette vil kun ta få minutter. Avslutningsvis bes dere besvare noen få spørsmål på et spørreskjema før dere kan reise hjem.

Retningslinjer før måling

- *Dagen før måling må du trene maksimalt 60 minutter med <u>lav eller moderat intensitet</u> (dette inkluderer både kondisjons- og styrketrening) og ingen trening siste 12 timer før måling*
- *Du må ikke spise eller drikke (annet enn vann) siste 12 timer før måling. Målingen skal altså utføres om morgenen før frokost («fastende»)*
- *Det er ikke tillatt å røyke, snuse eller innta alkohol de siste 12 timene før målingen*
- *Vi ber deg om å anstrenge deg så lite som mulig på morgenen. Det er derfor ønskelig at du kjører eller reiser kollektivt, ikke sykler eller går til Spicheren (hvis dette er umulig vil vi forsøke å hente deg). Du må heller ikke bære tungt eller anstrenge deg på annet vis før målingen.*

Dag 3 på SSHF: Måling av beinmineraltetthet (ca. 15 minutter)

DXA (dobbel rønten absorpsjonsmetri) er gullstandard måling for vurdering av din kroppssammensetning. Ved DXA måling vil du foruten å få målt muskelmasse og fettmasse også få målt din beinmineraltetthet (indikator på hvor sterkt skjelettet ditt er). Du vil få resultater både totalt for hele kroppen, men også i spesielt interessante områder som rygg og hofter. Selve målingen er helt smertefri og gjennomføres fullt påkledd ved å ligge på en benk/seng. Det vil kun ta ca. 15 minutter å gjennomføre målingen.

Retningslinjer før måling

- *Du må være fastende. Det vil si at du kan drikke vann om morgenen, men ikke kaffe/te eller spise frokost*
- *Du må ikke trene om morgenen før målingen*
- *Unngå hvis mulig å ha metall på deg, som smykker, piercing og knapper* l DXA *målingen)*

Gjøres «hjemme»: Registrering av kostholds- og aktivitetsvaner

Prinsippet i kostregistreringen er å beskrive når, hva og hvor mye du spiser og drikker så presist som mulig. Vi benytter et kostanalyseprogram som også benyttes av Olympiatoppen hvor vi kan få detaljerte opplysninger om eksempelvis hvilke næringsstoffer du inntar tilstrekkelig av og om du har eventuelle mangler knyttet til kostholdet ditt. Du vil få låne en kjøkkenvekt slik at du kan veie matvarene. På denne måten får vi nøyaktige data til å beregne blant annet energitilgjengelighet. Du vil måtte registrere kostholdet ditt to helgedager og to hverdager, aller helst en lørdag, søndag, mandag og tirsdag. Nødvendig veiledning vil bli gitt i forkant. Samme dager som du registrerer kostholdet ditt må du også ha på deg en aktivitetsmåler (eks Polar V 800 pulsklokke). Pulsbeltet må benyttes på alle treningsøkter disse fire dagene og du må føre treningsdagbok. Dette gjøres for at vi så nøyaktig som mulig skal kunne kartlegge energiforbruket til deltakerne. Dersom du ikke selv har egnet pulsklokke vil du kunne låne dette i de fire dagene registreringen foregår.

Laboratoriemålingene dag 1 og dag 2 gjennomføres i 2. etasje på Spicheren (arbeidsfysiologisk testlab) (fortell i resepsjonen at du skal testes i prosjektet og de vil vise deg vei). Er det noe du lurer på? Kontakt Monica eller Thomas (kontaktinfo nederst på siden).

Vel møtt!

Appendix 5

Samtykke til deltakelse i studien

«Energitilgjengelighet, helse og prestasjon»

Ved å si ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert på deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Ved å signere samtykkeerklæringen bekrefter du også at du ikke har kjent hjertesykdom eller andre lidelser/sykdom som medfører at din fastlege har frarådet deg å trene intensivt. Alle deltakere i studien er for øvrig forsikret via UIAs egen forsikringsordning for forskningsprosjekter.

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix 6

KOSTHOLDSREGISTRERING
Prinsippet med kostholdsregistreringen er at du <u>så nøyaktig som mulig</u> skal beskrive <u>når</u>, <u>hva</u> og <u>hvor mye</u> du spiser og drikker.

Ved hjelp av et elektronisk registreringsprogram kan alle disse opplysninger legges inn via din PC eller MAC (dessverre ennå ikke tilgjengelig på nettbrett eller smartphone).

Du vil få opplæring i bruk av programmet og det er pedagogisk fint oppbygget. For at informasjonen skal bli nøyaktig nok til bruk i forskning ber vi deg om å veie all mat/drikke du inntar. Du vil få utlevert en egen elektronisk kjøkkenvekt som du kan låne i registreringsperioden. Vi anbefaler å ha vekten tilgjengelig i løpet av dagen. For å gjøre det lettere for deg selv er det imidlertid en mulighet å veie mat/drikke du skal ha med ut av huset om morgenen, før du drar avgårde (eks. matpakke, mellommåltider som frukt, grønnsaker etc).

Som et <u>hjelpemiddel</u> i løpet av dagen kan du eventuelt notere alt ned på papirskjema og deretter føre informasjonen inn elektronisk ved anledning. Det er viktig at du har skjemaet/PC/Mac tilgjengelig ved hvert måltid slik at registreringen blir mest mulig reell.

På skjemaene skal du notere alt hva du spiser og drikker (**også vann fra kranen**) for hvert måltid eller mellommåltid eller "snacksing". Begynn hver dag med et nytt skjema. Notér kun én mat- eller drikkevare på hver linje. Det er svært viktig at **du spiser og drikker, som du pleier** og registrerer alt. Husk derfor å få med alle mellommåltider, snop og drikkevarer (som vann fra kranen, vann fra flaske, kaffe og te, vin, øl og sprit, juice, saft og mineralvann).

Slik fyller du ut registreringsskjemaet i papirform dersom du ønsker å bruke dette som et hjelpemiddel:

Kolonne 1: Noter **tidspunktet** hver gang du spiser og drikker noe.
Kolonne 2: Noter så nøyaktig som mulig **hva** du spiser og drikker.
Kolonne 3: Noter **hvordan** maten er tilberedt R=rå, K=kokt, S=stekt, B=bakt
Kolonne 4: Notere så nøyaktig som mulig, **hvor mye** du spiser og drikker. Angi mengdene i gram.
Benytt den utleverte kjøkkenvekt og husk å nullstille den før veiing. Har du ikke mulighet for å veie, angi mengden i kopper, skjeer og i porsjonsstørrelser som liten, stor, tykk eller tynn skive etc.
Dessuten skal du notere hvor mye du spiser av retten (se eksemplet på neste side).
Ved hjemmelaget mat skal du helst registrere oppskriften med mengdene av de forskjellige ingredienser som inngår i oppskriften. Husk å veie hver enkelt ingredisens på vekten og noter resultatet.

VEILEDNING TIL KJØKKENVEKT
Når du kostregistrerer, er det viktig at du får veid all mat og drikke du inntar (inkludert vann!).
Du bruker vekten på følgende måte:
- Sett vekten på et flatt/hardt underlag
- Start vekten ved å trykke på knappen ON/TARE
- Vent til displayet viser 0

Hvis du vil veie flere ting etterhverandre:
- Skal du veie matvarene f.eks i en skål, nullstill så vekten på ON/TARE etter at du har satt den tomme skålen på vekten
- Etter hver enkelt matvare nullstiller du så vekten hvor du deretter legger neste matvare på
- Apparatet slår seg av på egen hånd etter ca 2 minutter

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	TILBERED N.	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

Hvordan registrere elektronisk?
Gå inn på www.kostdata.se

- **Klikk på det <u>norske flagget</u> dersom det ikke automatisk kommer opp norsk språk**
- **Klikk på <u>Dietist Net</u>**

- **klikk på Dietist Net Gruppe**

- **Klikk på Universitetet i Agder – Fakultet for helse- og idrettsvitenskap**
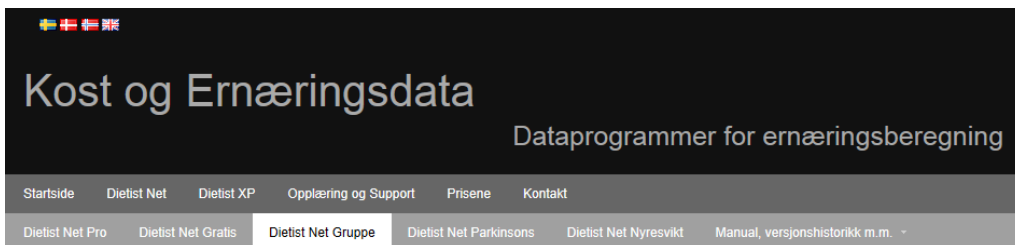
- **Velg hvilken datamaskin du har (PC eller Mac) og installer programmet**

- **For både PC og Mac finnes det to alternativer. Dersom du ikke vet om du har Java på din datamaskin er det enkelst å velge det første alternativet (da får du Java inkludert i installasjonen)**

Universitetet i Agder – Fakultet for helse- og idrettsvitenskap

Logg inn med gruppe passord

Man henter inn passordet som kreves av den som bestilt programmet, for eksempel skole, avdeling trener etc.

Dietist Net Gruppe lagrer beregninger du gjør på datamaskinen du sitter ved. Hvis du ønsker å åpne beregninger på en annen datamaskin, må du lagre dem på for eksempel en usb stick

Programmet startes forskjellig avhengig av hvilken type datamaskin du har:

For Windows-brukere

Installer programmet:
Last ned installasjonsprogram for Dietist Net Gruppe
⌄ Hjelp

Du kan også starte programmet ved å klikke på:
Starte Dietist Net Gruppe
Det krever at du har Java installert på datamaskinen.
⌄ Hjelp

For Mac-brukere

Installer programvaren:
Hvis du har en Mac, som er fra **2011 eller senere** (fra Mountain Lion-operativsystemet, versjon 10.8), så bruk denne l
Last ned installasjonsprogram for Dietist Net Gruppe
⌄ Hjelp

Hvis du har en eldre Mac, så bruk denne linken:
Last ned installasjonsprogram for Dietist Net Gruppe
⌄ Hjelp

Du kan også starte programmet ved å klikke på:
Starte Dietist Net Gruppe
Det krever at du har Java installert på datamaskinen.
⌄ Hjelp

Første alternativ inkludert Java

Andre alternativ ikke inkludert Java (hvis du har det fra før)

Første alternativ inkludert Java

Andre alternativ ikke inkludert Java (hvis du har det fra før)

- **Du skal så finne dette ikonet på ditt skrivebord**
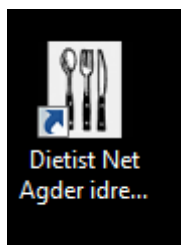
- **Trykk på ikonet og muligheter for å logge inn kommer opp**

PASSORD: XXXXXX

Begynne å bruke programmet

- **Så kommer du inn på forsiden i selve registreringsprogrammet**
- **Det er en liten blå knapp med et spørsmålstegn for hver funksjon i programmet ❓. Hvis du klikker på disse det vil åpne opp et hjelp vindu hvor funksjonen er forklart.**
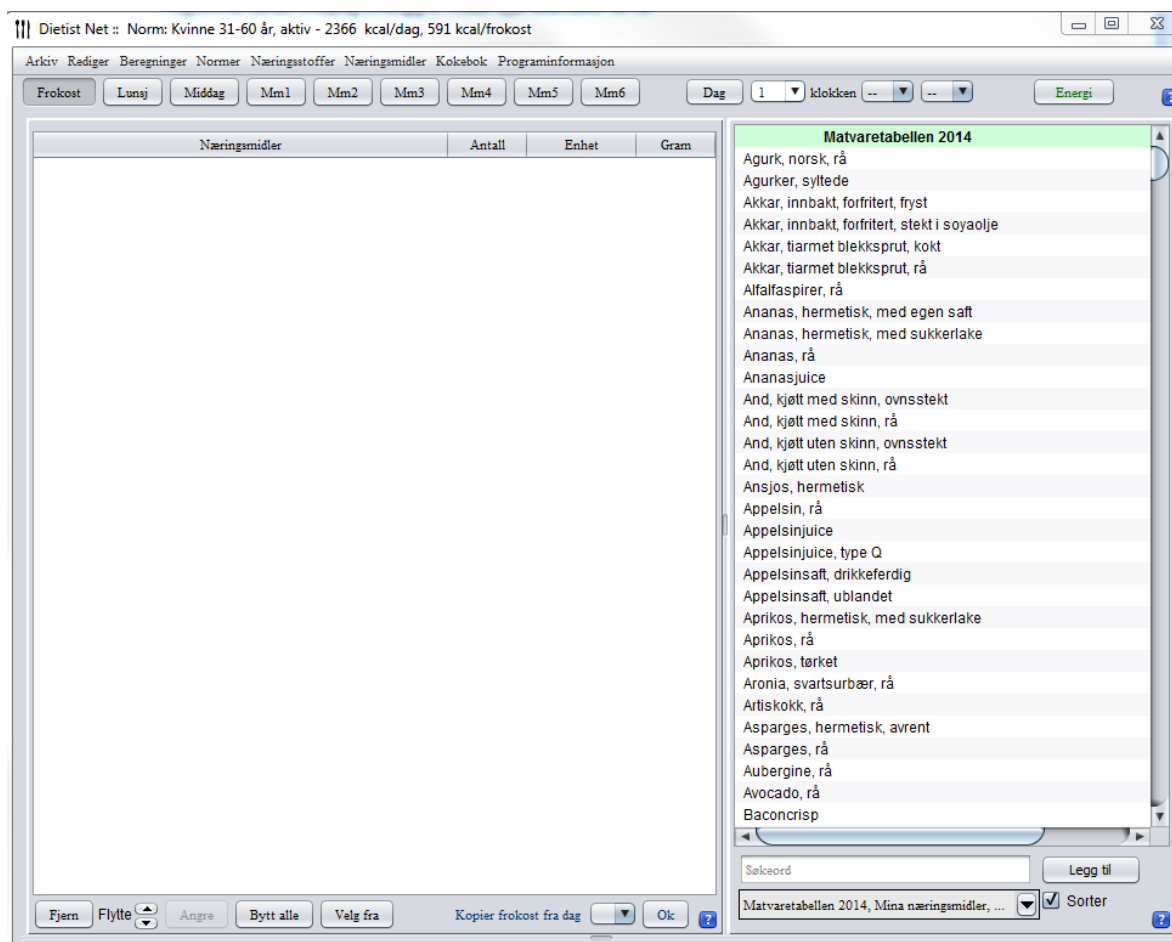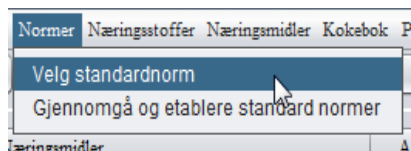
Grunnleggende innstillinger for en ernæringsberegning

I utgangspunktet er det flere komponenter som styrer ernæringsberegninger, men du skal kun justere på en komponent og det er "norm".

Sette opp normen:

Klikk på "Normer" og deretter "Velg standardnorm".
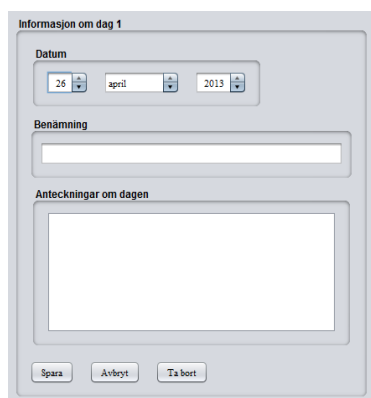
Velg normen i samsvar med den personen som beregningen utføres for (eks. mann 31-60 år, aktiv), og klikk deretter på "Lagre". Du bør beholde samme norm for alle dager i registreringsperioden.
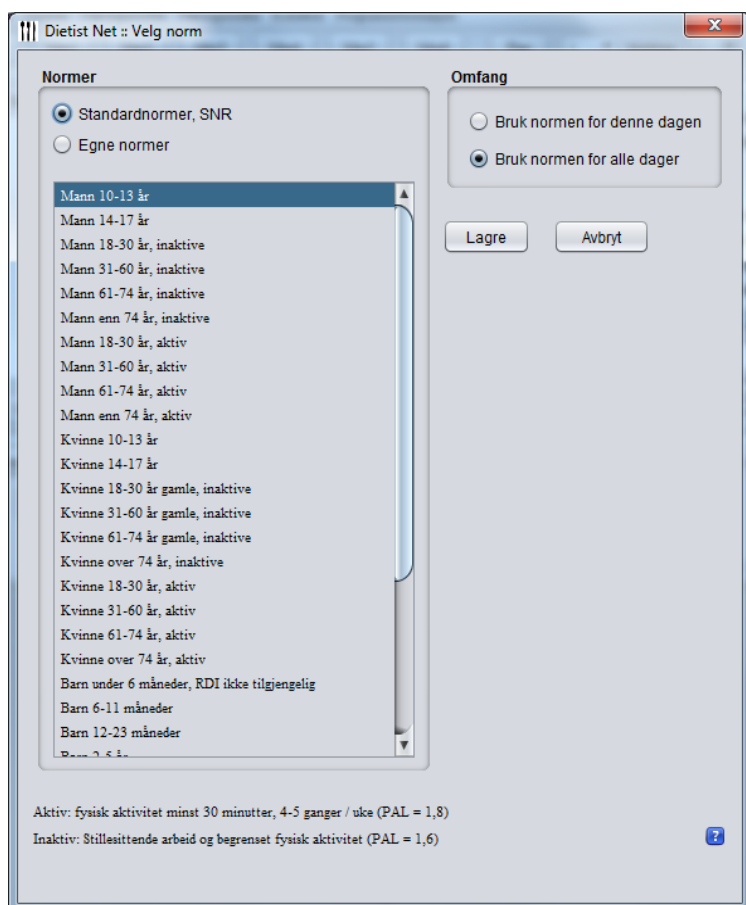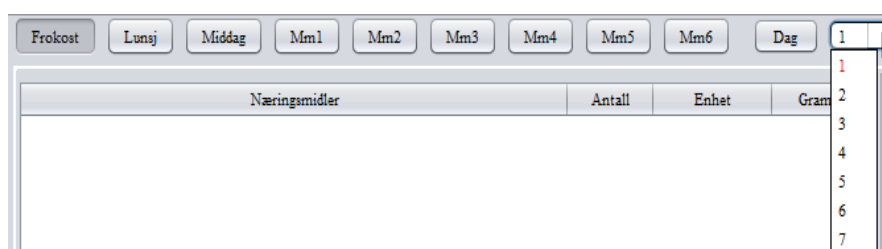
Legge til næringsmidler til ernæringsberegningen

Velge måltid og dag
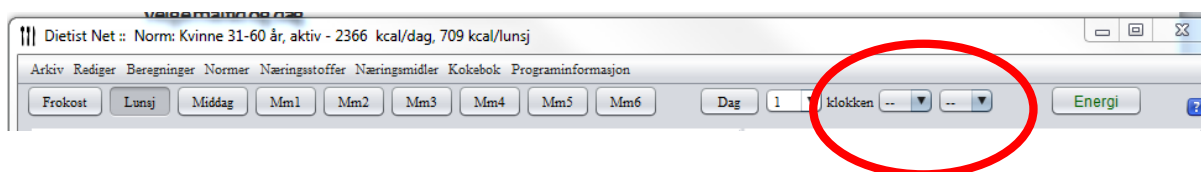
1. Velg **dag** og legg inn dato og fyll evt inn opplysninger av relevans for kostholdet (eks. syk, på reise eller annet). Du legger inn informasjon om dagen ved å klikke på knappen merket "Dag".

2. Velge **måltid**: Når programmet starter er frokost dag 1 valgt. Endre måltid ved å klikke på måltidsknapper og rullegardinmenyen litt lengre til høyre for dager (dag 1, 2 eller 3).

3. Når det gjelder måltider er hovedmåltider som frokost, lunsj og middag presisert via egne knapper. Kveldsmåltid/mat må registreres under mellommåltid (Mm). Det samme gjelder for andre mat/drikke inntak som regnes som mindre måltider/snacksing.


4. Husk å legge inn **klokkeslett** for hvert måltid.

Søk etter næringsmidler

Du søker etter matvarer ved å skrive inn noen bokstaver i navnet på mat, for eksempel melk. Programmet viser alle matvarer som inneholder ordet melk. Først er mat som begynner med melk, fulgt, i alfabetisk rekkefølge, matvarer med ordet melk i deres navn, som for eksempel helmelk. Du kan begrense antall alternativer ved å skrive inn to ord, f.eks "app ju" for å få akkurat de matvarene som inneholder begge bokstavkombinasjoner, for eksempel appelsinjuice.
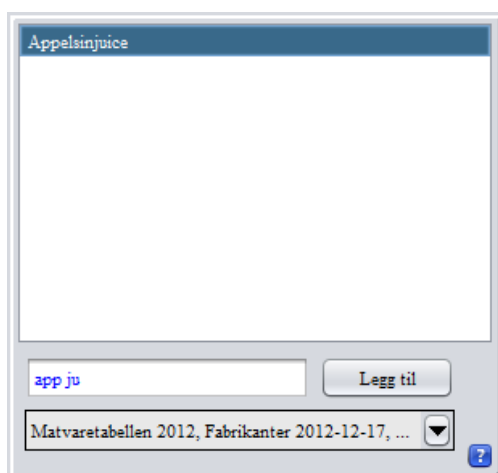
Når du har funnet den matvaren du leter etter så dobbeltklikk på den for å sende den til måltidet. Du kan også markere de ønskede næringsmidler og trykk på "Enter"-tasten, dra maten til måltidet, eller trykk på knappen "Legg til".

Kvantitet, enheter, gram

Du kan enten bruke pilene eller skrive inn antall enheter av næringsmiddel.

Næringsmidler	Antall	Enhet	Gram	Pris/kg
Helmelk, 3,5 % fett	1.1	glass	165	0

Velg enhet.

Næringsmidler	Antall	Enhet	Gram	Pris/kg
Helmelk, 3,5 % fett	1.1		165	0
		dl (100 g)		
		glass (150 g)		
		-		
		ss (15 g)		
		ts (5 g)		

Du kan enten bruke pilene eller skrive inn antall gram av næringsmiddel.

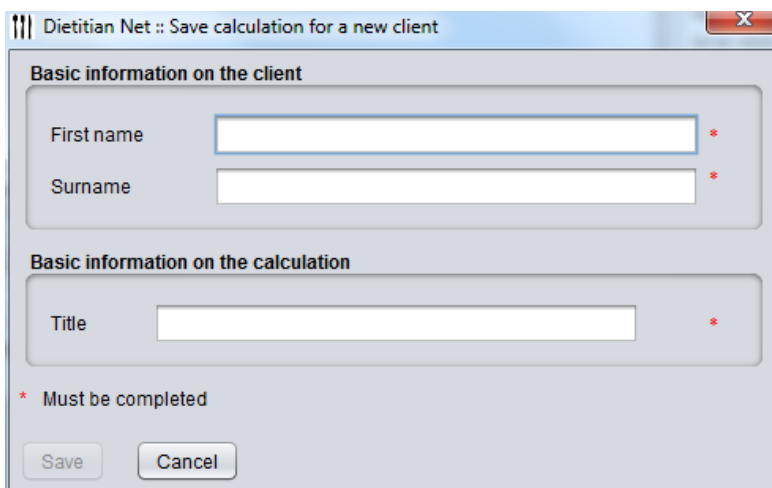| Næringsmidler	Antall	Enhet	Gram	Pris/kg
Helmelk, 3,5 % fett	-	-	166	0

Lagre, åpne og sende beregninger

Lagre beregninger
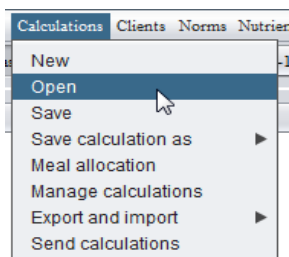
Du lagrer en beregning ved å klikke Beregninger -> Lagre
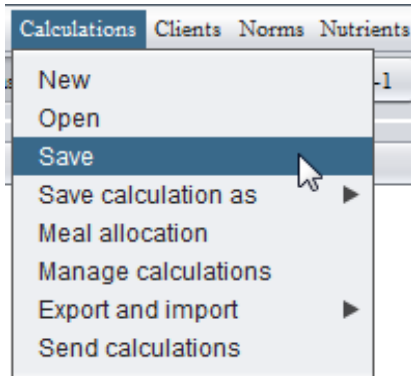
Hvis beregningen tidligere ikke er lagret, vil programmet ønske å lagre beregning med ditt navn.
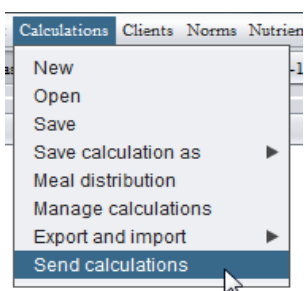
Åpne beregninger

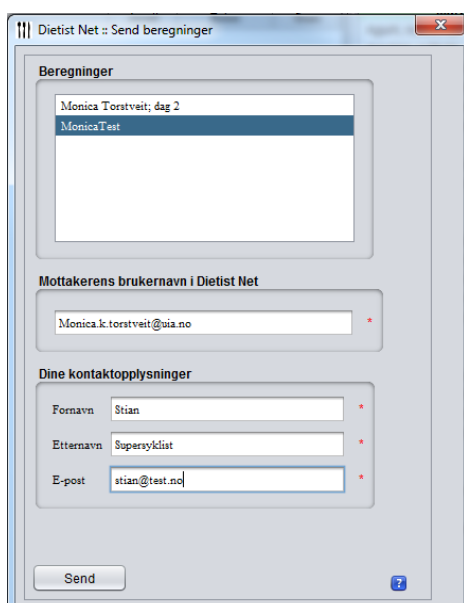Å åpne en tidligere lagret beregning klikk Beregninger -> Åpne.

Send beregninger

For å sende en beregning, klikk på Beregninger -> Send beregninger. For å sende en beregning, sender du til

Du merker beregningen skal sendes, fyll i mottakerens brukernavn (eller kode), og din kontaktinformasjon.

PAPIRSKJEMA TIL BRUK SOM HJELPEMIDDEL FØR ELEKTRONISK REGISTRERING (frivillig; starte evt. hver dag på nytt skjema)
DAG 1; dato:_____

Kl.	ANGIVELSER AV MAT OG DRIKKEVARER	TILBERED N.	MENGDE

PAPIRSKJEMA TIL BRUK SOM HJELPEMIDDEL FØR ELEKTRONISK REGISTRERING (frivillig; starte evt. hver dag på nytt skjema)
DAG 2; dato:_____

Kl.	ANGIVELSER AV MAT OG DRIKKEVARER	TILBERED N.	MENGDE

Kl.	ANGIVELSER AV MAT OG DRIKKEVARER	TILBERED N.	MENGDE

PAPIRSKJEMA TIL BRUK SOM HJELPEMIDDEL FØR ELEKTRONISK REGISTRERING (frivillig; starte evt. hver dag på nytt skjema)
DAG 3; dato: _____

Kl.	ANGIVELSER AV MAT OG DRIKKEVARER	TILBERED N.	MENGDE

Appendix 7

Kort veiledning i bruk av Polar M400 aktivitetsmåler og pulsklokke

Klokken og ladning av batteri:
Klokken har oppladbart batteri som lades via USB-porten på en datamaskin. Hvis klokken ikke brukes til trening m. Puls og GPS, vil batteritiden vare ca. 14 dager. Brukes puls og GPS vil batteritiden være ca. 24 timer.
Det kan derfor godt skje at du må lade klokken ila de 4 dager du skal gå med den. Viktig at du lader klokken når du sitter ned over en lengre periode (typisk kveldstid, foran TV´en osv).
Klokken lades med det medfølgende USB kabel og lades på baksiden av klokken (Se instruksjonsbok i esken).

Aktivitetsregistrering:
Inni klokken finnes en aktivitetsmåler, som måler akselerasjon. Det er derfor **VIKTIG** at du har klokken på deg 24 timer i døgnet når du registrerer kost (også når du sover). Klokken må derfor kun tas av *hvis* dere skal lade den! Alle aktivitetsmålere sliter med å registrere og "skjønne" når en sykler, men mindre en har på seg pulsbelte og lagrer dette som en økt. Skal du derfor levere barna i barnehage, sykle til jobb/skole, sykle ned å handle mat osv. **SKAL** du registrere dette med puls og som en treningsøkt! **Regelen er**: sykler du, registrerer du puls, uansett om du skal sykle 1,5km eller 150km. Klokken vil gi anmerkning når du har sittet for lenge i ro, og gi tilbakemelding på, hvor mye av dagens "anbefalte" aktivitet du har gjort. Ikke tenkt på hvor mange % av dagens aktivitet du har oppnådd. Dette er et fiktivt tall som Polar fremsetter og som ikke er relevant i denne sammenhengen!

Trening:
Når du trener **SKAL** du bruke det medfølgende pulsbelte og ta opp og lagre treningen som en økt på klokken. Klokken har predefinert 5 økt-typer. Disse er følgende:
Løping på tredemølle: Bruk **KUN** denne hvis du trener inne på tredemølle
Løping: Denne brukes på al type løping ute (langturer, intervaller, konkurranser, orientering osv.)
Sykling: Denne brukes til al type sykling ute.
Annen utendørs: Bruk denne om du skal en tur i skogen eller gå en fjelltur.
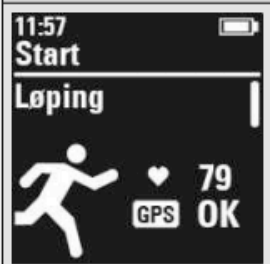**Annen innendørs**: Brukes til al annen trening som gjøres inne. (styrketrening, spinning, sirkeltrening osv.)

Har du egen pulsmåler du benytter under trening, må du i tillegg til denne bruke vår Polar M400 – da må du altså ha på deg 2 klokker og 2 pulsbelter under trening. Helst ser vi at du kun benytter vår måler under trening.

Starte en treningsøkt:

START EN TRENINGSØKT

Ha på deg pulssensoren, og kontroller at du har koblet den til M400.

Man, 9/8 **11:55**	**Trykk på START i tidsvisning for å gå til førtreningsmodus.**
11:57 **Start** **Løping** GPS 0%	**Velg sportsprofilen du vil bruke, med OPP/NED.** ⓘ *Hvis du vil endre sportsprofilinnstillingene før du starter økten (i førtreningsmodus), trykker du på og holder nede LYS for å gå til Hurtigmeny. For å gå tilbake til førtreningsmodus trykker du på TILBAKE.* Hvis du har aktivert GPS-funksjonen, begynner M400 automatisk å søke etter signalene.
11:57 **Start** **Løping** ♥ 79 GPS OK	**Hold deg i førtreningsmodus til M400 har funnet GPS-satellittsignalene og pulssensoren din for å sikre nøyaktige treningsdata.** For å fange opp GPS-satellittsignalene bør du være utendørs og i god avstand fra høye bygninger og trær. Ha på deg M400 på håndleddet med displayet i retning oppover for å oppnå best mulig GPS-ytelse. Hold M400 i horisontal posisjon foran deg og bort fra brystet. Hold armen i ro og hevet over brystnivå mens søket pågår. Stå i ro og hold posisjonen til M400 har funnet satellittsignalene. Prosentverdien ved siden av GPS-ikonet indikerer når GPS-funksjonen er klar. Når verdien når 100 %, vises OK og du er klar til å starte. M400 har funnet signalet fra pulssensoren når pulsen din vises. Når M400 har funnet alle signalene, trykker du på START. Registrering startet vises, og du kan begynne å trene. GPS-posisjonsbestemmelse går raskt ved hjelp av assistert GPS (A-GPS). A-GPS-datafilen oppdateres automatisk til M400 når du synkroniserer M400 med Flow-nettjenesten via FlowSync-programvaren. Hvis A-GPS-datafilen er utløpt eller du ennå ikke har synkronisert M400, kan det ta lengre tid å fastsette gjeldende posisjon. Du finner mer informasjon under Assistert GPS (A-GPS).

Når du er **ferdig med treningsøkten**, trykker du en gang på "tilbakeknappen" for å sette treningen på pause. Når treningen er i pause-modus trykker du og holder "tilbakeknappen" inne i min. 3 sekunder. Treningsøkten er lagret og du vil få en tilbakemelding på treningen. Trykke tilbake igjen for å gå i klokkemodus.

Synkronisering:

Klokken har begrenset lagringsplass for treningsøkter – Dette skal ifølge Polar være 24 timers trening. Det er derfor ikke sikkert du trenger å synkronisere klokken med Polar. Skulle dette likevel skje, synkroniserer du øktene med Polar flow tjenesten på internettet. Dette gjøres på følgende måte:

1. Gå inn på https://flow.polar.com/
2. Logg inn med brukernavn og passord (står på esken + det ligger lapp i esken)
3. Trykk på navnet (Testperson UIAXX) oppe i høyre hjørne.
4. Trykk på "produkter"
5. Trykk på "nedlastning" v. Flow-sync.
6. Innstaller programmet Polar Flow Sync.
7. Kople M400 til dataen med det medfølgende USB kabel.
8. Start flow-sync.
9. Synkroniseringen skal nå gå automatisk! Dette kan ta litt tid! Du må være på internettet for at dette skal fungere!
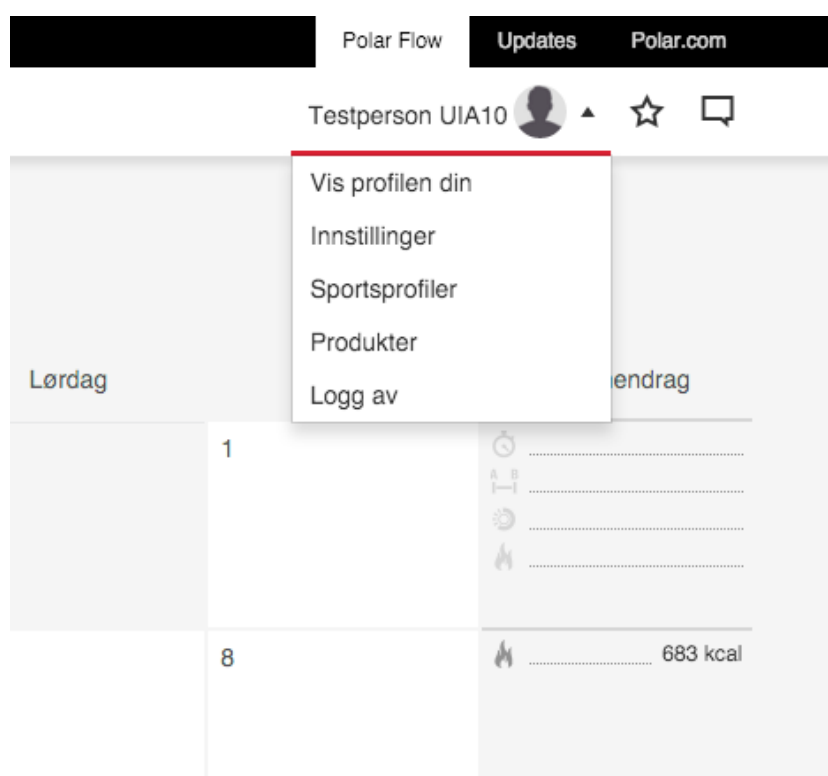
Går det hele opp i fisk, ring Thomas for eksperthjelp!
NB: Det er viktig at du IKKE retter eller stiller om på innstillingene på klokkene! Dette vil føre til feil tolkning av resultatene og gi et feil bilde av energiforbruk!!!

Manual finnes her:
http://www.polar.com/e_manuals/M400/Polar_M400_user_manual_Norsk/manual.pdf

Appendix 8

"Energitilgjengelighet, helse og prestasjon"; veiledning til bruk av senseware armband

Veiledning for bruk av senseware armband

Når skal jeg ha den på?

- Apparatet skal tas på kvelden før dere begynner å registrere kosthold, slik at vi får med alle døgnets minutter (fra 00.00 til 24.00) i 4 dager: _____

- Apparatet skal <u>ikke</u> tas av om natten.

- Ta av apparatet når du skal dusje, bade eller svømme. Legg det oppå håndkleet, så husker du å ta det på igjen etterpå.

Hvordan skal den sitte på?

- Fest apparatet på midt på **venstre** overarm som vist på bildet.

- Sensoren festes altså midt på triceps-muskelen.

- Apparatet skal være godt festet, men ikke for stramt.

Andre viktige opplysninger?

- Vær like aktiv som du pleier – apparatet skal måle «vanlige dager» for deg!

- Apparatet må ikke åpnes, vaskes eller lånes bort!

- Apparatet er svært kostbart, pass godt på det!

- Dersom du har nikkel allergi kan det være du reagerer på bruk av apparatet

- Apparatet skal tas av på morgningen etter dere er ferdige med å registrere kosthold den _____

- Apparatet leveres tilbake til prosjektansvarlig (sitter i resepsjonsområdet på Spicheren treningssenter) den <u>25/11 i tidsrommet mellom kl. 20.30-21.30 eller 2/12 i tidsrommet mellom kl. 07.30-08.30</u>

Lurer du på noe?

- Kontakt laboratorieansvarlig Thomas Stenqvist på telefon   eller epost   for å oppklare eventuelle spørsmål eller problemer.

Takk for ditt bidrag til forskningen! ☺