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Energy turnover - and aerobic fitness in endurance athletes and in the general population

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UNIL | Université de Lausanne
Faculté de biologie et de médecine

Institut des sciences du sport

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Thèse de doctorat ès sciences de la vie (PhD)

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List of publications

Articles PhD related (included in the thesis)

1. **Heydenreich J**, Kayser B, Schutz Y, Melzer K. Total energy expenditure, energy intake, and body composition in endurance athletes across the training season: A systematic review. *Sports Med Open*. 2017 Dec;3(1):8. Doi: 10.1186/s40798-017-0076-1

2. **Heydenreich J**, Melzer, K, Flury C, Kayser B. Low energy turnover of physically inactive participants as a determinant of insufficient mineral and vitamin intake in NHANES. *Nutrients*. 2017;9(7).pii:E754. Doi: 10.3390/nu9070754

3. **Heydenreich J**, Schutz Y, Kayser B, Melzer K. Validity of the Actiheart step test for the estimation of maximum oxygen consumption in endurance athletes and healthy controls. *Current Issues in Sports Science*. 2019;4:003. Doi: 10.15203/CISS_2019.003

4. **Heydenreich J**, Schutz Y, Kayser B, Melzer K. Comparison of conventional and individualized 1-MET values for expressing maximum aerobic metabolic rate and habitual activity related energy expenditure. *Nutrients*. 2019;11(2).458. Doi: 10.3390/nu11020458

Articles PhD related (not included in the thesis)

4. Melzer K, **Heydenreich J**, Schutz Y, Renaud A, Kayser B, Mäder U. Metabolic equivalent in adolescents, adults and pregnant women. *Nutrients.* 2016;8(7),438. Doi: 10.3390/nu8070438 **Contribution**: statistical analysis, preparation of tables and figures, interpretation of study findings, contribution to the manuscript

Articles and chapters in edited volumes not PhD related (since 2015)

5. **Heydenreich J**. Gewichtsmanagement im Kindes- und Jugendalter. *Sportunterricht.* 2015;64(6):178-182.

6. Kratzenstein S, Carlsohn A, **Heydenreich J**, Mayer F. Dietary supplement use in young elite athletes and school children aged 11 to 13 years: A cross-sectional study design. *Dtsch Z Sportmed*. 2016;67:13-17.

7. De Souza Silveira S, Otto C, **Heydenreich J**. Consistency of peak fat oxidation rates during treadmill ergometry: A pilot protocol and methodological elaboration. *Nutri Food Sci Int J*. 2017;2(5):555599. Doi: 10.19080/NFSIJ.2017.02.555599

8. **Heydenreich J**. Kohlenhydrate im Ausdauersport. (2017). In A Carlsohn (Ed.), *Sporternährung Fokus Ausdauersport. Ein Tagungsband* (pp. 13-26). Hamburg: Verlag Dr. Kovac.

9. **Heydenreich J**. Fette und Proteine im Ausdauersport. (2017). In A Carlsohn (Ed.), *Sporternährung Fokus Ausdauersport. Ein Tagungsband* (pp. 27-38). Hamburg: Verlag Dr. Kovac.

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11. **Heydenreich J**, Melzer K, Schutz Y, Kayser B, Mäder U. Energy expenditure, energy intake, and body composition in adult endurance athletes across the training season: A systematic review. *Int J Sport Nutr Exerc Metab* 2016;26:S7.

12. **Heydenreich J**, Melzer K, Schutz Y, Kayser B, Mäder U. Influence of aerobic fitness on resting metabolic rate in endurance athletes: A systematic review. *Int J Sport Nutr Exerc Metab* 2016;26:S11.

13. Melzer K, **Heydenreich J**, Schutz Y, Renaud A, Kayser B, Mäder U. Resting metabolic rate of specific population subgroups in comparison to the standard metabolic equivalent (MET). *Official Journal of the ACSM.* 2016;48 (Suppl 5): S424.

14. **Heydenreich J**, Kayser B, De Souza Silveira R, Schutz Y, Melzer K. Comparison of subjective and objective methods of activity energy expenditure in endurance-trained women athletes and healthy controls. *Advances and Controversies in Measurement of Energy Metabolism (RACMEM)*, 20-22 October 2017, Fribourg (CH).

15. **Heydenreich J**, Kayser B, Schutz Y, Melzer K. Aerobic fitness relates to resting metabolic rate in women. *Int J Sport Nutr Exerc Metab* 2018;28:S1-1.

16. **Heydenreich J**, Kayser B, Schutz Y, Melzer K. Revisiting the exclusion of the initial five min of indirect calorimetry measurement of RMR. *Int J Sport Nutr Exerc Metab* 2019;29:S1-9. Doi: 10.1123/ijsnem.2019-0057

Table of contents

Abstract

The impact of energy turnover on mechanisms relating to energy balance and energy metabolism in humans is still not fully understood. The objective of the present thesis is to partially clarify these mechanisms by identifying specific problems going along in individuals with high (e.g. endurance athletes) and low energy turnovers. In a first step, a systematic review assessing the energy expenditure, energy intake, and body composition in endurance athletes across the training season was conducted. We found that men and women endurance athletes showed important fluctuations in energy turnover across the training season. Secondly, the extent to which the physical activity levels (PAL) are associated with compliance to dietary micronutrient intake recommendations was explored. Results of the present thesis indicate that individuals with low energy turnover have a higher risk for the development of insufficient micronutrient intake, as those with higher PAL. We further showed by using a linear model that an increase in PAL up to levels of 2.0 and a concomitant linear increase in energy intake in order to cover for increased energy demands would reduce the prevalence of micronutrient deficiencies. Finally, our work demonstrated that for the valid assessment of maximum metabolic equivalent of task and energy expenditure the resting oxygen consumption should be measured or, if not possible, estimated by use of published validated equations. To conclude, the energy and nutrient requirements of individuals with high energy turnover (viz. endurance athletes) are not static over the training year and strongly dependent on daily PAL. In addition, promotion of physical activity is important not only for weight management but also for adequate energy and micronutrient intake. In general, caution must be taken when interpreting energy expenditure data, where the calculation is based on an estimated or a standard resting oxygen consumption value.

Résumé (Abstract in French)

L'impact du renouvellement énergétique sur les mécanismes liés à la balance énergétique et son métabolisme chez l'humain n'est encore pas totalement élucidé. L'objectif de cette thèse est de comprendre ces mécanismes en identifiant les problèmes spécifiques liés aux personnes ayant un métabolisme élevé (par exemple chez les athlètes d'endurance) ou bas. Dans un premier temps, des mesures de dépenses et apports énergétiques ainsi que la composition corporelle ont été effectuées systématiquement chez des athlètes de sports d'endurance durant la saison d'entrainement. Nos résultats montrent des fluctuations importantes dans le renouvellement énergétique chez les athlètes d'endurance (hommes et femmes) durant la saison. Dans un second temps, nous avons étudié la corrélation entre le niveau d'activité physique (NAP) et la discipline avec laquelle les conseils d'alimentation en micronutriments étaient suivis. Les résultats de cette thèse démontrent que les individus avec un NAP bas ont un risque plus élevé de développer une insuffisance d'apport en micronutriment comparé aux sujets ayant un NAP élevé. De plus, en utilisant un modèle linéaire, nous avons démontré qu'une augmentation du NAP jusqu'à un niveau de 2.0 en parallèle avec une augmentation linéaire de l'apport énergétique afin de couvrir l'augmentation de dépense énergétique réduirait la prévalence des déficits en micronutriments. Finalement, nos travaux montrent que la détermination du métabolisme maximal et la dépense énergétique nécessite la mesure du taux de consommation d'oxygène au repos. Si cela n'est pas possible, cette consommation devrait être estimée par des équations publiées et validées. En conclusion, notre étude démontre que les demandes énergétiques et en nutriments chez les personnes ayant un métabolisme élevé (viz. athlètes d'endurance) ne sont pas statiques durant l'année mais dépendent fortement du taux d'activité physique quotidien. En outre, la promotion de l'activité physique n'est pas uniquement importante pour la gestion du poids corporelle mais aussi pour un apport énergétique et en micronutriments adéquat. En général, les auteurs recommandent d'interpréter prudemment les mesures de dépense énergétique basées uniquement sur une estimation ou une valeur standard de consommation d'oxygène au repos.

Index of abbreviations

A

Scaling constant

AEE

Activity energy expenditure

ATH

Athletes

BMI

Body Mass Index

BMR

Basal metabolic rate

CI

Confidence interval

CON

Control subjects

DLW

Doubly labelled water

EB

Energy balance

EEmax

Maximum energy expenditure

E_{in}

Energy intake

END

Endurance trained athletes

FFM

Fat-free mass

FM

Fat mass

HRmax

Maximum heart rate

k

Scaling exponent

m

Body mass

MAE

Mean average error

MAPE

Mean average percentage error

MET

Metabolic equivalent of tasks

METmax

Maximum metabolic equivalent of tasks

O_2

Oxygen

PAL

Physical Activity Level

R^2

Coefficient of determination

RDA

Recommended Daily Allowance

RED-S

Relative Energy Deficiency in Sport

RMR

Resting metabolic rate

SD

Standard Deviation

SEE

Standard error of the estimate

SHR

Sleeping heart rate

TEE

Total energy expenditure

VO_2

Oxygen consumption

VO_2max

Maximum oxygen consumption

VO_2rest

Oxygen consumption at rest

Chapter One

Introduction

1. Introduction

In the past the human was repeatedly exposed to the risk of a negative energy balance. Nowadays, the risk is exposure to an environment leading to a positive energy balance and there is more overnutrition and obesity worldwide than there is famine. According to the World Health Organization in 2016 more than 1.9 billion adults (39%) were overweight worldwide and of these over 650 million adults (13%) were obese (1), whereas 462 million adults were underweight (2). The global prevalence of obesity nearly tripled between 1975 and 2016 and overweight and obesity are linked to more deaths worldwide than underweight (1). Energy (im)balance is not only the arrhythmic result of energy intake and expenditure but also of total daily energy expenditure. The latter varies greatly between individuals according to their physical activity levels (PAL). This has consequences for body composition, for micronutrient intake, for health and athletic performance. Much is known about the mechanisms regulating energy metabolism and energy balance and numerous influencing factors were identified. For example, evidence indicates that there is a weak coupling between energy intake and energy expenditure in individuals with low PAL, whereas in individuals with high energy turnover a strong coupling between both factors is observed (3). Promotion of physical activity, therefore, is important for weight management, not only by increasing total daily energy expenditure but also by stimulating a more sensitive appetite regulation (3). However, there are still many questions remaining to be answered regarding the impact of energy turnover on mechanisms relating to energy balance and energy metabolism. The objective of the present thesis is to partially answer some of those by identifying specific problems going along with high (e.g. in endurance athletes) and low energy turnovers.

In **Figure 1** a schematic overview about the included studies and their relationship is displayed. In short, profiles of energy metabolism at rest, during exercise, in athletes during training and in healthy non-athletes are investigated. The first part of the introduction provides an overview about basic principles dealing with the topic of energy balance, such as determinants of energy expenditure and energy intake, and their variations in humans with high energy turnover (e.g. endurance athletes) across the training season. In the second part, the relationship between low energy turnover and the risk of micronutrient deficiencies is displayed. In the last part, the pitfalls of current expression of aerobic capacity as an indicator of physical fitness and energy turnover in individuals of different body size, an alternative method, and the risks and benefits of submaximal exercise tests for determination of aerobic capacity are investigated.

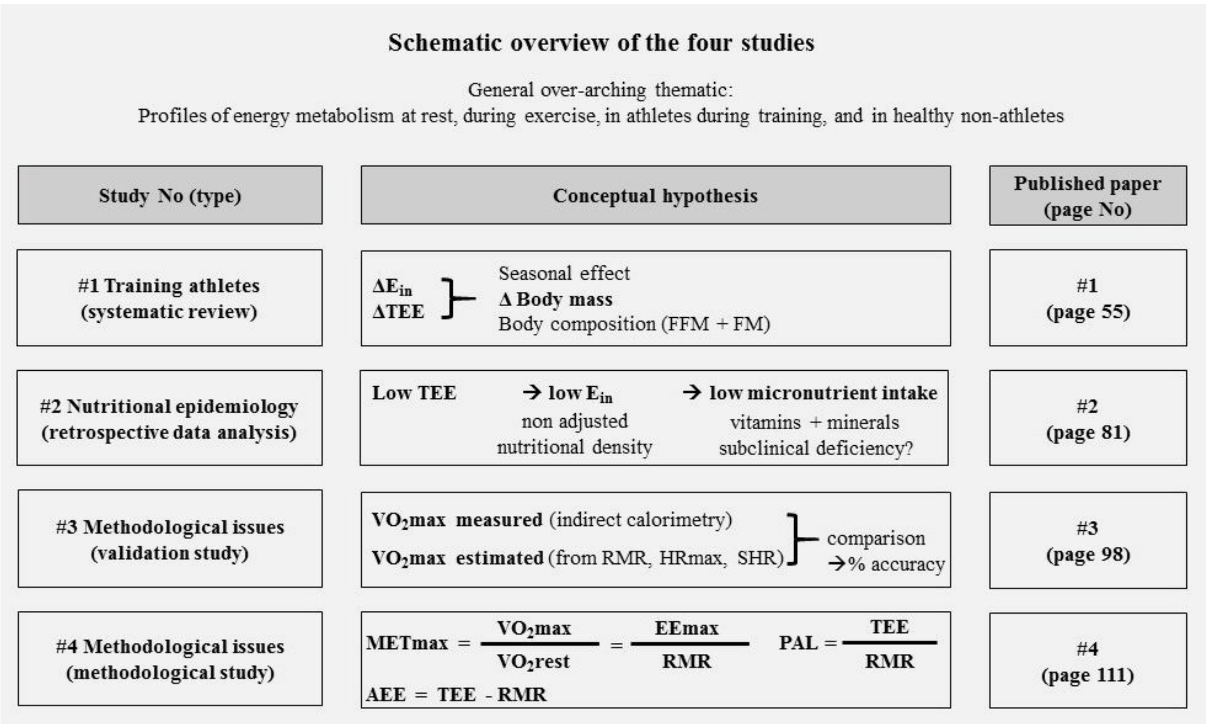

Figure 1 Schematic overview of the four studies. AEE = activity energy expenditure, EEmax = maximum energy expenditure, E_{in} = total energy intake, FFM = fat-free mass, FM = fat mass, HRmax = maximum heart rate, METmax = maximum metabolic equivalent of tasks, PAL = physical activity level, RMR = resting metabolic rate, SHR = sleeping heart rate, TEE = total energy expenditure, VO_2max = maximum oxygen consumption, VO_2rest = oxygen consumption at rest.

1.1. Variations of energy expenditure in humans with high energy turnover

The components of an individuals' total daily energy expenditure (TEE) comprise the energy costs of the processes essential for life (basal metabolic rate (BMR)), the energy expended in order to digest, absorb, and convert food (thermic effect of food, ~10% of TEE, depending on energy content and macronutrient composition of the diet), and the energy expended during physical activities (activity energy expenditure (AEE), ~15 – 30%) (4,5). BMR corresponds to the energy required to maintain the systems of the body and to regulate body temperature at rest. It is measured in the early morning in a temperature controlled, quiet and shaded room, using indirect calorimetry with subjects laying on a bed, being in an overnight fasted state, and no strenuous activity the day before measurement allowed. Since assessment of BMR requires the subject to stay overnight in the laboratory, very often the basal metabolism is measured under conditions meeting the terminology of resting metabolic rate (RMR) instead. Subjects are allowed to sleep at home and arrive at the laboratory after being awake, where they rest for a certain period of time before the measurement starts. Similar as for measurement of BMR the subjects must be fasted and with no strenuous activity allowed the day before measurement. RMR is approximately 10% higher compared to BMR and accounts for 60-80% of TEE in most sedentary healthy adults (4). A variety of factors are known to influence the subjects' RMR. These factors include age, sex, body size, fat-free mass (FFM), and fat mass. Age, sex and FFM generally explain about 80% of the variability in RMR (6).

The contribution of RMR on TEE is strongly dependent on a subjects' AEE. For example, athletes easily spend 4100 – 8300 kJ · d^{-1} (1000 – 2000 kcal · d^{-1}) in sport-related activities (7). Thompson and colleagues reported that RMR represented only 38 – 47% of TEE in 24 elite male endurance athletes (8), whereas in female endurance athletes values between 42% (9) and 54% (10) are reported. During days of repetitive, heavy competition, such as during a multistage cycle race, RMR might represent even less than 25% of TEE (11).

A low RMR for a given body composition has been identified as a risk factor for weight gain and obesity (12,13). Furthermore, in formerly obese persons a low RMR is likely to contribute to the risk for weight regain (14). In humans, during weight loss usually a fall in energy expenditure in all energy expenditure components, i.e. RMR, thermic effect of food and AEE, is observed, which is greater than predicted from the reduction of fat mass and FFM alone (15). This phenomenon supports the concept in humans of a regulatory or adaptive thermogenesis, whereby an "active" metabolic adaptation spares energy and hence contributes to limit further weight loss and predisposes to weight regain (16). Therefore, weight loss interventions aim to limit the loss of the more metabolically active component of the body, i.e. FFM. This can be achieved by performing a gradual weight loss at approximately 0.5 – 1 kg per week, which corresponds to a daily negative energy balance of about 500 – 1000 kcal, and additionally performing strength training in order to maintain or increase FFM and thus limit the decline of RMR (17).

The AEE is the most variable component of energy expenditure in humans. It comprises both involuntary and voluntary physical activities. Involuntary (or "non-exercise activity thermogenesis") is further subdivided into occupational/leisure activity and spontaneous physical activity (fidgeting, posture); the latter being essentially involuntary and subconscious (16). Volitional physical activity comprises the activities spent during exercise and sports, and is highly dependent on duration, frequency, kind, and intensity of the exercise performed. Elite endurance athletes are characterized by high fluctuations of TEE, which is mainly due to the variability of the energy expended during sporting activities. As seen in **Figure 2**, the training volume and training intensity are strongly dependent from the seasonal training phase. During the preparatory phase predominantly high training volumes at moderate intensity are performed, in order to improve endurance capacity and a more efficient use of fuel substrates (18). In transition to the competition phase the training volume is reduced while training intensity is gradually increased in order to reach peak performance and to transfer training effects into the competition phase. Immediate prior to competition the training volume and intensity is reduced (taper phase) to allow the athlete to start the competition in an optimally recovered state. In the days and weeks after competition, regeneration and mental/physical preparation for the next training cycle (transition phase) is the primary goal.

In general, training loads from 500 h · year^{-1} (19,20) up to 1000 h · year^{-1} (21–23) have been reported among elite senior endurance athletes, depending on the specific muscular loading characteristic of the sport. During heavy sustained exercise (e.g., during the Tour de France), TEE can be as high as fivefold the BMR over several weeks (11). In contrast, during recovery days, pre-competition tapers, or during the off-season, the energy expended in activities is much lower. Therefore, TEE is expected to be much less and may even reach levels comparable to that of a sedentary behavior.

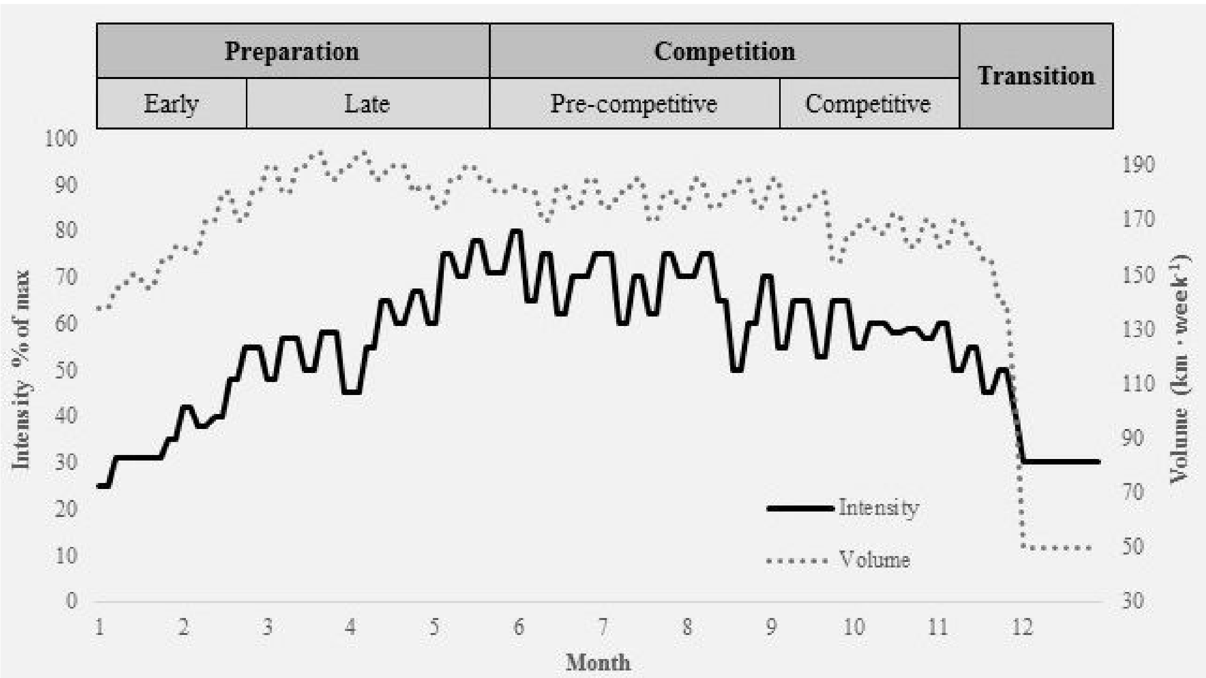

Figure 2 Periodization of the training year for a "one-peak annual year" of an elite runner. Adapted from Bompa & Haff (24).

In a recent study with 15 collegiate male rowers, the training intensity/volume, body composition and energy intake/expenditure in three different training phases (off-season, pre-season, in-season) was assessed (25). The authors found a significant higher time spent for high-intensity training during in-season compared to off-season, but also a significantly lower energy expenditure (measured by accelerometry) during pre-season/in-season and off-season. As a possible explanation for these surprising results the authors argue that the performance level of the athletes was low (collegiate athletes, most of whom started rowing during their time at the university), that the accelerometer probably did not accurately measure intense physical movements of the high-intensity training, and the relatively small sample size. However, there is a lack of longitudinal studies assessing TEE throughout the training season in elite endurance athletes so that the fluctuations in energy expenditure and energy balance throughout a training year remain unknown.

Controlling energy balance is a key goal for athletes. Energy balance occurs when metabolizable energy intake matches energy expenditure so that overall energy content of the body remains stable. A positive energy balance (energy intake higher than energy expenditure) is associated with a gain of body mass and fat mass, whereas a negative energy balance (energy intake lower than energy expenditure) is linked to loss of body and fat mass. There exist a variety of factors which are regulating and influencing energy balance, which are shown in **Figure 3**. Energy balance is a complex but highly coordinated system where peripheral signals of nutrient intake with long-term signals of energy status are integrated and mediated by multiple behavioral and societal factors (3).

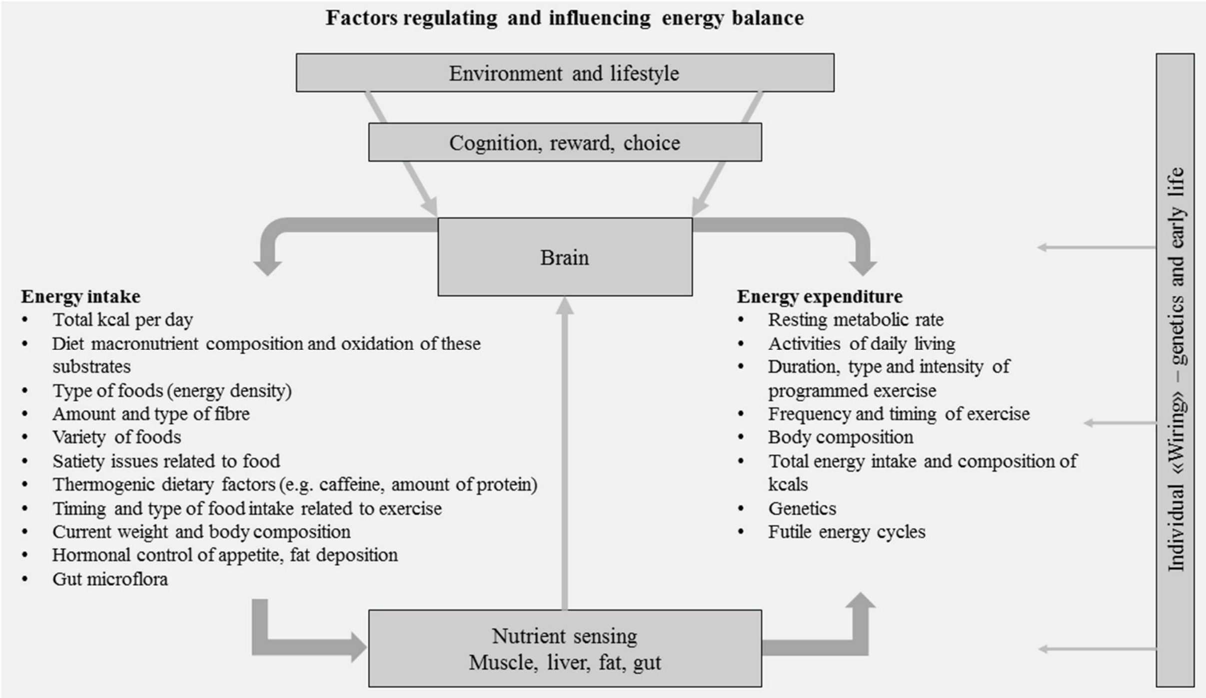

Figure 3 Factors that regulate and influence energy balance: changes in the environment that can influence subsequent generations (e.g. genetics and epigenetics) and current habitual lifestyle factors that influence diet and physical activity. Adapted from Manore & Thompson (7).

In order to be able to maximize performance, athletes do not only strive to achieve energy balance, but also to maintain their body mass and body composition at levels that are compatible with good health and athletic performance (26). For endurance athletes, it is a big challenge to appropriately match energy intake and TEE in order to achieve energy balance on the one side and on the other side to strive for a low body mass and/or body fat level for various advantages in their sports, specifically during the competitive season (27). However, the high fluctuations in TEE due to altering training loads require permanent adaptation of energy intake in order to match for differing energy costs and/or to achieve the goal of a certain body mass or body composition. These adaptations must be performed on a daily base, bearing in mind that an appropriate energy intake supports optimal body function, determines the capacity for intake of macro- and micronutrients, and positively influences performance in athletes (28). In the last decade, guidelines for optimal training diet have evolved from a universal prescription of a static energy and macronutrient intake to a personalized, periodized and practical plan (28). These specific day-to-day manipulations of the timing and amount of energy and macronutrients is called “dietary periodization” or “nutrient timing” (29). Nowadays, there exist periodized nutrition guidelines for several specific sports disciplines, such as for middle-distance (29), power sport (30), and combat sport (31) athletes. In addition, there exist general guidelines for carbohydrate, protein, and fat intake during training and competition, not exclusively focusing on endurance sports (28,32–34). Especially for carbohydrate intake, a variety of terms have emerged to describe new or nuanced versions of specific exercise-diet strategies (e.g., train low/high (glycogen) session, ketogenic low-carbohydrate high-fat diet, periodized carbohydrate availability diet), which are discussed in detail in a recent article (35). However, it remains unclear whether elite athletes adopted some of these “new” strategies into their diet. In a study with middle- and long-distance runners/race walkers it was shown that elite endurance athletes execute before and after key training sessions specific nutrition recommendations, but only very few athletes deliberately undertake some contemporary dietary periodization approaches, such as training in the fasted state or periodically restricting carbohydrate intake (36). The results of this study suggest that there is a great mismatch between practice and current and developing sports nutrition guidelines. Recently, several studies were conducted investigating different nutrition-training interventions and their implementations in athletes (e.g., (37,38)). These studies conclude that a successful implementation and monitoring of dietary interventions requires meticulous planning and the expertise of chefs and sports dietitians. In addition, more education is needed to assist elite endurance athletes to achieve guidelines which promote specific and periodized approaches to macronutrient intake around training sessions within phases of the training program.

Generally, assessment of energy and macronutrient intake is difficult since most of the available methods lack of accuracy and precision. A review of nine studies using doubly labelled water

(DLW) to validate self-reported energy intake revealed that under-reporting can amount to 10-45% of TEE in athletes (39). Since there is a linear relationship between the magnitude of under-reporting and increasing energy requirements (39), endurance athletes might suffer from an increased risk of under-reporting of their energy and macronutrient intakes during days and weeks of intensified training. Explanations for mis-reporting of dietary intake comprise, among other factors, limitations associated with memory and difficulty in estimating quantities, eating behavioral changes, and errors in nutrient-composition databases used for dietary data analysis (40). Some authors even conclude that self-reported dietary intake data should not be used as a measure of energy intake (40). Instead, according to these authors administration of DLW is the most useful tool for evaluating energy intake and energy expenditure, whereas the best measure of energy balance is the assessment of body mass stability.

Athletes from weight-bearing sports, such as runners or cross-country skiers, strive for a low body mass and/or body fat level for various advantages in their sports. These athletes benefit from a greater movement economy and better thermoregulatory capacity from a favorable ratio of body mass to surface area and less insulation from subcutaneous fat tissue (27). Therefore, many endurance athletes are characterized by a very low body fat levels and Body Mass Index (BMI). In male Kenyan endurance runners a body fat percentage of 7.1% was observed (41), which is only marginally above the recommended 5% minimum for males (17). In the same athletes, the BMI was 18.3 $kg \cdot m^{-2}$, which is according to the World Health Organization generally classified as being underweight (42). However, the investigators undertook their measurements when athletes were in peak physical condition (prior to competition). It is unclear whether or not elite endurance athletes are able to maintain their low body mass fat mass throughout the complete year, since there exist only few longitudinal studies and no systematic data analysis was performed yet. One must assume that there are also fluctuations in body mass and composition in consequence of altering training loads and TEEs and/or lacking adaptation of energy intakes. One article of the present PhD thesis (Article I) is addressing this theme.

1.2. Low energy turnover might relate to insufficient micronutrient intake

Energy balance is when energy expenditure equals energy intake. In healthy-weight individuals the primary mechanism to regulate energy balance on a day-to-day basis are adaptive fluctuations in energy intake, rather than energy expenditure (43,44). In **Figure 4** a model showing the relationship between PAL, body mass and food intake is displayed. According to the model of Melzer et al. (45) a reduced PAL below 1.7 – 1.8 does not induce a compensatory reduction of energy intake, which is further leading to a positive energy balance and increasing body fat storage (**Figure 4**, *zone 1*). However, it was shown that over the long-term an increase in physical activity of moderate to vigorous intensity for two or more hours per day in sedentary nonobese women and men is compensated by an increase in energy intake within a period of about three days (45,46). This range of activity is called "*responsive*" with respect to food consumption (**Figure 4**, *zone 3*). However, there is a limit to the performance ("*sustainable metabolic rate*") of an individual set by the energy intake and energy expenditure. In the general population the upper limit is approximately 2.2 – 2.5 (**Figure 4**, *zone 4*), whereas in endurance athletes it is approximately twice as high (**Figure 4**, *zone 5*), as a result of long-term exercise training inducing increased FFM and carbohydrate consumption during exercise (47,48). The human body is not able to cope with values above the *sustainable metabolic rate*, leading to a negative energy balance and concomitant body mass loss.

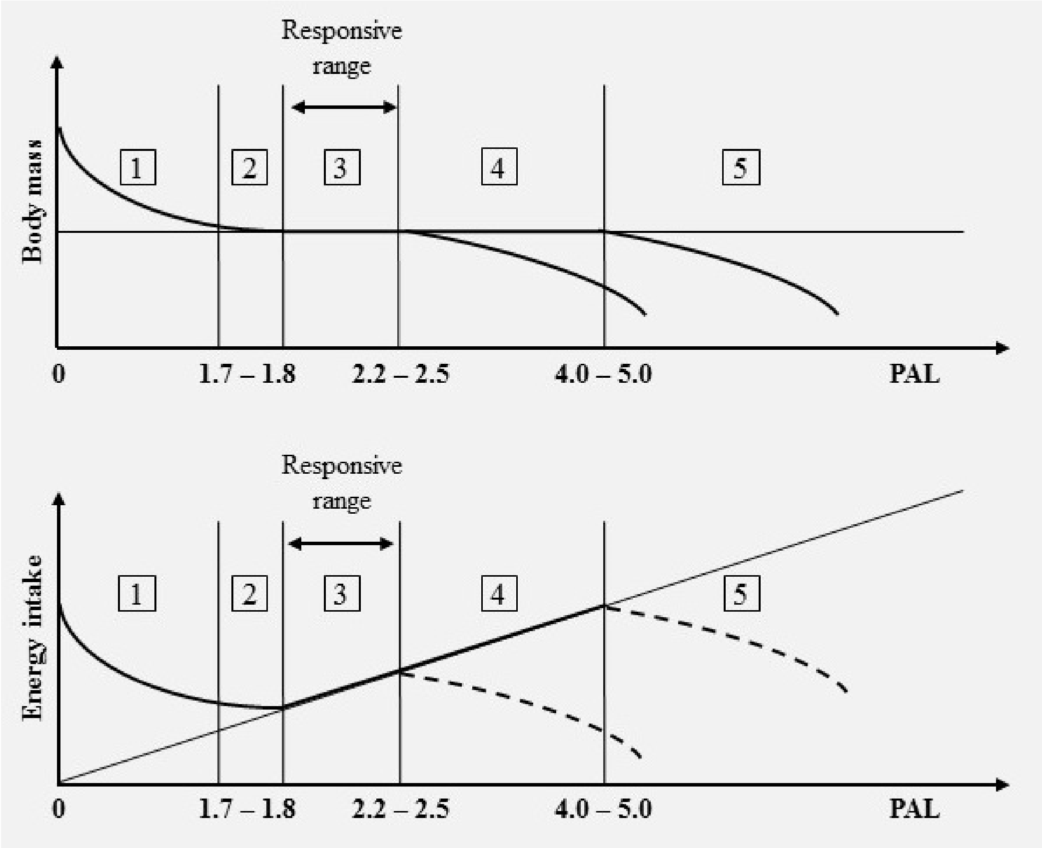

Figure 4 A model showing the relationship between Physical Activity Level (PAL), body mass and food intake. *Zone 1*: a decrease in PAL and increase in sedentariness does not induce compensatory reduction in food intake and leads to an increase in body mass; *zone 2*: introduction of acute physical activity on a short-term basis suppresses food intake, due to mobilization of stored fuels, and leads to a decrease in body mass; *zone 3* (responsive range): moderate to intense physical activity performed regularly and on a long-term basis by lean individuals increases food intake accordingly and maintains body mass; obese individuals, due to their excess energy storage, do not change significantly their food intake; the PAL level in the general population has an upper limit around 2.2 – 2.5 (*zone 4*); and in highly trained individuals around 4.0 – 5.0 (*zone 5*). Above these values, the human body is not capable to cover the high-energy expenditure, and loss of body mass occurs as a consequence. Adapted from Melzer et al. (45).

Micronutrients are essential nutrients which are required in small quantities and support numerous physiological functions, such as immune function, integrity of cell membranes, sperm production, nervous function, muscle contraction, and brain and muscle metabolism (49). There are several groups at risk for micronutrient deficiencies, including the elderly, pregnant women, vegans, people on a weight-reduction diet, and some groups of athletes (50). However, micronutrient deficiencies are very common, also in the general population (51). The increase in overweight and obesity rates in Western countries also causes a sharp increase in dieting attempts undertaken by the affected individuals with the intent to lose body mass and/or improve their health (52). Since individuals on a weight-reduction diet have a higher risk for micronutrient deficiency (50), the number of micronutrient insufficient individuals and concomitant health issues might be raising and will be a serious future concern. Several popular diet profiles have been analyzed according their micronutrient content showing high levels of micronutrient deficiencies (52,53). A food only approach, therefore, might promote the individuals' body mass loss, but may also increase the risk of micronutrient deficiencies and concomitant adverse health effects. Alternatively, an increase in energy expenditure might support body mass loss in obese individuals, since, in contrast to the lean, obese show no adaptive response to increasing physical activity with regard to energy and nutrient intake, maybe due to their excess energy storage in the form of adipose tissue (45). This hypothesis is confirmed by many studies, where an uncoupling of energy intake from energy expenditure in obese individuals was found (54–56). For a detailed review see Melzer et al. (45).

However, when increasing food intake in order to match increased energy expenditure and maintaining body mass, physical activity may also contribute to micronutrient deficiency coverage, by increasing intake of other food constituents like minerals and vitamins, at least in the lean (45). This hypothesis is confirmed by many studies examining the difference of energy and nutrient intake between individuals performing different levels of physical activity. In a cross-sectional study of Csizmadi et al. including $n = 5333$ healthy-weight adults, in individuals with lower levels of PAL (≤ 1.6) the prevalence of low micronutrient intake was higher than in individuals with a PAL > 1.6 (57). They also found a linear relationship between activity-related energy expenditure, represented by PAL, and increasing nutrient intakes. Another study examined the relationship between dietary intake and different levels and types of physical activity (58). The authors found a positive association between higher levels of physical activity in leisure time and higher micronutrient intakes, whereas for total and occupational physical activity similar nutrient intakes were observed between active and sedentary individuals. A review about the interaction between physical activity and micronutrient intakes pointed out that the micronutrient intake increases with increasing energy intake (49). This fact would explain why in physically highly active people, such as athletes, higher micronutrient intakes compared to untrained individuals were observed. However, the author pointed out two important aspects: first, that *moderate* physical activity does not necessarily affect daily micronutrient intake, and second, that the increase in micronutrient intakes is not as large as the increase in energy intake when compensating for increasing energy expenditure. The second hypothesis is supported by a very large study comprising 439 Dutch athletes (59). The authors regressed the intake of calcium and iron against energy intake and found that a 100% increase in energy intake was associated with a 70 – 80% increase in iron, and an 80 – 90% increase in calcium intake only. However, it is unclear how much of an increase in PAL, up to levels recommended for health, combined with a corresponding linear up-scaling of dietary intake without altering dietary composition, would improve compliance with recommended micronutrient intake in healthy individuals of various body size. Article II of the present PhD thesis is dealing with this topic.

1.3. Aerobic capacity as a determinant of energy turnover

Aerobic capacity (maximum oxygen consumption; VO_2max) is defined as the highest rate at which oxygen can be taken up and utilized by the body during intense large muscle groups exercise (60). It is frequently used to indicate the cardiorespiratory fitness of an individual and in the development of exercise prescriptions. Usually, VO_2max testing is performed on a treadmill or a cycle ergometer while oxygen uptake and expired carbon-dioxide are measured with a respiratory gas analyzer. By progressively increasing the workload the participant reaches volitional exhaustion after a minimum of 5 minutes (treadmill) or 7 minutes (cycle), up to 26 minutes (61). Since maximum exercise tests are (1) time consuming, expensive and depending on physiological expertise (62), (2) some of the individuals cannot achieve the maximal effort required for the determination of VO_2max, and (3) contraindications for performing maximum exercise tests exist (63), several indirect methods to estimate VO_2max have been developed. Most of these tests use the linear relationship between heart rate and oxygen uptake (VO_2). By performing a submaximal exercise testing the VO_2max is then predicted by extrapolation of the submaximal heart rate values to an estimated maximum heart rate. This allows the evaluation of cardiorespiratory fitness in a population, in which the direct assessment of VO_2max is not possible (62). There exist a multitude of submaximal exercise tests, including diverse step tests, such as the Chester step test, the STEP tool protocol, the modified YMCA 3-minute step test, and the Åstrand-Rhyming step test (64). The coefficient of determination (R^2) of these step tests for the estimation of VO_2max ranged between 0.22 and 0.88 (65–74). Another submaximal step test offers the software of the Actiheart (Cambridge Neurotechnology Ltd., Papworth, UK), a lightweight (10 g), waterproof combined heart rate and movement sensor (accelerometer), which was designed to noninvasively assess daily PAL. By extrapolating the heart rate vs. work rate regression line (obtained during the submaximal step test) to the estimated or measured individual maximum heart rate, an estimated VO_2max can be achieved. Although a good level of agreement between Actiheart and doubly labelled water measured daily TEE was found in adult men and women (75), in children and adolescents (76), and in lean and overweight men of various fitness levels (77), no study assessed the validity of the VO_2max estimation using the Actiheart step test. The Actiheart software enables to enter several parameters into the VO_2max prediction equations, e.g., individual RMR, sleeping heart rate (obtained during Actiheart long-term recordings), and maximum heart rate.

It can be assumed that these variables improve the estimation of VO_2max in comparison to the use of the standard settings (estimated RMR by use of the Schofield equation (78), estimated sleeping heart rate of 70 bpm, and estimated maximum heart rate by use of the Tanaka equation (79)). This issue will be investigated in one part of the present PhD thesis (Article III).

Traditionally, VO_2max is expressed as the ratio of maximum rate of oxygen consumption and body mass ($mL \cdot kg^{-1} \cdot min^{-1}$). This ratio has been used to facilitate the comparison of VO_2max measurements from individuals with different body size, since it is assumed that the difference in the physiological variable due to the individual's size will be removed (80). However, the use of this ratio can be problematic (1) because when simple ratio standards, e.g. VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$), are correlated with a body size dimension, e.g. body mass, the correlations are negative, i.e. the simple ratio standard fails to produce a dimensionless physiological performance variable, and (2) when the linear regression line between the two ratio variables is not passing the origin (80). Therefore, the use of a simple ratio standard imposes a penalty on heavier individuals (81), and is thus inappropriate for studies where VO_2max is compared between groups not matched for body size and mass, or when body mass changes over time (82,83). In **Figure 5** the VO_2max of animals ranging in size from a few grams to 250 kg is displayed. When VO_2max was adjusted for body mass (*m*), small animals have 8 – 10 times higher VO_2max/m values than large animals, which are made possible by an increased mitochondrial density as well as an increased capacity for oxygen transport. The difference in VO_2max seen in animals of different body mass is called "allometric variation", where VO_2max values increase with body mass to the power of 0.81 (60).

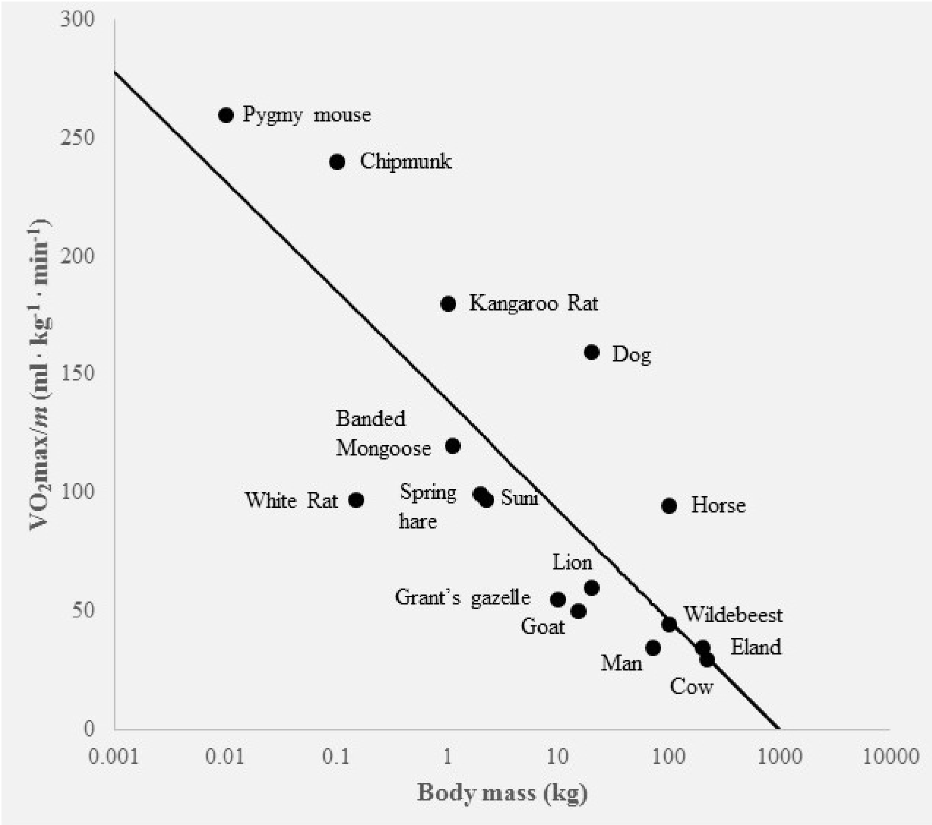

Figure 5 Maximum oxygen consumption (VO_2max/m in mL · kg^{-1} · min^{-1}) of various animal species in relation to body mass (*m*). Adapted from Bassett & Howley (60).

One possibility to remove the effects of *m* is to adjust VO_2max by using the power function relationship $VO_2max = a \cdot m^k$, where *a* is the scaling constant and *k* is the scaling exponent (82). However, there is a great debate as to the theoretical value this exponent should take (e.g., k = 2/3, 3/4 or >3/4) (84,85). Since the effect of *m* on metabolic rate is also a function of body composition, with muscle volume being the most important determinant of metabolic capacity whereas fat tissue is comparatively metabolically inert, fat mass changes would introduce a greater bias as compared to lean mass changes. In the study of Nevill et al. (82) the mass exponents associated with girth measurements were calculated in 279 athletic and 199 non-exercising participants. Their results indicate that the human adult physiques were not geometrically similar to each other, not even within their experimental groups (girth exponents should be approximately proportional to $m^{1/3}$). The thigh muscle girths of athletes and controls increased at a greater rate than predicted by geometric similarity, proportional to body mass ($m^{0.439}$ and $m^{0.377}$, respectively). Assuming that the thigh muscles make a major contribution to VO_2max performance, these findings highlight the danger of using body mass power laws to scale performance variables recorded on individuals of different body size.

One way to remove the effects of body size and composition on VO_2max is to express the aerobic capacity as maximum aerobic metabolic rate in a multiple of metabolic equivalent of tasks (MET), i.e. METmax. However, correct assessment of oxygen consumption at rest requires considerable expense for both participants and researchers. Therefore, researchers might be misled to use the conventional standard 1-MET value (per definition 3.5 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ (86) equivalent to ~1 kcal $\cdot kg^{-1} \cdot h^{-1}$ (87)) or other RMR predicting equations (e.g., Harris-Benedict (88), Cunningham (89)) instead of performing additional RMR measurements. So far there exist no study assessing the error in METmax calculation by using the conventional compared to an individual (measured) 1-MET value.

Although the concept of a MET has been used for quite some time (90), the exact derivation is unknown (91). The MET concept provides a useful way to describe and classify physical activities by expressing the specific level of AEE (under steady state conditions) in relative value, i.e. as a multiple of RMR: theoretically, 10 METs would then correspond to 35 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$, equivalent to ~10 kcal $\cdot kg^{-1} \cdot h^{-1}$. The Compendium of Physical Activities provides a useful five-digit coding scheme linking categories and types of physical activity with their corresponding intensity values in METs (87). Originally, the Compendium was developed for use in epidemiologic and surveillance studies to standardize the MET intensities for various types of physical activity used in questionnaires. However, it is also frequently applied outside of its original scope, i.e. for the determination of precise energy costs of activities. For example, in several studies where physical activity was assessed by questionnaire, the energy expenditure was estimated by using established MET codes from the Compendium of Physical Activity (92–97).

In the last decade, several authors started to question the widespread application of the conventional 1-MET value (91,98–100), since this value was derived from measurements of resting oxygen consumption of just one person, a 70-kg, 40-year old male. In several studies it was shown that the conventional 1-MET value over- (99–106) and underestimates (99) RMR for many types of individuals. In a review of McMurray and colleagues (100) scientific articles that measured RMR were identified to determine the relationship of age, sex, and obesity status to RMR as compared to conventional 1-MET value. They found a mean value for RMR of 0.86 $kcal \cdot kg^{-1} \cdot h^{-1}$ (95% CI = 0.85 – 0.87), higher for men than women, decreasing with increasing age, and lower in overweight than normal weight adults. The conventional 1-MET value *overestimated* RMR approximately by 10% in men and almost 15% in women. Therefore, the authors conclude that no single value for RMR is appropriate for all adults and that the use of the conventional 1-MET value may result in important miscalculations of energy expended during physical activity. In a study by Byrne and colleagues (91) RMR measurements of 642 women and 127 men with an age and body mass range of 18 – 74 years and 35 – 186 kg, respectively, were analyzed (**Figure 6**). The average oxygen consumption and energy cost at rest were 2.6 ± 0.4 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ or 0.85 $kcal \cdot kg^{-1} \cdot h^{-1}$, respectively, and significantly lower than the conventional 1-MET value, which overestimated RMR by 20%. They further found a significant relationship between resting oxygen consumption and sex, age, BMI, percentage of body fat, waist circumference, fat mass, and FFM. Multiple regression analysis revealed that body composition (fat mass and FFM) accounted for 62% of the variance in resting oxygen consumption compared with age, which accounted for only 14%.

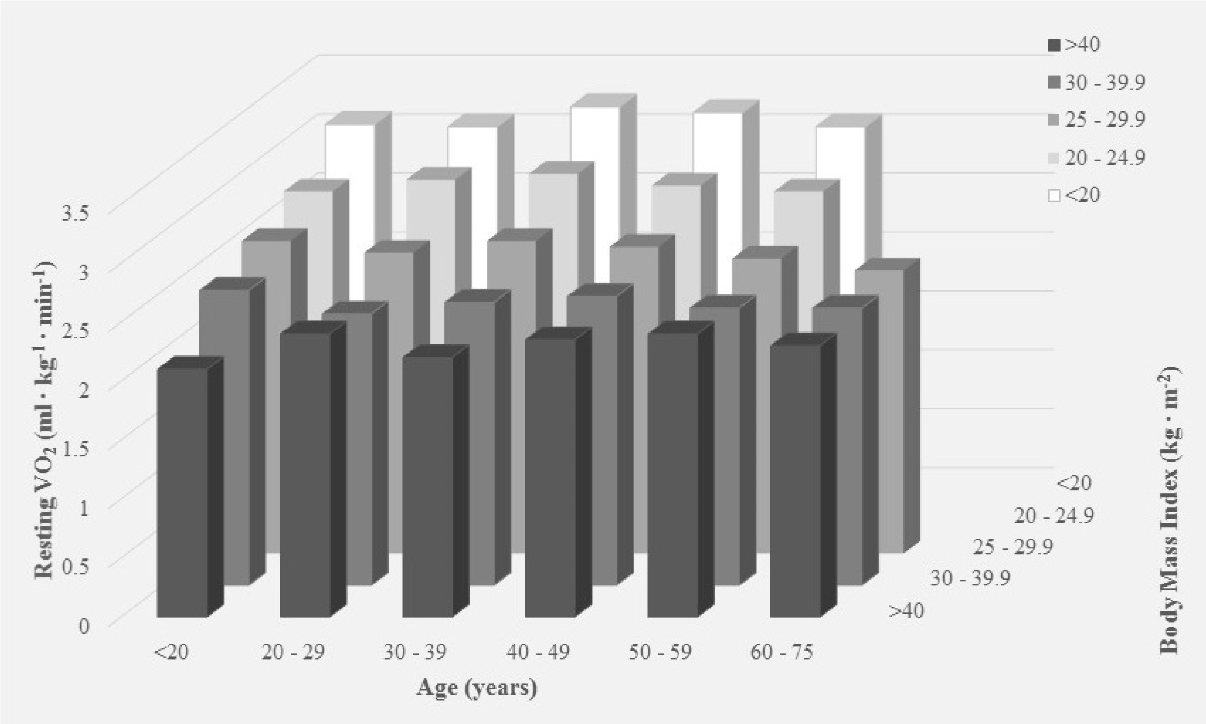

Figure 6 Measured resting oxygen uptake (VO_2; mL · kg^{-1} · min^{-1}) stratified by age and Body Mass Index. Adapted from Byrne et al. (91).

When reviewing data on endurance trained men and women, where RMR was measured using indirect calorimetry, a mean value of 1.11 kcal · kg^{-1} · h^{-1} for women and 1.13 kcal · kg^{-1} · h^{-1} for men can be calculated (107–115). Therefore, it must be assumed that, in contrast to the general population, an estimation of AEE by use of the conventional 1-MET value might lead to an *underestimation* of (true) energy costs. Since PAL and MET values are frequently used for estimation of energy requirements in athletes, there might be at higher risk for promotion of insufficient energy intake due to underestimation of AEE, especially in situations when athletes wish to control their energy balance (e.g., during weight loss or maintenance). This discrepancy between energy intake and energy expenditure would lead to a negative energy balance and thus to undesirable effects on body mass and body composition. In addition, a too low energy intake and further energy availability might lead to a higher risk of suffering from Relative Energy Deficiency in Sport (RED-S), a syndrome which 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 (116). Next to the negative effects on health, RED-S can have potential undesirable performance consequences, such as decreased endurance performance, increased injury risks, decreased glycogen stores, decreased muscle strength, and decreased training responses. However, so far there exists no study where the error of the use of the conventional 1-MET value for estimation of activity and total energy expenditure was quantified in athletes with high energy turnover. In Article IV of the present PhD thesis this issue will be investigated.

1.4. Aims of the thesis

Article I: To (1) systematically analyze TEE, energy intake, and body composition in highly trained athletes of various endurance disciplines and of both sexes with focusing on objective assessment methods, and (2) analyze fluctuations in these parameters across the training season.

Article II: To (1) explore the extent to which physical activity levels of a sample of the US adult population are associated with compliance with dietary intake recommendations for minerals and vitamins, and (2) to explore by how much of an increase in physical activity levels, up to levels recommended for health, combined with a corresponding linear up-scaling of dietary intake without altering dietary composition, would improve compliance with recommended micronutrient intake.

Article III: To estimate VO_2max using the Actiheart step test in two different modes (i.e., AHraw and AHcomplete) and to compare the results with measured VO_2max over a range of aerobic capacities.

Article IV: To quantify the error when the conventional compared to an individualized 1-MET value is used for (1) calculation of METmax, and (2) estimation of energy expenditure for various daily physical activities, in endurance trained women and men and active healthy controls.

Chapter Two

Summary of experimental results

2. Summary of experimental results

2.1. Article I: Total energy expenditure, energy intake, and body composition in endurance athletes across the training season: A systematic review

Contribution: literature research, data analysis, drafting and writing of the manuscript, preparation of tables and figures

Purpose of this systematic review was to: (1) analyze TEE, energy intake, and body composition in highly trained athletes of various endurance disciplines and of both sexes, and (2) analyze fluctuations in these parameters across the training season. Eighty-two articles meeting the inclusion criteria were analyzed. TEE of endurance athletes was significantly higher during the competition phase than during the preparation phase ($p < 0.001$) and significantly higher than energy intake in both phases ($p < 0.001$). During the competition phase, both body mass and fat-free mass were significantly higher compared to other seasonal training phases ($p < 0.05$). In a separate analysis of energy balance by including only studies where both energy intake and expenditure were assessed in parallel a significant energy deficit of 304 kcal · d^{-1} and 2,177 kcal · d^{-1} during the preparation and competition phase was found, respectively ($p < 0.05$; **Figure 7**). In female endurance athletes, a negative energy balance was also observed during the preparation and competition phase (-1,145 kcal · d^{-1} and -1,252 kcal · d^{-1}, respectively; $p < 0.0001$; **Figure 8**). Male and female endurance athletes show important training seasonal fluctuations in TEE, energy intake, and body composition. Therefore, dietary intake recommendations should take into consideration other factors including the actual training load, TEE, and body composition goals of the athlete.

Study or subgroup	Energy intake (kcal/d) Mean	SD	Total	Total energy expenditure (kcal/d) Mean	SD	Total	Weight	Mean difference IV, Fixed, 95% CI
1.1.1 Preparation phase								
Sjodin et al. 1994	7,218	1,099	4	7,218	1,004	4	2.4%	0 [-1,459, 1,459]
Boulay et al. 1994	3,872	382	7	4,063	956	7	8.9%	-191 [-954, 572]
Fudge et al. 2006	3,165	318	9	3,492	249	9	74.1%	-327 [-591, -63]
Subtotal (95% CI)			**20**			**20**	**85.4%**	**-304 [-549, -58]**
Heterogenity: $Chi^2 = 0.28$, df= 2 ($p = 0.87$); $I^2 = 0\%$								
Test for overall effect: Z = 2.42 ($p = 0.02$)								
1.1.2 Competition phase								
Bescós et al. 2012	5,549	2,127	8	10,253	1,625	8	1.5%	-4,704 [-6,559, -2,849]
Costa et al. 2014	5,497	2,868	19	13,862	2,390	19	1.8%	-8,365 [-10,044, -6,686]
Rehrer et al. 2010	6,525	908	4	6,549	478	4	5.1%	-24 [-1,030, 982]
Hulton et al. 2010	4,918	810	4	6,420	470	4	6.1%	-1,502 [-2,420, 584]
Subtotal (95% CI)			**35**			**35**	**14.6%**	**-2,177 [-2,772, -1,582]**
Heterogenity: $Chi^2 = 79.02$, df= 3 ($p < 0.00001$); $I^2 = 96\%$								
Test for overall effect: Z = 7.17 ($p < 0.00001$)								
Total (95% CI)			**55**			**55**	**100%**	**-577 [-804, -349]**
Heterogenity: $Chi^2 = 111.80$, df= 6 ($p < 0.00001$); $I^2 = 95\%$								
Test for overall effect: Z = 4.97 ($p < 0.00001$)								
Test for subgroup differences: $Chi^2 = 32.50$, df= 1 ($p < 0.00001$); $I^2 = 96.9\%$								

Mean difference IV, Fixed, 95% CI

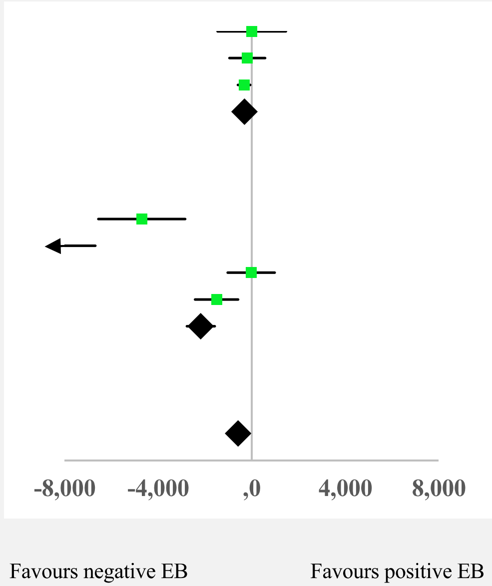

Figure 7 Energy balance (EB) of male endurance athletes during preparation and competition phase.

Study or subgroup	Energy intake (kcal/d) Mean	SD	Total	Total energy expenditure (kcal/d) Mean	SD	Total	Weight	Mean difference IV, Fixed, 95% CI
1.2.1 Preparation phase								
Hill & Davies 2002	2,214	313	7	3,957	1,219	7	6.2%	-1,743 [-2,675, -811]
Sjodin et al. 1994	4,350	454	4	4,374	526	4	11.6%	-24 [-705, 657]
Trappe et al. 1997	3,131	239	5	5,593	502	5	22.6%	-2,462 [-2,949, -1,975]
Schulz et al. 1992	2,193	466	9	2,826	312	9	40.1%	-633 [-999, -267]
Subtotal (95% CI)			**25**			**25**	**80.5%**	**-1,145 [-1,404, -887]**
Heterogenity: Chi² = 47.55, df= 3 ($p < 0.00001$); I² = 94%								
Test for overall effect: Z = 8.68 ($p < 0.00001$)								
1.2.2 Competition phase								
Costa et al. 2014	3,107	1,195	6	10,755	1,912	6	1.7%	-7,648 [-,9452, -5,844]
Winters et al. 1996	2,013	418	10	2,673	781	10	17.8%	-660 [-1,209, -111]
Subtotal (95% CI)			**16**			**16**	**19.5%**	**-1,252 [-1,778, -727]**
Heterogenity: Chi² = 52.75, df= 1 ($p < 0.00001$); I² = 98%								
Test for overall effect: Z = 4.67 ($p < 0.00001$)								
Total (95% CI)			**41**			**41**	**100%**	**-1,166 [-1,398, -934]**
Heterogenity: Chi² = 100.43, df= 5 ($p < 0.00001$); I² = 95%								
Test for overall effect: Z = 9.85 ($p < 0.00001$)								
Test for subgroup differences: Chi² = 0.13, df= 1 (p =0.72); I² = 0%								

Mean difference IV, Fixed, 95% CI

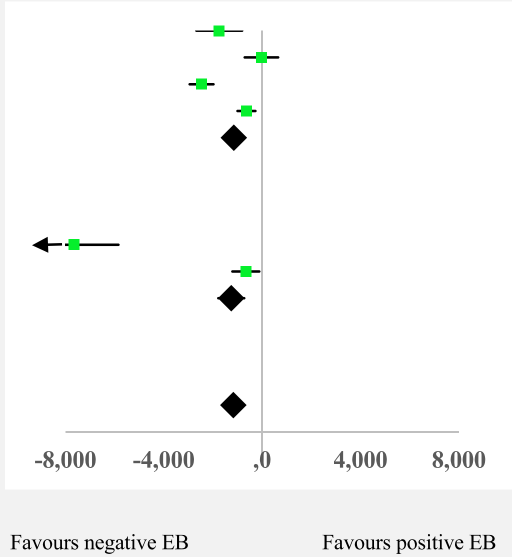

Figure 8 Energy balance (EB) of female endurance athletes during preparation and competition phase.

2.2. Article II: Low energy turnover of physically inactive participants as a determinant of insufficient mineral and vitamin intake in NHANES

Contribution: data analysis, drafting and writing parts of the manuscript, preparation of tables and figures

In this study, we extracted data from NHANES 2003-2006 on 4015 adults (53 ± 18 years (mean ± SD), 29 ± 6 kg · m^2, 48% women) with valid physical activity and food intake measures. In a first step, energy intake was scaled to match TEE assuming energy balance. In a second step, we increased all individual PALs that were < 2.0, up to a PAL of 2.0. In parallel, we linearly increased dietary intake, without changing diet composition. The resulting changes in micronutrient intake were quantified and compliance with recommendations for daily micronutrient intake was checked.

The NHANES population was physically insufficiently active (61% had a PAL < 1.4). The inactive vs. active had significantly lower intake for all micronutrients apart from vitamin A, B12, C, K, and copper ($p < 0.05$). The inactive had insufficient intake for 6 of 19 micronutrients, the active 5 of 19 ($p < 0.05$). Multiple linear regression indicated a lower risk for insufficient micronutrient intake for participants with higher PAL and BMI ($p < 0.001$). Symmetrical up-scaling of food intake with the same dietary composition to meet a theoretical PAL of 2.0 reduced the frequency of insufficient micronutrient intakes in males and females (**Figure 9**).

We conclude that symmetrical up-scaling of PAL and energy intake to recommended physical activity levels reduced the frequency of micronutrient insufficiencies. It follows that prevalence of insufficient micronutrient intake from food in NHANES might partly be determined by low energy turnover from insufficient PAL.

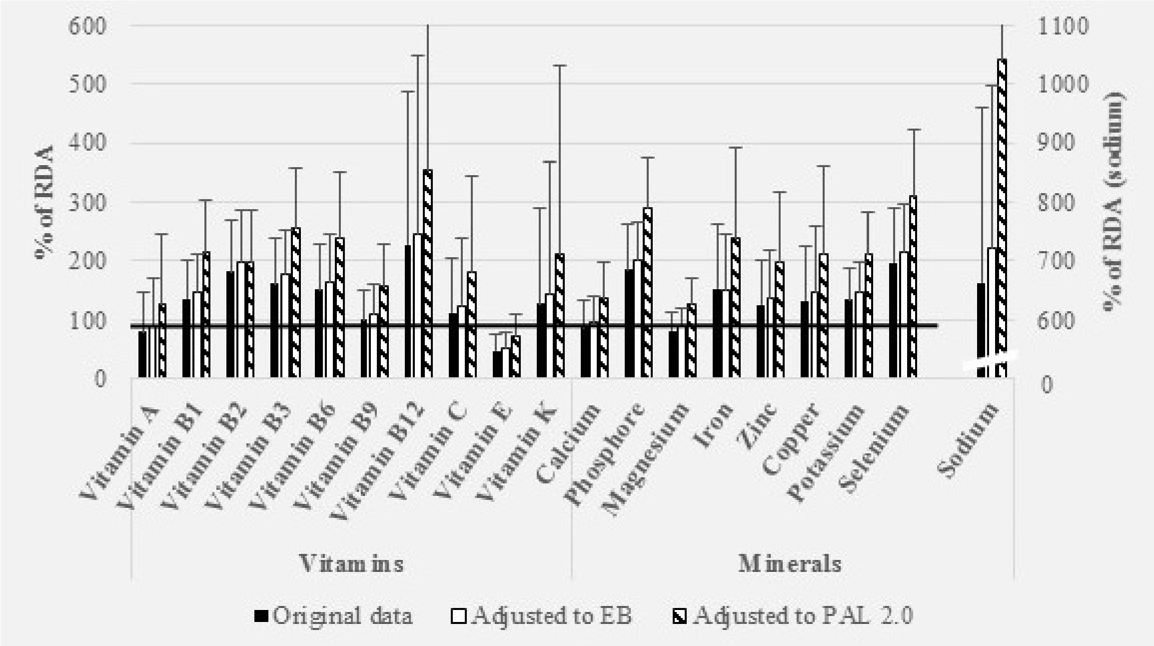

Figure 9 Vitamin and mineral intake in percentage of Recommended Daily Allowance (RDA) for original data (black bars), data adjusted for energy balance (EB, white bars), and data adjusted for Physical Activity Level (PAL) of 2.0 (grey bars). Solid line represents 100% of RDA. Data are shown as Mean ± SD.

2.3. Article III: Validity of the Actiheart step test for the estimation of maximum oxygen consumption in endurance athletes and healthy controls

Contribution: literature research, data assessment, data analysis, drafting and writing of the manuscript, preparation of tables and figures

In this study, we assessed the validity of the Actiheart step test for estimation of VO_2max in 68 endurance trained athletes (ATH; 54% men, 28.0 ± 5.4 years, 20.9 ± 1.7 kg · m^{-2}) and 63 healthy non-athletes (CON; 46% men, 27.6 ± 5.1 years, 22.1 ± 1.7 kg · m^{-2}). We compared two different entry modes of the Actiheart software: (1) AHraw (estimated RMR [Schofield] and maximum heart rate [HRmax; Tanaka], sleeping heart rate [SHR] = 70 bpm) and (2) AHcomplete (measured RMR, HRmax, and SHR).

VO_2max estimated by AHraw was significantly related to measured $\dot{V}O_2max$ in women CON ($R^2 = 0.22$; $p < 0.05$), whereas when VO_2max was estimated by AHcomplete the relation was significant in women ATH and CON, and in men CON ($R^2 = 0.17 - 0.24$; $p < 0.05$). AHraw significantly underestimated VO_2max in the total sample by 8% (51.4 ± 10.2 vs. 55.9 ± 7.6 mL · kg^{-1} · min^{-1}; $p < 0.0001$), whereas no significant difference between AHcomplete and the criterion method was found (57.0 ± 11.1 vs. 55.9 ± 7.6 mL · kg^{-1} · min^{-1}; $p = 0.26$). The range of the Mean Absolute Percentage Error (MAPE) across all groups was 11.4 – 17.7% and 10.8 – 14.7% for AHraw and AHcomplete.

Based on MAPE the Actiheart step test is not an acceptable tool for the estimation of VO_2max. However, accuracy of the VO_2max prediction is much improved when entering measured variables, such as RMR, SHR, and HRmax, into the software.

2.4. Article IV: Comparison of conventional and individualized 1-MET values for expressing maximum aerobic metabolic rate and habitual activity related energy expenditure

Contribution: literature research, data assessment, data analysis, drafting and writing of the manuscript, preparation of tables and figures

Purpose was to quantify the error when the conventional ($3.5\ mL \cdot kg^{-1} \cdot min^{-1}$) compared to an individualized 1-MET-value is used for calculation of METmax and estimation of AEE/TEE in 52 endurance-trained athletes (END; 46% male, 27.9 ± 5.7 years) and 53 active healthy controls (CON; 45% male, 27.3 ± 4.6 years).

There was a significant positive relationship between VO_2max and RMR in all subgroups ($p < 0.0001$). In women and men CON and men END, METmax was significantly higher when the conventional 1-MET-value was used for calculation in comparison to the use of the individual 1-MET value ($p < 0.01$), whereas in women END no difference was found ($p > 0.05$; **Table 1**). The range of MAPE was 6.6 – 11.3% across all groups.

The conventional 1-MET-value significantly underestimated AEE in men and women CON, and men END ($p < 0.05$), but not in women END ($p > 0.05$). Likewise, TEE was significantly underestimated in men and women CON, and men END when the conventional 1-MET value was used for estimation of RMR ($p < 0.05$).

The conventional 1-MET-value appears inappropriate for the determination of the aerobic metabolic capacity and AEE/TEE in active and endurance-trained persons.

Table 1 Values and concurrent validity of the maximum metabolic equivalent of tasks (METmax) by use of the individual (METmax_ind) and conventional (METmax_fix; 3.5 mL · kg^{-1} · min^{-1}) 1-MET-value for calculation in endurance trained participants (END) and healthy controls (CON). Data are presented as Mean ± SD.

	Women		Men	
	CON (*n* = 29)	**END (*n* = 28)**	**CON (*n* = 24)**	**END (*n* = 24)**
METmax_ind	13.3 ± 0.9[2)]	15.5 ± 1.0[1)]	14.9 ± 0.8[3)]	16.9 ± 0.7[1) 2)]
METmax_fix	13.8 ± 1.4	15.9 ± 1.2[1)]	16.3 ± 1.6	18.3 ± 1.8[1)]
r value	0.69[4)]	0.24	0.78[4)]	0.10
MAE	0.9 ± 0.8	1.0 ± 0.9	1.5 ± 0.9	1.9 ± 1.3
MAPE (%)	6.6 ± 6.2	6.8 ± 6.2	10.3 ± 5.9	11.3 ± 7.6
SEE	0.63	1.03	0.52	0.70

MAE = mean absolute error, MAPE = mean absolute percentage error, SEE = standard error of the estimate. [1)] Significantly different from CON of the same sex group ($p < 0.0001$). [2)] Significantly different from METmax_fix of the same sex and experimental group ($p < 0.01$). [3)] Significantly different from METmax_fix of the same sex and experimental group ($p < 0.0001$). [4)] Correlation significant at $p < 0.0001$.

Chapter Three

Discussion and perspectives

3. Discussion and perspectives

3.1. Variations of energy balance throughout the training season in athletes with high energy turnover

Article I provides new insights regarding the fluctuations in energy intake, energy expenditure and body composition in athletes with high energy turnover, i.e. endurance athletes. The main findings include that some, but not all, investigated outcomes depend on the time point of data assessment during seasonal training, e.g. during competition vs. preparation phase. For example, TEE was highest during the competition phase, whereas energy intake did not follow TEE alterations, at least in women athletes. Generally, in the included studies a high prevalence of under-reporting of energy intake was observed. Based on the citations to date upon the publication of the article in 2017 (*n* = 24; source: Google Scholar, date of search: 2019 17 Apr) it would seem that our analysis answered a need in the scientific community.

Although there seems to be reasonable evidence for fluctuations in energy expenditure due to altering training loads in endurance athletes, which should go along with adaptations in energy intake and/or body mass changes, only few studies report the time point of data assessment, i.e. the seasonal training phase. Therefore, only 2% of the articles matching the search strategy could be included into this systematic analysis. Future studies dealing with energy balance and/or body composition not exclusively focusing on endurance athletes, should always report the time point of data assessment with regard to the seasonal training phase. There was also a lack of longitudinal studies assessing either energy balance or body composition changes throughout the training season, and a lack of studies with focusing on the transition phase ("off-season") or when an athlete has to cover with a sudden stop of elite training, e.g. due to injury. Only few studies examined the effect of detraining on body composition in endurance athletes.

While LaForgia et al. found no difference in RMR and body fat percentage in male endurance athletes after three weeks of detraining (110), others found a decreased RMR and increasing fat and body mass after five weeks of detraining in men and women swimmers (117).

The separate analysis of studies where energy intake and expenditure were investigated at the same time highlighted an important apparent negative energy balance in men and women endurance athletes, independent of the seasonal training phase. The negative energy balance mounted up to 304 kcal · d^{-1} (4.7% of TEE) and 1145 kcal · d^{-1} (27.8%) for men and women athletes during the preparation phase, respectively, whereas during the competition phase even higher values of 2177 kcal · d^{-1} (32.5%) for males and 1252 kcal · d^{-1} (47.9%) for men and women were obtained. These findings can be explained by the high magnitude of under-reporting generally found in athletes (39), a low accuracy and precision of the dietary assessment tools used in these studies (39), and the fact that studies assessing energy balance during the competition phase investigated TEE during a competition and not during a habitual training day in the competition phase (viz. energy intake might be limited). There seems to be also a certain threshold of energy expenditure (probably around 20 MJ or 4780 kcal), where athletes are not further able to consume sufficient conventional food to provide the body adequate energy for compensation of the increased energy turnover (118). Nevertheless, the high magnitude of under-reporting supports the ongoing debate about the use of self-reported dietary intake data for estimation of energy intake (40).

When analyzing the few longitudinal studies assessing body composition during preparation and competition phase, both men and women endurance athletes showed a significant lower body fat percentage and higher FFM during the competition phase. These findings support the model of training periodization and adaptive responses of food intake and/or body composition due to differing training loads. It seems to be obvious that during the competition phase the athletes should be in “peak” condition, not only with regard to their performance but also with regard to their body composition. These findings are supported by the various body composition data assessed immediately before a competition, often showing extremely low body fat percentages in elite endurance athletes (41,119).

3.2. Low energy turnover as a determinant of insufficient micronutrient intake

The main findings of Article II are that (1) the actual energy intake was underestimated in the NHANES participants and that these data have to be adjusted before interpretation, (2) that individuals with higher PAL were more in line with current micronutrient recommendations compared to inactive individuals, and (3) that a linear up-scaling of food intake to meet energy balance with a PAL of 2.0 without altering diet composition led to an increased compliance with micronutrient intake recommendations.

These findings are in concordance with the available literature, indicating that a lack of physical activity increases the risk for micronutrient deficiencies, whereas an increase of PAL might be protective (49). In the present study those participants meeting the micronutrient intake recommendations at baseline had a significant higher PAL compared to those not meeting the recommendations for at least one mineral or vitamin. Similar findings were observed in the study of Csizmadi et al. (57), where individuals with lower PAL showed a higher prevalence of low micronutrients compared to active individuals, and in another study where the authors found a positive association between higher levels of physical activity in leisure time and higher micronutrient intakes (58). When we increased the PAL of the individuals of the present study to 2.0 and at the same time increased energy and nutrient intake in order to match for the additional energy costs, we found that the mean intakes of all vitamins and minerals increased by 56 – 66%. We also observed a lower number of participants with micronutrient deficiencies. These findings clearly indicate that additional physical activity and concomitant increasing food intake may support the adequacy of delivering essential nutrients to the body and may even improve health. However, according to Fogelholm (49) two main aspects have to be considered: First of all, a certain limit of physical activity has to be exceeded to affect micronutrient intake and, second, that the micronutrient increase might be not as large as the increase in energy expenditure when compensating for increasing energy expenditure. For the results of the present study this would mean that the effect of additional physical activity on the true/"real-life" micronutrient intake would be smaller than estimated in our theoretical model. However, there is future research in real-life conditions warranted to examine exactly this point: whether an increase in energy expenditure due to higher PAL would lead to an adaptation of energy and nutrient intake, and how big the difference is between increasing energy supply and intake of micronutrients.

When analyzing the baseline data of the present study we observed a great magnitude of under-reporting, which mounted up to an average of 176 and 109 kcal · d^{-1} for women and men, respectively, with greater under-reporting for those with higher BMI ($r = 0.34$, $p < 0.0001$) and higher PAL ($r = 0.12$, $p < 0.0001$). These findings are in line with previous published values of the NHANES population, showing a great magnitude of under-reporting, in particular in obese individuals (120,121). Reason for the under-reporting might be the use of the 2 x 24 h dietary recall method, a method lacking accuracy of quantifying eating habits with reported underestimations of intake up to 20% (122). Since we were able to quantify under-reporting because of the availability of physical activity data, we assumed in a first step energy balance and changed energy and nutrient intake in order to match the participants' individual energy expenditure. However, we still found a high percentage of subjects with insufficient micronutrient intake, where again inactive participants had a lower intake for all micronutrients apart from vitamin K compared to the active participants. These results support the statement that individuals with low energy turnover and related higher obesity rates are at a high risk for development of micronutrient deficiencies (50).

3.3. Aerobic capacity as an indicator of physical fitness and energy turnover

In Article III the validity of the Actiheart step test for estimation of VO_2max in men and women along a range of aerobic fitness levels was evaluated by comparing two different data entry methods (viz. AHraw and AHcomplete).

In this study a significant relationship between estimated and measured VO_2max for the total sample was observed, with R^2 0.24 for AHraw and 0.36 for AHcomplete; falling in the range of values for R^2 previously reported in the literature for step test estimated VO_2max (65–74). In addition, the absolute measures of agreement showed similar or slightly better validity of the Actiheart step test for the estimation of VO_2max compared to other studies (SEE: 3.98 – 6.31 $ml \cdot kg^{-1} \cdot min^{-1}$ for AHraw and 3.91 – 6.15 $ml \cdot kg^{-1} \cdot min^{-1}$ for AHcomplete). Previous studies reported an SEE of 6.9 – 8.76 $ml \cdot kg^{-1} \cdot min^{-1}$ for the modified YMCA 3-minute step test in healthy men and women (72) and 3.9 $ml \cdot kg^{-1} \cdot min^{-1}$ for the Chester step test in healthy adults (73). When looking at the absolute difference between measured and estimated VO_2max, in 28.3% of the athletes the value was smaller than 5 $ml \cdot kg^{-1} \cdot min^{-1}$ for AHraw, whereas when the AHcomplete entry method was used, 45.7% had a smaller absolute difference. These results indicate that in endurance trained individuals the AHcomplete entry method is more accurate than AHraw for prediction of aerobic capacity. If possible, researchers should manually enter additional values, such as measured RMR, HRmax and SHR into the Actiheart software in order to improve the accuracy. However, based on the MAPE values observed in the present study (AHraw: 11.4 – 17.7%, AHcomplete: 10.8 – 14.7%), it must be concluded that both data entry methods were not acceptable for estimation of VO_2max in endurance trained individuals and healthy controls and cannot replace a maximum exercise test.

In Article IV the absolute and relative errors using the conventional 1-MET value (1 $kcal \cdot kg^{-1} \cdot h^{-1}$) compared to an individualized RMR value for determination of METmax and estimation of AEE and TEE was quantified. In men and women controls, and endurance trained men the individual 1-MET value was significantly higher than the conventional 1-MET value ($p < 0.05$). These results are in contrast to the majority of published studies, where the individual 1-MET value was *lower* than 3.5 ml · kg^{-1} · min^{-1} or 1 kcal · kg^{-1} · h^{-1} (91,100). However, most of these studies included a very heterogeneous sample of various age and body size. In the present study, endurance athletes and active control subjects were analyzed, with relatively lower body fat levels, higher FFM, normal BMI, and higher aerobic capacities than usually observed in the general Swiss population (123,124). Since fat mass is the strongest predictor of the variability of resting VO_2 (91), these differences in body composition might explain the higher individual 1-MET value of our participants compared to the values reported in the literature.

Anyway, the use of the conventional 1-MET value led to a significant overestimation of METmax and underestimation of AEE and TEE in the subjects of the present study (apart for women endurance athletes). The range of the MAPE for METmax calculation by use of either the individual or the conventional 1-MET value was 6.6 – 11.3% across all groups. TEE and AEE were underestimated by average 1.5 – 7.5% across all groups when the conventional 1-MET value compared to the individual 1-MET value was used for estimation. Therefore, it can be concluded that the use of the conventional 1-MET value is inappropriate for determination of the aerobic capacity and estimation of daily activity related energy expenditure in active individuals and endurance trained athletes.

3.4. Strengths and limitations

Article I is the first systematic review assessing fluctuations in energy balance and body composition in endurance athletes across the training season. Only studies assessing body composition and energy expenditure by use of validated measures (e.g., dual x-ray absorptiometry for body composition, DLW/accelerometry for TEE) were included into the analysis, leading to a high robustness of the outcomes on the one side, but on the other to a limited number of included study estimates. Inclusion of for example the skinfold technique for estimation of body fat percentage would have increased the number of publications which would have been incorporated into the analysis and may have led to a higher explanatory power of the fluctuations across different training phases. However, since the results of skinfold measurements are highly variable when assessors with limited training and experience perform the measurements (125), the accuracy of measurements depends on the number of measurement sites and the formula used to calculate body fat (126), and many different techniques exist (127), we decided to not include these studies into our analysis.

In Article II a large dataset with objectively measured physical activity was analyzed for investigating the research question whether a linear up-scaling of energy and nutrient intake in order to match increased energy expenditure can decrease the magnitude of micronutrient insufficiencies. However, this study has several limitations. First of all, there is again the issue of the accuracy of self-reported dietary intake data, such as the 2 x 24 h dietary recall method used in the present study population, which has shown to underestimate energy intake up to 20% (122). Furthermore, in our theoretical model we assumed that all food constituents were underreported by the same degree, which might not be true in reality, such as shown in a study of Goris et al. where fat intake was more often underreported than other food constituents (128). Accelerometer data are also prone to error, especially they lack accuracy during low-intensity activities and are not able to detect static exercise (129). This might lead to underestimations of true energy costs. Finally, our modelling strategy applied the assumption that an increase on PAL would be automatically compensated by a reciprocal increase in food intake without changes in dietary composition. In reality, the associations between physical activity vs. linear up-scaling in diet quantity and composition might be non-linear, and also dependent on the obesity status. However, the chosen study model may serve as a baseline for future studies investigating the effect of increased PAL on adaptations of energy intake and diet composition.

Limitations of article III include a strong selection bias of participants in studies involving maximum exercise testing, leading to an inclusion of fitter individuals than usually observed in the general population. Second, performing a step test requires the person's ability to maintain a certain stepping tempo and technique. Alterations in stepping technique can affect the mechanical efficiency and further the physiological heart rate response and oxygen consumption (64). The step test was also performed using a pre-defined step height, which might introduce potential error since there is a high inter-individual difference in leg length, step length, body mass, and morphology leading to individual physiological responses. Another potential source of error is the assumption that there is a linear relationship between VO_2 and power output as shown by Åstrand and Rodahl (130). However, other authors found a non-linear relationship between both variables, which may affect VO_2max prediction by use of submaximal exercise tests (131–133). Strengths include high number of individuals at the upper range of aerobic capacities, and the availability of RMR, body composition and long-term Actiheart data for all participants.

Article IV is the first study with the purpose to assess the individual 1-MET value in endurance trained athletes. The results of this study show that expressing aerobic capacity as a ratio of maximum oxygen consumption divided by oxygen consumption at rest is a suitable measure in endurance athletes and healthy, active controls. However, this study has some limitations. First of all, we did not include overweight or obese individuals into the study, so the applicability of the METmax-concept and the quantification of the error of the conventional 1-MET value for estimation of AEE/TEE in these individuals remains unclear. Dietary intake was not analyzed in this study; however, objective assessment of energy intake would have given further information about the interplay with energy requirements and aerobic metabolic capacity.

3.5. Overall conclusion and perspectives

The present thesis demonstrated that men and women endurance athletes show important fluctuations in energy turnover across the training season. Therefore, periodized nutritional guidelines should be developed taking into consideration also the actual training load, TEE, and the body composition goal of the athlete, in order to provide the athlete with adequate nutrients and energy. Studies dealing with energy balance, body composition, and/or nutrient intake in endurance athletes should always mention the time point of data assessment (viz. seasonal training phase). The results of the present thesis once again demonstrate the uselessness of self-reported dietary intake data in endurance athletes, a well-known limitation of many energy balance studies. Future areas of research include the investigation of valid and applicable energy intake assessment methods in individuals with high energy turnover and the assessment of energy turnover during periods of low training intensity and volume (e.g. during transition phase).

Results of the present thesis also indicate that individuals with low energy turnover have a higher risk for the development of insufficient micronutrient intake, as those with higher levels of physical activity. As demonstrated using a linear model an increase in the population's PAL might also lead to increased energy intake in order to cover the increased energy expenditure and, at the same time, increased mineral and vitamin intake, reducing the prevalence of micronutrient deficiencies. However, future studies should investigate which level of additional physical activity is required to increase both daily energy and micronutrient intake in real-life conditions and verify the hypothesis that the increase of micronutrient intake might not be as large as the increase in energy intake when compensating for increasing energy expenditure (49).

For estimation of VO_2max as an indicator of physical fitness in young adults with good to superior aerobic capacity the present thesis highlighted that the Actiheart step test was, in general, not acceptable. The manual entry of additional measured variables, such as RMR, HRmax and SHR, improved the accuracy of VO_2max prediction. However, future studies are required to investigate the validity of the Actiheart step test for estimation of aerobic capacity in older and sedentary individuals, in the clinical setting, and to assess the effect of using different step heights. In addition, the effect on validity of entry of additional variables (e.g., heart rate and VO_2 obtained during the Actiheart step test) on VO_2max prediction should be investigated.

Furthermore, the use of the conventional 1-MET value appeared inappropriate for the determination of the aerobic metabolic capacity and estimation of daily energy expenditure in active and endurance trained individuals. For the valid assessment of METmax (calculated from VO_2max) the resting oxygen consumption should be measured or, if not possible, estimated by use of published validated equations considering the group characteristics, such as age, sex, body composition (FFM), physiological status (e.g., pregnancy), and ethnicity. Future studies should examine whether a direct assessment of resting VO_2 immediately prior to maximum exercise testing (e.g. standing still on the treadmill or sitting quietly on a bike) could serve as a proxy for RMR measurement. Energy expenditure should be either (1) measured directly by use of validated tools, or (2) estimated using measured (or at least estimated) RMR values and appropriate adjusted MET values (87) for determination of the energy costs of various structured exercises as well as for free-living daily physical activities.

Chapter Four

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4. References

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Appendix I

Total energy expenditure, energy intake, and body composition in endurance athletes across the training season: A systematic review

Appendix I: Total energy expenditure, energy intake, and body composition in endurance athletes across the training season: A systematic review

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SYSTEMATIC REVIEW Open Access

Total Energy Expenditure, Energy Intake, and Body Composition in Endurance Athletes Across the Training Season: A Systematic Review

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Abstract

Background: Endurance athletes perform periodized training in order to prepare for main competitions and maximize performance. However, the coupling between alterations of total energy expenditure (TEE), energy intake, and body composition during different seasonal training phases is unclear. So far, no systematic review has assessed fluctuations in TEE, energy intake, and/or body composition in endurance athletes across the training season.

The purpose of this study was to (1) systematically analyze TEE, energy intake, and body composition in highly trained athletes of various endurance disciplines and of both sexes and (2) analyze fluctuations in these parameters across the training season.

Methods: An electronic database search was conducted on the SPORTDiscus and MEDLINE (January 1990–31 January 2015) databases using a combination of relevant keywords.

Two independent reviewers identified potentially relevant studies. Where a consensus was not reached, a third reviewer was consulted. Original research articles that examined TEE, energy intake, and/or body composition in 18–40-year-old endurance athletes and reported the seasonal training phases of data assessment were included in the review. Articles were excluded if body composition was assessed by skinfold measurements, TEE was assessed by questionnaires, or data could not be split between the sexes.

Two reviewers assessed the quality of studies independently. Data on subject characteristics, TEE, energy intake, and/or body composition were extracted from the included studies. Subjects were categorized according to their sex and endurance discipline and each study allocated a weight within categories based on the number of subjects assessed. Extracted data were used to calculate weighted means and standard deviations for parameters of TEE, energy intake, and/or body composition.

Results: From 3589 citations, 321 articles were identified as potentially relevant, with 82 meeting all of the inclusion criteria. TEE of endurance athletes was significantly higher during the competition phase than during the preparation phase ($p < 0.001$) and significantly higher than energy intake in both phases ($p < 0.001$). During the competition phase, both body mass and fat-free mass were significantly higher compared to other seasonal training phases ($p < 0.05$).

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Conclusions: Limitations of the present study included insufficient data being available for all seasonal training phases and thus low explanatory power of single parameters. Additionally, the classification of the different seasonal training phases has to be discussed.

Male and female endurance athletes show important training seasonal fluctuations in TEE, energy intake, and body composition. Therefore, dietary intake recommendations should take into consideration other factors including the actual training load, TEE, and body composition goals of the athlete.

Key Points

- Endurance athletes show training seasonal fluctuations in TEE, energy intake, and body composition.
- Dietary recommendations should consider the actual training load, TEE, and body composition goals.

Background

Total energy expenditure (TEE) is composed of the energy costs of the processes essential for life (basal metabolic rate (BMR), 60–80% of TEE), of the energy expended in order to digest, absorb, and convert food (diet-induced thermogenesis, ~10%), and the energy expended during physical activities (activity energy expenditure, ~15–30%) [1, 2]. Elite endurance athletes are characterized by high fluctuations of TEE, mainly due to the variability of the energy expended during sporting activities. Among elite senior endurance athletes, training loads from 500 h/year [3, 4] up to 1000 h/year [5–7] have been reported, depending on the specific muscular loading characteristic of the sport. During heavy sustained exercise (e.g., during the Tour de France), TEE can be as high as fivefold the BMR over several weeks [8]. On the other hand, during recovery days, pre-competition tapers, or during the off-season, the energy expended in activities is far less. Therefore, TEE is expected to be much lower and may even reach levels comparable to that of sedentary behavior.

An appropriate energy intake supports optimal body function, determines the capacity for intake of macronutrients and micronutrients, and assists in manipulating body composition in athletes [9]. It is a challenge for each endurance athlete to appropriately match energy intake and TEE in order to achieve energy balance and thus, weight stability, both on a micro level (i.e., over 1 day or several days) and through the training and competitive season. Furthermore, endurance athletes in general strive for a low body mass and/or body fat level for various advantages in their sports, specifically during the competition season [10]. This allows runners and cyclists to reach greater economy of movement and better thermoregulatory capacity from a favorable ratio of weight to surface area and less insulation from subcutaneous fat tissue. Elite endurance athletes are therefore characterized by low body mass and body fat content. For example, in elite Kenyan endurance runners, the body fat percentage was 7.1% [11], which is only marginally above the recommended 5% minimum for males [12]. In the same athletes, body mass index (BMI) was 18.3 kg/m^2 [11], which is generally classified as being underweight [13]. However, these athletes were in peak physical conditions as the investigations were undertaken and a low body fat percentage and body weight might be an advantage for competition. Achieving a negative energy balance and a concomitant loss of body and fat masses in preparation for competition can be accomplished in phases with high daily TEE solely by the reduction of energy intake, since any further training load increases could cause overtraining [12]. Therefore, the nutritional goals and requirements of endurance athletes are not static over the training year. Since endurance athletes undertake a periodized training program and follow periodized body composition goals, the nutritional support also needs to be periodized [9].

Usually, the annual training schedule of an elite endurance athlete is divided into distinct phases, each with very specific objectives. This is necessary to maximize physiological adaptations for improved performance, usually scheduled to peak around the main competitions of the year [14]. The principle of training periodization was first introduced in the 1960s by the Soviet trainer Leo Matveyev [15] and has not fundamentally changed since then [14]. The basis of this model is to prepare the athlete for one or more major competitions during the year by separating the training into the following three main phases (macrocycles): preparatory, competitive, and transition phases [15]. An example for a "one-peak annual plan" for a runner is shown in Fig. 1. The preparatory phase is characterized by predominantly high-volume training at moderate intensities, which improves endurance capacity and provides a more efficient use of fuel substrates. During the late preparatory phase, training volume is reduced while intensity is gradually increased. The goal of this phase is to reach peak performance and to transfer the training effects into the competitive phase, where exercise intensity is the highest. In the week before an important competition, volume and intensity are typically decreased (taper phase) to allow the body to optimally

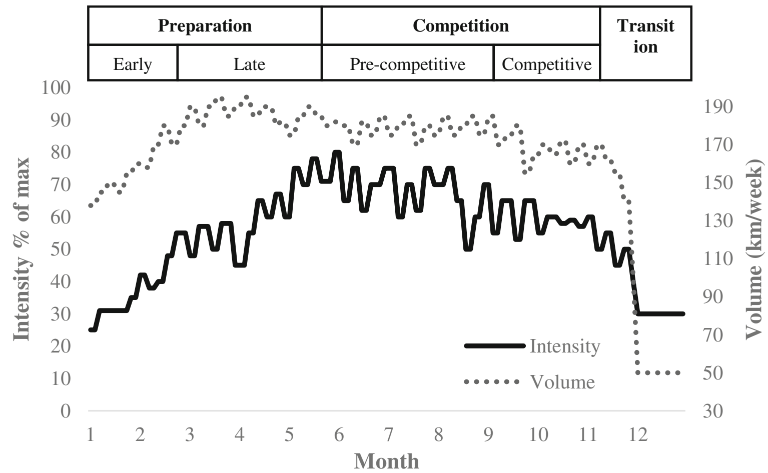


Fig. 1 Periodization of the training year for a "one-peak annual year" of an elite runner. Adapted from Bompa & Haff [16]

recover for competition. The days and weeks after a main competition are characterized by low-intensity and low-volume training, with goals to induce regeneration and to prepare the athlete mentally and physically for the next training cycle (transition phase) [14, 16].

Although the concept of training periodization in elite endurance sports has been established for a long time, the coupling of periodized training with nutrition and body composition has gained scientific awareness only recently [17]. Stellingwerff's group was one of the first to publish periodized nutrition guidelines for middle-distance athletes [17], they then expanded these recommendations for a multitude of power sports [18]. Nowadays, there are guidelines for carbohydrate, protein, and fat intake during training and competition phases, not exclusively focusing on endurance sports [19–21]. Meanwhile, for endurance athletes, sport-specific dietary intake recommendations were developed only for a few endurance disciplines (e.g., swimmers [22–25], distance runners [26], marathon/triathlon/road cycling [27]). But it remains unclear whether endurance athletes are actually following these nutrient guidelines across all seasonal training phases.

The validity of either body composition, energy intake, or TEE-determination in athletes strongly depends on the methods used. The measurement of body composition in general is prone to error. It has been shown that acute food or fluid ingestion [28], subject positioning [29], previous physical activity [30], and hydration status [31] have an impact on reliability of body composition measurement. Since endurance athletes often train several times per day, it might be difficult to assure best conditions for body composition assessment. According to a recent methodology review performed by Nana et al., only few of the studies, where body composition of athletes was measured with dual X-ray absorptiometry (DXA), provided details about their subject and device standardization [30]. However, other methods like skinfold measurements require highly experienced investigators [32] and strongly depend on the number of measurement sites and the formula used to calculate the percentage of body fat [33]. Therefore, it is important to report standardization protocols in order to evaluate the quality of data assessment. One main issue in assessing energy intake in athletes is the magnitude of under-reporting, which can amount to 10–45% of TEE [34]. It was shown that the magnitude of under-reporting increases as energy requirements increase [34]. Since endurance athletes are often characterized by high TEE, we must assume that these athletes are very prone to a high percentage of under-reporting. For determination of TEE objective methods such as doubly labelled water (DLW) or heart frequency measurements are available. However, in many studies subjective methods such as activity records and activity questionnaires are used in order to assess the activity level and TEE of subjects. These methods *estimate* TEE or activity level and their validity strongly depends on the breadth of the activity dimensions analyzed.

There exist some longitudinal studies that have assessed fluctuations in body composition, dietary intake, and/or TEE of endurance athletes across the training seasons [35–52], but no systematic reviews have been performed. Therefore, the purpose of this study was to (1) systematically analyze TEE, energy intake, and body composition in highly trained athletes of various endurance disciplines and of both sexes with focusing on objective assessment methods and (2) analyze fluctuations in these parameters across the training season. We hypothesized that endurance athletes show large fluctuations of TEE during different seasonal training phases due to differing exercise loads, and concomitant alterations in energy intake and body composition.

Methods

The review protocol was developed according to the Meta-analysis of Observational Studies in Epidemiology Guidelines for meta-analyses and systematic reviews of observational studies [53].

Search Strategy

A systematic literature search was performed to retrieve articles pertaining to body composition, energy intake, and TEE in endurance athletes across the training season. One researcher (JH) conducted the search for publications on 31 January 2015 in the electronic databases MEDLINE (via PubMed) and SPORTDiscus with Full Text (via EBSCOHost). A hand search of relevant reviews was performed to obtain additional articles missed by the database search. No individual or organization was contacted to receive further publications. To identify the population of endurance athletes, the following keywords connected with the Boolean operator "OR" were searched: endurance athletes, endurance-trained, endurance trained, aerobically trained, runners, swimmers, triathletes, skiers, cyclists, and rowers. To identify the outcome of body composition, TEE, and energy intake, the following keywords connected with the Boolean operator "OR" were searched: body composition, fat mass, fat-mass, fat free mass, fat-free mass, body fat, metabolic rate, energy expenditure, dietary intake, food intake, energy intake, food consumption, and macronutrient*. Terms for the study population and outcomes were combined by the use of the Boolean operator "AND". Limits included articles published in the English language, human studies, and publishing date limits between 1990 and January 2015. Keywords were searched as free text in the title, abstract, and subject heading. A detailed overview of search strategies in the two databases can be obtained in Additional file 1: Table S1.

Literature Selection

Two researchers independently assessed the eligibility of the records by screening the title, abstract, and keywords for inclusion and exclusion criteria. An agreement between the two researchers was quantified by kappa statistics [54]. The full texts of all abstracts meeting the eligibility criteria were retrieved and subjected to a second assessment for relevance performed by one author (JH).

The inclusion criteria included (1) articles reporting original data in peer-reviewed journals; (2) in vivo, human analyses; (3) adult endurance athletes (highly aerobically trained individuals who were engaged in a competitive endurance sport) with a mean age of 18–40 years; (4) reporting of training seasonal phase of data assessment; and (5) assessment of body composition and/or ad libitum daily energy intake and/or daily TEE. Articles were excluded from the review if (1) the article was only in abstract form or a case report, (2) data could not be split between the sexes (where both male and female subjects were analyzed), (3) body composition was assessed by skinfold measurements, (4) daily TEE was assessed by the use of questionnaires, and (5) descriptive quantitative results were not reported in a text or tabular form. Any difference in assessments between the two researchers was discussed in the first instance or resolved by a third author (KM).

Methodological Quality Assessment

All relevant articles were examined for full methodological quality using a modified version of the Downs and Black [55] checklist for the assessment of the methodological quality of randomized and non-randomized studies of health care interventions. According to Fox et al. [56], 10 of the 27 criteria that logically applied to all of the types of studies included in this review were used. The maximum possible total score was 10. Two researchers assessed the study quality independently, with differences resolved by consensus or by a third author (KM). The agreement between the two researchers was quantified by kappa statistics [54]. Based on the assessment of the methodological study quality, no studies were excluded and no additional analyses were undertaken. The methodological quality of the included studies is shown in Additional file 2: Table S2.

Data Extraction

Body composition, energy intake, and/or TEE data were extracted from all studies included in the review by the first author (JH). Demographic and methodological data were also extracted for the following confounding factors: age, sex, sports discipline, competition level, seasonal phase, and methods for assessing body composition, energy intake, and/or TEE. If the same subjects were analyzed during different time points in the same seasonal phase (e.g., energy intake before three different races, or assessment of energy intake at three time points during the training period), the first time point was chosen for data analysis to facilitate data entry and to avoid selection bias. If studies reported any intervention leading to a non-habitual behavior of athletes' nutrient intakes (e.g., dietary supplementation), the baseline and/or control group data were used. To enable comparisons between studies, reported units were converted into standard units. These conversions were performed by using the reported mean values of the outcomes. Energy intake and TEE were reported in either absolute (kcal/day) or relative values (energy intake or TEE in relation to body weight [kcal/kg·day]). Body composition was converted into fat mass (%, kg) and fat-free mass (kg). According to the definition by Wang et al. [57], the terms lean body mass and fat-free mass (FFM) were considered synonymous. Duplicate publications from the same data set were identified according to the criteria published in the Cochrane Handbook for Systematic Reviews of Intervention [58]. The most complete record was then used for data extraction.

According to the traditional periodization model, the reported seasonal training phases of data assessment were clustered into three groups that included the preparation phase, the competition phase, and the transition phase [14–16]. A detailed overview of the clustering can be obtained in Table 1.

Statistical Analysis

The main outcome measures were body composition (fat mass, FFM), energy intake, and TEE of endurance athletes across the season. Once all of the relevant data were extracted, the weighted mean and standard deviation of the weighted mean were calculated for the main outcome variables. Based on the number of subjects examined within the study, relative to the total number of subjects examined for the specific variable, a percentage weight (w) was allocated to each result within each outcome variable and used for the calculation of the overall weighted mean (X_w) and standard deviation of the weighted mean (SD_w) for each variable [59]. A capital "N" denotes the number of separate studies, while a small "n" denotes the number of included individual subjects.

Statistical analyses were performed using the statistical software SPSS statistics version 22 for Windows (IBM Corp., Chicago, IL, USA). p values < 0.05 were considered statistically significant. Kolmogorov-Smirnov tests were performed to check for normal distributions. All parameters were normally distributed except body mass, fat mass, and FFM. To test for comparisons of subgroups, one-factorial analyses of variance (ANOVAs) with Scheffé post hoc tests (parametric) and Kruskal-Wallis tests (H-test) with Mann-Whitney U post hoc tests (non-parametric) were performed. When multiple non-parametric post hoc tests were applied, Bonferroni-adjusted alpha levels were applied. Since parameters for body composition were not normally distributed, we abstained from multiple statistical comparisons between seasonal training phases and endurance disciplines to reduce the risk of type I errors. For comparisons of energy intake and TEE during different seasonal training phases, paired t-tests were used. The separate analysis of studies, where energy intake and TEE were assessed in parallel, and longitudinal studies that reported energy intake during different training season phases, were performed using the free software for meta-analysis Review Manager 5 version 5.3.5 for Windows (Cochrane Collaboration, Copenhagen, Denmark). The results were then presented as means and 95% confidence intervals (95% CI).

Results

Description of Studies and Assessment Methods

The flow chart for the study selection process is shown in Fig. 2. Data were extracted from 82 studies in endurance athletes, with 53 studies assessing body composition, 48 energy intake, and 14 TEE. The kappa value of 0.47 for the agreement between the two researchers who assessed the eligibility of records was considered to reflect a "fair agreement", whereas "excellent agreement" (kappa value of 0.96) was obtained for the assessment of the methodological quality of included studies [54].

The characteristics of the included studies for body composition, energy intake, and TEE are shown in Table 2. In Additional file 3: Table S3, an overview of excluded studies and the reasons for their exclusion can be found.

The cumulative number of subjects included in the analysis was 1674 (71.4% male). Runners (27.8%), cyclists (18.7%), and swimmers (16.4%) comprised the largest proportion of subjects. All athletes for whom an endurance sports discipline was not described or for whom multiple endurance disciplines were mentioned were grouped into "other endurance athletes" (13.5%). On average, the mean age, VO_{2max}, and training volume of study estimates were 26.3 ± 6.7 years, 61.8 ± 6.0 mL/kg min, and 12.0 ± 6.9 h/week, respectively ($X_w \pm SD_w$). A detailed overview of physical characteristics of included study estimates is shown in Table 3.

Body composition was assessed by DXA in 32.1% of studies, by bioelectrical impedance analysis (BIA) in

Table 1 Clustering of seasonal training phases for body composition, energy intake, and total energy expenditure

Preparation phase	Competition phase	Transition phase
Training/preparation/conditioning/peak training period	Before/during/after race/competition	Detraining
Beginning/early/middle/ end of training season	Taper phase	Off-season
Beginning of season	Peak-season, in-season	Post-season
Before/pre-season	Top of performance	After/between season
High/low volume weeks	Early/start/during/end of competitive season	Recreation
Before/during/after high intensity/exhaustive training periods/training camps	Pre-competition	Resting period
Intensified/overloaded/heavy training	Mid/late season	
End of preparatory training phase	Beginning of competition preparatory period	
Habitual/basic/normal training phase		
Non-competitive season		

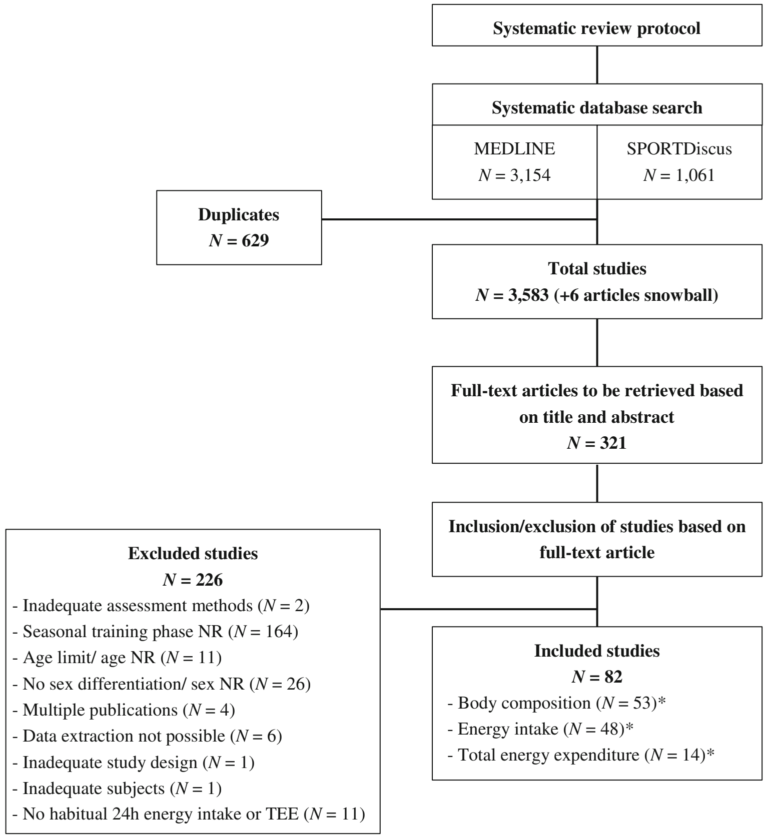


Fig. 2 Flow chart for the present systematic review. NR = not reported. *Sum of studies not equal to total as multiple parameters were assessed in certain studies. *N* = number of studies

25.6% of studies, and by hydrostatic weighing in 25.6% of studies. In 71.7% of the studies, where body composition was measured, no details of standardization were provided. Ten studies (18.9%) reported some standardization details, whereas only three studies (5.7%) reported satisfactory details about their standardization. For determination of energy intake, dietary records (95.1%) with a mean observation time of 4.7 ± 4.1 days were most often utilized. Dietary recall (3.3%) and food frequency questionnaires (FFQs, 1.6%) played secondary roles in energy intake assessments. Half of the studies (50.0%) used DLW for determination of TEE. Other methods included heart rate monitoring (33.3%) and accelerometers (16.7%). The studies using heart rate monitoring for estimation of TEE used individual derived linear relationships between heart rate and oxygen consumption (HR–VO_2) during different tasks to estimate the oxygen cost and energy expenditure during the observation period. Two third of the studies used the 24-h heart rate recordings and the individual HR–VO_2 relationship to estimate TEE (gross calculation). Two studies calculated TEE by summation of activity energy expenditure (based on individual HR–VO_2 relationship) and resting metabolic rate (RMR; net calculation).

Total Energy Expenditure and Energy Intake

In total, 14 studies where TEE was assessed during various seasonal training phases were identified by the literature search. Since no study assessed TEE during the transition phase, only data during the preparation phase ($N = 8$) and the competition phase ($N = 6$) are shown. In addition, due to limited data, no separations between the sexes and endurance disciplines of TEE were performed.

Absolute and relative TEE were significantly higher during the competition phase than during the preparation phase (9869 ± 4129 vs. 4345 ± 1062 kcal/day, and 98.9 ± 46.5 vs. 68.5 ± 11.4 kcal/kg·day, respectively, all $p < 0.001$). Most of the studies assessing TEE during the competitive phase were conducted during an ultra-endurance competition ($N = 5$), such as during a 24-h team relay cycling race [60], during a 6-day cycling stage race [61], or during a 4851-km team relay cycling race [62]. The maximum TEE amounted to 13,862 kcal/day and 156.0 kcal/kg·day, respectively, observed in male ultra-endurance runners during a 24-h ultra-marathon [63]. The absolute and relative TEE were significantly higher than the energy intake in the preparation phase (4345 ± 1062 vs. 2915 ± 761 kcal/day, and 68.5 ± 11.4 vs. 42.8 ± 10.5 kcal/kg·day, respectively, all $p < 0.001$) and

Table 2 Characteristics of the studies included in the review of body composition (BC), energy intake (EI), and total energy expenditure (TEE)

Reference	Study design	*n* (sex)	Discipline (distance), level	Age (years)	Ethnicity, country	Assessment methods			Seasonal phase[a]	Quality rating
						BC	EI	TEE		
Armstrong et al. 2012 [80]	Observational study	42 (M)	Cyclists, nonelite	38 ± 6	NR, USA		24 h DR		2	8
Barr & Costill 1992 [43]	Observational study	24 (M)	Swimmers, tertiary	19.4 ± 0.4	NR, USA		2d DR		1, 2	8
Bemben et al. 2004 [35]	Observational study	11 (F)	Cross-country runners, tertiary	19.5 ± 0.4	NR, USA	DXA	3d DR		1, 3	8
Berg et al. 2008 [81]	Observational study	9 (M) 7 (F)	Athletes (UE), elite	27 [25–35] (M) 32 [26–42] (F)	NR, Sweden	BIA			2	8
Berg et al. 2008 [81]	Observational study	6 (M)	Athletes (UE), elite	27 [25–35]	NR, Sweden			HR	2	8
Bescós et al. 2012 [60]	Observational study	8 (M)	Cyclists (6), triathletes (2), non-professional	36.7 ± 4.7	NR, Spain		DR	HR	2	8
Boulay et al. 1994 [66]	Cross-sectional study	7 (M)	Cross-country skiers, provincial/national	21 ± 5	NR, Canada	UW	3d DR	HR	1	8
Brewer et al. 2013 [82]	RCT	9 (M)	Cyclists, NR	32.6 ± 7.4	NR, Australia	DXA			2	8
Brinkworth et al. 2002 [83]	RCT	6 (F)	Rowers, international	20.6 ± 2.3	NR, Australia		DR		2	7
Carbuhn et al. 2010 [36]	Observational study	16 (F)	Swimmers, tertiary	19 ± 1	NR, USA	DXA			1, 3	9
Costa et al. 2014 [63]	Cross-sectional study	19 (M) 6 (F)	Runners (UE), NR	39 ± 7	NR, UK		24 h recall	Accelerometry	2	8
Couzy et al. 1990 [44]	Observational study	6 (M)	Runners (MD), national/international	21.5 ± 0.7	NR, France		7d DR		1, 2	8
Decombaz et al. 1992 [84]	Observational study	17 (M)	Endurance skiers, NR	34.1 ± 1.4	NR, Switzerland		14d DR		1	8
Dellavalle & Haas 2014 [85]	RCT	28 (F)	Rowers, NR	19.8 ± 1.1 (PLA) 19.7 ± 0.9 (CON)	NR, USA		7d DR		1	8
Desgorces et al. 2004 [45]	Observational study	11 (M)	Rowers, NR	21.5 ± 0.8	NR, France		3d DR		1, 3	7
Desgorces et al. 2008 [86]	Observational study	13 (M)	Rowers, NR	21.5 ± 0.8	NR, France		3d DR		1	7
Drenowatz et al. 2012 [87]	Observational study	15 (M)	Endurance athletes (LD/UE), NR	23.6 ± 3.4	NR, USA	BodPod	FFQ		1	8
Drenowatz et al. 2013 [88]	Observational study	15 (M)	Endurance athletes, NR	23.6 ± 3.4	NR, USA			HR	1	8
Emhoff et al. 2013 [89]	Cross-sectional study	6 (M)	Cyclists/triathletes, competitive	24 ± 2	NR, USA		3d DR		2	8

Table 2 Characteristics of the studies included in the review of body composition (BC), energy intake (EI), and total energy expenditure (TEE) *(Continued)*

Enqvist et al. 2010 [90]	Observational study	6 (M)	Endurance athletes (UE), NR	31 ± 4	NR, Sweden	BIA			2	8
Fudge et al. 2006 [11]	Observational study	9 (M)	Runners (MD/LD), national/ international	21 ± 2	Kalenjin, Kenya	BIA	7d DR	DLW	1	8
Fudge et al. 2008 [91]	Cross-sectional study	14 (M)	Runners (MD/LD), national/ international	22 ± 3	NR, Kenya	BIA	5d DR		2	8
Garcia-Roves et al. 1998 [92]	Cross-sectional study	10 (M)	Cyclists, international	27.6 ± 2.0	NR, Spain		3d DR		2	8
Garcia-Roves et al. 2000 [46]	Observational study	6 (M)	Cyclists, international	27.0 ± 1.9	NR, Spain		3d DR		1, 2	8
Gorsuch et al. 2013 [93]	RCT	10 (M) 10 (F)	Cross-country runners, tertiary	19.2 ± 0.4 (M) 19.9 ± 0.4 (F)	NR, USA	BodPod			3	8
Griffith et al. 1990 [94]	Observational study	6 (M)	Endurance athletes, NR	28	NR, USA	UW			1	8
Hassapidou & Manstrantoni 2001 [47]	Observational study	11 (F)	Runners (MD), regional	22.7 ± 2	NR, Greece		7d DR		1, 2	7
Hassapidou & Manstrantoni 2001 [47]	Observational study	9 (F)	Swimmers, regional	18.5 ± 1.1	NR, Greece		7d DR		1, 2	7
Havemann & Goedecke 2008 [95]	Observational study	45 (M)	Cyclists, NR	39 ± 10	NR, South Africa		3d DR		2	8
Heinonen et al. 1993 [96]	Cross-sectional study	30 (F)	Orienteers, NR	23.3 ± 3.1	NR, Finland	BIA			1	8
Heinonen et al. 1993 [96]	Cross-sectional study	29 (F)	Cyclists, NR	24.0 ± 5.7	NR, Finland	BIA			1	8
Heinonen et al. 1993 [96]	Cross-sectional study	28 (F)	Cross-country skiers, NR	21.3 ± 3.2	NR, Finland	BIA			1	8
Herring et al. 1992 [97]	Observational study	9 (F)	Endurance runners, NR	25.9 ± 2.4	NR, USA	UW	3d DR		1	9
Hill & Davies 2002 [69]	Cross-sectional study	7 (F)	Lightweight rowers, elite	20.0 ± 1.1	NR, Australia	DLW	4d DR	DLW	1	9
Hulton et al. 2010 [62]	Cross-sectional study	4 (M)	Cyclists (UE), non-professional	37 ± 4	NR, USA		6.5d DR	DLW	2	9
Jensen et al. 1992 [48]	Observational study	14 (M)	Cyclists, tertiary	23.1 ± 2.4	NR, USA		5d DR 3d DR		1, 2	7
Jones & Leitch 1993 [98]	Cross-sectional study	5 (M) 3 (F)	Swimmers, tertiary	19.8 (M) 20.7 (F)	NR, Canada	DLW			2	8
Jurimae et al. 1999 [99]	Cross-sectional study	10 (M)	Rowers, tertiary	21.6 ± 4.2	NR, Estonia	BIA			1	8
Jurimae et al. 2006 [100]	Cross-sectional study	8 (M)	Rowers, tertiary	21.5 ± 4.5	NR, Estonia	BIA			1	8

Table 2 Characteristics of the studies included in the review of body composition (BC), energy intake (EI), and total energy expenditure (TEE) *(Continued)*

Jurimae & Jurimae 2004 [101]	Cross-sectional study	10 (F)	Rowers, tertiary	19.4 ± 1.6	NR, Estonia	DXA			2	8
Jurimae et al. 2007 [102]	Observational study	12 (M)	Rowers, national/international	20.8 ± 3	NR, Estonia	BIA			1	8
Jurimae et al. 2011 [103]	Cross-sectional study	9 (M)	Rowers, national	20.1 ± 1.6	NR, Estonia	DXA	3d DR		2	8
Kabasakalis et al. 2007 [37]	Observational study	4 (M)	Swimmers (sprint/MD), international	18.4 ± 1.2	NR, Greece	BIA			1, 2	8
Koshimizu et al. 2012 [104]	Cross-sectional study	24 (M)	Endurance athletes, elite	21.5 ± 3.4	NR, Japan	BodPod	3d DR		1	8
LaForgia et al. 1999 [38]	Observational study	16 (M)	Endurance athletes, NR	23.1 ± 4.7	NR, Australia	DXA			1, 3	8
Lazzer et al. 2012 [105]	Cross-sectional study	10 (M)	Runners (UE), amateur	38.2 ± 12.4	NR, Italy	BIA			2	8
Loftin et al. 1992 [39]	Observational study	5 (M) 5 (F)	Cross-country runners, tertiary	20.8 ± 1.1 (M) 20.8 ± 1.8 (F)	NR, USA	UW			2, 3	8
Maestu et al. 2010 [106]	Observational study	9 (M)	Rowers, international	19.7 ± 1.0	NR, Estonia	DXA			2	8
Magkos et al. 2007 [107]	Cross-sectional study	7 (M)	Endurance swimmers, national/ international	19.4 ± 1.9	Caucasian, Greece	DXA			2	8
Magkos et al. 2007 [107]	Cross-sectional study	10 (M)	Endurance runners, national/ international	23.4 ± 3.8	Caucasian, Greece	DXA			2	8
Maïmoun et al. 2003 [108]	Cross-sectional study	11 (M)	Cyclists, national	27.4 ± 5.8	NR, France	DXA			2	8
Maïmoun et al. 2003 [108]	Cross-sectional study	14 (M)	Triathletes, regional	25.7 ± 6.6	NR, France	DXA			2	8
Maïmoun et al. 2003 [108]	Cross-sectional study	13 (M)	Swimmers (sprint/MD), tertiary	25.4 ± 6.5	NR, France	DXA			2	8
Margaritis et al. 2003 [49]	Observational study	9 (M)	Triathletes (LD), NR	32.6 ± 10.5	NR, France		28d/14d DR		1, 2	8
Martin et al. 2002 [109]	Observational study	8 (F)	Cyclists, international	25.1 ± 4.0	NR, Australia		8–9d DR		2	8
Medelli et al. 2009 [110]	Cross-sectional study	23 (M)	Cyclists, international	28.5 ± 3.9	NR, France	DXA			1	7
Moses & Manore 1991 [111]	Observational study	17 (M)	Runners (LD), elite	25.7 ± 3.9	NR, USA		3d DR		2	8
Moses & Manore 1991 [111]	Observational study	9 (F)	Runners, NR	34.8 ± 6	NR, USA		3d DR		1	8
Motonaga et al. 2006 [112]	Cross-sectional study	6 (M)	Runners, sub-elite	19-21	NR, Japan	BIA		HR	1	8

Table 2 Characteristics of the studies included in the review of body composition (BC), energy intake (EI), and total energy expenditure (TEE) *(Continued)*

Muoio et al. 1994 [113]	Cross-sectional study	6 (M)	Runners (LD), tertiary	21 ± 0.7	NR, USA	UW	4d DR		1	8
Noland et al. 2001 [40]	Observational study	12 (F)	Swimmers, tertiary	19.8 ± 0.1	NR, USA	UW			1, 2	7
Ousley-Pahnke et al. 2001 [114]	Cross-sectional study	15 (F)	Swimmers, tertiary	19.6 ± 1.2	NR, USA		4d DR		2	7
Palazzetti et al. 2004 [115]	Observational study	7 (M)	Triathletes, NR	32.9 ± 9.9	NR, France		28d DR		1	8
Palm et al. 2005 [116]	Cross-sectional study	11 (M)	Rowers, national	19.1 ± 3.8	NR, Estonia	DXA			2	8
Papadopoulou et al. 2012 [50]	Observational study	23 (M) 10 (F)	Cross-country skiers, international	20 ± 6 (M) 20 ± 5 (F)	NR, Greece	BIA	3d/1d DR		1 (BC/EI), 2 (EI)	8
Penteado et al. 2010 [117]	Cross-sectional study	31 (M)	Cyclists, NR	24.7 ± 3.2	NR, Brazil	DXA	4d DR		3	9
Peters & Goetzsche 1997 [51]	Observational study	151 (M) 22 (F)	Runners (UE), NR	37 ± 9.2 (M) 36 ± 6.1 (F)	NR, South Africa		24 h DR		1, 2	8
Phillips et al. 1993 [118]	Cross-sectional study	6 (M) 6 (F)	Runners, tertiary	23.3 ± 3.9 (M) 23.0 ± 4.9 (F)	NR, Canada	UW			1	8
Rehrer et al. 2010 [61]	Observational study	4 (M)	Cyclists, national/international	20 ± 3	NR, New Zealand	DXA	6d DR	DLW	2	8
Roberts & Smith 1992 [119]	Observational study	9 (M)	Swimmers, international	23 ± 2	NR, Canada		2d DR		1	8
Santos et al. 2014 [120]	Cross-sectional study	36 (M)	Swimmers, NR	19.1 ± 3.4 (M)	NR, Portugal	DXA			2	8
Santos et al. 2014 [120]	Cross-sectional study	38 (M) 10 (F)	Triathletes, NR	22.9 ± 5.4 (M) 20.4 ± 3.1 (F)	NR, Portugal	DXA			2	8
Santos et al. 2014 [120]	Cross-sectional study	11 (M) 16 (F)	Athletic athletes, NR	20.1 ± 3.0 (M) 21.3 ± 4.1 (F)	NR, Portugal	DXA			2	8
Sato et al. 2011 [121]	Observational study	6 (M) 13 (F)	Swimmers, tertiary	19.5 ± 1.0 (M) 19.4 ± 1.0 (F)	NR, Japan	BIA	3d DR		1	9
Schena et al. 1995 [122]	Cross-sectional study	73 (M)	Cross-country skiers, NR	26.9 ± 4.4	NR, Italian		7d DR		1	8
Schena et al. 1995 [122]	Cross-sectional study	33 (M)	Roller skiers, NR	25.6 ± 4.1	NR, Italian		7d DR		1	8
Schena et al. 1995 [122]	Cross-sectional study	35 (M)	Runners, NR	26.8 ± 3.7	NR, Italian		7d DR		1	8
Schena et al. 1995 [122]	Cross-sectional study	18 (M)	Cyclists, NR	30.1 ± 5.1	NR, Italian		7d DR		1	8
Schenk et al. 2010 [123]	Cross-sectional study	25 (M)	Mountain bikers, amateur	38 ± 10	NR, Austria	BIA			2	8

Table 2 Characteristics of the studies included in the review of body composition (BC), energy intake (EI), and total energy expenditure (TEE) *(Continued)*

Schulz et al. 1992 [68]	Cross-sectional study	9 (F)	Runners (LD), national/international	26.0 ± 3.3	NR, USA	UW	6d DR	DLW	1	8
Sherman et al. 1993 [124]	Cross-sectional study	18 (M)	Cyclists, NR	30 ± 3 (n = 9) 25 ± 3 (n = 9)	NR, USA	UW			1	7
Sherman et al. 1993 [124]	Cross-sectional study	18 (M)	Runners, NR	30 ± 3 (n = 9) 34 ± 3 (n = 9)	NR, USA	UW			1	7
Siders et al. 1991 [41]	Observational study	6 (M) 11 (F)	Swimmers, tertiary	19.5 ± 1.0 (M) 19.2 ± 1.0 (F)	NR, USA	UW			1, 2	8
Siders et al. 1993 [42]	Observational study	31 (M) 43 (F)	Swimmers (sprint), tertiary	20.5 ± 1.9 (M) 19.7 ± 1.4 (F)	NR, USA	UW			1, 2	8
Simsch et al. 2002 [125]	Cross-sectional study	6 (M)	Rowers, NR	18.7	NR, Germany	Near infrared			1	7
Sjodin et al. 1994 [67]	Cross-sectional study	4 (M) 4 (F)	Cross-country skiers, international	26 ± 2 (M) 25 ± 2 (F)	NR, Sweden	DLW	4d DR (M) 5d DR (F)	DLW	1	8
Sundby & Gorelick 2014 [126]	Cross-sectional study	10 (F)	Runners, tertiary	25.7 ± 4.7	NR, USA	BodPod			1	8
Taylor et al. 1997 [52]	Observational study	7 (F)	Swimmers, national	19 ± 2	NR, South Africa		7d DR		1, 2	8
Tomten & Hostmark 2006 [127]	Cross-sectional study	20 (F)	Runners, recreational/national	34.8 ± 1.7 (R) 26.0 ± 1.8 (IR)	Caucasian, Norway	DXA	3d DR		2	8
Trappe et al. 1997 [70]	Cross-sectional study	5 (F)	Swimmers, international	19 ± 1	NR, USA		2d DR	DLW	1	8
Vaiksaar et al. 2011 [128]	Observational study	11 (F)	Rowers, national	18.4 ± 1.9	Caucasian, Estonia	DXA	3d DR		1	8
Winters et al. 1996 [71]	Cross-sectional study	10 (F)	Runners (LD), tertiary	19.7 ± 1.7	Caucasian, USA	UW	3d DR	HR	2	8
Witard et al. 2011 [129]	Cross-sectional study	8 (M)	Cyclists, NR	27 ± 8	NR, UK		3d DR		1	8
Yeater et al. 1996 [130]	Cross-sectional study	8 (M)	Cross-country runners, tertiary	21 [18–30]	NR, USA	UW			1	8
Zajac et al. 2014 [131]	Observational study	8 (M)	Cyclists, NR	28.3 ± 3.9	NR, Poland	BIA			1	8
Zalcman et al. 2007 [132]	Cross-sectional study	18 (M) 6 (F)	Adventure racers, national/international	30.9 ± 5.8 (M) 30.3 ± 7.8 (F)	NR, Brazil	BodPod	3d DR		1	8

Note. Age is given as *M* ± *SD* or *M* [range]

F female, *M* male, *UE* ultra-endurance, *MD* middle distance, *LD* long distance, *NR* not reported, *RCT* Randomized Controlled Trial, *R* regular menstrual function, *IR* irregular menstrual function, *PLA* placebo group, *CON* control group, *DXA* dual-energy X-ray absorptiometry, *BIA* bioelectrical impedance analysis, *UW* underwater/hydrostatic weighing, *DR* dietary record, *FFQ* Food Frequency Questionnaire, *HR* heart rate monitoring, *DLW* doubly labelled water

[a](1) = preparation phase, (2) = competition phase, (3) = transition phase

Table 3 Physical characteristics of included study estimates

Endurance discipline (*N*)	*n*	Age [years]	Height [cm]	Body mass [kg]	BMI [kg/m^2]	VO_2max [mL/kg min]	Train load [h/week][b]
Cyclists							
Total (18)	313	30.9 ± 6.1	177 ± 5	75.4 ± 5.9	23.4 ± 1.6	62.4 ± 6.2	14.0 ± 8.5
Male (16)	276	31.8 ± 5.6	179 ± 3	74.4 ± 5.5	23.6 ± 1.6	65.0 ± 4.8	15.2 ± 9.6
Female (2)	37	24.2 ± 0.5	166 ± 1	61.2 ± 1.1	22.1 ± 0.6	55.8 ± 4.0	–
Runners							
Total (23)[a]	465	30.3 ± 7.1	172 ± 5	64.1 ± 7.4	20.3 ± 1.3	61.7 ± 7.2	8.6 ± 4.2
Male (16)	330	31.4 ± 6.9	175 ± 3	67.9 ± 5.5	20.6 ± 1.4	64.3 ± 6.7	8.6 ± 4.3
Female (13)	135	27.4 ± 6.7	167 ± 3	55.6 ± 2.2	19.9 ± 1.0	57.3 ± 5.8	8.7 ± 4.0
Swimmers							
Total (16)[a]	275	19.9 ± 1.5	176 ± 6	69.5 ± 5.9	22.4 ± 0.7	–	17.2 ± 10.3
Male (10)	141	20.3 ± 1.9	181 ± 3	74.3 ± 3.2	22.7 ± 0.7	–	13.4 ± 5.6
Female (10)	134	19.4 ± 0.4	170 ± 4	63.9 ± 2.5	22.0 ± 0.5	–	23.1 ± 12.8
Rowers							
Total (14)	151	20.2 ± 1.0	180 ± 9	76.1 ± 10.3	23.5 ± 1.0	54.6 ± 8.5	7.2 ± 2.4
Male (9)	89	20.6 ± 1.0	188 ± 3	85.4 ± 5.0	24.0 ± 0.9	–	7.2 ± 2.4
Female (5)	62	19.6 ± 0.6	171 ± 2	66.3 ± 2.2	22.9 ± 0.7	–	–
Cross-country skiers							
Total (6)[a]	166	25.0 ± 4.3	175 ± 5	65.9 ± 4.5	21.5 ± 0.7	61.9 ± 4.3	11.5 ± 0.5
Male (5)	124	26.2 ± 4.2	177 ± 2	68.1 ± 1.4	21.7 ± 0.6	–	11.7 ± 0.4
Female (3)	42	21.3 ± 1.3	168 ± 2	59.2 ± 3.5	21.0 ± 0.8	–	–
Triathletes							
Total (4)[a]	78	25.1 ± 4.2	175 ± 3	66.2 ± 3.6	21.6 ± 0.7	65.3 ± 0.4	11.4 ± 2.0
Male (4)	68	25.8 ± 4.0	176 ± 0	67.5 ± 1.8	21.8 ± 0.5	65.3 ± 0.4	11.6 ± 2.1
Female (1)	10	–	–	–	–	–	–
Other endurance athletes							
Total (13)[a]	226	25.2 ± 4.0	176 ± 6	69.1 ± 6.7	22.5 ± 1.1	61.7 ± 4.7	10.5 ± 3.8
Male (12)	167	25.5 ± 4.0	178 ± 3	72.7 ± 3.4	22.9 ± 0.9	63.8 ± 3.8	11.2 ± 4.5
Female (4)	59	24.5 ± 3.7	168 ± 1	59.3 ± 1.8	21.3 ± 0.6	56.8 ± 2.3	9.1 ± 0.7
Total							
Total (82)[a]	1674	26.3 ± 6.7	176 ± 6	68.7 ± 8.0	22.2 ± 1.5	61.8 ± 6.0	12.0 ± 6.9
Male (63)	1195	27.7 ± 6.8	179 ± 4	72.1 ± 6.5	22.6 ± 1.5	64.4 ± 4.8	11.6 ± 5.6
Female (34)	479	22.9 ± 5.1	169 ± 3	60.5 ± 4.5	21.4 ± 1.2	56.6 ± 4.6	12.8 ± 9.0

Note. Data are shown in weighted mean and standard deviation of the weighted mean ($\overline{X}_w \pm SD_w$)
N = number of studies, *n* = cumulative number of subjects, *BMI* body mass index, – = insufficient data
[a]Sum of male and female studies not equal to total as in certain studies both sexes were assessed
[b]Calculated as the following: 1 h of training = 25 km cycling or 10 km running or 2 km swimming

competition phase (9869 ± 4129 vs. 3156 ± 967 kcal/day, and 98.9 ± 46.5 vs. 43.5 ± 11.3 kcal/kg·day, respectively, all $p < 0.001$).

Absolute and relative energy intake was higher in males compared to females in the preparation phase (3111 ± 717 vs. 2291 ± 525 kcal/day, and 44.0 ± 10.6 vs. 39.0 ± 9.1 kcal/kg·day, respectively, all $p < 0.001$) and competition phase (3405 ± 940 vs. 2337 ± 483 kcal/day, and 44.8 ± 11.9 vs. 39.3 ± 7.9 kcal/kg·day, respectively, all $p < 0.001$, Figs. 3 and 4).

In males, the absolute energy intake was higher during the competition phase compared to the preparation phase ($p < 0.001$), whereas relative energy intake was unchanged ($p = 0.553$). In females, neither the absolute ($p = 0.735$) nor relative ($p = 0.951$) energy intake was different between the two seasonal training phases.

Table 4 provides a detailed overview of the absolute and relative energy intakes differentiated by sex, endurance discipline, and seasonal training phase. Energy intake was significantly higher in male runners, swimmers,

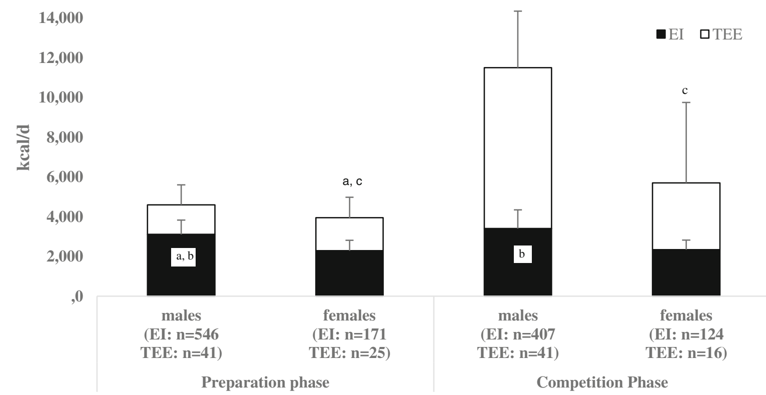
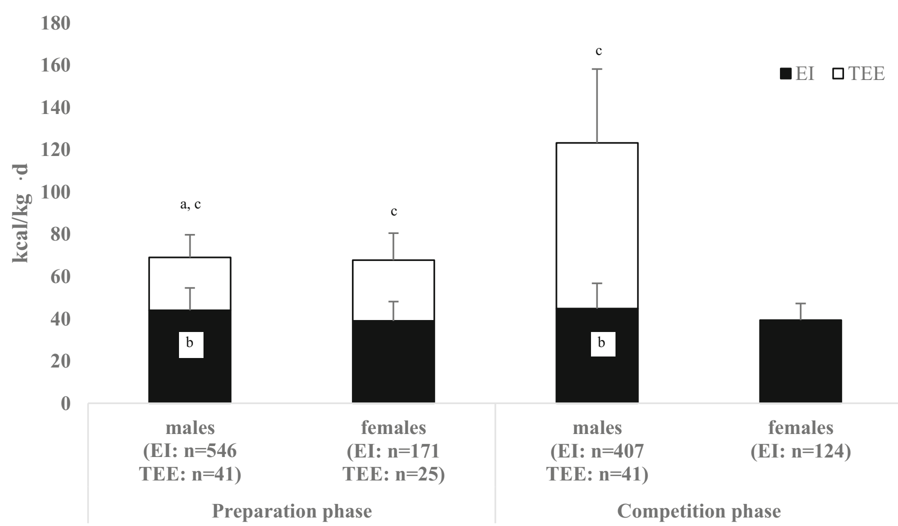


Fig. 3 Energy intake (EI) and total energy expenditure (TEE) in kcal/day of endurance athletes. Data are shown in weighted mean and standard deviation of the weighted mean ($\overline{X}_w \pm SD_w$). n = number of cumulative subjects

and rowers compared to their female counterparts during both the preparation and competition phases (all $p < 0.01$). In male and female runners, male endurance athletes, and combined male and female rowers and cross-country skiers, the energy intake was higher during the competition phase compared to the preparation phase, whereas for male and female swimmers, energy intake was higher during the preparation phase (all $p < 0.01$). The energy intake of female runners and rowers during the preparation phase was significantly lower than that of all other endurance athletes (all $p < 0.05$). Reasons for the lower energy intake in female rowers might be that during preparation phase the athletes often reduce their energy intake in order to reduce concomitantly their body weight to start in the lightweight category. During pre-season, body mass may reduce by as much as 8% among lightweight rowers [64]. Runners, in general, profit from a low body mass since greater economy of movement and better thermoregulatory capacity from a favorable ratio of weight to surface area and less insulation from subcutaneous fat tissue is reached [10].

A separate analysis of energy balance was performed by including only studies where both energy intake and expenditure were assessed in parallel. Male endurance athletes showed a significant energy deficit of 304 kcal/day (95% CI −549, −58, $p = 0.02$) during the preparation phase and 2177 kcal/day (95% CI −2772, −1582, $p < 0.0001$) during the competition phase (Fig. 5). In female endurance athletes, a negative energy balance was also observed during the preparation phase (−1145 kcal/day,



[a]significantly different from competition phase ($p < 0.001$)
[b]significantly different from females in the same seasonal training phase ($p < 0.001$)
[c]significantly different from EI of the same sex and seasonal training phase ($p < 0.01$)

Fig. 4 Energy intake (EI) and total energy expenditure (TEE) in kcal/kg·day of endurance athletes. Data are shown in weighted mean and standard deviation of the weighted mean ($\overline{X}_w \pm SD_w$). n = number of cumulative subjects. No data for TEE of females during competition phase available

Table 4 Energy intake in kcal/day and kcal/kg/day of endurance athletes in preparation and competition phase

	Preparation			Competition		
Endurance discipline	*n*	Energy intake [kcal/day]	Energy intake [kcal/kg·day]	*n*	Energy intake [kcal/day]	Energy intake [kcal/kg·day]
Cyclists						
Total	46	3789 ± 764[d,e,f]	52.3 ± 13.3[d,e]	133	3600 ± 1102[d]	46.9 ± 17.7[d,f]
Male	46	3789 ± 764[d,e]	52.3 ± 13.3[d,e]	125	3603 ± 1137	45.9 ± 18.0
Female	–	–	–	–	–	–
Runners						
Total	278	2489 ± 425[a]	38.2 ± 7.8[a]	272	3042 ± 788	42.7 ± 4.7
Male	207	2640 ± 366[a,b,f]	38.3 ± 8.6[a]	203	3298 ± 713[b]	43.8 ± 3.2[b]
Female	71	2046 ± 230[a]	38.0 ± 4.6[c]	69	2291 ± 443	39.4 ± 6.4
Swimmers						
Total	73	3366 ± 902[a,d,e,g]	48.7 ± 9.6[a,d,e]	55	2769 ± 681[g,h]	40.1 ± 7.7[g]
Male	39	3963 ± 762[a,b]	53.2 ± 9.5[a,b,d,e]	24	3462 ± 341[b]	46.2 ± 6.5[b]
Female	34	2683 ± 450[a,d,e]	43.6 ± 6.9[a,e]	31	2234 ± 256	35.4 ± 4.7
Rowers						
Total	70	2426 ± 448[a]	33.9 ± 4.5[a]	15	3633 ± 1097	46.8 ± 10.9
Male	24	2921 ± 326[b,f]	36.0 ± 0.1[b]	–	–	–
Female	46	2168 ± 330	32.8 ± 5.2[c]	–	–	–
Cross-country skiers						
Total	138	3224 ± 917[a,d,e,g]	48.3 ± 12.7[a,d,e]	33	2091 ± 53.2[d,e,f,g]	32.7 ± 2.9[c]
Male	124	3287 ± 876[d,f,g]	48.3 ± 11.6[d,e]	–	–	–
Female	14	2663 ± 1107[d,e]	49.1 ± 20.3	–	–	–
Triathletes						
Total	16	3162 ± 159[d,e]	45.7 ± 2.6[e]	–	–	–
Male	16	3162 ± 159[f,g]	45.7 ± 2.6	–	–	–
Female	–	–	–	–	–	–
Other endurance athletes						
Total	96	3261 ± 282[a,d,e,g]	46.5 ± 5.1[a,d,e]	14	4656 ± 1070	–
Male	90	3274 ± 286[a,d,f,g]	46.3 ± 5.2[a,d,e,f]	14	[d,f,g,h]	–
Female	–	–	–	–	4656 ± 1070[c] –	–
Total						
Total	717	2915 ± 761[a]	42.8 ± 10.5	531	3156 ± 967	43.5 ± 11.3
Male	546	3111 ± 717[a,b]	44.0 ± 10.6[b]	407	3405 ± 940[b]	44.8 ± 11.9[b]
Female	171	2291 ± 525	39.0 ± 9.1	124	2337 ± 483	39.3 ± 7.9

Note. Data are shown in weighted mean and standard deviation of the weighted mean ($\overline{X}_w \pm SD_w$)
n = cumulative number of subjects, – = insufficient data
[a]Significantly different from athletes of the same endurance discipline and sex during competition phase ($p < 0.01$)
[b]Significantly different from females of the same endurance discipline and seasonal training phase ($p < 0.01$)
[c]Significantly different from all other endurance disciplines of the same sex and seasonal training phase ($p < 0.05$)
[d]Significantly different to runners of the same sex and seasonal training phase ($p < 0.05$)
[e]Significantly different to rowers of the same sex and seasonal training phase ($p < 0.05$)
[f]Significantly different to swimmers of the same sex and seasonal training phase ($p < 0.05$)
[g]Significantly different to cyclists of the same sex and seasonal training phase ($p < 0.05$)
[h]Significantly different to cross-country skiers of the same sex and seasonal training phase ($p < 0.05$)

95% CI −1404, −887, $p < 0.0001$) and the competition phase (−1252 kcal/day, 95% CI −1778, −727, $p < 0.0001$, Fig. 6). The relative energy deficit was 6.6% of TEE during the preparation phase and 18.9% during the competition phase in males, and 29.0% of TEE during the preparation phase and 22.0% during the competition phase in females. When comparing energy intake during the preparation and competition phases by solely including studies where energy intake

Study or subgroup	Energy intake (kcal/d) Mean	SD	Total	Total energy expenditure (kcal/d) Mean	SD	Total	Weight	Mean difference IV, Fixed, 95% CI
1.1.1 Preparation phase								
Sjodin et al. 1994	7,218	1,099	4	7,218	1,004	4	2.4%	0 [-1,459, 1,459]
Boulay et al. 1994	3,872	382	7	4,063	956	7	8.9%	-191 [-954, 572]
Fudge et al. 2006	3,165	318	9	3,492	249	9	74.1%	-327 [-591, -63]
Subtotal (95% CI)			**20**			**20**	**85.4%**	**-304 [-549, -58]**
Heterogenity: $Chi^2 = 0.28$, df= 2 ($p = 0.87$); $I^2 = 0\%$								
Test for overall effect: Z = 2.42 ($p = 0.02$)								
1.1.2 Competition phase								
Bescós et al. 2012	5,549	2,127	8	10,253	1,625	8	1.5%	-4,704 [-6,559, -2,849]
Costa et al. 2014	5,497	2,868	19	13,862	2,390	19	1.8%	-8,365 [-10,044, -6,686]
Rehrer et al. 2010	6,525	908	4	6,549	478	4	5.1%	-24 [-1,030, 982]
Hulton et al. 2010	4,918	810	4	6,420	470	4	6.1%	-1,502 [-2,420, 584]
Subtotal (95% CI)			**35**			**35**	**14.6%**	**-2,177 [-2,772, -1,582]**
Heterogenity: $Chi^2 = 79.02$, df= 3 ($p < 0.00001$); $I^2 = 96\%$								
Test for overall effect: Z = 7.17 ($p < 0.00001$)								
Total (95% CI)			**55**			**55**	**100%**	**-577 [-804, -349]**
Heterogenity: $Chi^2 = 111.80$, df= 6 ($p < 0.00001$); $I^2 = 95\%$								
Test for overall effect: Z = 4.97 ($p < 0.00001$)								
Test for subgroup differences: $Chi^2 = 32.50$, df= 1 ($p < 0.00001$); $I^2 = 96.9\%$								

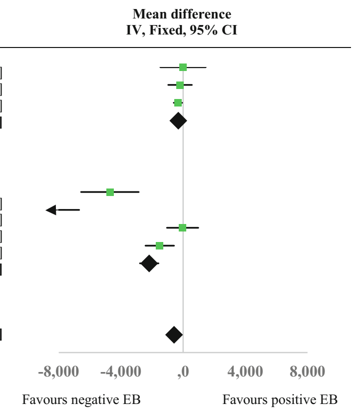


Fig. 5 Energy balance (EB) of male endurance athletes during preparation and competition phase

was assessed in both phases ($N = 8$), the energy intake was higher during the competition phase, being significant in males (+106 kcal/day, $p = 0.03$), but not in female endurance athletes (+134 kcal/day, $p = 0.20$, Fig. 7).

In more than half (53.7%) of the female study populations, where TEE was assessed, the menstrual status was not reported. 24.4% of the female study populations were eumenorrheic, whereas in 22.0% menstrual irregularities were reported. However, a separate statistical analysis assessing seasonal training phase differences of TEE between eumenorrheic and amenorrheic athletes could not be performed, since the cumulative number of subjects was too low in the single training phases.

Body Composition

For the total sample during the competition phase, both body mass and FFM were significantly higher compared to the preparation and transition phases ($p < 0.05$, Table 5). For the percentage of fat mass, no differences were detected between the seasonal training phases ($p > 0.05$). Since the percentage of female data on total data varies between the seasonal training phases, we further split the data by sex. In males, the body mass was lowest during the transition phase ($p < 0.05$) and absolute and relative fat mass were highest during the competition phase (all $p < 0.05$). FFM was lowest during the transition phase ($p < 0.001$, Fig. 8). For females, absolute and relative body fat were higher during the preparation phase compared to those during the transition phase ($p < 0.01$, Fig. 8). Neither body mass nor FFM differences between seasonal training phases were observed (all $p > 0.05$). When separately analyzing the few studies where body mass and composition were assessed during both the preparation and competition phases ($N = 5$), male and female endurance athletes showed a significantly lower percentage of body fat and higher absolute FFM during the competition phase compared to the preparation phase (18.2 ± 5.0% vs. 19.6 ± 5.0%, and 56.6 ± 8.7 kg vs. 54.0 ± 8.7 kg, respectively, all $p < 0.0001$).

In more than one third (34.5%) of the female study populations, where body composition was assessed, the menstrual status was not reported. 39.7% of the female study populations were eumenorrheic, whereas 16.4% menstrual irregularities were reported. However, a separate analysis between eumenorrheic and amenorrheic athletes

Study or subgroup	Energy intake (kcal/d) Mean	SD	Total	Total energy expenditure (kcal/d) Mean	SD	Total	Weight	Mean difference IV, Fixed, 95% CI
1.2.1 Preparation phase								
Hill & Davies 2002	2,214	313	7	3,957	1,219	7	6.2%	-1,743 [-2,675, -811]
Sjodin et al. 1994	4,350	454	4	4,374	526	4	11.6%	-24 [-705, 657]
Trappe et al. 1997	3,131	239	5	5,593	502	5	22.6%	-2,462 [-2,949, -1,975]
Schulz et al. 1992	2,193	466	9	2,826	312	9	40.1%	-633 [-999, -267]
Subtotal (95% CI)			**25**			**25**	**80.5%**	**-1,145 [-1,404, -887]**
Heterogenity: $Chi^2 = 47.55$, df= 3 ($p < 0.00001$); $I^2 = 94\%$								
Test for overall effect: Z = 8.68 ($p < 0.00001$)								
1.2.2 Competition phase								
Costa et al. 2014	3,107	1,195	6	10,755	1,912	6	1.7%	-7,648 [-,9452, -5,844]
Winters et al. 1996	2,013	418	10	2,673	781	10	17.8%	-660 [-1,209, -111]
Subtotal (95% CI)			**16**			**16**	**19.5%**	**-1,252 [-1,778, -727]**
Heterogenity: $Chi^2 = 52.75$, df= 1 ($p < 0.00001$); $I^2 = 98\%$								
Test for overall effect: Z = 4.67 ($p < 0.00001$)								
Total (95% CI)			**41**			**41**	**100%**	**-1,166 [-1,398, -934]**
Heterogenity: $Chi^2 = 100.43$, df= 5 ($p < 0.00001$); $I^2 = 95\%$								
Test for overall effect: Z = 9.85 ($p < 0.00001$)								
Test for subgroup differences: $Chi^2 = 0.13$, df= 1 (p =0.72); $I^2 = 0\%$								

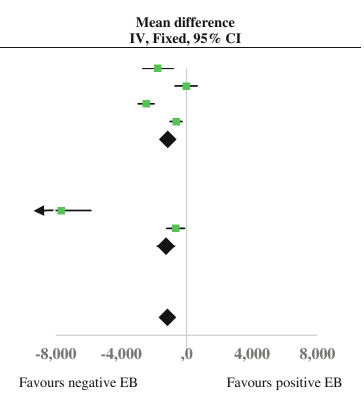


Fig. 6 Energy balance (EB) of female endurance athletes during preparation and competition phase

Study or subgroup	Preparation phase (kcal/d) Mean	SD	Total	Competition phase (kcal/d) Mean	SD	Total	Weight	Mean difference IV, Fixed, 95% CI
2.1.1 Males								
Margaritis et al. 2003	3,298	717	9	3,155	693	9	1.7%	143 [-509, 795]
Jensen et al. 1992	4,162	703	14	4,460	681	14	2.8%	-298 [-811, 215]
Garcia-Roves et al. 2000	5,354	406	6	5,473	359	6	3.9%	-119 [-553, 315]
Papadopoulou et al. 2012	2,255	790	23	2,125	639	23	4.3%	130 [-285, 545]
Barr & Costill 1992	3,609	287	13	3,155	215	13	19.4%	454 [259, 649]
Couzy et al. 1990	2,935	46	6	2,791	227	6	21.4%	144 [-41, 329]
Barr & Costill 1992	3,729	215	11	3,824	167	11	28.5%	-95 [-256, 66]
Subtotal (95% CI)			**82**			**82**	**82.0%**	**106 [11, 201]**
Heterogenity: Chi^2 = 21.85, df= 6 (p = 0.001); I^2 = 73%								
Test for overall effect: Z = 2.19 (p = 0.03)								
2.1.2 Females								
Hassapidou & Manstrantoni 2001	2,015	542	9	1,890	709	9	2.2%	125 [-458, 708]
Taylor et al. 1997	3,170	199	7	2,586	618	7	3.2%	584 [103, 1065]
Hassapidou & Manstrantoni 2001	1,816	549	11	1,679	546	11	3.5%	137 [-321, 595]
Papadopoulou et al. 2012	1,988	319	10	2,011	330	10	9.1%	-23 [-308, 262]
Subtotal (95% CI)			**37**			**37**	**18.0%**	**134 [-69, 336]**
Heterogenity: Chi^2 = 4.53, df= 3 (p = 0.21); I^2 = 34%								
Test for overall effect: Z = 1.29 (p = 0.20)								
Total (95% CI)			**119**			**119**	**100%**	**111 [25, 197]**
Heterogenity: Chi^2 = 26.44, df= 10 (p = 0.003); I^2 = 62%								
Test for overall effect: Z = 2.53 (p = 0.01)								
Test for subgroup differences: Chi^2 = 0.06, df= 1 (p = 0.81); I^2 = 0%								

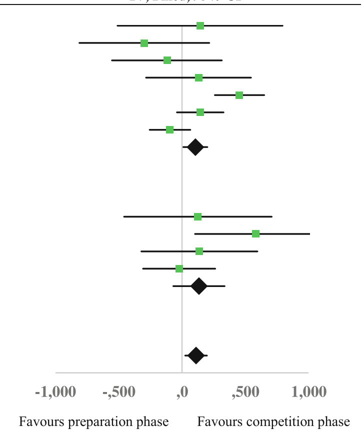


Fig. 7 Forest plot for comparison of energy intake during preparation and competition phase in endurance athletes

could not be performed, since the cumulative number of subjects during the different seasonal training phases was too low.

Discussion

In this systematic review, we examined fluctuations in TEE, energy intake, and/or body composition in endurance athletes across the training season. We found that some, but not all, of the investigated outcomes depended on the time point of data assessment during seasonal training. TEE was highest during the competition phase and higher than energy intake in all seasonal training phases. Alterations in TEE did not lead to adaptations of energy intake in females, whereas in males, a higher absolute energy intake during the competition phase was observed. The finding that male endurance athletes demonstrated the highest fat mass values during the competition phase and the lowest FFM during the transition phase seems to be an anomaly from the pooling of data.

Our systematic search initially yielded many studies where TEE, energy intake, or body composition in endurance athletes were investigated. Only a few (2%) reported the time point of data collection with regard to the training season and could thus be included in this review. This is unfortunate since our analysis clearly illustrates how training volume and related TEE vary importantly with seasonal training phases. Specifically and expectedly, both absolute and relative TEEs were significantly higher during the competition phase compared to the preparation phase. Interestingly, these differences were only partly in agreement with alterations in energy intake and/or body composition of endurance athletes.

During the transition phase, limited data for TEE and energy intake of endurance athletes was available. Only for body composition, it was possible to compare with other seasonal training phases, although the number of study estimates and therefore, explanatory power, was weak. Future research on elite athletes should focus on the effects of a sudden stop or reduction in TEE on body composition (e.g., because of injury). There exist only a few studies (with conflicting results) where this question has been examined. Ormsbee and Arciero investigated the effects of 5 weeks of detraining on body composition and RMR in eight male and female swimmers [65]. RMR decreased, whereas fat mass and body weight increased with detraining. In contrast, LaForgia et al. showed that after 3 weeks of detraining, no differences in RMR and percentage of fat mass occurred in male endurance athletes [38]. Unfortunately, energy intake was not reported in either of these studies. Thus, it remains unclear when, whether, and to what extent the body adapts (through changes in energy intake and/or body composition) for the decrease in TEE caused by detraining.

Our analysis highlights an important apparent negative energy balance in endurance athletes, both in the preparation and competition phases, when separately examining the energy balance in articles where both energy intake and TEE were assessed (N = 11). Negative energy balance was reported during the preparation phase in male [66, 67] and female [67] cross-country skiers, male [11] and female [68] runners, and female lightweight rowers [69] and swimmers [70], and amounted to a mean of 304 kcal/day (4.7% of TEE) for males and 1145 kcal/day (27.8%) for females. During the competition phase, a negative energy balance was reported in male cyclists and triathletes [60], male [63] and female [63, 71] runners, and male cyclists [61, 62], averaging 2177 kcal/day (32.5%) for male and 1252 kcal/day (47.9%) for female endurance athletes. The most obvious explanation for these energy deficits is likely the classical issue of under-reporting energy intake through self-

Table 5 Body composition of included study estimates across the season

	Preparation				Competition				Transition			
Endurance discipline	*n*	Body mass [kg]	Body fat [%]	Fat-free mass [kg]	*n*	Body mass [kg]	Body fat [%]	Fat-free mass [kg]	*n*	Body mass [kg]	Body fat [%]	Fat-free mass [kg]
Cyclists [a]												
Total	60	67.8 ± 6.5	16.7 ± 6.8	55.4 ± 9.2	49	75.3 ± 3.3	15.1 ± 1.3	62.5 ± 4.7	–	–	–	–
Male	31	73.3 ± 4.2	11.6 ± 1.7	64.1 ± 2.7	49	75.3 ± 3.3	15.1 ± 1.3	62.5 ± 4.7	–	–	–	–
Female	–	–	–	–	–	–	–	–	–	–	–	–
Runners [a]												
Total	77	58.0 ± 5.7	12.5 ± 4.5	50.7 ± 7.2	74	60.7 ± 6.4	14.5 ± 5.2	50.4 ± 6.8	40	58.4 ± 5.3	15.6 ± 4.7	49.4 ± 7.3
Male	35	62.3 ± 5.3	9.2 ± 2.4	57.1 ± 5.8	39	63.4 ± 7.8	10.3 ± 3.6	55.7 ± 4.6	15	64.8 ± 2.1	9.6 ± 0.9	58.5 ± 2.5
Female	42	54.4 ± 2.6	16.7 ± 2.7	45.3 ± 1.7	35	57.7 ± 1.5	19.2 ± 0.7	44.4 ± 2.3	25	54.5 ± 1.4	19.1 ± 0.5	44.0 ± 1.0
Swimmers [a]												
Total	166	69.1 ± 6.0	18.3 ± 5.6	54.8 ± 8.0	93	69.9 ± 6.5	16.0 ± 5.0	57.5 ± 8.2	–	–	–	–
Male	83	73.5 ± 2.7	12.9 ± 1.3	63.1 ± 2.4	56	75.5 ± 2.8	12.2 ± 1.2	64.4 ± 4.7	–	–	–	–
Female	83	63.5 ± 2.5	23.7 ± 1.4	47.6 ± 1.1	37	63.5 ± 2.2	21.8 ± 2.2	49.5 ± 1.3	–	–	–	–
Rowers [a]												
Total	54	78.1 ± 10.7	16.1 ± 7.1	65.8 ± 13.8	39	80.7 ± 10.1	14.3 ± 6.5	66.0 ± 12.2	–	–	–	–
Male	36	84.7 ± 5.6	11.3 ± 1.1	75.1 ± 4.9	29	86.2 ± 4.0	10.5 ± 1.0	72.9 ± 3.4	–	–	–	–
Female	18	64.8 ± 3.2	25.8 ± 2.5	47.4 ± 0.4	–	–	–	–	–	–	–	–
Cross-country skiers [a]												
Total	76	63.7 ± 5.9	15.7 ± 5.7	53.9 ± 7.7	–	–	–	–	–	–	–	–
Male	34	69.3 ± 2.3	10.3 ± 1.6	62.2 ± 1.7	–	–	–	–	–	–	–	–
Female	42	59.2 ± 3.5	20.1 ± 3.6	47.1 ± 0.9	–	–	–	–	–	–	–	–
Triathletes [a]												
Total	48	64.2 ± 3.3	13.6 ± 3.3	54.8 ± 5.2	–	–	–	–	–	–	–	–
Male	–	–	–	–	–	–	–	–	–	–	–	–
Female	–	–	–	–	–	–	–	–	–	–	–	–
Other endurance athletes [a]												
Total	142	67.9 ± 6.8	15.7 ± 4.2	57.5 ± 8.0	22	71.8 ± 11.0	18.5 ± 2.5	58.8 ± 10.6	–	–	–	–
Male	90	72.6 ± 3.2	13.0 ± 2.7	62.8 ± 4.3	15	79.2 ± 0.2	16.8 ± 0.2	65.8 ± 0.3	–	–	–	–
Female	52	59.8 ± 1.5	20.3 ± 1.6	48.2 ± 2.0	–	–	–	–	–	–	–	–

Table 5 Body composition of included study estimates across the season *(Continued)*

Total											
Total	623	67.5 ± 7.1[b]	15.9 ± 5.7	55.8 ± 9.2[b]	291	70.8 ± 8.6	15.2 ± 4.8	57.6 ± 9.5	95	65.3 ± 7.1[b]	15.1 ± 4.8	54.0 ± 7.2[b]
Male	347	72.0 ± 6.7[b,c]	11.8 ± 2.3[b,c]	63.0 ± 5.9[c]	202	74.5 ± 8.1	12.6 ± 2.8	62.9 ± 6.9	54	69.7 ± 3.4[b]	11.2 ± 1.7[b]	59.8 ± 1.9[b]
Female	276	60.5 ± 4.1	21.6 ± 3.6[c]	47.0 ± 1.6	89	60.2 ± 4.4	21.2 ± 2.4	47.0 ± 3.0	41	59.4 ± 6.4	20.2 ± 1.4	46.2 ± 2.9

Note. Data are shown in weighted mean and standard deviation of the weighted mean ($\bar{X}_w \pm SD_w$)
n = cumulative number of subjects, – = insufficient data
[a]Data not normal distributed. To limit the risk of type I error no statistical comparison between seasonal training phases differentiated by sex and endurance discipline were performed
[b]Significantly different from competition phase ($p < 0.05$)
[c]Significantly different from transition phase ($p < 0.05$)

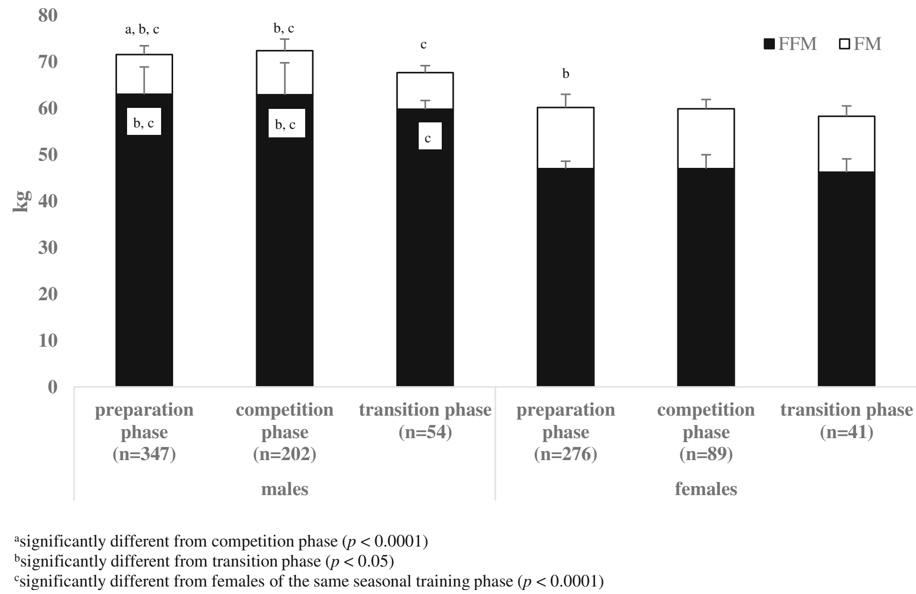


Fig. 8 Fat-free mass and fat mass of endurance athletes during preparation, competition, and transition phase. Data are shown in weighted mean and standard deviation of the weighted mean ($\bar{X}_w \pm SD_w$). n = number of cumulative subjects

assessment in human studies. A review of nine studies using DLW to validate self-reported energy intake in athletes revealed that under-reporting can amount to 10–45% of TEE [34]. Since under-reporting increases in magnitude as energy requirements increase [34], we must assume that under-reporting in the present study estimates was more important during the competition phase. Even when 45% was added to the energy intake of all athletes included in our review, there still remained a negative energy balance of 118 kcal (2.7% of TEE) in the preparation and 5293 kcal (53.6%) in the competition phase. Another explanation for the negative energy balance might be the low accuracy and precision of methods used to estimate energy intake in athletes in the articles included in our review. For example, mostly dietary records with a mean observation time of 4.7 ± 4.1 days were used. According to Magkos and Yannakoulia, for athletes, a 3–7-day diet-monitoring period would be enough for reasonably accurate and precise estimations of habitual energy and macronutrient consumption [34]. However, other methods like FFQs and dietary recalls were also used for energy intake estimations. These methods are both memory-dependent and show lower accuracy and precision than prospective methods like dietary records [72]. However, even when only articles were considered where energy intake was assessed by the use of dietary records, the error remained high (2.5% of TEE during the preparation phase and 54.9% during the competition phase). Finally, the high negative energy balance during the competition phase may also be explained by the fact that, apart from one study, all included studies investigated the TEE during the days with actual competition and not during habitual training days in the competition phase. Thus, it is likely that the TEE during this phase was over-estimated. During the preparation phase, a negative energy balance leading to increased energy store utilization might be desirable by coaches and athletes to reach a sport-specific body composition, but during the competition phase, body composition should not be modified anymore since it is typically already at its optimum. There was one study in which dietary intake was strictly controlled since the subjects were in confinement. Brouns et al. simulated a Tour de France race in a metabolic chamber and calculated the daily energy balance from the energy expended and energy intake as calculated from daily food and fluid consumption [73]. They found a positive energy balance during active rest days whereas during the exercise days, a significant negative energy balance was observed. The authors concluded that if prolonged intensive cycling increases energy expenditure to levels above a certain threshold (probably around 20 MJ or 4780 kcal), athletes are unable to consume enough conventional food to provide adequate energy to compensate for the increased energy expenditure. The authors of a recent review addressing the criticisms regarding the value of self-reported dietary intake data reasoned that these should not be used as a measure of energy intake [74]. Our analysis supports this statement since, for athletes, relative energy deficits amounted up to 48% of TEE in female athletes and 33% in male athletes during the competition phase. Thus, there is an urgent need for better methods of dietary intake quantification, such as dietary biomarkers and automated image analysis of food and drink consumption [74]. The classical concept of energy balance, defined as dietary energy intake minus TEE, has been criticized, since according to this definition energy balance is the amount of dietary energy added to or lost from the body's energy stores after the body's physiological systems have done their work for the day [75]. Thus, energy balance is an *output* from those systems. In contrast, energy availability, defined as the dietary energy intake minus the energy expended during exercise, is an *input* to the body's physiological systems, since energy availability is the amount of dietary energy remaining for all other metabolic processes [75]. Endurance athletes, especially female athletes, show low energy availability (<30 kcal/kg FFM/day) [76] and increased risk for changes of the endocrine system affecting energy and bone metabolism, as well as in the cardiovascular and reproductive systems [77]. In healthy young adults, energy balance = 0 kcal/day when energy availability = 45 kcal/kg FFM/day [75]. Since the results of the present study indicate a high negative energy balance in endurance athletes, we must assume that the athletes also demonstrate low energy availability. However, due to the limited data, it was not possible to account for other clinical markers (e.g., bone mineral density), menstrual status, or prevalence of eating disorders in the athletes. We recommend that energy balance-related studies in endurance athletes should also assess and report clinical markers, such as bone mineral density and menstrual status, in order to assess the clinical consequences of the mismatch of TEE and energy intake.

The aggregate analysis yielded a surprising finding. In male endurance athletes, the absolute and relative fat mass was highest during the competition phase. In contrast, during the transition phase, FFM was lowest, which goes along with our expectations with a decrease in exercise volume and intensity. For the female athletes, we did not find these fluctuations in body composition, except for a higher body fat content during the preparation phase compared to the transition phase. We believe that these findings are due to the paucity of data and to the fact that the number and type of athletes varied between seasonal training phases. Indeed, when separately analyzing the few studies where body mass and composition were assessed during both the preparation and competition phases ($N = 5$), both male and female endurance athletes showed a significantly lower percentage of body fat and higher FFM during the competition phase. Further studies with longitudinal assessments of body composition are required to support these findings. However, in only 5.7% of the studies, where body composition was assessed, satisfactory details about standardization were provided. According to Nana et al., studies involving DXA scans of body composition should report details of the DXA machine and software, subject presentation and positioning protocols, and analysis protocols [30]. It has been shown that the use of a non-standardized protocol increased the variability for total and fat-free soft tissue mass compared to a standard protocol, which might include a loss in ability to detect an effect of an intervention that might have relevance for sports performance [78]. The use of non-standardized protocols and the concomitant higher variability might explain some of the unexpected findings of body composition changes in athletes of the present study.

In male endurance athletes, absolute energy intake was higher during the competition phase compared to the preparation phase. The relative energy intake was not different, which can be explained by the apparent significant increase of body mass during the competition phase, and is likely an artifact of the aggregation of data from various studies. In female athletes, neither absolute nor relative energy intake was different between seasonal phases. When focusing on longitudinal studies that assessed energy intake during different training seasons in the same cohort, there was a tendency for male athletes to show greater fluctuations in energy intake. In female cross-country skiers, the energy intake was higher during the preparation phase [50], whereas in female runners and swimmers, the energy intake was higher during the competition phase [47]. However, summing up both studies, no significant differences between training season phases were found. In contrast, male endurance athletes showed a significantly higher energy intake during the competition phase, as seen in male runners [44], cross-country skiers [50], swimmers [43], and triathletes [49]. Although some of the included studies showed greater energy intake in male endurance athletes during the preparation phase (cyclists [46, 48], swimmers [43]), the power of these studies was too low to change the results. However, since energy intake varies in male endurance athletes depending on the training season phase, it indeed seems appropriate to adapt dietary recommendations according to the different training season phases, as proposed by Stellingwerff et al. [17, 18].

Strengths and Limitations

This is, to our knowledge, the first systematic review focusing on fluctuations in TEE, energy intake, and body composition in endurance athletes. To increase the robustness of the outcomes of our systematic review, we excluded articles where body composition was estimated by skinfold measurements and equations. The accuracy of skinfold measurements depends on the number of measurement sites and the formula used to calculate the percentage of body fat [33]. Since there are many different techniques [79], it is impossible to compare results accurately between studies. Furthermore, skinfold measurements cannot be used to assess intra-abdominal adipose tissue and are highly variable when assessors with limited training and experience perform the measurements [32]. Of course, since skinfolds are very often used for body composition assessments, the exclusion of these articles reduced the total number of articles measuring body composition, which were included in the present systematic review. The inclusion of articles with skinfold body composition determination would have led to a higher number of study estimates and comparisons of different seasonal training phases would have a higher explanatory power. The same is true for estimations of TEE. We included only articles measuring TEE in a more objective way (such as DLW) and excluded articles where TEE was assessed by questionnaires or activity records. This led to the inclusion of a limited number of high-quality studies.

Limitations of the present study relate to the limited cumulative number of subjects, which provided a low explanatory power, and the classification of the different seasonal training phases. In the literature, several similar-sounding terms have been used to describe time points of data collection in athletes. However, assigning the appropriate classification into one of the three seasonal training phases is essential and has a great impact on the final analysis. Furthermore, if articles reported several time points of data collection within one seasonal training phase, we included only the first time point into the analysis in order to assure standardization and avoid selection bias. The exclusion of other time points might have led to the loss of interesting data.

Conclusions

Our analysis highlights the important seasonal fluctuations in TEE, energy intake, and body composition in male and female endurance athletes across the training season. Therefore, dietary intake recommendations should take into consideration other factors including the actual training load, TEE, and body composition goals of the athlete. The present review supports the statement of the current position stand of the American College of Sports Medicine (ACSM) that energy and nutrient requirements are not static and that periodized dietary recommendations should be developed [9]. Importantly, our analysis again shows the uselessness of self-reported dietary intake, a well-known limitation to energy balance studies, in endurance athletes. The important underreporting suggested by our analysis again raises the question of whether self-reported energy intake data should be used for the determination of energy intake and illustrates the need for more valid and applicable energy intake assessment methods in free-living humans [74]. Since we observed a lack of data during the transition phase, future research should focus on the assessment of TEE, energy intake, and body composition on a reduction in training intensity and volume, such as at the end of the competitive season. In addition, future studies dealing with energy balance and nutrient intake in elite endurance athletes should always mention the time point of data assessments (e.g., seasonal training phase).

Additional files

Additional file 1: Search strategies in SPORTDiscus and MEDLINE. (PDF 140 kb)

Additional file 2: Results of methodological quality assessment undertaken on included studies. (PDF 276 kb)

Additional file 3: List of excluded references and reason for exclusion. (PDF 490 kb)

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Authors' contribution
JH participated in the design of the study; carried out the data acquisition, analysis and interpretation of the results; and drafted the manuscript. BK, YS, and KM participated in the conception and design; analysis and interpretation of the results; drafting and revisions of the manuscript for important intellectual content. All authors read and approved the final manuscript.

Competing Interests
Juliane Heydenreich, Bengt Kayser, Yves Schutz, and Katarina Melzer declare that there are no conflicts of interests regarding the publication of this paper.

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Appendix II

Low energy turnover of physically inactive participants as a determinant of insufficient mineral and vitamin intake in NHANES

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Low Energy Turnover of Physically Inactive Participants as a Determinant of Insufficient Mineral and Vitamin Intake in NHANES

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Abstract: Micronutrient requirements do not scale linearly with physical activity-related energy expenditure (AEE). Inactive persons may have insufficient micronutrient intake because of low energy intake (EI). We extracted data from NHANES 2003–2006 on 4015 adults (53 ± 18 years (mean ± SD), 29 ± 6 kg/m^2, 48% women) with valid physical activity (accelerometry) and food intake (2 × 24 h-dietary recall) measures. Total energy expenditure (TEE) was estimated by summing the basal metabolic rate (BMR, Harris-Benedict), AEE, and 10% of TEE for the thermic effect of food, to calculate the physical activity levels (PAL = TEE/BMR). Energy intake (EI) was scaled to match TEE assuming energy balance. Adjusted food intake was then analyzed for energy and micronutrient content and compared to estimated average requirements. The NHANES population was physically insufficiently active. There were 2440 inactive (PAL < 1.4), 1469 lightly to moderately active (PAL1.4 < 1.7), 94 sufficiently active (PAL1.7 < 2.0), and 12 very active participants (PAL ≥ 2.0). The inactive vs. active had significantly lower intake for all micronutrients apart from vitamin A, B12, C, K, and copper ($p < 0.05$). The inactive participants had insufficient intake for 6/19 micronutrients, while the active participants had insufficient intake for 5/19 ($p < 0.05$) micronutrients. Multiple linear regression indicated a lower risk for insufficient micronutrient intake for participants with higher PAL and BMI ($p < 0.001$). Symmetrical up-scaling of PAL and EI to recommended physical activity levels reduced the frequency of micronutrient insufficiencies. It follows that prevalence of insufficient micronutrient intake from food in NHANES might be partly determined by low energy turnover from insufficient PAL.

Keywords: total energy expenditure; physical activity level; micronutrients; adults; energy turnover; energy intake; minerals; vitamins

1. Introduction

Micronutrients are essential nutrients, required in small quantities for numerous physiological functions [1,2]. They include trace minerals, such as iron, chromium, cobalt, copper, iodine, magnesium, manganese, molybdenum, selenium, and zinc, and also vitamins, which are organic compounds that the organism cannot produce by itself. Micronutrients are essential for health [1,3–5], but sub-optimal intake of certain minerals and vitamins is common [5,6]. Micronutrient deficiency can impair cognitive and physical capacities, jeopardize the immune system, and compromise health, in general [1,3,4,7].

Previous studies investigated the adequacy of diet and micronutrient intake recommendations (RDA: recommended daily allowance) [1–8]. An analysis of 70 diets of athletes and non-athletes revealed non-compliance with regard to many compounds [5]. In a European census, several micronutrient-deficient risk groups were identified, including the elderly, pregnant women, vegans, people on a weight reduction diet, and some groups of athletes [3]. In addition, hospitalized and institutionalized people, patients with a chronic inflammatory disorder, participants with chronic administration of certain drugs, and specific clinically-defined patient groups are also considered to be at risk [3]. Although more than two-thirds of the US population is overweight or obese, micronutrient intakes are often found to be below the RDA [9]. Physical activity levels and associated daily energy turnover are recognized to influence micronutrient intake [8]. Csizmadi et al. found that participants with higher physical activity levels have a higher micronutrient intake. They hypothesized that the benefits of higher PALs may extend beyond the usual benefits attributed to physical activity to include having a more favorable impact on adequate nutrient intake [10].

Analyzing the National Health and Nutrition Examination Survey (NHANES) data, Kimmons et al. [6] reported that overweight and obese participants had lower micronutrient intake in comparison with normal weight participants. Since obesity is associated with low physical activity-related energy expenditure [11], this finding raises the question of whether increased energy expenditure, in conjunction with increased energy intake, would improve compliance with micronutrient intake recommendations. Physical activity bouts can, depending on fitness level, increase energy expenditure up to >20 times the basal metabolic rate (BMR) [12]. Regular physical activity is, therefore, accompanied by increased total energy expenditure and, in order to achieve energy balance, with increased dietary intake [1,13–15]. According to Melzer et al. [16], over longer periods, energy intake normally follows moderate to vigorous physical activity energy requirements for activities cumulatively lasting two or more hours per day.

Contrary to the energy requirements, the micronutrient requirements of inactive and physically active persons are quite similar [14]. For athletes, who typically have high energy expenditure and intake, even though there may be an increased need for some compounds, there is generally no need for supplementation [14,17]. This is essentially due to greater overall dietary intake, to cover the increased physical activity-related energy expenditure, coupled with an often enhanced food quality observed in more active participants [18–20].

In this study, we explored the extent to which physical activity levels of a sample of the US adult population are associated with compliance with dietary intake recommendations for minerals and vitamins. We also explored by how much of an increase in physical activity levels, up to levels recommended for health, combined with a corresponding linear up-scaling of dietary intake without altering dietary composition, would improve compliance with recommended micronutrient intake.

2. Materials and Methods

We extracted data from NHANES, a continuing population-based survey conducted by the Centers for Disease Control and Prevention, that uses a complex, stratified, multi-stage probability sample design in order to create a representative sample of the civilian, non-institutionalized U.S. population [21,22]. The National Center for Health Statistics ethics review board approved the protocols, and written informed consent was obtained from all NHANES participants. Anonymous data are freely available for analysis on the NHANES repository [23]. For our study, we needed quantification of energy and micronutrient intakes and an objective measurement of physical activity. Two data collection periods satisfied these conditions and were used for the analysis: NHANES 2003–2004 and NHANES 2005–2006.

2.1. Analytical Sample

We combined NHANES 2003–2004 and NHANES 2005–2006 data files to obtain a first sample with 20,470 participants. Of this sample, 10,081 participants were asked to wear an accelerometer, and 7139 provided valid measures of physical activity by use of accelerometry. We then excluded participants younger than 21 years (n = 2778, to exclude any late growth), pregnant women (n = 180) [24,25], participants without anthropometrical measurements (n = 25), and participants without dietary recall

(n = 129). According to Westerterp [26], in free-living humans, the physical activity level (PAL) ranges between 1.1 and 2.5. Technical artifacts from accelerometry can lead to erroneously extreme PALs. To minimize the errors, we excluded 12 participants with a PAL lower than 1.1 and greater than 2.5 from the sample. Thus, the analytical sample contained 4015 participants (53 ± 18 years (mean ± SD), 81 ± 20 kg, 29 ± 6 kg/m^2), of which 1945 (48%) were women. The datasets analyzed during the current study are available from the corresponding author on reasonable request.

2.2. Dietary Intake

The nutritional assessment component of NHANES included two 24 h dietary recalls. The first was conducted in person by trained dieticians in a mobile examination center using a standard set of measuring guides to help the respondent report the volume and dimensions of the food items consumed. Upon completion of the in-person interview, participants were given measuring cups, spoons, a ruler, and a food model booklet to use for reporting food amounts for a second 24 h recall through telephone interview. The telephone interviews were collected 3–10 days following the in-person interview, on a different day of the week. Dietary macro and micronutrient compositions and quantities were calculated with standard food tables (USDA Food and Nutrient Database for Dietary Studies, 2.0). The processed data (in SAS format) were downloaded from the NHANES website [23]. The average energy and nutrient intake over the two days for each participant was used for the present analysis. The NHANES sodium intake included all sources of salt, including that from table salt.

2.3. Energy Expenditure

Activity energy expenditure was measured with an accelerometer (Actigraph AM-7167, Pensacola, FL, USA) in a one-minute epoch setting. The device was carried on the right hip attached to an elastic band. Participants were asked to carry the device for seven days, to keep the device dry (i.e., remove it before swimming or bathing), and to remove the device at bedtime. Data collection occurred between the first and during and/or after the second 24 h dietary intake recalls. We downloaded the raw accelerometer count data (in SAS format) from the NHANES website and used the SAS programs published by the National Cancer Institute to reduce the data [27]. Energy expenditure from physical activity was then estimated with the Williams transformation [28]:

$$\text{Kcals} = \text{CPM} \times 0.0000191 \times \text{BM} \quad (1)$$

where Kcals = total calories for a single epoch, CPM = counts per minute, and BM = body mass (kg). The mean wearing time of the accelerometers was 14.3 ± 1.8 h per day (range: 10–23 h per day).

BMR was calculated using the Harris-Benedict equation [29]. We estimated total energy expenditure (TEE) by summing BMR and daily physical activity energy expenditure estimated from the accelerometer data, adding a further 10% to account for the thermic effect of food [30]. We then calculated physical activity level (PAL = TEE/BMR). The data were analyzed separating the participants into groups according to their PAL: inactive (PAL < 1.4), moderately active (PAL $1.4 \leq 1.7$). and active participants (PAL > 1.7). The chosen classification was adapted from the established classification provided by the World Health Organization (WHO) [31].

2.4. Micronutrients

We considered 19 micronutrients: 10 vitamins (A, B1, B2, B3, B6, B9, B12, C, E, and K) and nine minerals (calcium, phosphorus, magnesium, iron, zinc, copper, sodium, potassium, and selenium).

Daily intakes were compared to the dietary reference intakes provided by the Food and Nutrition Board of the Institute of Medicine in the USA [32–36]. The individual intake was compared to the estimated average requirement (EAR) for most of the micronutrients. For those micronutrients where no EAR is established (vitamin K, potassium, and sodium) the individual intake was compared to the adequate intake (AI). Individual micronutrient intake was also compared to the tolerable upper intake levels (UL). Fortification of certain foods with vitamins B12 and E was included in the total vitamin intake. Any supplements were not taken into account in order to only describe micronutrient intake from food sources.

2.5. Data Analysis

In a first step, we analyzed original dietary intake data and compared it to US dietary intake recommendations. Since we found that the reported energy intakes did not, on average, cover the estimated energy expenditures, we corrected for the estimated energy deficits, assuming energy balance and under-reporting by NHANES participants, as suggested by Archer et al. [37]. Energy balance was expressed as energy intake (kcal/day) minus TEE (kcal/day) and as the quotient between energy intake and BMR. We linearly increased (or decreased) nutrient intake data so that energy intake matched TEE, without changing diet composition. The corrected values were then compared to the dietary intake recommendations again. Finally, we increased all individual PALs that were <2.0, up to a PAL of 2.0. In parallel, we linearly increased dietary intake, without changing diet composition, to quantify the resulting changes in micronutrient intake and compliance with recommendations for daily micronutrient intake. For those participants, where the initial PAL was $\geq$2.0 (n = 12) the dietary intake was decreased in order that energy intake matched energy expenditure with a PAL of 2.0.

Lastly, assuming a fixed energy cost of 0.93 kcal/kg per km for level brisk walking, we transformed the necessary increase in individual energy expenditure to bring PAL up to 2.0 into an increased daily walking distance, since walking is the principal means for increasing physical activity in inactive people [38].

2.6. Statistical Analysis

Accelerometer data was transformed using SAS version 9.3 (SAS Institute, Cary, NC, USA) using the code developed by the National Cancer Institute [27]. All further data analysis was performed using SPSS Statistics version 23.0 (IBM Corporation, Armonk, NY, USA). Normality was checked using the Kolmogorov-Smirnov-test. Not all data were normally distributed and their analysis was performed with non-parametric tests. Mann-Whitney U-tests were used to perform sex comparisons, and Kruskal-Wallis tests were used to assess differences between PAL groups. Spearman-Rho correlations were performed to assess the relationships between various variables. We used multiple linear regression analysis with the forced entry method in order to quantify the relationship between chosen independent (number of insufficient vitamin, mineral, and micronutrient intake) and dependent variables (age, sex, PAL, and BMI). The alpha level cut-off was set at 0.05.

3. Results

3.1. Characteristics of the Participants

The participants' characteristics are described per sex (Table 1) and per PAL (Table 2). The weight, height, and BMI of males were significantly higher compared to females (p < 0.01).

Table 1. Characteristics of included participants differentiated by sex.

Participants	n	Age (Years)	Weight (kg)	Height (cm)	BMI (kg/m^2)
Males	2070	52.5 ± 17.9	86.5 ± 18.7 [a]	175 ± 8 [a]	28.3 ± 5.3
Females	1945	52.8 ± 17.5	74.8 ± 19.0	161 ± 7	28.9 ± 6.9
Total	4015	52.7 ± 17.7	80.8 ± 19.7	168 ± 10	28.6 ± 6.1

Data are shown as mean ± SD. BMI = body mass index; [a] significantly different to females (p < 0.001).

The adult NHANES population is on average insufficiently physically active. There were 2440 (very) inactive (PAL < 1.4; 52.7% females), 1469 lightly to moderately active (PAL 1.4 - < 1.7; 43.3% females), 94 sufficiently active participants (PAL 1.7 - < 2.0; 20.2% females), and 12 very active (PAL ≥ 2.0; 25.0% females). Inactive participants were significantly older than moderately active and active participants ($p < 0.05$). There was a significant negative correlation between age and PAL ($r = -0.44$, $p < 0.0001$).

Table 2. Characteristics of included participants differentiated by physical activity level (PAL).

PAL	*n*	Age (Years)	Weight (kg)	Height (cm)	BMI (kg/m^2)
<1.4	2440	57.2 ± 18.3 [a]	79.4 ± 20.0 [b]	167 ± 10 [a]	28.4 ± 6.2 [b]
1.4- <1.7	1469	45.9 ± 14.2	83.1 ± 19.0	169 ± 10	28.4 ± 5.1
≥1.7	106	42.5 ± 13.9	82.1 ± 19.1	170 ± 9	28.9 ± 6.1

Data are shown as mean ± SD. BMI = body mass index; [a] significantly different to PAL groups 1.4 ≤ 1.7 and ≥ 1.7 ($p < 0.05$); [b] significantly different to PAL group 1.4 ≤ 1.7 ($p < 0.01$).

3.2. Energy Balance

Sufficiently and very active participants (PAL ≥ 1.7) showed a greater absolute and relative negative energy balance compared to inactive and lightly to moderately active participants ($p < 0.05$; Table 3). There was a significant negative correlation between PAL and absolute and relative energy balance ($r = -0.15$ and $r = -0.12$, respectively; all $p < 0.0001$). The ratio of energy intake and BMR was higher in sufficiently and very active participants compared to inactive and lightly to moderately active participants ($p < 0.05$).

Table 3. Energy balance in kcal/day and percentage of total energy expenditure (TEE) differentiated by physical activity level (PAL).

PAL	*n*	Energy Intake (kcal/Day)	Energy Balance		EI/BMR
			kcal/Day	% of TEE	
<1.4	2440	1942 ± 731 [a]	−78 ± 690 [a]	−2.5 ± 33.8 [a]	1.26 ± 0.44 [a]
1.4- <1.7	1469	2286 ± 904	−216 ± 847	−8.0 ± 33.2	1.37 ± 0.50
≥1.7	106	2589 ± 1003 [b]	−574 ± 1041 [b]	−16.9 ± 31.6 [b]	1.52 ± 0.58 [b]

Data are shown as mean ± SD. Energy balance was calculated as energy intake (EI; kcal/day)–TEE (kcal/day). Basal metabolic rate (BMR) was calculated by use of Harris-Benedict equation [27]. [a] significantly different to PAL groups 1.4 ≤ 1.7 and ≥ 1.7 ($p < 0.0001$); [b] significantly different to PAL group 1.4 ≤ 1.7 ($p < 0.05$).

Obese participants (BMI ≥ 30) showed a higher absolute and relative negative energy balance and a lower ratio of energy intake and lower BMR compared to all other BMI subgroups ($p < 0.0001$; Table 4). There was a significant negative correlation between BMI and absolute and relative energy balance ($r = -0.37$ and $r = -0.35$, respectively; all $p < 0.0001$). In addition, a significant negative correlation between BMI and the ratio of energy intake and BMR was observed ($r = -0.34$, $p < 0.0001$).

Those participants whose baseline micronutrient intakes were compliant with the recommendations, defined as having micronutrient intakes above the EAR or AI ($n = 130$), having a significantly higher ratio of energy intake and BMR than those participants with at least one micronutrient intake not meeting the requirements (1.9 ± 0.6 vs. 1.3 ± 0.5, $p < 0.0001$).

Table 4. Energy balance in kcal/day and percentage of total energy expenditure (TEE) differentiated by Body Mass Index (BMI).

BMI (kg/m^2)	n	Energy Balance		EI/BMR
		kcal/Day	% of TEE	
<18.5	55	529 ± 816 [a]	31.9 ± 46.9 [a]	1.74 ± 0.63 [a]
18.5- <25	1144	175 ± 712 [b]	9.4 ± 35.6 [b]	1.49 ± 0.49 [b]
25- <30	1462	−136 ± 693 [c]	−6.0 ± 30.1 [c]	1.30 ± 0.43
≥ 30	1354	−443 ± 762	−17.2 ± 29.2	1.14 ± 0.41

Data are shown as mean ± SD. Energy balance was calculated as energy intake (EI; kcal/day)–TEE (kcal/day). Basal metabolic rate (BMR) was calculated by use of the Harris-Benedict equation [29]. BMI was classified according to standard WHO classification [39]. [a] Significantly different to all other BMI groups ($p < 0.05$); [b] significantly different to BMI groups 25 - < 30 and ≥ 30 ($p < 0.01$); [c] significantly different to BMI group ≥ 30 ($p < 0.01$).

A linear up-scaling in energy intake to cover a theoretical increase in PAL to 2.0 for all participants with a PAL < 2.0 would require an increase of an additional 13.4 ± 3.1 km (range: 0.1–23.9 km) of daily brisk walking, on average.

3.3. Micronutrient Intake

Female participants had a significantly lower intake of all micronutrients, apart from vitamin K, compared to male participants ($p < 0.01$, Table 5). Male participants had also a lower total number of insufficient micronutrient intakes compared to female participants (5.2 ± 3.2 micronutrients (3.5 ± 2.1 vitamins and 1.6 ± 1.5 minerals) vs. 5.9 ± 3.9 micronutrients (3.9 ± 2.4 vitamins and 2.0 ± 1.8 minerals); $p < 0.001$).

Inactive participants had a lower intake of all micronutrients compared to lightly to moderately active participants, with significant differences for all micronutrients apart from vitamin A and vitamin K (all $p < 0.05$; Table 6). Furthermore, inactive participants had a lower intake of all micronutrients compared to sufficiently and very active participants, with significant differences for all micronutrients, apart from vitamins A, B12, C, K, and copper (all $p < 0.05$). Sufficiently and very active participants showed a lower total number of insufficient micronutrient intakes compared to inactive participants (4.9 ± 3.6 micronutrients (3.4 ± 2.2 vitamins and 1.5 ± 1.6 minerals) vs. 5.8 ± 3.6 micronutrients (3.9 ± 2.3 vitamins and 2.0 ± 1.6 minerals); $p < 0.05$).

When nutrient intake was adapted so that energy intake matched estimated total energy expenditure, inactive participants had a lower intake of all micronutrients compared to moderately active participants, with significant differences for all micronutrients apart from vitamin K (all $p < 0.01$), and lower intake compared to active participants, with significant differences for all micronutrients apart from vitamins K and B12 (all $p < 0.01$). Inactive participants had less insufficient micronutrient intakes compared to moderately active and active participants (4.9 ± 2.6 micronutrients (3.4 ± 1.8 vitamins and 1.5 ± 1.2 minerals) vs. 3.7 ± 2.2 micronutrients (2.8 ± 1.6 vitamins and 0.9 ± 1.0 minerals) and 3.1 ± 1.9 micronutrients (2.5 ± 1.4 vitamins and 0.6 ± 0.9 minerals); $p < 0.05$). Male participants had less insufficient micronutrient intakes compared to female participants (4.3 ± 2.4 micronutrients (3.1 ± 1.6 vitamins and 1.2 ± 1.1 minerals) vs. 4.6 ± 2.7 micronutrients (3.2 ± 1.9 vitamins and 1.3 ± 1.2 minerals); $p < 0.001$).

Table 5. Micronutrient intake (original data without dietary supplement intake) of included participants differentiated by sex.

			Males (n = 2070)		**Females (n = 1945)**	
			Intake	**DRI ***	**Intake**	**DRI ***
Vitamins	Vitamin A	[µg/day]	677 ± 660 [a]	625	584 ± 454	500
		[µg/MJ]	71.6 ± 78.4 [a]		83.2 ± 66.8	
	Vitamin B1	[mg/day]	1.9 ± 0.9 [a]	1.0	1.9 ± 0.8	0.9
		[mg/MJ]	0.19 ± 0.07 [a]		0.20 ± 0.07	
	Vitamin B2	[mg/day]	2.5 ± 1.2 [a]	1.1	1.9 ± 0.8	0.9
		[mg/MJ]	0.26 ± 0.10 [a]		0.26 ± 0.10	
	Vitamin B3	[mg/day]	28.2 ± 12.9 [a]	12	20.1 ± 8.4	11
		[mg/MJ]	2.9 ± 1.0		2.8 ± 1.0	
	Vitamin B6	[mg/day]	2.2 ± 1.1 [a]	1.1	1.7 ± 0.8	1.1
		[mg/MJ]	0.23 ± 0.10		0.24 ± 0.11	
	Vitamin B9	[µg/day]	446 ± 222 [a]	320	354 ± 173	320
		[µg/MJ]	45.8 ± 18.5 [a]		49.8 ± 22.0	
	Vitamin B12	[µg/day]	6.4 ± 7.5 [a]	2.0	4.5 ± 4.2	2.0
		[µg/MJ]	0.66 ± 0.84		0.63 ± 0.59	
	Vitamin C	[mg/day]	96.5 ± 83.5 [a]	75	86.9 ± 72.6	60
		[mg/MJ]	10.1 ± 8.7 [a]		12.5 ± 11.1	
	Vitamin E	[mg/day]	7.6 ± 4.4 [a]	12	6.3 ± 3.9	12
		[mg/MJ]	0.77 ± 0.35 [a]		0.87 ± 0.49	
	Vitamin K	[µg/day]	105 ± 141	120	99 ± 121	90
		[µg/MJ]	11.2 ± 16.8 [a]		14.5 ± 21.7	
Minerals	Calcium	[mg/day]	948 ± 509 [a]	800	789 ± 398	800
		[mg/MJ]	96.2 ± 40.1 [a]		110 ± 48	
	Phosphorus	[mg/day]	1473 ± 586 [a]	580	1112 ± 419	580
		[mg/MJ]	149 ± 34 [a]		154 ± 37	
	Magnesium	[mg/day]	321 ± 131 [a]	350	254 ± 105	265
		[mg/MJ]	33.0 ± 9.9 [a]		35.7 ± 11.7	
	Iron	[mg/day]	18.0 ± 8.5 [a]	6.0	13.7 ± 6.3	8.1
		[mg/MJ]	1.9 ± 0.7 [a]		1.9 ± 0.8	
	Zinc	[mg/day]	13.9 ± 9.0 [a]	9.4	10.0 ± 5.3	6.8
		[mg/MJ]	1.4 ± 0.9		1.4 ±0.7	
	Copper	[mg/day]	1.5 ± 1.1 [a]	0.7	1.2± 0.7	0.7
		[mg/MJ]	0.15 ± 0.13 [a]		0.16 ± 0.09	
	Potassium	[mg/day]	2990 ± 1152 [a]	4700	2365 ± 878	4700
		[mg/MJ]	309 ± 92 [a]		334 ± 105	
	Selenium	[µg/day]	124 ± 55 [a]	45	92 ± 40	45
		[µg/MJ]	12.6 ± 3.8		12.8 ±4.1	
	Sodium	[mg/day]	3781 ± 1644 [a]	1500	2825 ± 1101	1500
		[mg/MJ]	382 ± 113 [a]		392 ± 104	

DRI = dietary reference intake. Data are shown as mean ± SD. * For all micronutrients, apart from vitamin K, potassium, and sodium the estimated average requirement (EAR) for the age group 31–50 years is displayed. For vitamin K, potassium, and sodium the average intake is shown. [a] significantly different from females ($p < 0.01$).

Table 6. Micronutrient intake (original data without dietary supplement intake) of included participants differentiated by PAL.

Micronutrient Intake		**PAL**		
		<1.4 (n = 2440)	**1.4 ≤ 1.7 (n = 1469)**	**≥1.7 (n = 106)**
Vitamins	Vitamin A [µg/day]	625 ± 516	644 ± 658	636 ± 503
	Vitamin B1 [mg/day]	1.6 ± 0.7 [a]	1.8 ± 0.9	1.9 ± 0.8 [b]
	Vitamin B2 [mg/day]	2.1 ± 1.0 [a]	2.3 ± 1.1	2.4 ± 1.1
	Vitamin B3 [mg/day]	22.9 ± 11.1 [a]	26.5 ± 12.2	27.6 ± 11.4
	Vitamin B6 [mg/day]	1.9 ± 1.0 [a]	2.1 ± 1.1	2.2 ± 1.1
	Vitamin B9 [µg/day]	385 ± 197 [a]	424 ± 215	459 ± 219
	Vitamin B12 [µg/day]	5.3 ± 5.9 [b]	5.8 ± 6.8	5.7 ± 3.9
	Vitamin C [mg/day]	88 ± 74 [b]	97 ± 84	107 ± 97
	Vitamin E [mg/day]	6.6 ± 4.0 [a]	7.5 ± 4.5	7.8 ± 4.7
	Vitamin K [µg/day]	100 ± 124	104 ± 135	121 ± 220

Table 6. *Cont.*

	Micronutrient Intake	PAL		
		<1.4 (n = 2440)	1.4 ≤ 1.7 (n = 1469)	≥1.7 (n = 106)
Minerals	Calcium [mg/day]	824 ± 431 [a]	942 ± 502	968 ± 555
	Phosphorus [mg/day]	1219 ± 496 [a]	1412 ± 578	1543 ± 703
	Magnesium [mg/day]	273 ± 115 [a]	310 ± 129	344 ± 163
	Iron [mg/day]	15.3 ± 7.3 [a]	16.9 ± 8.4	17.3 ± 8.5
	Zinc [mg/day]	11.5 ± 8.3 [a]	12.8 ± 6.6	13.4 ± 6.5
	Copper [mg/day]	1.3 ± 0.8 [a]	1.5 ± 1.2	1.5 ± 0.7
	Potassium [mg/day]	2580 ± 994 [a]	2831 ± 1146	3167 ± 1433
	Selenium [µg/day]	103 ± 47 [a]	117 ± 54	133 ± 63 [b]
	Sodium [mg/day]	3141 ± 1377 [a]	3578 ± 1598	3766 ± 1670

Data are shown as mean ± SD. PAL = physical activity level. [a] significantly different from PAL groups 1.4 ≤ 1.7 and ≥ 1.7 ($p < 0.05$); [b] significantly different from PAL group 1.4 ≤ 1.7 ($p < 0.05$).

In Figure 1, the vitamin and mineral intake in percentage of the dietary reference intake is displayed. The mean intakes of vitamin E (58.3%), vitamin K (98.2%), magnesium (93.9%), and potassium (57.2%) were below the recommendations. When data were adjusted to reach energy balance, the mean intake of vitamin E (62.7%) and potassium (62.6%) were still below recommendations. When data were adjusted to a PAL of 2.0, intakes were still below recommendations for vitamin E (84.5%) and potassium (83.0%).

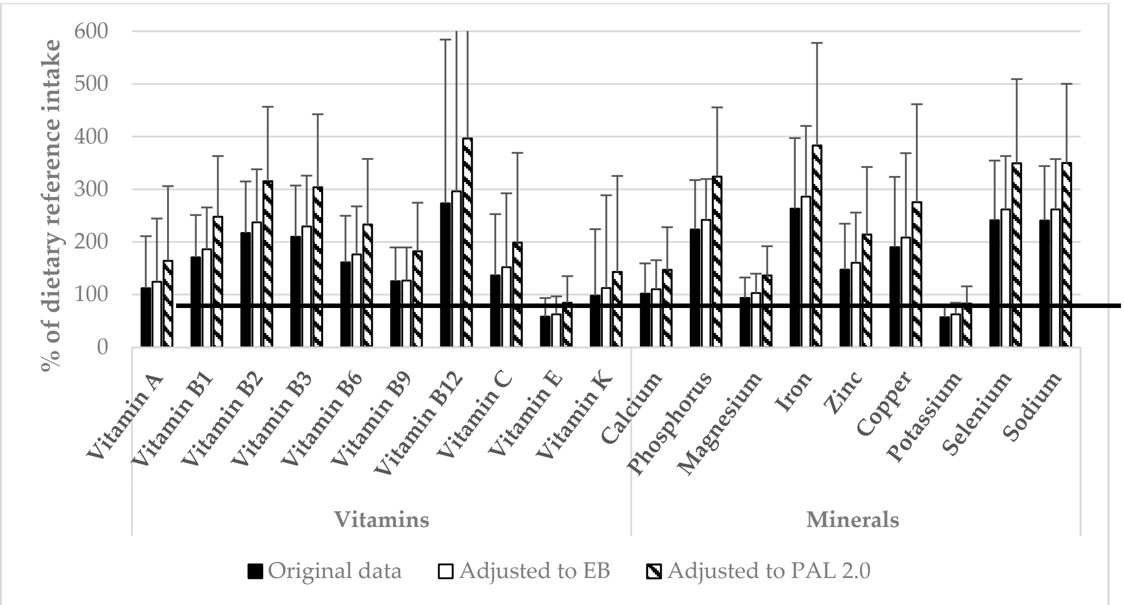

Figure 1. Vitamin and mineral intake in percentage of dietary reference intake (adequate intake for vitamin K, potassium, and sodium; estimated average requirement for the remaining micronutrients) for original data (black bars), data adjusted for energy balance (EB, white bars), and data adjusted for physical activity level (PAL) of 2.0 (shaded bars). The solid line represents 100% of the dietary reference intake. Data are shown as mean ± SD.

For some micronutrients intake greatly exceeded recommendations. For the EB-adjusted dataset, mean intake of sodium was 3606 ± 1375 mg/day (262% of AI), while it reached 5205 ± 1884 mg/day (350%) after the adjustment to a PAL of 2.0. More than 80% of participants (85.6%, n = 3436) had an intake above the UL in the EB-adjusted dataset, whereas when the data was adjusted to a PAL of 2.0 the intake was above UL for 93% of participants (n = 3715). For vitamin B3, 17% of the participants

(n = 697) had an intake above UL (EB-adjusted dataset), and when data were adjusted to a PAL of 2.0 intake was above UL for 42% of the participants (n = 1704).

For vitamin E, vitamin K, magnesium, and potassium only 10.2%, 27.4%, 36.5%, and 4.8% of participants had sufficient intake, respectively (Figure 2). When original data was adjusted to a PAL of 2.0, intake of vitamin E, vitamin K, magnesium, and potassium was sufficient in 27.0%, 44.2%, 74.4%, and 26.3% of participants, respectively. There was no sex difference in the average sum of insufficient micronutrient intakes (p = 0.76).

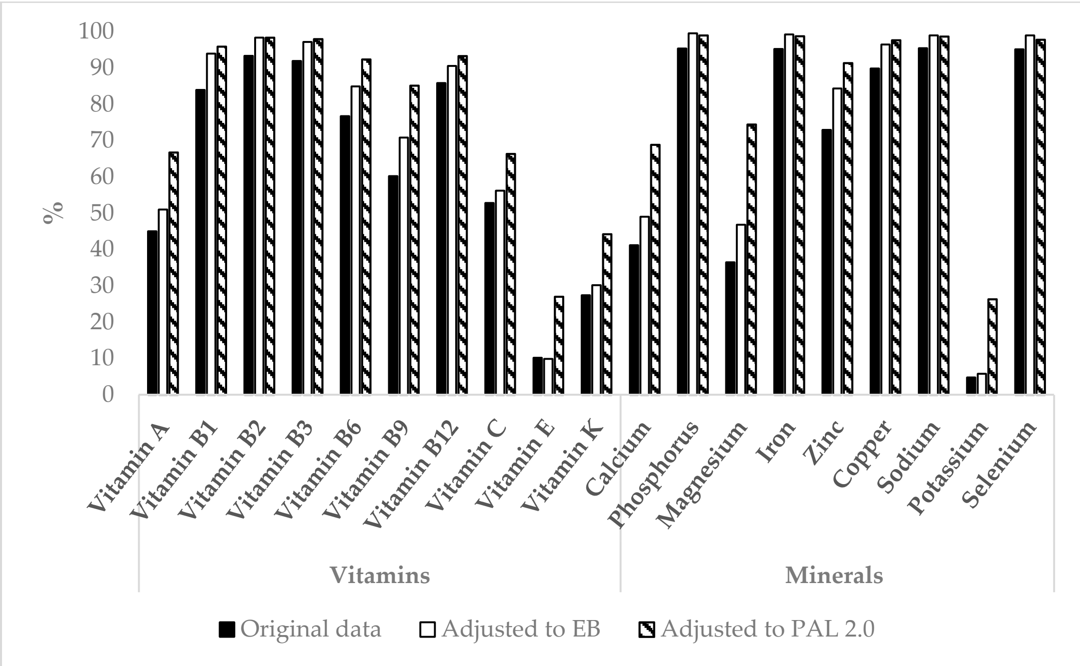

Figure 2. Percentage of participants with sufficient vitamin and mineral intake. Black bars indicate original data, white bars show adjusted data for energy balance (EB), and shaded bars indicate data adjusted for physical activity level (PAL) of 2.0.

When energy and nutrient intake was adjusted so that energy intake matched total energy expenditure, the sum of insufficient vitamin intakes was significantly associated with age (β = –0.04, p = 0.03), BMI (β = −0.22, p < 0.001), and PAL (β = −0.21, p < 0.001), but not with sex (β = 0.01, p = 0.47). The adjusted R^2 for the model was 0.09. The sum of insufficient vitamin intakes was lowest in older participants with a higher BMI and a higher PAL. In addition, the sum of insufficient mineral intakes was significantly associated with sex (β = 0.04, p < 0.05), age (β = 0.14, p < 0.001), BMI (β = −0.27, p < 0.001), and PAL (β = −0.26, p < 0.001), with an adjusted R^2 for the model of 0.20; and was lowest in younger, male participants with a higher BMI and a higher PAL.

4. Discussion

The main findings of this study are (1) NHANES nutritional intake data underestimate actual intake and need to be adjusted before interpretation; (2) NHANES participants with higher physical activity levels were more in line with recommendations for mineral and vitamin intake compared to insufficiently active participants; and (3) modeling an increase in physical activity to higher levels, together with a linear up-scaling of food intake with the same dietary composition, to compensate for the increased energy expenditure, increased compliance with recommendations for micronutrient intake. These findings underline how levels of physical activity, through the effect on energy intake, impact on the intake of non-energy constituents for a given diet composition. The lack of physical activity comes with an increased risk of mineral and vitamin deficiencies, as hypothesized. Our modelling would further suggest that increasing physical activity levels might be protective.

There was a negative correlation between PAL and the number of insufficient micronutrient intakes ($r = -0.14$, $p < 0.0001$). The participants whose baseline micronutrient intakes were compliant with the recommendations, defined as having an intake above the EAR or AI (n = 130), had a significantly higher ratio of energy intake and BMR compared to those with at least one micronutrient intake not meeting the recommendations. Non-compliance with the recommendations might be related to a higher magnitude of underreporting (low ratio of energy intake and BMR). On the other hand, those participants with complete compliance of micronutrient intake had PALs ranging from 1.13 to 2.03. This suggests that not only PAL and, hence, energy intake, play a role, but also diet composition. In that respect, the increased energy turnover due to increased physical activity could have an additional favorable impact on nutrient adequacy if it is accompanied by changes in dietary composition and/or supplementation with certain minerals and vitamins. Similar conclusions were drawn by other large-scale studies as well [10].

In an analysis of NHANES III data (1988–1994), Kimmons et al. [6] reported that participants who were overweight or obese, particularly premenopausal women, were more likely to report low levels of micronutrient intake (particularly vitamins E, C, and D, beta-carotene, selenium, and folate) than were normal-weight participants in the same sex/age category. These results are in line with findings from other studies that assessed the relationship between obesity and micronutrient intake [6,40–49].

NHANES dietary intake values might not be accurate, because of the data collection method used a 2 × 24 h dietary recall method. This, and other techniques to quantify eating habits, lack accuracy, with reported underestimations of intake up to 20%, in particular in obese individuals [50]. Briefel et al. [51] analyzed the NHANES III data (1988–1994) and reported that dietary intake was probably underestimated in up to 18% of men and 28% of women. Archer et al. [37] analyzed NHANES data (from 1971 through 2010) by using physiologically-credible energy intake values, and estimated an average under-reporting of 281 and 365 kcal/day for men and women, respectively, with greater under-reporting for participants with a greater BMI. In our NHANES data sample, we were able to actually estimate under-reporting of intake, since the objective measurement of daily physical activity with accelerometers in NHANES 2003–2006 allowed us to estimate the physical activity-related energy expenditure and to calculate the energy balance. Our results suggest NHANES 2003–2006 dietary intake data are underreported by an average of 176 and 109 kcal/day for women and men, respectively, with greater under-reporting for those with a higher BMI ($r = 0.34$, $p < 0.0001$) and higher physical activity levels ($r = 0.12$, $p < 0.0001$).

An increase in physical activity levels is not necessarily immediately compensated by an energetic equivalent increase in food intake. The type and duration/intensity of physical activity, as well as the body composition of individuals, when they engage in more physical activity, be it in the form of physical activity integrated into daily life (walking, cycling, stair climbing), exercise (jogging, working out), or sports, affect food intake regulation and its changes over time. We previously reported that overweight or obese untrained participants who engage in a long-term physical activity program do not necessarily increase energy intake during the first months [16,52]. This absence of an immediate compensatory increase in food intake in the obese might be due to their excess energy stores. Fully compensatory responses in intake to altered levels of exercise energy expenditure might not begin before a certain amount of the excess adipose tissue is depleted. Conversely, more active and lean individuals would have to increase their energy intake in response to a further increase in physical activity to prevent weight loss.

We chose to model the effect of a linear up-scaling of dietary intake to cover the energy requirements of a PAL= 2.0. A PAL of 1.7 identifies participants who can be considered to be minimally sufficiently physically active while a PAL of 1.9 may be necessary to prevent weight gain over time [53]. However, a PAL around 2.0 is more representative of typical behavior observed in modern hunter-gatherers and may reflect habitual *Homo sapiens* activity for most of its history [54].

It likely is a level sufficiently high to lead to eventual compensatory responses in food intake, but is obviously challenging to implement in modern, everyday life. It would imply a change of an entire lifestyle that is in contrast to the one supported by "modern" life in motorized and food-abundant surroundings. We calculated that, on average, the NHANES population would need to walk briskly for an additional 13 ± 3 km per day, something difficult to envisage in the USA at present. Other means to increase PAL, such as more non-exercise physical activity into daily occupational, transportation and household routines, were proven to be a useful strategy for increasing energy expenditure in otherwise inactive participants [55–57], although it is acknowledged that a meaningful increase in energy turnover is plausible only at high PALs in lean participants performing physical activity on a regular basis [16,52].

Increasing PAL to 2.0 increased mean intakes of all vitamins and minerals by 56–66%. However, the higher energy turnover did not fully correct imbalances of all minerals and vitamins. For example, the baseline sodium intake of our sample was, on average, more than double that of the EAR [36]. A linear up-scaling of sodium intake further increased the already worrisome sodium intake levels, which could jeopardize health and lead to an potential increase in cardiovascular risk [58]. More than 90% of the participants would have a sodium intake above the UL after data adjustment. On the other hand, intake of vitamin E and potassium still remained below recommendations when linear up-scaling was performed.

Our study has limitations. The participants were not equally distributed when they were divided into the three PAL subgroups (PAL < 1.4, PAL 1.4 - < 1.7, and PAL ≥ 1.7), but were composed of 60.8% (n = 2440), 36.6% (n = 1469), and 2.6% (n = 106) of the participants, respectively. Furthermore, the determination of PAL was based on the results from accelerometer data, which are prone to recording and/or analysis errors. Accelerometers have low sensitivity in low-intensity activities and are unable to register static exercise nor the activities that do not involve a transfer of the center of mass at a rate relative to the energy expended (e.g., weight lifting, uphill walking, walking and carrying a load) [59]. In addition, there is currently no consensus related to the selection of cut-off points to define activity intensities despite a number of proposed cut-offs for some devices. Furthermore, the TEE was estimated using formulas and not objectively measured with methods, such as doubly-labeled water.

Published studies using NHANES 2003–2004 data have reported that 5% of adults performed 30 minutes or more of physical activity on a daily basis [60]. Our analyses show that only 2.6% of participants were compliant with a PAL ≥ 1.7, which corresponds to a daily physical activity of moderate intensity of approximately 45–60 min, in order to prevent unhealthy weight gain [53].

Evaluation of nutritional intake has some methodological weaknesses, such as misreporting or under-reporting, that limit the interpretation of dietary record data. The NHANES dietary intake was analyzed using the 2 × 24 h dietary recall technique, which is subject to bias. In order to be able to exclude data that might not be authentic, Archer et al. [37] suggested using a ratio of energy intake and BMR that is less than 1.35 to identify the values that seem implausible. Our analyses showed energy intake to BMR ratios of 1.26 ± 0.44, 1.37 ± 0.50, and 1.52 ± 0.58 for inactive, moderately active, and active participants, respectively. However, 59.8% of all participants had a ratio of energy intake and BMR of less than 1.35. This, again, raises the question of whether memory-based dietary assessment methods should be used for the assessment of energy and nutrient intake [61–63].

We further did not take into account that under-reporting of dietary intake is not necessarily consistent across the various constituents of a diet. For instance, it was reported that fat may be more under-reported than other food constituents [64], which would be of relevance for fat-soluble vitamins. In addition, the nutritional analysis in NHANES derives mineral and vitamin intake from food tables according to the declared intake and not from a direct analysis of daily food intake.

Finally, our modeling strategy applied the assumption that an increase in physical activity-related energy expenditure would be automatically compensated by a reciprocal increase in food intake without changes to dietary composition. This theoretical model likely oversimplifies the true associations (physical activity vs. linear up-scaling in diet quantities and composition), which may be non-linear, and also dependent on the obesity status of participants.

The strength of our study lies in the fact that we used a large dataset in which physical activity was measured objectively. The chosen study model may serve as a baseline for future studies, which can deal with the aforementioned limitations and investigate them in more detail using a longitudinal study design.

5. Conclusions

Even after correcting for inadequate dietary intake reporting there is a high prevalence of insufficient micronutrient intake in the adult NHANES population. Prevalence is higher in participants with lower PALs. Insufficient mineral and vitamin intake thus seems partly determined by low energy turnover from insufficient PALs. An increase in the population's PALs might lead to increased energy intake to cover the increased expenditure and, at the same time, increased intake of the non-energy compounds in food, like minerals and vitamins, reducing the prevalence of insufficient mineral and vitamin intake.

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix III

Validity of the Actiheart step test for the estimation of maximum oxygen consumption in endurance athletes and healthy controls

Appendix III: Validity of the Actiheart step test for the estimation of maximum oxygen consumption in endurance athletes and healthy controls

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Validity of the Actiheart step test for the estimation of maximum oxygen consumption in endurance athletes and healthy controls

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ABSTRACT

Submaximal step tests are often used for estimation of maximum oxygen consumption (VO_2max) in humans. The validity of the Actiheart step test for VO_2max estimation was not fully studied yet. Therefore, purpose of the study was to estimate VO_2max using the Actiheart step test and to compare the data with measured VO_2max in endurance trained athletes (ATH) and healthy non-athletes (CON).
68 ATH (54% men, 28.0±5.4 yrs, 20.9±1.7 kg·m^{-2}) and 63 CON (46% men, 27.6±5.1 yrs, 22.1±1.7 kg·m^{-2}) performed the Actiheart step test and a spiroergometry for assessment of VO_2max. In addition, resting metabolic rate (RMR; indirect calorimetry), maximum heart rate (HRmax; heart rate monitoring system during spiroergometry), and sleeping heart rate (SHR; Actiheart 6-day long term measurement) were determined. Validity of two different Actiheart software entry modes was assessed: (1) AHraw (estimated RMR [Schofield] and HRmax [Tanaka], SHR = 70 bpm) and (2) AHcomplete (measured RMR, HRmax, and SHR). Validity was investigated using linear regression (R^2 and standard error of the estimate (SEE)) and repeated-measures ANOVA with a Bonferroni post-hoc correction. The level of significance was set to α=0.05.
VO_2max estimated by AHraw was significant related to measured VO_2max in women CON (R^2=0.22; p<0.05), whereas when VO_2max was estimated by AHcomplete the relation was significant in women ATH and CON, and in men CON (R^2=0.17-0.24; p<0.05). AHraw significantly underestimated VO_2max in the total sample by 8% (51.4±10.2 vs. 55.9±7.6 ml·kg^{-1}·min^{-1}; p<0.0001), whereas no significant difference between AHcomplete and the criterion method was found (57.0±11.1 vs. 55.9±7.6 ml·kg^{-1}·min^{-1}; p=0.26).
The Actiheart step test is an acceptable tool for the estimation of VO_{2max} if an error within 8% can be tolerated. However, accuracy of the VO_2max prediction is much improved when entering measured variables, such as RMR, SHR, and HRmax, into the software.

Keywords:
Maximum oxygen consumption – athletes – cardiorespiratory fitness – exercise testing – metabolic equivalent

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Introduction

Maximum oxygen consumption (VO_2max), a key indicator of cardiorespiratory fitness, is used in both athletic and health settings, as a determinant of physical performance (Bassett & Howley, 2000) or as a predictor of health risk and longevity (Kodama et al., 2009). VO_2max-testing is usually performed on a treadmill or a cycle ergometer while oxygen uptake and expired carbon-dioxide are measured with a respiratory gas analyzer. The workload is progressively increased until the participant reaches volitional exhaustion (after a minimum of 5 min (treadmill) or 7 min (cycle), up to 26 min (Midgley, Bentley, Luttikholt, McNaughton, & Millet, 2008)). However, maximum exercise tests are time consuming, expensive and depend on physiological expertise (Björkman, Ekblom-Bak, Ekblom, & Ekblom, 2016). In addition, some individuals from the general population cannot achieve the maximal effort required for the determination of VO_2max. Furthermore, there are contraindications for maximum exercise tests (American Thoracic Society & American College of Chest Physicians, 2003). These contraindications include uncontrolled asthma, syncope, acute myocardial infarction, and respiratory failure. Therefore, several indirect methods to estimate VO_2max have been developed, where VO_2max is predicted from submaximal exercise results. Most of these tests use the linear relationship between heart rate (HR) and oxygen uptake (VO_2). The VO_2max is then predicted by extrapolation of the submaximal values to an estimated maximum heart rate (HRmax). Submaximal tests thus allow evaluation of cardiorespiratory fitness in a population, in which the direct determination of VO_2max is not possible (Björkman et al., 2016).
The Actiheart is a lightweight (10 g), waterproof combined HR and movement sensor (accelerometer) designed to non-invasively assess daily physical activity levels (Cambridge Neurotechnology Ltd., Papworth, UK). The Actiheart was shown to give accurate estimations of activity energy expenditure against indirect calorimetry during a wide range of activities in men and women in both laboratory (Thompson, Batterham, Bock, Robson, & Stokes, 2006) and field (Crouter, Churilla, & Bassett, 2008) settings. A good level of agreement between Actiheart and doubly labelled water measured daily total energy expenditure was found in adult men and women (Brage et al., 2015), in children and adolescents (Butte et al., 2010), and in lean and overweight men of various fitness levels (Villars et al., 2012). The Actiheart needs to be individually calibrated for each person with a standard step test, a built-in function of the Actiheart software. During stepping, the relationships between actimetry and HR vs. work rate are assessed. The step test also yields estimated VO_2max values, obtained by extrapolating the HR vs. work rate regression line to the estimated or measured HRmax for the individual. No studies assessed the validity of the VO_2max estimation using the Actiheart step test. The purpose of the study was to estimate VO_2max using the Actiheart step test in AHraw and AHcomplete modes (see "Methods") and to compare the results with measured VO_2max over a range of aerobic capacities. The results were expected to fill a gap in the knowledge regarding the practical use and precision of the Actiheart modes for estimating VO_2max in comparison to the VO_2max ergometer measurements for athletic and non-athletic populations.

Methods

Participants

We recruited by advertisement 68 competitive endurance athletes (ATH; 31 women, 37 men; regular endurance training volume ≥ 300 min · wk^{-1} and participation in competitions) and 63 healthy, non-endurance-trained nonsmoking controls (CON; 34 women, 29 men; max. 150 min · wk^{-1} moderate endurance training). Most but not all participants were workers, students or athletes located on the campus of the Swiss Federal Institute of Sports Magglingen. All participants had to be weight-stable (< 2 kg of weight difference in the last 3 months) with a Body Mass Index (BMI) between 18.5 and 25 kg · m^{-2}, and aged between 18 and 40 years. Participants were excluded if they were smoking, pregnant, lactating, dieting, suffering from metabolic disease and/or eating disorders, or taking medication (apart from contraceptives). Athletes were excluded from the study if they had changed their training habits within the last four weeks before the experiments (e.g., due to injury or disease).
All experimental procedures were approved by the Regional Ethics Committee in Berne, Switzerland (KEK-number 090/15), and the study was carried out according to the recommendations of the Helsinki Declaration. Written informed consent of the participants was obtained before any testing.

Study design

The participants were recruited by advertisement. They came to the testing center on two separate testing days, having refrained from strenuous physical activity ≥ 24 h. After providing written informed consent, on the first day the participants completed, in a fasted state (≥ 12 h absence of any food or fluid intake, ≥ 36 h absence of alcohol or caffeine intake) measurements in the following order: 1) anthropometry and body composition; 2) resting metabolic rate (RMR); and 3) a step test using the in-built function of the Actiheart. One week after the first measurement day, the participants did an incremental exercise test (VO_2max) in a non-fasted state. In the days between the two testing days the participants wore an Actiheart for at least 7 days. All tests were carried out in Magglingen (Switzerland) at an altitude of 950 m.

Estimation of VO_2max with the Actiheart

The Actiheart was clipped onto two standard ECG electrodes (3M™ Red Dot™ Electrode 2560; 3M Health Care, St. Paul, USA) on the chest of the participant according to manufacturer's instructions and worn day and night (Brage et al., 2006). The device was individually calibrated for each participant using the standard step test, a built-in function of the Actiheart software version 4.0.92 (Cambridge Neurotechnology Ltd., Papworth, UK). In short, the participants stepped up and down a 195 mm high step for eight minutes while stepping rate was ramped linearly from 15 step cycles · min^{-1} (one step cycle is "up, up, down, down") to 33 step cycles · min^{-1} at the end (rate of change 2.5 body lifts · min^{-1}). Participants were advised to stop the test if they felt uncomfortable. After the end of the test, participants were requested to stand still for two minutes with the Actiheart still fitted while not speaking during which recovery HR was assessed. The mechanical power of the step test (mass-specific lift work rate) was calculated as 9.81 m · s^{-2} x step height (m) x lift frequency (number of body weight lifts · min^{-1}) and expressed in J · kg^{-1} · min^{-1}. The Actiheart software uses linear regression to model the relationship between work rate and HR during stepping. To estimate the VO_2max, the regression line was extrapolated to the estimated HRmax for each participant. The predicted VO_2max in L · kg^{-1} · min^{-1} was then calculated as a maximum mechanical power in J · kg^{-1} · min^{-1} divided by a factor of 20.2 (assuming an invariant net efficiency of 20%). The HRmax was defined in two ways: (1) estimated by use of the Tanaka equation (Tanaka, Monahan, & Seals, 2001); and (2) measured during a maximum exercise test (see section "Measurement of VO_2max").

Total energy expenditure (TEE) and activity energy expenditure were estimated by analyzing 6 full-day (24 h) recordings of HR and body movement with a 15-second averaging epoch setting. During the recording period participants were requested to continue their habitual life routine and physical activities. Sleeping heart rate (SHR) was measured as a mean of the 120 lowest HR recordings during 24 h, the SHR for a full six-day period was determined as the mean value of the six SHR recordings. TEE was calculated as sum of RMR (measured by indirect calorimetry, see section "Resting metabolic rate"), activity energy expenditure, and diet-induced thermogenesis (estimated as 10% of TEE (Manore & Thompson, 2010)). Physical activity level (PAL) was calculated as TEE/RMR.

For the validity testing, two inbuilt scenarios of the Actiheart step test were looked at: (1) "AHraw". In this mode SHR is set to 70 bpm, RMR is estimated by the Schofield equation (Schofield, 1985), and HRmax is estimated by use of the Tanaka formula (Tanaka et al., 2001); and (2) "AHcomplete". In this mode SHR, measured during the long-term recordings of the Actiheart, RMR, measured using indirect calorimetry, and HRmax, measured during a maximum exercise test, are manually entered into the Actiheart software.

Measurement of VO_2max

Before exercise testing each participant filled out the German (Marti, Villiger, Hintermann, & Lerch, 1998) or French (Société canadienne de physiologie de l'exercice, 2002) version of the Physical Activity Readiness Questionnaire (PAR-Q). Only if all items were answered with "no", participants were allowed to start the exercise testing. The test was performed on a treadmill (women: model mercury; men: model venus, h/p/Cosmos Sports & Medical GmbH, Traunstein, Germany). Treadmill inclines were set at 4° throughout the test. After a 5-min warm-up jog, non-athletic participants begun running at 7 km · h^{-1}, whereas participants from the athlete group started at 9 km · h^{-1}. The speed was increased by 1 km · h^{-1} every minute for the first 3 minutes of the test and thereafter by 0.5 km · h^{-1} every 30 s until exhaustion (Steiner & Wehrlin, 2011). Gas exchange was measured breath-by-breath with an open-circuit system (Quark CPET; COSMED Srl, Rome, Italy), which was calibrated before each test according to manufacturer's instructions. VO_2 data was processed using 10-second time averages. VO_2max was determined as the highest 30-second VO_2 average for the test (Robergs, Dwyer, & Astorino, 2010). Heart rate was continuously registered with a wireless HR monitoring system (model SZ990; COSMED Srl, Rome, Italy). If the primary criteria of a plateau in oxygen uptake (defined as an increase of VO_2 < 2.1 mL · kg^{-1} · min^{-1}) was not reached by the participant ($n = 4$), then the secondary criteria of a RQ value ≥ 1.10, and a HR close (± 10 bpm) to the age-predicted HRmax (Tanaka et al., 2001) were used to determine whether the participant reached maximal effort and thus VO_2max. VO_2max-tests were carried out at a mean room temperature of 21.8 ± 1.0°C, a humidity of 39.8 ± 10.1%, and an air pressure of 914 ± 7 hpa. In general, a temperature range of 20 to 22°C in a cool, dry environment (< 50% humidity) is considered comfortable for exercise testing (Myers et al., 2009).

Anthropometric data and body composition

Body weight was measured to the nearest 0.1 kg on a calibrated beam scale (Seca 877, Seca, Hamburg, Germany) and body height was measured to the nearest 0.5 cm with a height rod (Seca 213, Seca, Hamburg, Germany), with the participants in light clothing and without shoes.

Body composition was assessed using Lunar iDXA (GE Healthcare, Madison, WI, USA). Calibration of the iDXA was performed and checked on a daily basis before testing using a calibration phantom. Participants voided their bladder before the scan. The participants were in underwear and all metal artefacts were removed. During measurement, participants were in supine position on the scanning table with their ankles and legs fixed using supports. Arms were positioned to the side with the palms flat on the table. Participants were requested to stay still during the measurement. Whole body scans were performed according to the manufacturer's instructions, and adipose tissue mass, lean tissue mass, and bone mineral content were derived (enCore software v. 11.10; GE Healthcare, Madison, WI, USA). Total body composition estimates with the Lunar iDXA have been reported to be excellent in other studies (Carver, Christou, & Andersen, 2013; Hind, Oldroyd, & Truscott, 2011).

Resting metabolic rate

RMR was assessed by indirect calorimetry using a ventilated hood system (Quark CPET; COSMED Srl, Rome, Italy). Calibrations of the gas analyzer and flowmeter were carried out before each test according to manufacturer's instructions. After acclimatization and relaxing for 30 min on a bed, a ventilated hood was placed over the participant's head and measurements were started. VO_2 and carbon dioxide production (VCO_2) were measured for 30 min at 10-second intervals with participants remaining motionless in supine position in a thermoneutral environment (20 - 25 °C (Compher, Frankenfield, Keim, Roth-Yousey, & Evidence Analysis Working Group, 2006)). The first 5 min were eliminated as acclimatization artifact. From the remaining 25 min an interval of 5 consecutive minutes with a < 10% coefficient of variation of VO_2 and VCO_2 was considered as steady state. VO_2 and VCO_2 were then used to calculate RMR using the abbreviated Weir equation (Weir, 1949). Since respiratory quotient (RQ, defined as the ratio of VCO_2 and VO_2) measures at rest < 0.70 and > 1.00 suggest protocol violations or inaccurate gas measurement (Compher et al., 2006), participants with RQ-values outside of the plausible range should be excluded from data analysis ($n = 1$). The obtained RQ for all measurements was 0.76 ± 0.04 and the mean coefficients of variation of VO_2 and VCO_2 were 3.8 ± 1.5% and 5.0 ± 1.9%. RMR measurements took place at a mean temperature of 21.9 ± 1.2°C, 40.0 ± 10.2% humidity, and an air pressure of 914 ± 7 hpa.

Statistics

Statistical analyses were performed using the statistical software SPSS version 24 for MS-Windows (IBM Corp., Chicago, IL, USA). Mean values and standard deviations (SD) were calculated and data was checked for normality using the Shapiro-Wilk-test. All parameters were normal distributed with the exception of age, body fat (%), fat-free mass (kg), RMR ($kcal \cdot d^{-1}$), VO_2max ($L \cdot min^{-1}$), recovery HR, and PAL. Group differences were tested by independent *t*-tests and Mann-Whitney-U-tests ($\alpha = 0.05$). The validity of the Actiheart step test was first investigated using Pearson's Product moment correlation analysis. The correlation coefficients (r) were classified according to Cohen (Cohen, 1988). An r between 0.10-0.29 was considered a small, between 0.30-0.49 a moderate and between 0.50-1.0 a strong association. The data were further analyzed using a repeated-measures ANOVA with a Bonferroni post-hoc correction to compare measured VO_2max, estimated VO_2max by AHraw, and estimated VO_2max by AHcomplete. In addition, the mean absolute error (MAE) and the mean absolute percentage error (MAPE) of AHraw and AHcomplete compared to the criterion measure were computed. Since no standardized threshold exists for high or low MAPE, we considered a MAPE ≥ 10% as an indicator of inaccuracy as suggested by other authors (Boudreaux et al., 2018; Lee, Kim, & Welk, 2014; Nelson, Kaminsky, Dickin, & Montoye, 2016; Roos, Taube, Beeler, & Wyss, 2017). The R^2 and standard error of the estimate (SEE) were calculated by linear regression, where measured VO_2max was entered as dependent variable and estimated VO_2max as independent variable. Levels of agreement between Actiheart and measured values were further expressed as mean difference with Limits of Agreement (mean difference ± 1.96 SD) (Bland & Altman, 1999).

Results

Participants

Data of in total 50 women (24 ATH and 26 CON) and 41 men (22 ATH and 19 CON) with valid Actiheart, VO_2max, and RMR measurements were included into the analysis. The Table 1 presents the anthropometric data, body composition, RMR and VO_2max of the participants. The VO_2max ranged from 39.1 – 65.4 $ml \cdot kg^{-1} \cdot min^{-1}$ in women and 42.8 – 78.4 $ml \cdot kg^{-1} \cdot min^{-1}$ in men.

Validity of the Actiheart step test for estimation of VO_2max

The Pearson's correlation analyses revealed moderately correlated data of AHraw and the criterion method only in women CON ($r = 0.46$, $p < 0.05$), whereas the correlation between AHcomplete and measured VO_2max was significant in women ATH, women CON, and men CON ($r = 0.41 – 0.49$, all $p < 0.05$; Table 2), and r was interpreted as moderate association. The MAE, MAPE, and SEE for all groups are presented in Table 2.

In women and men ATH the AHraw significantly underestimated VO_2max ($p < 0.05$), whereas in the CON groups no significant differences between estimated and measured VO_2max were found. For AHcomplete, VO_2max was significantly underestimated in women ATH ($p = 0.03$), and overestimated in men CON ($p = 0.03$), whereas no significant differences were obtained in women CON and men ATH. When looking at the total sample, significant differences between AHraw and the measured VO_2max (51.4 ± 10.2 vs. 55.9 ± 7.6 $ml \cdot kg^{-1} \cdot min^{-1}$; $p < 0.0001$) were observed, whereas the difference between AHcomplete and VO_2max was non-significant (57.0 ± 11.1 vs. 55.9 ± 7.6 $ml \cdot kg^{-1} \cdot min^{-1}$; $p = 0.26$). The range of the MAPE across all groups was 11.4 – 17.7% and 10.8 – 14.7% for AHraw and AHcomplete, respectively (Table 2). The absolute difference between measured and estimated VO_2max by use of AHraw was smaller than 5 $ml \cdot kg^{-1} \cdot min^{-1}$ in 29% ($n = 7$), 54% ($n = 14$), 27% ($n = 6$), and 58% ($n = 11$) in women ATH, women CON, men ATH, and men CON, respectively. When VO_2max was estimated by AHcomplete, the absolute difference between measured and estimated VO_2max was smaller than 5 $ml \cdot kg^{-1} \cdot min^{-1}$ in 42% ($n = 10$), 39% ($n = 10$), 50% ($n = 11$), and 37% ($n = 7$) of women ATH, women CON, men ATH, and men CON, respectively.

The results of the linear regression analyses are presented in Table 3. In women CON a statistically significant relationship between VO_2max estimated by AHraw and the measured val-

Table 1: Participant characteristics of the men and women athletes (ATH) and controls (CON).

	Women		Men	
	ATH (n = 24)	**CON (n = 26)**	**ATH (n = 22)**	**CON (n = 19)**
Age (years)	30.0 ± 5.5	27.4 ± 4.9	25.8 ± 4.4	27.1 ± 5.4
Body mass (kg)	57.0 ± 5.9 [a]	61.6 ± 6.6	69.9 ± 6.8	72.4 ± 7.3
Height (cm)	168 ± 5	168 ± 7	180 ± 4	179 ± 7
BMI ($kg \cdot m^{-2}$)	20.3 ± 1.6 [b]	21.8 ± 1.6	21.6 ± 1.6	22.5 ± 1.7
Body fat (%)	23.3 ± 4.8 [a]	26.0 ± 4.8	14.1 ± 4.1 [a]	17.0 ± 5.0
Fat-free mass (kg)	44.4 ± 4.3	45.7 ± 5.2	60.9 ± 6.6	60.8 ± 6.7
RMR				
$kcal \cdot d^{-1}$	1436 ± 121	1500 ± 153	1868 ± 162	1835 ± 257
$kcal \cdot kg^{-1} \cdot d^{-1}$	25.4 ± 2.5	24.4 ± 1.4	26.8 ± 1.6 [a]	25.3 ± 2.5
$kcal \cdot kg^{-1} \cdot h^{-1}$	1.06 ± 0.10	1.02 ± 0.06	1.12 ± 0.07 [a]	1.06 ± 0.10
VO_2max				
$L \cdot min^{-1}$	3.2 ± 0.3 [a]	3.0 ± 0.4	4.5 ± 0.4 [a]	4.1 ± 0.6
$ml \cdot kg^{-1} \cdot min^{-1}$	56.1 ± 4.5 [c]	48.4 ± 4.4	64.0 ± 6.2 [c]	56.6 ± 5.0
Maximum metabolic equivalent of task	15.2 ± 1.4 [c]	13.7 ± 1.2	16.3 ± 1.2 [a]	15.3 ± 1.4
HRmax (bpm)	181 ± 9 [a]	186 ± 7	189 ± 8	189 ± 7
SHR (bpm)	49 ± 7 [a]	53 ± 6	48 ± 6	50 ± 7
Recovery HR (bpm)	87 ± 14	90 ± 17	85 ± 11	85 ± 12
PAL	2.1 ± 0.3 [b]	1.9 ± 0.2	1.9 ± 0.3	1.7 ± 0.2

Legend: Results are expressed as mean ± standard deviation. [a] significantly different from CON of the same sex ($p < 0.05$), [b] significantly different from CON of the same sex ($p < 0.01$), [c] significantly different from CON of the same sex ($p < 0.0001$)

Table 2: Concurrent validity (tested mode vs. criterion measure) of the two Actiheart modes to estimate VO_2max in men and women athletes (ATH) and controls (CON).

	Women		Men	
	ATH (n=24)	**CON (n=26)**	**ATH (n=22)**	**CON (n=19)**
r value				
AHraw	0.225	0.463 [a]	-0.069	0.431
AHcomplete	0.410 [a]	0.488 [a]	0.235	0.480 [a]
MAE [$ml \cdot kg^{-1} \cdot min^{-1}$]				
AHraw	8.8 ± 9.2	2.5 ± 7.2	5.2 ± 10.0	1.0 ± 9.1
AHcomplete	3.0 ± 6.5	-1.2 ± 8.0	-1.5 ± 10.0	-5.4 ± 9.8
MAPE [%]				
AHraw	17.7 ± 13.7	11.4 ± 10.4	13.6 ± 8.6	11.4 ± 10.6
AHcomplete	10.8 ± 6.5	13.6 ± 8.9	11.1 ± 10.5	14.7 ± 12.2
SEE [$ml \cdot kg^{-1} \cdot min^{-1}$]				
AHraw	4.47	3.98	6.31	4.66
AHcomplete	4.19	3.91	6.15	4.53

Legend: Results are expressed as mean ± standard deviation. [a] correlations significant at $p < 0.05$

Table 3: Parameters of linear regression for maximum oxygen consumption (VO_2max; ml · kg^{-1} · min^{-1}) in men and women athletes (ATH) and controls (CON). Measured VO_2max was entered as dependent variable and estimated VO_2max by Actiheart was the independent variable.

	Women		**Men**	
	ATH (*n*=24)	**CON (*n*=26)**	**ATH (*n*=22)**	**CON (*n*=19)**
R^2				
AHraw	0.051	0.215	0.005	0.186
AHcomplete	0.168	0.238	0.055	0.230
p-value				
AHraw	0.291	**0.017**	0.760	0.065
AHcomplete	**0.047**	**0.011**	0.293	**0.038**
Slope				
AHraw	0.11	0.251	-0.057	0.216
AHcomplete	0.266	0.234	0.153	0.217
Intercept				
AHraw	50.914	36.881	67.394	44.632
AHcomplete	42.004	36.782	54.011	43.183

Legend: Significant *p*-values are highlighted in bold.

ues was detected ($p < 0.05$). The relationship between VO_2max estimated by AHcomplete and the criterion method was significant in women ATH and CON and in men CON ($p < 0.05$).
In Figure 1, the data of AHraw and AHcomplete and the reference method are presented using Bland-Altman plots. For the total sample, the mean bias (± 1.96 SD) was 4.5 (-13.6; 22.6) ml · kg^{-1} · min^{-1} for AHraw, whereas for the AHcomplete no systematic errors were observed. Likewise, no systematic errors were found in the single groups for both AHraw and AHcomplete, except for women ATH when VO_2max was estimated by AHraw ($p < 0.0001$; Figure 1).

Discussion

We evaluated the validity of the Actiheart step test for the estimation of VO_2max in men and women along a range of aerobic fitness levels, comparing two different data entry modes, AHraw (estimated SHR, HRmax and RMR) and AHcomplete (measured SHR, HRmax and RMR). Based on MAPE, the Actiheart step test is not an acceptable method for estimation of VO_2max.
There exist several submaximal step tests for the estimation of VO_2max. The most common are the Chester step test, the STEP tool protocol, the modified YMCA 3-minute step test, and the Åstrand-Rhyming step test (Bennett, Parfitt, Davison, & Eston, 2016). The R^2 of previous reported step tests for the estimation of VO_2max ranged between 0.22 and 0.88 (Francis & Brasher, 1992; Francis & Culpepper, 1989; Hansen, Jacobs, Thijs, Dendale, & Claes, 2016; Knight, Stuckey, & Petrella, 2014; McArdle, Katch, Pechar, Jacobson, & Ruck, 1972; Perroni, Cortis, Minganti, Cignitti, & Capranica, 2013; Petrella, Koval, Cunningham, & Paterson, 2001; Santo & Golding, 2003; Sykes & Roberts, 2004; Webb, Vehrs, George, & Hager, 2014). In our study we found a significant relationship between estimated and measured VO_2max for the total sample, with R^2 0.24 for AHraw and 0.36 for AHcomplete. However, when dividing the groups by sex and experimental group (ATH and CON), the R^2 ranged from 0.01 to 0.22 for AHraw (with being significant only for women CON), and 0.06 to 0.24 for AHcomplete (significant for women ATH and CON, and men CON).
Compared to other studies the absolute measures of agreement showed similar or slightly better validity of the Actiheart step test for the estimation of VO_2max (SEE: 3.98 – 6.31 ml · kg^{-1} · min^{-1} for AHraw and 3.91 – 6.15 ml · kg^{-1} · min^{-1} for AHcomplete). Previous studies reported a SEE of 6.9 – 8.76 ml · kg^{-1} · min^{-1} for the modified YMCA 3-minute step test in healthy men and women (Santo & Golding, 2003) and 3.9 ml · kg^{-1} · min^{-1} for the Chester step test in healthy adults (Sykes & Roberts, 2004). In well-trained men a SEE of 0.28 L · min^{-1} was detected for the Åstrand-Ryhming step test (Åstrand & Ryhming, 1954). Assuming a body mass of 70 kg (not reported in that study) this would amount to a SEE of 3.6 ml · kg^{-1} · min^{-1}.
In the present study, we found a systematic bias of 4.5 ml · kg^{-1} · min^{-1} for the total sample for AHraw, indicating that estimated VO_2max was significantly lower compared to the measured VO_2max values. When VO_2max was estimated by AHcomplete, no systematic errors were observed (-1.1 ml · kg^{-1} · min^{-1}, non-significant). Knight et al. (2014) observed a systematic bias of -6.4 ml · kg^{-1} · min^{-1} for the STEP tool protocol in 40 healthy men and women, with higher VO_2max values in the predictive vs. maximal test. For the Chester step test a mean systematic bias of 2.8 ml · kg^{-1} · min^{-1} was reported in university students,

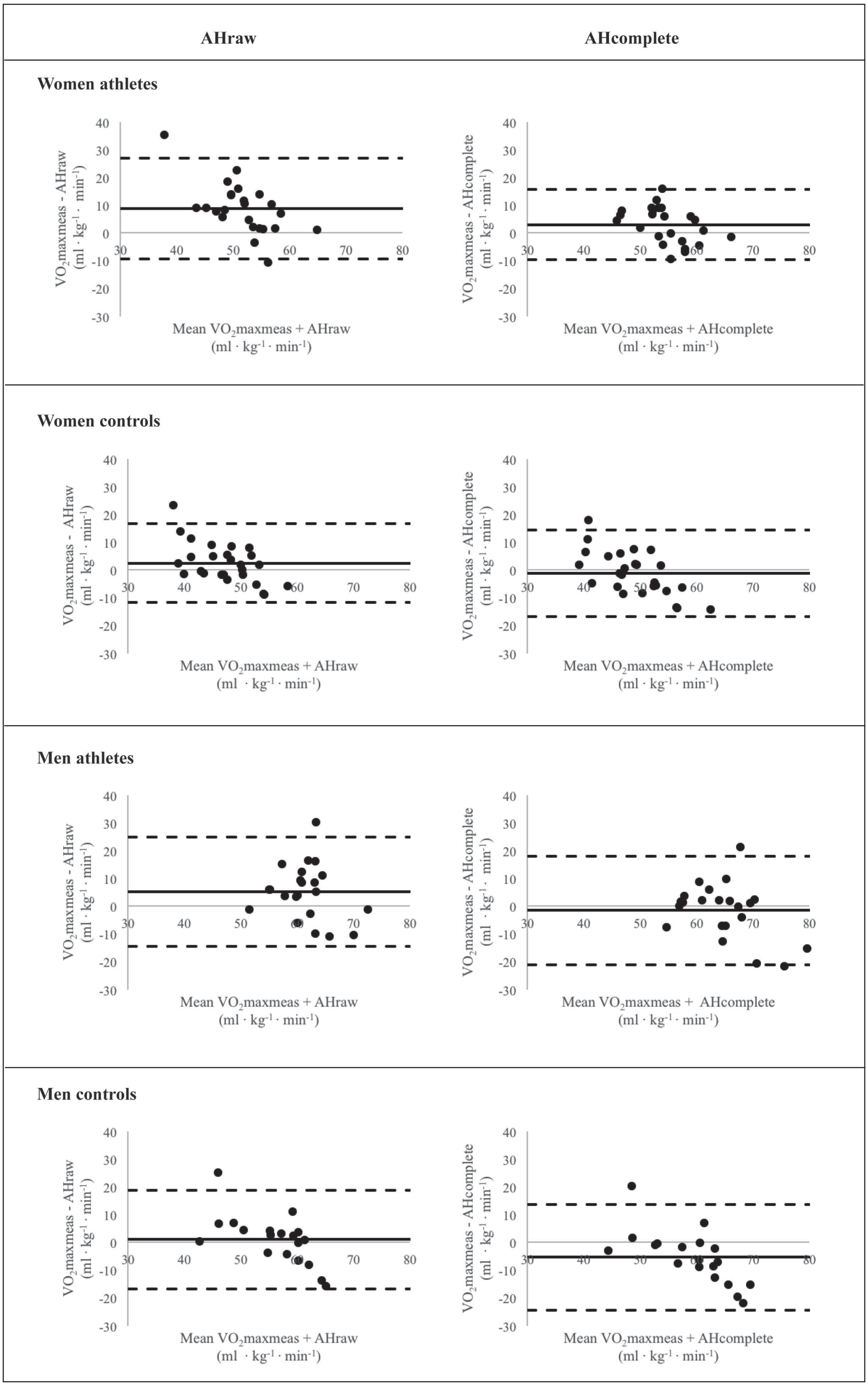

Figure 1: Bland-Altman plots of AHraw and AHcomplete and the reference method (VO_2maxmeas) for men and women athletes and controls.

indicating that predicted VO_2max was significantly lower compared to the measured VO_2max during a maximal treadmill test (Buckley, Sim, Eston, Hession, & Fox, 2004). We also found a significant negative relationship between the mean of measured and estimated VO_2max and the difference between both methods, indicating that with increasing VO_2max the difference between actual and estimated VO_2max increased ($r = -0.33$ for AHraw and $r = -0.45$ for AHcomplete, $p < 0.01$). This means that for participants with very high (such as endurance athletes) or very low aerobic capacity (untrained) the difference between estimated VO_2max by the Actiheart step test and measured VO_2max is larger. In participants with high aerobic capacity the Actiheart step test thus *overestimates* actual VO_2max, whereas in participants with low aerobic capacity VO_2max is *underestimated*, independent of which entry mode is used.

In our study, the absolute difference between measured and estimated VO_2max was smaller than 5 ml · kg^{-1} · min^{-1} in 41.8% of the participants for both AHraw and AHcomplete. However, when VO_2max was estimated by use of AHraw, only 28.3% (n = 13) of the athletes had a smaller than 5 ml · kg^{-1} · min^{-1} absolute difference between measured and estimated VO_2max, whereas when VO_2max was estimated by AHcomplete 45.7% (n = 21) of the participants were within 5 ml · kg^{-1} · min^{-1}. These results indicate that in endurance trained participants the AHcomplete for estimating VO_2max is more accurate than the AHraw, whereas in persons with a good VO_2max the AHraw setting is sufficient to predict aerobic capacity.

We assume that the underlying equations of the manufacturer were originally developed for the estimation of VO_2max of the general population with a limited range of aerobic capacity and not especially for endurance athletes nor for persons with low aerobic capacity. Based on our results it would thus seem necessary to adapt these equations in order to provide more valid VO_2max values for a broader range of aerobic capacities.

Strengths and limitations

A strength of the study is the number of included men and women covering the upper range of aerobic capacities. In addition, body composition, RMR, and long-term Actiheart data (such as SHR and PAL) were available for all participants and could be entered into the Actiheart software in order to evaluate the effect on the estimation of VO_2max. We also put effort into measuring "real" VO_2max (and not VO_2peak) by applying current criteria for maximal exercise testing (Scharhag-Rosenberger 2010, Midgley et al. 2008), including the recommendation that the duration of VO_2max tests should last between 5 and 26 minutes (Midgley et al., 2008). The specific maximal exercise test in our study was performed on a motorized treadmill and the chosen protocol for the determination of VO_2max was previously shown to induce exhaustion in athletic and non-athletic persons after 5 - 9 min (Steiner & Wehrlin, 2011).

A common issue in studies involving maximal exercise testing is the selection bias, often including fitter individuals than the general population. In a recent study examining the aerobic capacity in the Swiss working population VO_2max values of 33 and 45 ml · kg^{-1} · min^{-1} for women and men were reported (Mundwiler et al., 2017). In our control group, the mean values for VO_2max were 48.4 and 56.6 ml · kg^{-1} · min^{-1} for women and men, indicating a higher fitness level in the included participants compared to the general population.

Step tests, in general, are prone to error. For example, performing a step test requires the person's ability to maintain a certain stepping tempo and technique. Alterations in stepping technique can affect mechanical efficiency and therefore physiological responses in HR and oxygen consumption (Bennett et al., 2016). In addition, performing a step test with a pre-defined fixed step height, might introduce potential error since leg length, step length, body mass, and morphology vary between individuals and these differences might result in different individual physiological responses.

The mode AHcomplete requires the measurement of RMR, a long-term assessment of SHR, and a maximum exercise test for determination of HRmax. These measurements have higher financial and time costs, and require the same or even more expertise compared to the direct assessment of VO_2max. However, HRmax can be also measured using a maximum exercise test without performing a spiroergometry, which makes its application possible in settings where the equipment for a respiratory gas analysis is not available. Another limitation of the AHcomplete mode is that performing a maximum exercise test (with or without respiratory gas analysis) is not possible in every population, which limits the application of this mode. In summary, the mode AHcomplete is only applicable in settings without respiratory gaz analysis equipment and in subjects who can perform a maximum exercise test for determination of HRmax. We did not verify the validity of an Actiheart mode where RMR and SHR are entered as measured variables, and HRmax is estimated. However, using an estimated HRmax as endpoint for the estimation of VO_2max, such as for AHraw, might introduce error since it was shown that estimations of HRmax can have a considerable prediction error (Tanaka et al., 2001). Another potential source of error is the reliance on the linear relationship between VO_2 and power output, as shown by Åstrand and Rodahl (1970). However, Zoladz, Rademaker and Sargeant (1995) found a non-linear relationship between VO_2 and power output, which may affect the prediction of VO_2max by use of submaximal tests. Although the non-linearity might occur predominantly at high intensities above the anaerobic threshold (Majerczak et al., 2012; Zoladz, Szkutnik, Majerczak, & Duda, 1998) this underlines the importance of choosing an individual work rate which is not too high. In addition, it was shown that there is not always a linear relationship between HR and VO_2 (Buckley et al., 2004).

Conclusion

Based on MAPE, in young adults with good to superior aerobic capacity the Actiheart step test was not acceptable for estimation of VO_2max. The mode AHraw significantly underestimated VO_2max in men and women endurance trained athletes, and AHcomplete significantly underestimated VO_2max in women athletes. In endurance trained participants one should manually enter RMR, HRmax, and SHR into the Actiheart software in order to increase the accuracy of the VO_2max prediction. Areas of future investigation include the repetition of analyses of this study when performing the Actiheart step test with other step heights, evaluation of the reliability of the step test, inclusion of older and more sedentary persons and use in a clinical setting, and assessing factors such as HR and VO_2 during the Actiheart step test to improve the estimation of VO_2max.

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Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

All relevant data are included in the paper. Further data can be made available upon request.

Abbreviations

ATH = athletes, BMI = body mass index, CON = controls, HR = heart rate, HR_{max} = maximum heart rate, MAE = mean absolute error, MAPE = mean absolute percentage error, PAL = physical activity level, RMR = resting metabolic rate, RQ = respiratory quotient, SEE = standard error of the estimate, SHR = sleeping heart rate, TEE = total energy expenditure, VCO_2 = expired carbon dioxide, VO_2 = oxygen consumption, VO_2max = maximum oxygen consumption

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Appendix IV

Comparison of conventional and individualized 1-MET values for expressing maximum aerobic metabolic rate and habitual activity related energy expenditure

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Comparison of Conventional and Individualized 1-MET Values for Expressing Maximum Aerobic Metabolic Rate and Habitual Activity Related Energy Expenditure

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Abstract: The maximum aerobic metabolic rate can be expressed in multiple metabolically equivalent tasks (MET), i.e., METmax. The purpose was to quantify the error when the conventional (3.5 mL·kg^{-1}·min^{-1}) compared to an individualized 1-MET-value is used for calculating METmax and estimating activity energy expenditure (AEE) in endurance-trained athletes (END) and active healthy controls (CON). The resting metabolic rate (RMR, indirect calorimetry) and aerobic metabolic capacity (spiroergometry) were assessed in 52 END (46% male, 27.9 ± 5.7 years) and 53 CON (45% male, 27.3 ± 4.6 years). METmax was calculated as the ratio of VO_2max over VO_2 during RMR (METmax_ind), and VO_2max over the conventional 1-MET-value (METmax_fix). AEE was estimated by multiplying published MET values with the individual and conventional 1-MET-values. Dependent *t*-tests were used to compare the different modes for calculating METmax and AEE ($\alpha = 0.05$). In women and men CON, men END METmax_fix was significantly higher than METmax_ind ($p < 0.01$), whereas, in women END, no difference was found ($p > 0.05$). The conventional 1-MET-value significantly underestimated AEE in men and women CON, and men END ($p < 0.05$), but not in women END ($p > 0.05$). The conventional 1-MET-value appears inappropriate for determining the aerobic metabolic capacity and AEE in active and endurance-trained persons.

Keywords: resting metabolic rate; maximum oxygen consumption; energy expenditure; endurance athletes

1. Introduction

The aerobic capacity (maximum oxygen consumption, VO_2max) is defined as the highest rate at which oxygen can be taken up and utilized by the body during an intense large muscle group exercise [1]. It is used in both athletic and health settings, as a determinant of physical performance [1] or as a predictor of health risk and longevity [2]. Traditionally, VO_2max is expressed as the ratio of maximum rate of oxygen consumption and body mass (mL·kg^{-1}·min^{-1}). However, expressing VO_2max by normalizing for body mass (m) can be problematic since an m-based ratio is negatively correlated with m [3] and, therefore, imposes a penalty in heavier subjects, especially since the actual scaling with m is not linear [4]. This ratio is, thus, inappropriate for studies where VO_2max is compared between groups that are not matched for body size and mass, or when body mass changes over time [5,6]. One way to remove the effects of m is to adjust VO_2max by using the power function relationship $VO_2\text{max} = am^k$ where a is the scaling constant and k is the scaling exponent [5]. However, there is considerable controversy regarding the theoretical value this exponent should take (e.g., k = 2/3, 3/4 or >3/4) [7,8]. In addition, the effect of m is also a function of body composition since muscle volume is an important determinant of metabolic capacity while fat tissue is comparatively metabolically inert. This would imply that fat mass changes would introduce a greater bias as compared to lean mass changes.

Alternatively, the aerobic capacity can be expressed as the maximum aerobic metabolic rate in a multiple of metabolic equivalent of tasks (MET), i.e., METmax. One MET is defined as the energy expended by a subject at rest (resting metabolic rate, RMR) of ~1 kcal·kg^{-1}·h^{-1} [9], and is equivalent to a volume of oxygen consumed of 3.5 mL O_2 kg^{-1}·min^{-1} [10]. The MET provides a useful way to describe and classify physical activities by expressing the specific level of activity energy expenditure (AEE) (under steady state conditions) in relative value, i.e., as a multiple of RMR. Theoretically, 10 METs would then correspond to 35 mL O_2 kg^{-1} ·min^{-1}, which is equivalent to ~10 kcal·kg^{-1}·h^{-1}.

The Compendium of Physical Activities provides a five-digit coding scheme linking categories and types of physical activity with their respective intensity values in METs [9]. It was originally developed for use in epidemiologic and surveillance studies to standardize the MET intensities for various types of physical activity used in questionnaires. However, the Compendium is also frequently applied for determining precise energy costs of activities outside of its original scope. In several studies, where physical activity questionnaires were applied, the energy expenditure was estimated by using established MET codes from the Compendium of Physical Activity [11–16].

Several authors have questioned the widespread application of the conventional 1-MET value [17–20]. The value was derived from measurements of resting oxygen consumption (resting metabolic rate, RMR) of just one person, who is a 70-kg, 40-year old male, and it was shown that this value over-estimates [19–26] or underestimates [19] RMR for many types of individuals. RMR is lower in overweight subjects, declines with age, and is lower in females compared to males [20]. Therefore, estimation of AEE using the conventional 1-MET value might misrepresent actual energy expenditure. This might lead to inaccurately estimating energy requirements resulting in a positive or negative energy balance and undesirable and unexpected weight fluctuations. In addition, the maximum aerobic metabolic rate expressed as METmax might be erroneous when the conventional 1-MET value is used instead of the actual metabolic rate at rest. However, the correct assessment of oxygen consumption at rest (resting metabolic rate; RMR) requires considerable expense for both participants and researchers. Therefore, several prediction equations were developed to estimate RMR (e.g., Harris-Benedict [27], Cunningham [28]), but these also have their limitations [29,30].

Purpose of the study was to quantify the error when the conventional compared to an individualized 1-MET value is used for (1) calculating METmax, and (2) estimating energy expenditure for various daily physical activities, in endurance trained women and men, and active healthy controls. It was further investigated whether the use of a predicted RMR by the Harris-Benedict equation would reduce such an error. It was hypothesized that the use of the conventional 1-MET value would lead to relevant errors in both calculating METmax and estimating energy expenditure in comparison with an individualized approach.

2. Materials and Methods

2.1. Participants

After a public announcement, 68 competitive endurance athletes (31 women, 37 men; regular endurance training volume ≥300 min·wk^{-1} and participation in competitions) and 63 healthy, non-endurance-trained active controls (34 women, 29 men; max. 150 min·wk^{-1} moderate endurance training) were recruited for this study. Inclusion criteria for all participants were weight stability (<2 kg of weight difference in the last 3 months), a Body Mass Index (BMI) between 18.5 and 25 kg·m^{-2}, and an age between 18 and 40 years. All participants were non-smokers, not pregnant or lactating, not dieting, not suffering from metabolic disease and/or eating disorders, and not taking medication (apart from contraceptives). Athletes did not change their training habits within the last four weeks before the experiments (e.g., due to injury or disease).

The Regional Ethics Committee of the Canton Berne, Switzerland (KEK-number 090/15) approved all experimental procedures, and the study was carried out according to the recommendations of the latest Helsinki Declaration. Written informed consent of the participants was obtained before any testing.

2.2. Study Design

The participants arrived to the testing center on two separate testing days and had refrained from strenuous physical activity for at least 24 h. On the first testing day, the participants completed, in a fasting state (≥12 h absence of any food or fluid intake, ≥36 h absence of alcohol or caffeine intake) measurements in the following order: (1) anthropometry and body composition, (2) RMR, and (3) individual calibration of a combined heart rate (HR) and movement sensor (see below). One week after the first testing day, the participants performed an incremental exercise test (VO_2max) in a non-fasted state. On the days between the two testing days, the participants wore the HR and movement sensor for at least 7 days. All tests were carried out in Magglingen (Switzerland) at an altitude of 950 m.

2.3. Anthropometric Data and Body Composition

Height and body mass were measured to the nearest 0.5 cm and 0.1 kg using a height rod (Seca 213, Seca, Hamburg, Germany) and a calibrated beam scale (Seca 877, Seca, Hamburg, Germany), respectively, with the participants in light clothing and without shoes.

Body composition was assessed using Lunar iDXA (GE Healthcare, Madison, WI, USA). The iDXA was calibrated on a daily basis using a calibration phantom before any testing. The participants were in underwear, bladder-voided, and all metal artefacts were removed. During the measurement, participants were in a supine position on the scanning table with their ankles and legs fixed using supports. Arms were positioned to the side with the palms flat on the table. Participants were requested not to move during the measurement. Whole body scans were performed, according to the manufacturer's instructions. Adipose tissue mass, lean tissue mass, and bone mineral content were derived with the accompanying software (enCore software v. 11.10, GE Healthcare, Madison, WI, USA). Estimation of total body composition with the Lunar iDXA has been reported to be excellent in other studies [31,32].

2.4. Resting Metabolic Rate

Following the body composition assessment, RMR was measured by indirect calorimetry using a ventilated hood system (Quark CPET, COSMED Srl, Rome, Italy). Calibrations of the flowmeter and gas analyzer were carried out before each test, according to the manufacturer's instructions. Participants were acclimatizing and relaxing for 30 min on a bed before the hood was placed over the participant's head and measurements were started. VO_2 and carbon dioxide production (VCO_2) were measured for 30 min at 10-s intervals with participants remaining motionless in a supine position in a thermo-neutral environment (20–25 °C [33]). The first 5 min were eliminated as the acclimatization artifact. From the remaining 25 min, the interval of 5 consecutive minutes with the lowest means of the coefficients of variation (CV) for VO_2 and VCO_2 was chosen. By use of the abbreviated Weir equation, RMR was calculated [34]. Pre-hoc exclusion criteria were values of CV of VO_2 and VCO_2 ≥10% and respiratory quotient (RQ, defined as the ratio of VCO_2 and VO_2) <0.70 and >1.00, since values outside the plausible range for RQ suggest protocol violations or inaccurate gas measurements [33]. Average RQ during RMR measurement was 0.76 ± 0.04 and the CV of VO_2 and VCO_2 were 3.6 ± 1.5% and 4.5 ± 1.9%, respectively. RMR measurements took place at a mean temperature of 21.9 ± 1.1 °C, 39.1 ± 10.3% humidity, and an air pressure of 914 ± 8 hpa.

2.5. Measurement of VO_2max

Before the test, each participant filled out the German [35] or French [36] version of the Physical Activity Readiness Questionnaire (PAR-Q). Only if participants answered all items with "no," the exercise testing was started. The test was performed on a treadmill (women: model Mercury, men: model Venus, h/p/Cosmos Sports & Medical GmbH, Traunstein, Germany). After a 5-min warm-up jog, non-athletic participants began running at 7 km·h^{-1}, whereas participants from the athletic group started at 9 km·h^{-1}. The speed was increased by 1 km·h^{-1} every minute for the first 3 min of the test, and, thereafter, by 0.5 km·h^{-1} every 30 s until exhaustion. Treadmill inclines were set at 4° throughout the test [37]. Gas exchange was measured breath-by-breath with an open-circuit system (Quark CPET, COSMED Srl, Rome, Italy). Calibration was performed before each test, according to the manufacturer's instructions. VO_2 data was processed using 10-s time averages and VO_2max was determined as the highest 30-s VO_2 average for the test [38]. HR was continuously registered with a wireless HR monitoring system (model SZ990, COSMED Srl, Rome, Italy). The participants' rating of perceived exertion (RPE) was assessed immediately after the test with Borg's RPE scale [39]. If the primary criteria of a plateau in oxygen uptake (defined as an increase of VO_2 <2.1 mL·kg^{-1}·min^{-1} [40]) was not reached by the participant (n = 4), then the secondary criteria of a RQ value ≥1.10, and an HR close (± 10 bpm) to the age-predicted maximum HR [41] were used to determine whether the participant reached maximal effort and VO_2max [42]. VO_2max-tests were carried out at a mean room temperature of 21.7 ± 1.2 °C, a humidity of 39.2 ± 9.8%, and an air pressure of 914 ± 7 hpa. In general, a temperature range of 20 to 22 °C in a cool, dry environment (<50% humidity) is considered comfortable for exercise testing [43].

2.6. Calculation of METmax and Estimation of Energy Expenditure

METmax was calculated in two modes, which are the ratio of (1) VO_2max (mL·min^{-1}) over VO_2 during RMR measurement (METmax_ind), and (2) the VO_2max (mL·min^{-1}) over the conventional 1-MET value (3.5 mL·kg^{-1}·min^{-1}, METmax_fix). For calculating activity energy expenditure (AEE) activities, different MET intensities were chosen: light (<3 METs), moderate (3–5.99 METs), vigorous (6–8.99 METs), and very vigorous (≥9 METs) activity [44]. MET values for the different activities were achieved by using the Compendium of Physical Activities [9]. AEE of the different activities was estimated by multiplying the MET value (1) with the individual 1-MET value (AEE_ind), (2) with the conventional 1-MET value (AEE_fix), and with the predicted RMR by using the Harris-Benedict equation (AEE_pred). For estimating the total energy expenditure (TEE), three different physical activity levels (PAL) were chosen including a sedentary or light activity lifestyle (PAL 1.53), a moderately active or active lifestyle (PAL 1.76), and a vigorously active lifestyle (PAL 2.25) [45]. The different PAL values were then multiplied (1) with the individual 1-MET value (TEE_ind), (2) with the conventional 1-MET value (TEE_fix), and (3) with the predicted RMR by use of the Harris-Benedict equation (TEE_pred).

2.7. Physical Activity Level (PAL)

The PAL of the participants was assessed using a combined HR and movement sensor (Actiheart; Cambridge Neurotechnology Ltd., Papworth, UK). The Actiheart was clipped onto two standard ECG electrodes (3M™ Red Dot™ Electrode 2560; 3M Health Care, St. Paul, USA) on the chest of the participant, according to the manufacturer's instructions, and worn day and night [46]. The device was calibrated for each participant using a standard step test, which is a built-in function of the Actiheart software version 4.0.92 (Cambridge Neurotechnology Ltd., Papworth, UK). AEE was estimated by analyzing 6 full-day (24 h) recordings of HR and body movement with a 15-s averaging epoch setting. Participants were requested to continue their habitual life routine and physical activities during the recording period. TEE was calculated as the sum of RMR, AEE, and diet-induced thermogenesis (estimated as 10% of TEE [47]). PAL was then calculated as TEE/RMR. The Actiheart was shown to give accurate estimations of AEE during a wide range of activities in male and female subjects of various ages, body mass, and fitness levels [48–52].

2.8. Statistics

Statistical analyses were performed with SPSS statistics version 24 for MS-Windows (IBM Corp., Chicago, IL, USA). Mean values and standard deviations (SD) were calculated and data was checked for normality using the Shapiro-Wilk-test. All parameters were normally distributed with the exception of age, body mass, body mass index (BMI), body fat (%), fat-free mass (FFM; kg), RMR (kcal·day^{-1}), VO_2max

(L·min^{-1}), RQrest, and AEE/TEE calculated either by use of the conventional, predicted, or individual 1-MET value. Group differences were tested by independent *t*-tests and Mann-Whitney-U-tests ($\alpha = 0.05$). The relationship between the two modes for calculating METmax and the relationship between VO_2max and RMR were first investigated using the Pearson's Product moment correlation analysis. The correlation coefficients (*r*) were classified, according to Cohen [53]. An *r* between 0.10–0.29 was considered small, between 0.30–0.49 was considered moderate, and between 0.50–1.0 was considered showing a strong association. The data were further analyzed using dependent *t*-tests. In addition, the mean absolute error (MAE) and the mean absolute percentage error (MAPE) of METmax_fix compared to METmax_ind were calculated. Since no standardized threshold exists for high or low MAPE, a MAPE ≥10% was considered an indicator of inaccuracy as suggested by other authors [54–57]. The standard error of the estimate (SEE) was calculated by linear regression, where METmax_ind was entered as a dependent variable and METmax_fix as an independent variable. For differences in estimating AEE/TEE by use of the individual, predicted, and conventional 1-MET value, the Wilcoxon signed-rank test for dependent samples was applied.

3. Results

3.1. Participants

Nine participants who did not meet the pre-defined inclusion criteria (e.g., BMI <18.5 or >25.0 kg·m^{-2}, age <18 or >40 years, not weight stable), 9 participants with invalid RMR tests (e.g., RQ <0.70 or >1.00, CV of VO_2 and VCO_2 >10%), and 7 subjects without a valid VO_2max test (e.g., no plateau or other criteria for the maximal effort reached) were excluded from the analysis. One participant withdrew from the study due to personal reasons. In total, data of 57 women and 48 men were included in the analysis. The subjects were grouped, according to their aerobic fitness level (METmax_ind). Male and female participants with a METmax_ind above the 50th percentile were classified as endurance trained participants (END, $n = 24$ and $n = 28$, respectively). Subjects with a METmax_ind below the 50th percentile served as healthy, non-endurance trained active controls (CON, 24 men and 29 women). In Table 1, anthropometric data, body composition, RMR, and VO_2max of the participants are displayed. Women END had a significantly lower body fat percentage and BMI and higher PAL than CON ($p < 0.05$). In men, no significant differences between groups were obtained for body composition, RMR, and PAL ($p > 0.05$). The individual 1-MET value was significantly higher than 1 kcal·kg^{-1}·h^{-1} in men and women CON, and men END ($p < 0.05$). When RMR was predicted by the use of the Harris-Benedict equation, the RMR was significantly lower than 1 kcal·kg^{-1}·h^{-1} in women CON, and higher in men CON and END ($p < 0.05$). The range of VO_2max was 2.2–3.9 L·min^{-1} or 34.4–62.0 mL·kg^{-1}·min^{-1} for women and 2.5–5.3 L·min^{-1} or 42.8–78.4 mL·kg^{-1}·min^{-1} for men, respectively. Men and women END had significantly higher aerobic capacity compared to CON ($p < 0.01$). In Figure 1, the relationship between VO_2max and RMR is displayed. There was a significant positive relationship between VO_2max and RMR in all subgroups ($p < 0.0001$).

Table 1. Overview about included endurance trained participants (END) and healthy controls (CON) with a valid resting metabolic rate (RMR) and maximum oxygen consumption (VO_2max) measurements. Data are presented as Mean ± SD.

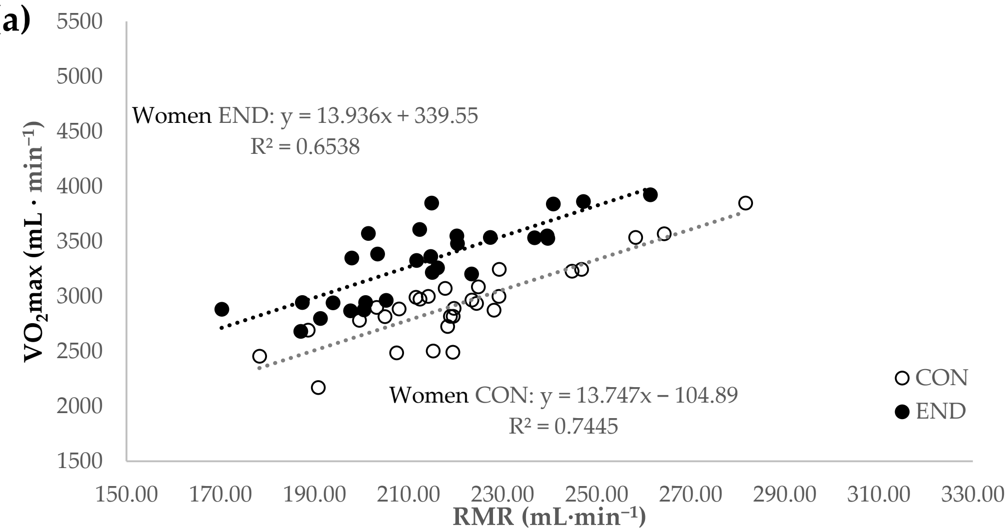
	Women		Men	
	CON ($n = 29$)	END ($n = 28$)	CON ($n = 24$)	END ($n = 24$)
Age (years)	27.6 ± 4.1	39.0 ± 6.1	27.0 ± 5.2	26.6 ± 5.0
Body mass (kg)	60.7 ± 6.7	59.6 ± 6.3	72.0 ± 7.4	70.8 ± 7.3
Height (cm)	167 ± 6	169 ± 6	178 ± 6	180 ± 6
BMI (kg·m^{-2})	21.7 ± 1.6	20.8 ± 1.5 [2]	22.7 ± 2.2	21.8 ± 1.8
Fat mass (%)	27.1 ± 5.5	23.7 ± 4.4 [2]	15.9 ± 5.5	15.2 ± 4.7
FFM (kg)	45.0 ± 4.9	46.2 ± 4.9	61.3 ± 5.7	60.8 ± 7.2
PAL [1]	1.8 ± 0.2	2.1 ± 0.2 [4]	1.8 ± 0.3	1.9 ± 0.3
RMR				
(kcal·day^{-1})	1505 ± 155	1457 ± 148	1873 ± 186	1824 ± 198
(kcal·kg^{-1}·day^{-1})	24.9 ± 1.9	24.5 ± 2.1	26.1 ± 1.8	25.9 ± 2.5

Table 1. *Cont.*

	Women		Men	
	CON (n = 29)	**END (n = 28)**	**CON (n = 24)**	**END (n = 24)**
($kcal \cdot kg^{-1} \cdot h^{-1}$)	1.04 ± 0.08 [5]	1.02 ± 0.09	1.09 ± 0.07 [7]	1.08 ± 0.10 [6]
RMRpred ($kcal \cdot kg^{-1} \cdot h^{-1}$)	0.98 ± 0.05 [5]	0.99 ± 0.05	1.03 ± 0.05 [5]	1.04 ± 0.04 [7]
RQrest	0.76 ± 0.04	0.76 ± 0.04	0.76 ± 0.06	0.75 ± 0.04
VO_2max				
($L \cdot min^{-1}$)	2.9 ± 0.4	3.3 ± 0.4 [3]	4.1 ± 0.5	4.5 ± 0.5 [3]
($mL \cdot kg^{-1} \cdot min^{-1}$)	48.3 ± 5.1	55.3 ± 4.1 [4]	56.8 ± 5.7	64.1 ± 6.1 [4]

BMI = body mass index, FFM = fat-free mass, PAL = physical activity level, RMRpred = RMR predicted by use of the Harris-Benedict equation, RQrest = respiratory quotient at rest. [1] Valid Actiheart data available for 46 females (22 CON and 24 END) and 35 males (16 CON and 19 END). [2] Significantly different from CON of the same sex group ($p < 0.05$). [3] Significantly different from CON of the same sex group ($p < 0.01$). [4] Significantly different from CON of the same sex group ($p < 0.0001$). [5] Significantly different from the value of 1 $kcal \cdot kg^{-1} \cdot h^{-1}$ ($p < 0.05$). [6] Significantly different from the value of 1 $kcal \cdot kg^{-1} \cdot h^{-1}$ ($p < 0.01$). [7] Significantly different from the value of 1 $kcal \cdot kg^{-1} \cdot h^{-1}$ ($p < 0.0001$).

(a)



(b)

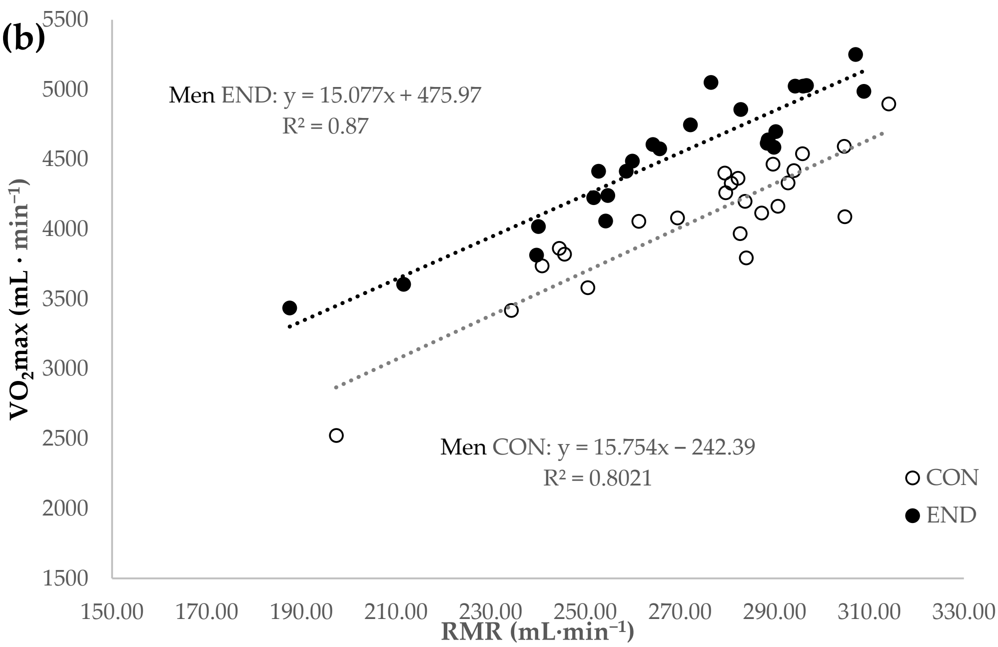


Figure 1. Resting metabolic rate (RMR) and maximum oxygen consumption (VO_2max) in (**a**) women and (**b**) men who were endurance trained subjects (END) and healthy controls (CON).

3.2. Calculation of METmax

METmax_ind and METmax_fix in women and men CON correlated (r = 0.69 and r = 0.78, respectively, $p < 0.0001$, Table 2). The MAE, MAPE, and SEE are presented for all groups in Table 2. In women and men CON, and men END METmax_fix significantly overestimated METmax ($p < 0.01$), whereas, in the women END, there was no difference ($p > 0.05$). When looking at the total sample, METmax_fix significantly overestimated METmax, compared to the use of the individual 1-MET value for its calculation (16.0 ± 2.2 vs. 15.1 ± 1.6, $p < 0.0001$). The range of MAPE was 6.6% to 11.3% across all groups. METmax_ind was significantly higher in men and women END compared to their non-athletic counterparts ($p < 0.0001$, Figure 2).

Table 2. Values and concurrent validity of the maximum metabolic equivalent of tasks (METmax) by use of the individual (METmax_ind) and conventional (METmax_fix, 3.5 mL·kg^{-1}·min^{-1}) 1-MET-value for calculating in endurance trained participants (END) and healthy controls (CON). Data are presented as Mean ± SD.

	Women		**Men**	
	CON (n = 29)	**END (n = 28)**	**CON (n = 24)**	**END (n = 24)**
METmax_ind	13.3 ± 0.9 [2]	15.5 ± 1.0 [1]	14.9 ± 0.8 [3]	16.9 ± 0.7 [1,2]
METmax_fix	13.8 ± 1.4	15.9 ± 1.2 [1]	16.3 ± 1.6	18.3 ± 1.8 [1]
r value	0.69 [4]	0.24	0.78 [4]	0.10
MAE	0.9 ± 0.8	1.0 ± 0.9	1.5 ± 0.9	1.9 ± 1.3
MAPE (%)	6.6 ± 6.2	6.8 ± 6.2	10.3 ± 5.9	11.3 ± 7.6
SEE	0.63	1.03	0.52	0.70

[1] Significantly different from CON of the same sex group ($p < 0.0001$). [2] Significantly different from METmax_fix of the same sex and experimental group ($p < 0.01$). [3] Significantly different from METmax_fix of the same sex and experimental group ($p < 0.0001$). [4] Correlation significant at $p < 0.0001$.

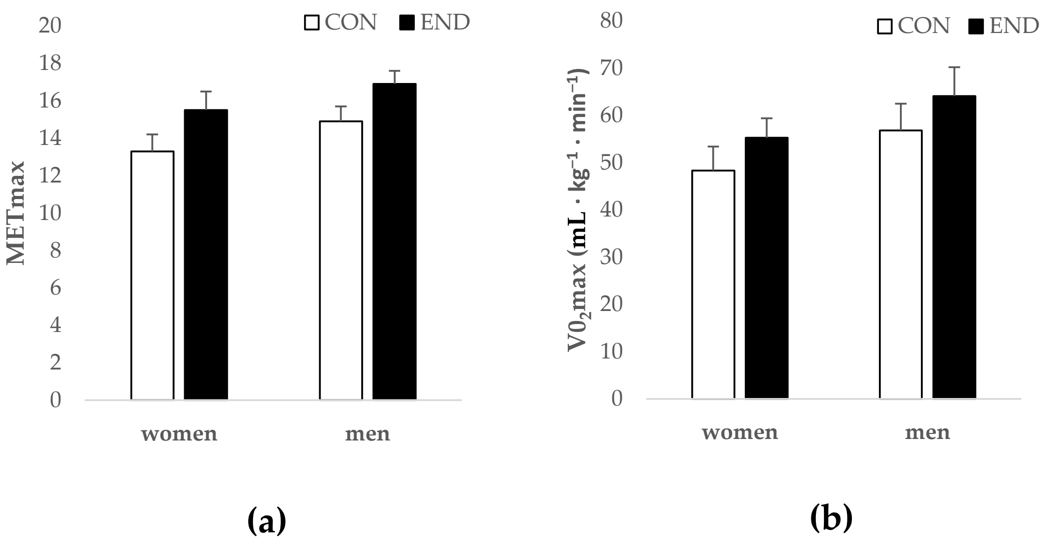


Figure 2. (**a**) Maximum metabolic equivalent of task (METmax) and (**b**) maximum oxygen consumption (VO_2max) in women and men who were endurance trained subjects (END) and healthy controls (CON).

3.3. Estimation of AEE/TEE

The conventional 1-MET value significantly underestimated the energy expenditure of all activities in men and women CON and men END ($p < 0.05$), whereas, in women END, no difference was observed ($p > 0.05$, Table 3). For example, when the energy expenditure during one hour of running was estimated by use of the individual 1-MET value, the AEE was in the mean 32 kcal·h^{-1}, 97 kcal·h^{-1}, and 84 kcal·h^{-1} higher in women and men CON, and men END, respectively, compared to the use of the conventional 1-MET value for its calculation. When AEE was calculated based on an RMR estimated by use of the Harris-Benedict equation in all subgroups, estimated AEE was significantly lower than AEE_ind for all activities ($p < 0.05$).

Table 3. Calculation of activity energy expenditure (AEE) for one hour of activity (either light, moderate, vigorous, and very vigorous) by multiplication of the individual (AEE_ind), conventional (AEE_fix), and predicted (AEE_pred) 1-MET value with published MET values of specific activities [9]. Data are presented as Mean ± SD.

	Women		**Men**	
	CON (*n* = 29)	**END (*n* = 28)**	**CON (*n* = 24)**	**END (*n* = 24)**
Light activity (e.g., sitting tasks, Code 11580, 1.5 METs)				
AEE_ind ($kcal·h^{-1}$)	94 ± 10	91 ± 9	117 ± 12	114 ± 12
AEE_fix ($kcal·h^{-1}$)	91 ± 10 [1]	89 ± 10	108 ± 11 [3]	106 ± 11 [2]
Mean difference AEE_ind—AEE_fix ($kcal·h^{-1}$)	3 ± 7	2 ± 8	9 ± 8	8 ± 10
AEE_pred ($kcal·h^{-1}$)	89 ± 5 [2]	88 ± 5 [1]	110 ± 8 [2]	110 ± 8 [2]
Mean difference AEE_ind—AEE_pred ($kcal·h^{-1}$)	6 ± 7	3 ± 8	7 ± 8	4 ± 8
Moderate activity (e.g., organizing room, Code 05125, 4.8 METs)				
AEE_ind ($kcal·h^{-1}$)	301 ± 31	291 ± 30	375 ± 37	365 ± 40
AEE_fix ($kcal·h^{-1}$)	292 ± 32 [1]	286 ± 30	346 ± 36 [3]	340 ± 35 [2]
Mean difference AEE_ind – AEE_fix ($kcal·h^{-1}$)	10 ± 23	5 ± 24	29 ± 26	25 ± 33
AEE_pred ($kcal·h^{-1}$)	283 ± 16 [2]	281 ± 17 [1]	353 ± 26 [2]	352 ± 27 [2]
Mean difference AEE_ind—AEE_pred ($kcal·h^{-1}$)	18 ± 24	11 ± 24	22 ± 24	13 ± 27
Vigorous activity (e.g., stair climbing, Code 17130, 8.0 METs)				
AEE_ind ($kcal·h^{-1}$)	502 ± 52	486 ± 49	625 ± 62	608 ± 66
AEE_fix ($kcal·h^{-1}$)	486 ± 54 [1]	477 ± 51	576 ± 60 [3]	566 ± 58 [2]
Mean difference AEE_ind—AEE_fix ($kcal·h^{-1}$)	16 ± 38	9 ± 41	48 ± 43	42 ± 55
AEE_pred ($kcal·h^{-1}$)	472 ± 27 [2]	468 ± 29 [1]	589 ± 43 [2]	587 ± 44 [2]
Mean difference AEE_ind—AEE_pred ($kcal·h^{-1}$)	30 ± 39	18 ± 40	36 ± 41	21 ± 45
Very vigorous activity (e.g., running 11 mph, Code 12130, 16 METs)				
AEE_ind ($kcal·h^{-1}$)	1004 ± 103	971 ± 99	1249 ± 124	1216 ± 132
AEE_fix ($kcal·h^{-1}$)	972 ± 107 [1]	953 ± 101	1152 ± 119 [3]	1132 ± 117 [2]
Mean difference AEE_ind—AEE_fix ($kcal·h^{-1}$)	32 ± 75	18 ± 81	97 ± 85	84 ± 110
AEE_pred ($kcal·h^{-1}$)	944 ± 54 [2]	934 ± 57 [1]	1177 ± 85 [2]	1174 ± 89 [2]
Mean difference AEE_ind—AEE_pred ($kcal·h^{-1}$)	59 ± 78	36 ± 80	72 ± 81	42 ± 89

[1] Significantly different from AEE_ind of the same experimental and sex group ($p < 0.05$). [2] Significantly different from AEE_ind of the same experimental and sex group ($p < 0.01$). [3] Significantly different from AEE_ind of the same experimental and sex group ($p < 0.0001$).

TEE was significantly underestimated in men and women CON, and men END when the conventional 1-MET value was used for estimating RMR and a PAL of 1.53, 1.76, or 2.25 was applied for calculating TEE ($p < 0.05$, Table 4). The range of the mean difference between TEE_ind and TEE_fix was 41–222 $kcal·day^{-1}$ for a PAL of 1.53, 47–255 $kcal·day^{-1}$ for a PAL of 1.76, and 60–326 $kcal·day^{-1}$ for a PAL of 2.25 across all groups. When TEE was calculated based on an RMR estimated by use of the Harris-Benedict equation, estimated TEE was still significantly lower than TEE_ind for all PAL values in all subgroups ($p < 0.05$).

Table 4. Total energy expenditure (TEE) calculated with the individual (TEE_ind), conventional (TEE_fix), and predicted (TEE_pred) 1-MET value for a sedentary or light activity lifestyle (PAL 1.53), an active or moderately active lifestyle (PAL 1.76), and a vigorous or vigorous active lifestyle (PAL 2.25) [45].

	Women		**Men**	
	CON (*n* = 29)	**END (*n* = 28)**	**CON (*n* = 24)**	**END (*n* = 24)**
Sedentary or light activity lifestyle (PAL 1.53)				
TEE_ind ($kcal·day^{-1}$)	2303 ± 237	2229 ± 226	2866 ± 285	2790 ± 303
TEE_fix ($kcal·day^{-1}$)	2230 ± 246 [1]	2188 ± 232	2645 ± 273 [3]	2598 ± 268 [2]
Mean difference TEE_ind—TEE_fix ($kcal·day^{-1}$)	73 ± 173	41 ± 186	222 ± 196	192 ± 253
TEE_pred ($kcal·day^{-1}$)	2167 ± 124 [2]	2146 ± 131 [1]	2702 ± 195 [2]	2694 ± 204 [2]
Mean difference TEE_ind—TEEpred ($kcal·day^{-1}$)	136 ± 180	83 ± 183	164 ± 186	96 ± 205

Table 4. *Cont.*

	Women		Men	
	CON (n = 29)	END (n = 28)	CON (n = 24)	END (n = 24)
Active or moderately active lifestyle (PAL 1.76)				
TEE_ind (kcal·day^{-1})	2649 ± 272	2564 ± 260	3297 ± 327	3210 ± 348
TEE_fix (kcal·day^{-1})	2565 ± 283 [1]	2517 ± 267	3042 ± 314 [3]	2989 ± 308 [2]
Mean difference TEE_ind—TEE_fix (kcal·day^{-1})	84 ± 199	47 ± 214	255 ± 225	221 ± 291
TEE_pred (kcal·day^{-1})	2493 ± 143 [2]	2468 ± 151 [1]	3108 ± 224 [2]	3099 ± 234 [2]
Mean difference TEE_ind—TEEpred (kcal·day^{-1})	157 ± 207	95 ± 211	189 ± 215	111 ± 236
Vigorous or vigorous active lifestyle (PAL 2.25)				
TEE_ind (kcal·day^{-1})	3387 ± 348	3277 ± 332	4215 ± 418	4102 ± 445
TEE_fix (kcal·day^{-1})	3279 ± 361 [1]	3217 ± 342	3889 ± 402 [3]	3821 ± 394 [2]
Mean difference TEE_ind—TEE_fix (kcal·day^{-1})	108 ± 254	60 ± 273	326 ± 288	282 ± 372
TEE_pred (kcal·day^{-1})	3186 ± 183 [2]	3156 ± 193 [1]	3974 ± 287 [2]	3962 ± 300 [2]
Mean difference TEE_ind—TEEpred (kcal·day^{-1})	201 ± 265	122 ± 270	241 ± 274	141 ± 301

[1] Significantly different from TEE_ind of the same experimental and sex group ($p < 0.05$). [2] Significantly different from TEE_ind of the same experimental and sex group ($p < 0.01$). [3] Significantly different from TEE_ind of the same experimental and sex group ($p < 0.0001$).

4. Discussion

Aims of the study were to quantify the absolute and relative errors when the conventional 1-MET estimated value (defined as a constant viz. 1 kcal·kg^{-1}·h^{-1}) was compared to an individualized RMR value measured by indirect calorimetry. Both values were used as the baseline for calculating METmax and they were compared to determine whether the use of a measured (rather than predicted) RMR would reduce the relative and absolute error of prediction of METmax.

In endurance trained men of the present study, METmax was significantly overestimated and predicted that the resting energy expenditure was slightly underestimated when the conventional standard 1-MET value was used for their calculation. In the endurance trained women, no differences between the conventional vs. individual 1-MET value were found so that the estimation of METmax was marginally higher by 0.4 METs only, as compared to the measured value (Table 2).

In men and women controls, and endurance trained men, the individual 1-MET value was significantly higher than the conventional and fixed 1-MET value ($p < 0.05$). Therefore, it can be concluded that the use of the conventional 1-MET value is inappropriate for determining the aerobic metabolic capacity and estimating the daily activity related energy expenditure (using a METs table) in active people and endurance trained athletes.

These findings are in contrast to the majority of published studies, where the measured individual 1-MET value in women was mostly lower than 3.5 mL·kg^{-1}·min^{-1} or 1 kcal·kg^{-1}·h^{-1}. For example, in a study of Byrne et al., the mean resting energy cost was 2.56 mL·kg^{-1}·min^{-1} or 0.84 kcal·kg^{-1}·h^{-1} [17]. However, they measured RMR in a large, heterogeneous sample, comprising many women and less men, with a wide age range (18–74 years) as well as the BMI range (13.8–57.5 kg·m^{-2}). Indirect calorimetry measurements were made (1) with a comfortable hood system (and not with face mask or mouthpiece, which are known to generate slightly higher RMR values, [58]), (2) under strictly standardized conditions with participants fasting for at least 12 h and no exercise allowed the day preceding testing, and (3) a 25-min period at steady state of a total 45 min RMR measurement was chosen for analysis. They also found that fat mass and FFM accounted for 62% of the variance in resting VO_2. In a review of McMurray et al. examining RMR in healthy adults, the mean value for RMR was 0.86 kcal·kg^{-1}·h^{-1}, as expected, which is higher for men than women, decreases with increasing age, and is less in overweight/obese than normal weight adults [20]. Adults with a BMI ≥30 kg·m^{-2} had the lowest RMR (<0.74 kcal·kg^{-1}·h^{-1}).

In a previous study, the RMR of adolescents, pregnant and post-pregnant women, and active men was measured [19]. A significantly higher relative RMR in adolescents compared to the conventional

1-MET value was found, whereas, in the other subgroups, no differences were observed. When reviewing data on endurance trained men and women whose RMR was measured using indirect calorimetry, similar results to the present study were obtained: women were expending, on average, 1.11 kcal·kg^{-1}·h^{-1}, and men 1.13 kcal·kg^{-1}·h^{-1} [59–67]. In these experimental studies, the use of the conventional fixed 1-MET value led to considerably greater error in endurance trained men and women compared to the measured RMR value. In the present study, endurance trained men also demonstrated a significantly higher individual RMR value compared to the conventional theoretical 1-MET value. In addition, participants in the control groups also had significantly higher individual 1-MET values. Incidentally, it should be noted that the participants who were assigned to the control group are not representative of the general population of Switzerland, which comprises a majority of moderately active and sedentary individuals. In other words, the individuals of the present sample were more physically active and had a "normal" BMI. For comparison purposes, Swiss men with a mean age of 42 years had a relative body fat percentage of 21.0% [68], which is a slightly elevated value. In a sample of Swiss women (n = 64) with a mean age of 27 years, more than two-thirds (70.3%) had a body fat percentage ≥30% [69], which indicates the presence of a high percentage of plump women. Since fat mass is the strongest predictor of the variability of resting VO_2 [17], such differences in body composition (higher relative FFM in the present study) might explain the higher individual 1-MET value of the controls compared to the values reported in the literature. In any case, these findings underline the limits of using a fixed standard 1-MET value.

4.1. Calculation of METmax

The present study addressed how much relative and absolute error the use of the conventional 1-MET value would introduce when used for calculating METmax. In 79% of the women and 88% of the men CON, and 83% of the endurance trained men (END), the conventional 1-MET value overestimated METmax, which resulted in a significant overestimation of the mean aerobic metabolic capacity in these groups. The MAPE was 6.6%, 10.3%, and 11.3% in both women and men CON, and men END, respectively, and 35%, 50%, and 46% of the women and men CON, and men END had a MAPE ≥10%, respectively. Generally, a MAPE ≥10% can be considered to be a marker for inaccurate measurements [54–57]. Therefore, the authors of the present study strongly encourage researchers and any other person, who wants to determine the aerobic metabolic capacity of active subjects, to measure RMR before a maximum exercise test is conducted. Since proper assessment of RMR requires further expertise, equipment, and time, and is somewhat cumbersome for the participant, RMR should be at least *estimated* using established equations, such as the Harris-Benedict [27] or the Cunningham [28] formulas. Another possibility would be the direct assessment of resting VO_2 of the subject prior to exercise testing while standing still on the treadmill or sitting quietly on a bike. However, it is unclear whether this resting VO_2 is more appropriate for calculating METmax than using a conventional or estimated value for RMR.

4.2. Estimation of AEE, TEE, and Physical Activity Level (PAL = TEE/RMR)

As the second purpose, the error when the conventional 1-MET value was used for estimating daily energy expenditures was investigated. Large differences using the individual and conventional 1-MET values for estimating energy expenditures were observed. In women and men CON, and men END, the conventional 1-MET value significantly underestimated the energy expenditure of several physical activities and total daily energy expenditure. For example, using a PAL of 2.25 for estimation of TEE, reflecting a rather active lifestyle, led to an underestimation of energy requirements of 108 kcal·day^{-1}, 326 kcal·day^{-1}, and 282 kcal·day^{-1} for women and men CON, and men END, respectively, when the conventional 1-MET value of 1 kcal·kg^{-1}·h^{-1} was used. In the endurance trained women of this study, no difference between the individual and conventional 1-MET value was found when energy requirements were calculated. This is because the difference between the individual and conventional 1-MET value was not significant.

PAL and MET values are frequently used for estimating energy requirements in athletes. However, using the conventional 1-MET value for their calculation might underestimate (true) energy costs of their activities and might promote insufficient energy intake, especially in situations when athletes wish to control their energy balance (e.g., for weight loss or maintenance). Besides the possible undesirable effects on body mass and body composition, the underestimation of energy costs and further advised erroneous energy intakes might also lead to a higher risk of suffering from Relative Energy Deficiency in Sport (RED-S), its concomitant symptoms, and a decrease in endurance performance [70]. Therefore, it can be recommended to either (1) measure directly the energy costs of physical activities or daily energy requirements using validated and objective measures, (2) measure RMR and use the individual 1-MET value for estimating energy requirements, or, in the case that both options are not possible, to (3) estimate RMR using established formulae and use a corrected 1-MET value for estimating the energy expenditure.

Most often in the general population, the individual 1-MET value is significantly lower than the conventional 1-MET value [19–26]. Therefore, in the general population, the use of the conventional 1-MET value is mostly *overestimating* energy costs of activities, as shown previously by others [21,23]. This overestimation of energy requirements might, thus, promote a positive energy balance and could contribute to a higher risk for obesity and concomitant diseases. Several authors recommend the use of corrected MET values to account for personal variation in sex, body mass, height, and age in order to estimate the individual physical activity level more accurately [17,18]. Hereby, the standard 1-MET value of 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ is divided by a predicted RMR obtained from the Harris-Benedict equation [27]. The authors found a significant reduction of underestimation and misclassification of the MET values when a corrected 1-MET value was used. Howley (2011) stated that the ratio of the work metabolic rate to measured RMR should not be called "METs," since METs are, by definition, restricted to a denominator of 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ [71]. In the present study the use of a predicted 1-MET value by using the Harris-Benedict equation reduced the mean difference in energy expenditure estimation between the use of a measured and estimated RMR in men, whereas, in women, the mean difference was even higher than the use of the conventional 1-MET value. Therefore, the use of corrected METs might be useful for estimating individual energy costs in some cases, whereas the standard MET values can help classify the intensity of physical activities, when different studies are compared. Lastly, it must be stated that neither the standard nor the use of a corrected 1-MET value can replace the direct assessment of energy expenditure by either measuring oxygen consumption during physical activities or by using the doubly labeled water technique.

4.3. Strengths and Limitations

This is the first study with the purpose to assess the individual 1-MET value in endurance trained athletes. Expressing aerobic capacity as a ratio of maximum oxygen consumption divided by oxygen consumption at rest is a suitable measure in endurance trained athletes and healthy, active controls. A big advantage of the METmax calculation is that the denominator (RMR) already takes into account the metabolic and physiological characteristics of the individual at the baseline, so that inter-individual (e.g., comparisons between groups of different age, sex, body composition, physiological status, ethnicity) and intra-individual (e.g., change of body composition throughout different observation time points) comparisons of METmax will not be biased by a difference or change of these characteristics. The readjustment of RMR (upward or downward) will not bias the validity of the new METmax recalculation. Another advantage of the present study is the focus on strict protocols for assessment of the variables, e.g., RMR was measured in the early morning after an overnight fast with subjects abstaining from vigorous exercise for $\geq$24 h. Assessment of body composition was performed with a gold standard method viz. dual x-ray absorptiometry.

However, some limitations must be addressed. First of all, the control participants do not reflect the typical, less physically active Swiss population. For example, the METmax of the control participants was "only" two units less when compared to their endurance-trained counterparts.

In addition, the fat mass percentage might be lower in the control participants compared to the general population. On the other hand, the physical activity level, body composition, and the aerobic capacity of the control participants might reflect the recommended "normal" human phenotype, i.e., physically active and "normal" BMI. Nevertheless, inclusion of a sedentary overweight (or obese) control group would give additional insights about the error when using the conventional 1-MET value for both determining the aerobic metabolic capacity and estimating the energy expenditure in these populations. Generally, it must be stated that estimating the energy expenditure using published MET values of the Compendium of Physical Activities must be taken with caution since the published MET values are often based on only one reference. Therefore, it can be expected that there is a wider variance in estimated AEE compared to the direct assessment of oxygen consumption during various physical activities [9]. In the present study, the dietary intake data (diary) were not analyzed, since the validity of self-reported energy intake data is highly questionable [72]. However, assessment of the total energy expenditure by use of objective validated tools (e.g., doubly labelled water) would have given further information about the interplay with energy requirements and aerobic metabolic capacity.

5. Conclusions

The use of a conventional 1-MET value appears inappropriate for determining the aerobic metabolic capacity and estimating the daily energy expenditure in active and endurance-trained persons. When the conventional standard 1-MET value was used, the predicted resting energy expenditure was slightly but significantly underestimated (above all in men). As a result, the calculation of METmax was significantly overestimated due to the underestimation of the denominator. Furthermore, the energy costs of non-maximal physical activities should also be underestimated when the conventional 1-MET value is used and this might lead to an underestimation of energy requirements for a given physical activity. For valid assessment of METmax (calculated from VO_2max), measuring RMR by indirect calorimetry is recommended or, if not possible, estimating RMR is recommended using published validated equations tailored to the characteristics of the group studied in terms of age, gender, body composition (FFM), physiological status (i.e., pregnancy), and ethnicity. For estimating energy requirements, it can be recommended to (1) either measure directly the energy expenditure by use of validated tools, or (2) measure (or at least estimate) RMR and use appropriately adjusted MET values published in the literature [9] for estimating the energy costs of various structured exercises as well as free-living daily physical activities.

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