Impact of energy turnover on fat balance in healthy young men during energy balance, caloric restriction and overfeeding

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Key words: energy turnover, metabolic chamber, fat oxidation, physical activity, energy expenditure

Abbreviations:

CHO_I, carbohydrate intake; CHO_{OX}, carbohydrate oxidation;, CR, caloric restriction; EB, energy balance; EE, energy expenditure; ET, energy turnover; E_I, energy intake; FAT/CD36, fatty acid translocase; FFA, free fatty acids; F_I, fat intake; FMI, fat mass index; F_{OX}, fat oxidation; OF, overfeeding; PAL, physical activity level; P_I, protein intake; P_{OX}, protein oxidation; $rCHO_B$, relative carbohydrate balance; rE_B, relative energy balance; rF_B, realtive fat balance; rP_B, relative protein balance; SEE, sleeping energy expenditure; tAUC, total area under the curve; TEE, total energy expenditure; $\dot{V}O_2$, rate of oxygen concentration; $\dot{V}CO_2$, rate of carbon dioxide concentration; VO_2max, maximal oxygen uptake

Trial Registration: ClinicalTrials.gov as NCT03361566

Abstract

Body weight control is thought to be improved when physical activity and energy intake are both high (high energy turnover). The aim of this study was to investigate the short-term impact of energy turnover (ET) on fat balance during zero energy balance, caloric restriction and overfeeding. In a randomized crossover study, 9 healthy men (BMI: 23.0 ±2.1 kg/m^2, 26.6 ±3.5 y) passed 3x3 days in a metabolic chamber: 3 levels of ET (low, medium and high; physical activity level = 1.3-1.4, 1.5-1.6 and 1.7-1.8) were performed at zero energy balance (EB), caloric restriction (CR), and overfeeding (OF) (100%, 75%, 125% of individual energy requirement). Different levels of ET were obtained by walking (4 km/h) on a treadmill (0, 165, 330 min). 24-h macronutrient oxidation and relative macronutrient balance (oxidation relative to intake) were calculated and free fatty acids, 24-h insulin and catecholamine secretion were analyzed as determinants of fat oxidation. During EB and OF, 24-h fat oxidation increased with higher ET. This resulted in a higher relative fat balance at medium ET (EB: +17%, OF: +14%) and high ET (EB: +23%, OF: +17%) compared to low ET (all $p<0.05$). In contrast, CR led to a stimulation of 24-h fat oxidation irrespective of ET (no differences in relative fat balance between ET levels, $p>0.05$). In conclusion, under highly

controlled conditions a higher energy turnover improved relative fat balance in young healthy men during overfeeding and energy balance compared to a sedentary state.

Introduction

According to the concept of energy balance, energy intake must equal energy expenditure to maintain body weight [1]. As a result, to lose body weight preferably in the form of body fat, caloric restriction is the prevalent therapeutic strategy [2]. The theory of an asymmetric body weight regulation proposed by Mayer et al. in 1956 [3] extends this paradigm by proposing that body weight control is more effective when energy balance is reached at a high energy expenditure and corresponding high energy intake (i.e. at a high energy turnover, ET). This theory is based on the analysis of dietary intake of 213 workers in West Bengal who had large differences in their physically demanding occupational activities. At a higher level of physical activity, a positive relationship was observed between energy intake and energy expenditure, whereas below a certain threshold of physical activity, a decrease in activity did not lead to a corresponding decrease in food intake. A low ET at a sedentary lifestyle may therefore pose a risk for weight gain. In line with this hypothesis, a recent observational study found that increasing energy expenditure rather than decreasing energy intake is more effective for reducing body fat [4]. This study was, however, challenged because of methodological issues such as that the level of energy turnover was defined only by energy expenditure in weight stable individuals and no adjustment of total energy expenditure (TEE) by resting metabolic rate was performed [5]. Other authors, who adjusted TEE by resting metabolic rate did not find that a high ET provides protection against fat gain [6,7].

Besides energy balance, fat balance is crucial for body weight control [8] and may be improved by a higher ET. Correspondingly, subjects with medium or high fitness level had a better fat utilization (lower respiratory quotient) than subjects with a low fitness level [9]. It is well known that an improved aerobic fitness positively affects the capacity to oxidize fat (maximal fat oxidation) during exercise [10,11]. However, little is known about the impact of acute changes in ET on 24-h fat oxidation and fat balance. Previous studies that investigated the acute effect of physical activity on fat balance under tightly controlled energy balance conditions in a metabolic chamber, found no impact of exercise (≤ 60 min or 400-530 kcal/day) at the intensity of 40-70% maximal oxygen uptake (VO_2max), performed in the postprandial state, on 24-h fat oxidation [12–14]. It is known that in particular physical activity at a medium intensity up to 55-65% VO_2max is able to enhance whole body fat oxidation [15]. A higher fat oxidation was found with low-intensity (33% VO_2max) and long duration (90 min) activity compared to moderate-intensity (66% VO_2max) and shorter duration

(45 min) exercise of similar energy expenditure [16]. These observations were, however, made during physical activity performed in the fasted state.

The impact of a high ET obtained by prolonged physical activity (> 60 min/d) with low intensity on 24-h fat oxidization, fat utilization and fat balance in the postprandial state remains unclear.

Improved regulation of fat balance is especially important in the condition of a positive energy balance. On a day to day basis or even shorter time periods, there is a continuous change between overfeeding and compensatory underfeeding or, conversely, between underfeeding and compensatory overfeeding [17]. Overfeeding for example at weekends or holidays may lead to long-term weight gain if not compensated by subsequent underfeeding [18,19] or by an increased energy expenditure."

Therefore, it is particularly interesting not only to investigate the impact of ET during zero energy balance but also during short-term over- and underfeeding periods.

We hypothesize, that a higher ET obtained by long-duration physical activity of low intensity (walking at 4 km/h), beneficially affects 24-h fat oxidation and fat balance.

Thus, the aim of this study was to investigate the impact of different levels of ET (obtained by different periods of low intensity physical activity) on 24-h fat oxidation and fat balance (fat oxidation as a percentage of fat intake) during zero energy balance, caloric restriction (-25% of energy requirement) and overfeeding (+25% of energy requirement). Therefore, a tightly controlled intervention study in a metabolic chamber was performed with three different levels of ET.

Subjects and Methods

The present analysis included nine healthy men and was part of a larger trial, that investigated the impact of energy turnover on macronutrient balance, appetite control and glucose metabolism (clinicaltrials.gov as NCT03361566). Participants were recruited by notice board postings at the University of Hohenheim and Stuttgart as well as on the social media platform Facebook between December 2016 and January 2018. Exclusion criteria were food allergies or intolerances, alternative nutrition habits (e.g. vegetarian or low carbohydrate diet), competitive sports, smoking, chronic diseases and regular use of medications. The study protocol was approved by the ethics committee of the Medical Council of Baden-Württemberg, Germany (F-2016-099) in accordance with the Declaration of Helsinki. All subjects provided written informed consent before participation.

Study protocol

The randomized crossover trial was conducted at the University of Hohenheim. An outline of the study protocol is given in **Figure 1**. Participants underwent 3 x 3 24-h interventions in a caloric chamber with 3 different levels of ET: (i) low, physical activity level (PAL) = 1.3 - 1.4 (ii) medium, PAL = 1.5 - 1.6 and (iii) high, PAL = 1.7 - 1.8. Each ET level was carried out at 3 levels of energy balance: zero energy balance (EB), caloric restriction (CR) and overfeeding (OF) (100%, 75% or 125% of individual energy requirement). Thus, in total, the study protocol consisted of nine intervention days. Different levels of ET were accomplished by walking on a treadmill (Kettler Track 9, KETTLER GmbH, Ense, Germany) with 4 km/h (2.49 mi/h) for different time periods. During low ET participants were sedentary and did not walk on the treadmill. During medium ET, they walked for 3 x 55 min and during high ET for 3 x 110 min. The three interventions with different ET levels were separated by one washout-day and the three energy balance conditions were also separated by at least one washout-day to avoid carry-over effects. A three-day run-in period with controlled diet preceded the intervention phase. Low, medium and high ET levels as well as OF and CR were randomized by block randomization. Therefore, the sequence of the three energy balance conditions was either CR-EB-OF or OF-EB-CR. Block randomization was conducted by using computer-generated random numbers.

The 24h-interventions took place between 0600 in the morning to 0600 the following morning. Participants were admitted to the institute at 1830 on the day before the 24-h intervention and spent the night before the intervention in the metabolic chamber. Participants left the morning after the intervention day (36 h length of stay in the metabolic chamber for one intervention day). On the washout day, they were allowed to go home for 12 h. During chamber days, participants followed a constant daily routine: wake up at 0600; meals at 0700, 1300, 1900; and bedtime at 2230. Prescribed physical activity at medium and high ET was performed after each meal at 0740, 1340 and 1940. Blood samples were taken every 2 h during the intervention-days between 0700 and 2100 using an intravenous catheter. Twenty-four-hour urine was collected during the intervention days.

Control of energy intake

All food during the study period was provided by the Institute of Nutritional Medicine. Participants were instructed to only consume the provided food and to only drink water and unsweetened herbal or fruit tea. Throughout the whole study period, macronutrient composition was kept constant with 50% carbohydrate, 35% fat and 15% protein for each day and each meal. During the 24-h interventions, participants received the same food items on each day and were asked to eat all the provided food within half an hour. Individual energy intake was based on individual energy requirement for the three distinct ET levels. Individual energy expenditure for each ET was therefore measured in a pre-study-test prior to the actual study period by 3 x 24-h room calorimetry and by performing the actual prescribed physical activities for each ET level. On washout-days, during the 3-day run-in period and during the pre-study test, food intake was *ad libitum* and leftovers were back-weighed to calculate dietary intake. Under the condition of caloric restriction, energy intake was calculated to be 25% less than the energy requirement for the respective ET level and under the condition of overfeeding 25% greater than energy requirement. Diet composition was calculated by using Prodi®6 software (Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany).

Control of physical activity

In advance of the study, it was tested which walking time and walking speed were appropriate to reach the predetermined PALs. The walking speed was set at 4 km/h (2.49 mi/h). On medium ET, participants walked 165 (3 x 55) minutes and on high ET 330 (3 x 110) minutes. Hence, they covered a distance of 11 km during medium and 22 km during high ET. Walking time, distance and speed were controlled with the software Kettler World Tours 2.0 (KETTLER GmbH, Ense, Germany).

Participants were asked to stay sedentary and to spend their time sitting at the desk or lying in bed during all interventions except for the walking session at medium and high ET. However, they were not allowed to sleep during the day. On the washout-days and during the three-day run-in period, participants were asked to refrain from exercise to avoid any impact of such activity on the outcome parameters [20,21].

Throughout the entire study period, the step count per hour was continuously measured using a triaxial activity monitor (ActivPAL, Paltechnologies Ltd., Glasgow, UK). The data were analyzed with the Software activPAL Professional v7.2.32. The ActivPAL was worn at the mid-line of the thigh, one-third of the way between hip and knee fixed with a waterproof tape according to the recommendation of the manufacturer. Because of technical problems with the ActivPAL device, the data of two participants are missing.

Anthropometry and body-composition analysis

Baseline anthropometry and body composition were obtained at baseline before the 3-day run-in period after an overnight fast. Height was measured with a stadiometer (seca 274, seca

GmbH&Co.KG, Hamburg, Germany). Body weight was measured on a calibrated impedance scale (seca mBCA 515, seca GmbH&Co.KG, Hamburg, Germany). Fat mass (FM) was assessed using Air Displacement Plethysmography via the BodPod Body Composition System (COSMED, Rome, Italy). Fat mass index (FMI) and fat-free mass index (FFMI) were calculated as FM or FFM divided by the square of height (kg/m^2).

Energy expenditure and macronutrient oxidation

The two respiratory chambers at the Institute of Nutritional Medicine at the University of Hohenheim each have a base area of 9 m^2 and a total volume of 21,000 L (D&S Consulting Services, Inc.). They are furnished with a day bed, chair and desk, computer with internet access, telephone, toilet, and sink. Air locks are used for the exchange of food and equipment (Life Science Technologies International LSTi, Leun, Germany). Fat oxidation and energy expenditure were determined at a constant flow of 120 l/min fresh air through the metabolic chamber and by continuously measuring rates of oxygen ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) concentrations on the exhaust side of the system using the Promethion integrated whole room indirect calorimeter system (Sable Systems International, Las Vegas, USA). The system consists of a GA-3m2 gas analyzer and an FG-250 flow generator. Oxygen and CO_2-concentrations (%) are measured to 0.001% by two distinguished gas analyzer chains, allowing “background baselining” in order to compensate for analyzer drift [22]. O_2- and CO_2 concentrations were measured by a galvanized fuel cell analyzer (Maxtec, Salt Lake City, USA) and a non-dispersive infrared analyzer (Sable Systems International, Las Vegas, USA). Water vapor pressure of the sample gas stream was measured directly to 0.001 kilopascals by a capacitive humidity sensor and the results are utilized to continuously correct the $\dot{V}O_2$ and $\dot{V}CO_2$, along with mass air flow (L). The rates of oxygen consumption and carbon dioxide production were calculated using equations, originated by Brown et al. [23]. Response time correction of the metabolic chambers to metabolic changes of the participant was performed by a z-Transformation mathematical model [24]. Data processing is performed using Sable Systems ExpeData software (version 1.9.51). Mean values were obtained from minute-to-minute intervals. Macronutrient oxidation and energy expenditure were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ and nitrogen excretion. Nitrogen was calculated from urinary urea (1 g urea contains 46.7% nitrogen), which was measured photometrically from 24-h urine and obligate nitrogen losses by feces and skin were assumed to be +2.5 g nitrogen/d. Macronutrient oxidation was computed according to Jéquier and Felber [25] by calculation of the nonprotein respiratory quotient, whereby protein oxidation was calculated as 6.25 x g urinary nitrogen. Total energy expenditure (TEE) was calculated using the Weir equation ($3.941 \times \dot{V}O_2 + 1.106 \times \dot{V}CO_2 - 2.17$ x g urinary nitrogen) [26]. Sleeping energy expenditure (SEE) was measured as reported by Schrauwen et al. as the lowest energy expenditure value of three consecutive hours during sleep between 2400-0600 [27]. Relative energy (rE_B) and macronutrient balances (%) were calculated as percent energy intake (E_I) of respective TEE ($\frac{EI}{TEE} \times 100$) and as percent 24-h fat oxidation of respective macronutrient intake ($\frac{\text{24-h oxidation}}{\text{intake}} \times 100$). In order to examine macronutrient utilization (fuel partitioning), macronutrient oxidation as a percentage of TEE, was calculated ($\frac{\text{24-h oxidation}}{\text{TEE}} \times 100$). Physical activity level was determined as TEE divided by resting energy expenditure ($PAL = \frac{TEE}{\text{resting energy expenditure}}$; resting energy expenditure = SEE + SEE x 0.05).

Determinants of fat oxidation

Free fatty acids in serum were measured photometrically, and total area under the curve (tAUC) was calculated for 14 h (0700–2100).

Twenty-four-hour insulin secretion was derived from 24-h urinary C-peptide excretion by using the luminescence immunoassay method.

Epinephrine and norepinephrine excretions in 24-h urine were measured using liquid chromatography-mass spectrometry. 24-h urine was acidified with hydrogen chloride within 6-7 h after the beginning of the 24-h sampling period.

Statistical analyses

Data are reported as means ± SDs. The statistical software R (2017) was used to evaluate the data using an appropriate statistical mixed model [28,29]. The data were assumed to be normally distributed and to be heteroscedastic with respect to the different conditions of energy balance and levels of ET. These assumptions are based on a graphical residual analysis. The statistical model included the 3 energy balance conditions (EB, CR, OF) and the 3 ET levels (low, medium, high), as well as their interaction term as fixed factors. The ID was regarded as a random factor. The correlations of the measured values between several intervention days were taken into account (auto-correlation). Based on this model, a Pseudo R^2 was calculated [30] and an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests (e.g., see [31]) in order to compare the several levels of the influence factors, respectively. Comparisons were made between different levels of ET within the same energy balance condition as well as between different energy balance conditions within the same level of ET. For the three ET levels, all possible comparisons were considered (low to medium, low to high, medium to high). For the comparison of energy balance conditions, zero energy balance (EB) was considered as control and comparisons between CR or

OF and EB were made. Deviations of the relative energy balances (%) from the values, that were predetermined by the study protocol (100, 75 and 125 %) and deviations of relative macronutrient balances (%) from 100 (intake = oxidation) were tested by one-sample *t* test. Significance was set at $P < 0.05$. In order to calculate the appropriate sample size to detect differences in fat oxidation using a 2-sided paired t-test, data of Iwayama et al. (12) were used, who examined the impact of exercise timing on fat oxidation (means ±SD for fat oxidation, control: 456 ±193 kcal/d; intervention: 717 ±202). A total sample size of n = 9 is required to assess these differences in fat oxidation at a α-level of 0.05 and a power of 80% (hypothesized effect size = 1.3).

Results

Baseline characteristics of the study population are shown in **Table 1.** Nine men aged 20-32 y participated in this study. BMI ranged between 19.7 to 26.1 kg/m² and FMI ranged between 2.1 to 8.1 kg/m², respectively. According to WHO criteria two participants were overweight. Body weight and fat mass did not change during the course of the study (baseline: 74.6 ±10.2 kg and 19.1 ± 4.5 % fat mass compared to 75.1 ±10.1 and 18.7 ± 4.2 % fat mass at the end of the study, p>0.05)

Comparisons of energy metabolism parameters and physical activity between conditions of energy balance and between ET levels are presented in **Table 2**. As determined by study design, energy intake (E_I, kcal/d) differed between the 3 ET levels and between the energy balance conditions and TEE (kcal/d) increased with increasing ET level. E_I was calculated to match TEE during EB. This was achieved at medium and high ET (rE_B = 100%, p>0.05), but at low ET, E_I was slightly higher than TEE (+83 kcal, p<0.05). Daily step count (steps/d) and PAL increased with higher ET within all energy balance conditions (**Table 2**). There was no difference in step count and PAL at the same ET level between the three energy balance conditions. Sleeping energy expenditure was higher with medium and high ET compared to low ET during EB and OF. During OF, SEE was also higher at high ET when compared to medium ET. During CR, SEE did not differ between the ET levels (**Table 2**).

Components of macronutrient metabolism are shown in **Table 3.** As determined absolute intake of fat-, carbohydrate and protein (F_I, CHO_I, $P_{I;}$ g/d) increased with higher ET and differed between energy balance conditions, whereas macronutrient composition was constant by study design.

There were no differences in physical activity (steps/d) and food intake (energy content and macronutrient composition) on washout-days between the study conditions (p > 0.05, data not shown).

Impact of energy turnover on fat oxidation

Figure 2 shows the 24-h fat oxidation profile during all intervention days. Twenty-four-hour fat oxidation (24-h F_{OX}, g/d) increased with higher ET during EB and OF (**Table 3**). During CR, 24-h F_{OX} was higher at high compared to low ET only.

During EB and OF, relative fat balance (rF_B) was higher at medium and high ET compared to low ET (**Table 3**). Fat oxidation as a percentage of TEE (F_{OX}/TEE; %) was higher at high ET compared to low ET during EB and OF and compared to medium ET during OF. During CR, there was no difference in rF_B between ET levels. F_{OX}/TEE also did not differ between all ET levels (**Table 3**).

During EB and OF, fat balance was positive (fat intake (F_I) was higher than 24-h F_{OX}) at all ET levels (relative fat balance, $rF_B > 100\%$, $p < 0.05$). During CR, F_I equaled 24-h F_{OX} at all ET levels ($rF_B = 100\%$, $p > 0.05$).

Determinants of fat oxidation are shown in **Table 4**. Total area under the curve for free fatty acids (mg/dl × 14h) did not differ between the ET levels during EB and CR. By contrast during OF, FFA_{tAUC} were higher at high ET compared to low ET. Twenty-four-hour insulin secretion (C-peptide excretion) did not differ between levels of ET during EB and OF, whereas during CR 24-h insulin secretion was lower at high ET compared to medium and low ET. There was no impact of ET on 24-h epinephrine and norepinephrine excretion at all energy balance conditions.

Impact of energy turnover on carbohydrate oxidation

Twenty-four-hour carbohydrate oxidation (24-h CHO_{OX}; g/d) increased with increasing ET during EB, CR and OF (**Table 3**). Relative carbohydrate oxidation ($rCHO_B$, %) did not differ between ET levels at all energy balance conditions. Carbohydrate oxidation as a percentage of TEE (CHO_{OX}/TEE; %) did not differ between ET levels during EB, CR and OF (**Table 3**). Twenty-four-hour carbohydrate balance was negative (higher oxidation than CHO_I; $rCHO_B > 100\%$, $p < 0.05$) at all ET levels during EB and CR and at low ET during OF. During OF, 24-h CHO_{OX} was equal to CHO_I at medium and high ET ($rCHO_B = 100\%$, $p > 0.05$).

Impact of energy turnover on protein oxidation

Twenty-four-hour protein oxidation (24-h P_{OX}; g/d) did not differ between ET levels during EB (**Table 3**). During CR and OF, 24-h P_{OX} was higher at high ET compared to low and medium ET.

Relative protein balance (rP_B; %) was lower at medium and high ET compared to low ET at all energy balance conditions. Protein oxidation as a percentage of TEE (P_{OX}/TEE; %) was decreased at medium and high ET compared to low ET as well as at high ET compared to medium ET during EB and OF. During CR, no difference was observed in P_{OX}/TEE between ET levels (**Table 3**).

During EB, protein balance was negative at low ET (higher oxidation than intake; $rP_B > 100\%$, $p < 0.05$) and positive at a high ET (lower oxidation than intake; $rP_B < 100\%$, $p < 0.05$). Protein balance was negative during CR and positive during OF irrespective of the level of ET (all $p < 0.05$). Twenty-four-hour protein oxidation was equal to protein intake only at medium ET during EB ($rP_B = 100\%$, $p > 0.05$).

Discussion

In accordance with the proposed hypothesis fat oxidation in percentage of intake was increased at medium ET (EB: +17%, OF: +14%) and high ET (EB: +23 %, OF: +17%) compared to low ET during EB and OF (Table 3).

In contrast to the findings of the current study, several metabolic chamber studies of Melanson et al. showed no increase in 24-h fat oxidation with increased physical activity, when participants were in energy balance [13,14,32,33]. Because physical activity was performed in the postprandial state, the absence of an increase in 24-h fat oxidation in these studies was explained by an insulin-mediated decrease in lipolysis that diminished the supply of FFA [34]. FFA availability in plasma is a major determinant of fat oxidation in muscle during and after physical activity [35]. In addition, it has been suggested that increased insulin concentrations can directly inhibit the transfer of fat through muscle cell and mitochondrial membranes and hence inhibit intramuscular triacylglycerol oxidation [36,37]. This is supported by a recent meta-analysis, that has shown a higher fat oxidation during aerobic exercise (≤ 120 min) performed in the fasted state when compared with aerobic exercise after the ingestion of a meal [38]. Another study found that only when exercise was performed before breakfast (fasted state), 24-h fat oxidation was increased [12]. Furthermore, Schrauwen et al. investigated 12 healthy subjects in a metabolic chamber and showed that when participants underwent a glycogen lowering exercise session the day prior to the stay in the metabolic chamber, fat oxidation rapidly increased in response to a high-fat diet during energy balance [39]. In line with these findings, activation of AMP-activated protein kinase, that induces catabolic pathways, is inversely correlated to glycogen content [40].

These findings emphasize the importance of the timing of physical activity in relation to food intake and indicate that 24h-fat oxidation can only be enhanced when physical activity is performed in a fasted state when glycogen stores in the muscle are depleted. In the present study, despite a higher food intake at higher ET (corresponding to a higher carbohydrate intake), 24-h insulin excretion was not increased (Table 4) presumably because of improved non-insulin mediated glucose uptake by higher physical activity (41). Therefore, although all physical activity sessions were performed during the postprandial phase (within 40 min after the beginning of the meal) lipolysis was not impaired as suggested by the fact that FFA_{tAUC} was not diminished at higher ET during EB but even increased at high ET during OF compared to low ET (Table 4). In summary, the increased 24-h fat oxidation and relative fat balance at higher ET occurred with physical activity in the postprandial state despite higher energy intake and is a unique finding that to the best of our knowledge has not been reported before.

The studies by Melanson et al. compared a day with a single exercise bout of 40-60 min or of 400 kcal energy expenditure and 40-70% VO_2max to a sedentary control day. The exercise was performed by cycling on a stationary ergometer and participants were lean sedentary, endurance-trained as well as obese sedentary men and woman [(13,14,32,33)]. The main difference between these studies and the current protocol is the duration (40-60 vs. 165 min - 330 min/d), the mode of physical activity (cycling vs. lower intensity walking) and the frequency of physical activity throughout the day (one vs. 3 sessions). The discrepant findings suggest that not only exercise intensity and meal timing relative to the activity period are important factors to consider, but also the frequency and duration of physical activity may affect 24-h fat oxidation and subsequent fat balance. It can be hypothesized, that the duration of physical activity in the other studies was too short to affect 24-h fat oxidation. This assumption is supported by Smith et al., who investigated the adaptation to a high fat diet and observed an accelerated increase in 24-h fat oxidation to a high fat diet after 1 day, when physical activity was performed by walking 2-3 times a day on a treadmill with 4.8 km/h to reach a PAL of 1.8 [(42)]. The frequency, mode, duration, and intensity of physical activity in this study is very similar to the current study. Newsom et al. have shown that an exercise session with 50% VO_2max and longer duration (70 min vs. 55min) of the same energy expenditure performed in the afternoon was more effective to improve insulin sensitivity as compared to higher intensity (65% VO_2max) [(43)]. Thus, maybe the combination of low intensity and long duration physical activity in the present study was superior to affect the impact of insulin on fat oxidation. Nevertheless, the current evidence on the impact of the intensity of physical activity on insulin sensitivity is inconclusive (see review [(44)]) Melanson et al. found no difference in 24-h fat oxidation between different intensities of physical activity (40% VO2max vs. 70% VO2max) of the same energy expenditure performed in the morning [(13)]. In addition, it can be presumed that timing of physical activity in the present study had further protective effects in respect to the diminishing impact of insulin on fat oxidation. Circadian variations in insulin sensitivity occurs with the highest insulin sensitivity in the morning and a decrease later during the day [45]. Therefore, especially the physical activity sessions after lunch and dinner had a higher potential to attenuate the insulin-derived inhibition of fat oxidation. In fact, postprandial insulin secretion after lunch decreased with higher ET during all conditions of energy balance (Buesing et al. Nutrition & Diabetes, in revision). Besides, the timing of physical activity, the frequency of physical activity in each postprandial period, might led to a cumulative attenuating effect of the inhibition of insulin on fat oxidation.

To address the mode of physical activity, walking as compared to cycling has shown to have a greater impact on fat oxidation (higher fat oxidation rates), maybe because walking involves the recruitment of a larger muscle mass than cycling [46]. This larger recruitment of muscle mass itself may led to higher rates of fat oxidation as fatty acid translocase (FAT)/CD36, a fatty acid binding protein is suggested to play an important role in particular for the short-term regulation of exercise-induced fat oxidation [40]. This protein was found to be located not only in the plasma membrane but also in the mitochondrial membrane and mitochondrial content of FAT/CD36 was increased after 30 min of electrical stimulation of muscle compared with a non-stimulated control [47]. Thus, walking may also have a greater impact on insulin-independent glucose uptake by muscle contraction and may result in more effective insulin lowering.

Since the magnitude of fat oxidation during physical activity seems to depend on insulin [34] and glycogen levels [48], it can be speculated, that the observed effects of a higher ET on relative fat balance would be more pronounced, when physical activity sessions are performed in a fasted state e.g. before breakfast.

The present study showed, that a medium ET increases relative fat oxidation during EB and OF. However, there was no further acute beneficial effect of a high ET, although TEE increased by about 400 kcal between medium and high ET. The duration of 24-h might have been too short to measure the full extent of the impact of high ET on fat oxidation, because the adaptation of fat oxidation to a higher fat intake needs several days [49]. Thus, the dose-response relationship between physical activity of low intensity under the condition of varying ET and relative fat balance remains unclear.

In addition, a limitation of the current study is, that the differences in relative fat balance were calculated compared to a very sedentary state (low ET), that may occur rarely in real life. Therefore, step count and fat oxidation for the low ET days were relatively low with 415 - 482 steps/day (Table 2) and 41 ±11 - 51 ±14 g/d (Table 3). Nevertheless, small differences in fat oxidation were also observed between medium and high ET during EB and OF.

Remarkably, 24-h fat oxidation during EB increased with medium and high ET to the same level as during CR (Table 3). Because fat intake was still higher than fat oxidation at high ET during EB, the impact of increased ET on body fat mass, needs to be evaluated in a long-term intervention.

Another important limitation of the study is that we investigated only young healthy men. Dynamics of fat oxidation in response to physical activity are different in women than in men [(50)] and may differ in obese compared to lean subjects [(51)] and with higher age [(52)]. Therefore, the study results cannot be transferred to these population groups.

Because the intensity of physical activity was not objectively assessed, it cannot be excluded that at least for some of the participants the intensity of physical activity was rather medium than low. Fat oxidation is known to increase from low to medium intensity with a peak at 45% to 65% of VO_2max [(35)]. Thus, based on low intensity physical activity, fat oxidation would have been lower as in the present results. Since the participants are all healthy, young and apparently fit men, it is however unlikely that the walking speed of 4 km/h, which is even slower than brisk walking, reached a medium intensity.

In contrast to EB and OF, during CR 24-h fat oxidation was equal to fat intake independent of ET and relative fat balance was not further improved at higher ET (Table 3). Thus, there was no synergistic effect between caloric restriction and physical activity on relative fat balance.

Relative carbohydrate balance and carbohydrate oxidation as a percentage of TEE did not differ between the ET levels at all energy balance conditions (Table 3). Therefore, carbohydrate oxidation was still the main source of energy supply without a shift in contribution at medium and high ET.

A higher 24-h protein oxidation than intake, was observed at low ET during EB. Relative protein balance shifted from a negative (> 100%) to a positive (<100%) balance as ET became higher. This is in accordance with the study of Melanson et al., in which a higher protein balance has been found when the participants were exercising compared to a sedentary control day [(14)]. The increase in fat oxidation with higher ET was therefore accounted for by a decrease in protein oxidation. Partly, the concomitant increase in protein intake along with a higher ET accounts for the improvement in relative protein oxidation. During CR, relative protein balance was negative with all ET levels but was less negative with higher ET. These results suggest that muscle mass losses during short-term caloric restriction can be decreased with a high ET. This is in line with a 12-week and a 4-month intervention study showing that aerobic exercise attenuated the loss of skeletal muscle during caloric restriction [(53,54)].

In addition to increased fat oxidation at higher ET, we observed a higher SEE with high and medium ET compared with low ET during EB (medium ET: +48 kcal/d, high ET: +80 kcal/d) and OF (medium ET: +76 kcal/d, high ET: +112 kcal/d). This suggests that a high ET facilitates a state of zero energy balance or reduces the magnitude of a positive energy balance during short-term overfeeding. Consistently, other studies have shown increased REE values with a higher ET level [55–58]. Chronically elevated ET led to increased ß-adrenergic receptor stimulation that may be mediated by a higher skeletal muscle sympathetic nerve activity [58]. Norepinephrine was found to be higher at high ET compared to low ET and was positively correlated with REE after excluding one single outlier [55]. By contrast, in the current study no difference in 24-h norepinephrine excretion was found between ET levels (Table 4). Higher SEE values with increasing ET may therefore rather be due to a protracted diet-induced thermogenesis at a higher energy content of the meals with higher ET. In line with this supposition, the magnitude and duration of diet-induced thermogenesis (DIT, in kcal) increases linearly with energy intake [59,60]. After consumption of a single mixed liquid meal (about 981 - 1,127 kcal) DIT lasted over 8 h [60]. In the current study, the average energy content of a single meal in the evening was 967 kcal at medium ET and 1,105 kcal at high ET during EB. Therefore, it can be presumed, that the increase in SEE with a higher ET (during energy balance and overfeeding) is a confounding effect of prolonged DIT. However, Paris et al. have shown that a high compared to a low ET led to a higher DIT as a percentage of energy intake in five out of six participants following a standardized breakfast [57]. This suggests, that energy dissipation in the DIT component of TEE might exist in response to a higher ET and could help to improve the regulation of energy balance. The extent of DIT (% of energy intake) was linked to SNS-activity [61] and there is further evidence, that DIT can be enhanced when exercise is performed after the meal compared to exercise before a meal [62].

The medium and high ET interventions describe physical activity levels of 1.5-1.6 and 1.7-1.8, respectively, which can be considered as feasible in daily life. In this study physical activity was achieved solely by walking, because other spontaneous physical activities (i.e. householding) are limited in the confined space of the metabolic chamber. Therefore, the step count at medium and high ET (~17500 and ~34500 steps/d) is very high in respect to the PAL. Nevertheless, the study shows that a long duration physical activity of low intensity has an impact on fat oxidation.

Conclusion

The study results suggest that a higher energy turnover obtained by prolonged physical activity of low intensity (walking with 4 km/h) has beneficial effects on body weight regulation when compared to a very sedentary state during energy balance and overfeeding in young healthy men because relative fat oxidation was increased at the expense of protein oxidation. In contrast, during caloric restriction relative fat balance was not further improved at higher energy turnover. Thus, a higher energy turnover can attenuate the adverse effects in particular of short-term overfeeding on fat balance."

Acknowledgments

Author`s contributions were as follows: AB-W designed the research study; AN, FB, FAH, AB-W performed the research; AN analyzed the data; AN, FB, FAH, MJM and AB-W discussed the results; MH provided statistical support; AN and A-BW wrote the manuscript, AN and AB-W had primary responsibility for final content. All authors read and approved the final manuscript.

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Conflicts of interest: None

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Tables

Table 1 Baseline characteristics of the study population[1]

	MW	SD
Age (y)	26.6	3.5
Height (m)	1.79	0.06
Body weight (kg)	74.4	10.1
BMI (kg/m²)	23.0	2.1
Fat mass (%)	18.3	6.2
FMI (kg/m²)	4.3	1.8
FFMI (kg/m²)	18.7	1.3

FMI, fat mass index; FFMI, fat-free mass index

[1]Values are means ±SDs, n=9.

Table 2 Comparison of energy expenditure variables and physical activity between the ET levels and conditions of energy balance[1]

	energy balance						caloric restriction						overfeeding					
	low		medium		high		low		medium		high		low		medium		high	
	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD
E_I (kcal/d)	2425	256	2906	381[a]	3342	496[a, b]	1819	230[c]	2195	288[a, c]	2507	400[a, b, c]	3033	382[c]	3658	479[a, c]	4184	638[a, b, c]
TEE (kcal)	2342	256	2909	298[a]	3304	395[a, b]	2308	345	2727	401[a, c]	3181	444[a, b]	2380	307	2926	357[a]	3349	461[a, b]
rE_B (%)	103	4	100	5	101	6	79	6[c]	81	3[c]	79	4[c]	128	6[c]	125	7[c]	125	6[c]
SEE (kcal/3h)	215	19	221	18[a]	225	21[a]	218	33	213	26	217	20[c]	210	20	224	18 [a]	232	25 [a, b]
Activity[2] (steps/d)	357	104	17480	1074[a]	34503	1768[a, b]	419	135	17387	934[a]	34334	2117[a, b]	415	205	17516	1197[a]	34521	2199[a, b]
PAL	1.30	0.04	1.57	0.06[a]	1.75	0.08[a, b]	1.26	0.06	1.52	0.08[a]	1.74	0.10[a, b]	1.34	0.06	1.55	0.08[a]	1.71	0.07[a, b]

ET, energy turnover; E_I, energy intake; TEE, total energy expenditure; rE_B, relative energy balance; SEE, sleeping energy expenditure; PAL, physical activity level.

[1]Values are means ±SDs; n = 9.

[2]n = 7.

[a]p<0.05 for comparison with low ET within the energy balance condition, [b]p<0.05 for comparison with medium ET within the energy balance condition, [c]p<0.05 for comparison of the same ET level with energy balance. Linear mixed model with multiple contrast tests.

Table 3 Comparison of macronutrient intake and oxidation variables between the ET levels and between conditions of energy balance[1]

	energy balance						caloric restriction						overfeeding					
	low		medium		high		low		medium		high		low		medium		high	
	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD
24-h npRQ	0.92	0.02	0.91	0.01	0.90	0.03[a]	0.88	0.02[c]	0.89	0.03	0.89	0.02	0.94	0.02[c]	0.93	0.02[c]	0.92	0.01[a, c]
F_I (g/d)	95	12	114	15[a]	132	20[a, b]	72	9[c]	86	11[a, c]	98	17[a, b, c]	119	15[c]	144	18[a, c]	165	25[a, b, c]
24-h F_{OX} (g/d)	51	14	80	12[a]	102	27[a, b]	78	25[c]	93	35	107	28[a]	41	11	71	20a	86	20[a, b]
rF_B (%)	54	15	71	7[a]	77	13[a]	108	24[c]	105	28[c]	109	19[c]	35	10[c]	49	10[a, c]	52	6[a, c]
F_{OX}/TEE (%)	20	5	26	2	28	5[a]	31	6[c]	31	8	31	5	16	4	22	4[a, c]	24	3[a, c]
CHO_I (g/d)	296	39	356	48[a]	408	61[a, b]	223	29[c]	269	36[a, c]	309	48[a, b, c]	372	48[c]	448	60[a, c]	512	78[a, b, c]
24-h CHO_{OX} (g/d)	378	55	451	45[a]	500	39[a, b]	304	43[c]	378	42[a, c]	456	55[a, b]	409	60	474	52[a, c]	541	71[a, b]
$rCHO_B$ (%)	128	13	127	9	124	14	137	19	142	20	149	10[c]	110	9[c]	106	9[c]	106	10[c]
CHO_{OX}/TEE (%)	66	6	64	2	62	5	54	6[c]	58	8	59	5	70	5	67	4[c]	66	4
P_I (g/d)	90	11	108	14[a]	124	17[a, b]	67	8[c]	81	10[a, c]	93	14[a, b, c]	112	14[c]	135	18[a, c]	154	23[a, b, c]
24-h P_{OX} (g/d)	101	16	103	16	104	17	95	14	96	17[c]	101	16[a, b]	103	15	107	12	113	17[a, b]
rP_B (%)	111	7	95	9[a]	84	7[a, b]	142	9[c]	118	8[a, c]	110	7[a, c]	92	9[c]	80	4[a, c]	74	7[a, c]
P_{OX}/TEE (%)	18	1	14	1[a]	13	1[a, b]	14	4[c]	13	2	12	1	18	2	15	1[a]	14	1[a, b]

npRQ, nonprotein respiratory quotient; F_I , fat intake, F_{OX}, fat oxidation; rF_B, relative fat balance; F_{OX}/TEE, percent fat oxidation of total energy expenditure; CHO_I, carbohydrate intake; CHO_{OX}, carbohydrate oxidation; $rCHO_B$, relative carbohydrate balance; CHO_{OX}/TEE, percent carbohydrate oxidation of total energy expenditure; P_I, protein intake; P_{OX}, protein oxidation; rP_B, relative protein balance; P_{OX}/TEE, percent protein oxidation of total energy expenditure.

[1]Values are means ± SDs; n = 9.

[a]p<0.05 for comparison with low ET within the energy balance condition, [b]p<0.05 for comparison with medium ET within the energy balance condition, [c]p<0.05 for comparison of the same ET level with energy balance, Linear mixed model with multiple contrast tests.

Table 4 Comparison of determinants of fat oxidation between the three ET levels within the energy balance condition[1]

	energy balance						caloric restriction						overfeeding					
	low		medium		high		low		medium		high		low		medium		high	
	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD	MW	SD
FFA$_{tAUC}$ (mg/dl*14h)	86	20	95	15	105	31	113	22	114	17	117	15	81	20	93	23	99	25[a]
C-peptide (µg/d)	78	50	88	36	74	37	82	42	78	34	60	27[a, b]	118	56	102	50	116	49
Epinephrine (µg/d)	11	4	10	3	11	6	10	2	10	1	11	5	10	4	10	2	9	2
Norepinephrine (µg/d)	40	9	39	9	48	10	37	10	39	10	40	11	35	5	41	8	42	10

ET, energy turnover; FFA$_{tAUC}$, total area under the curve for free fatty acids.

[1]Values are means ±SDs; n = 9.

[a]p<0.05 for comparison with low ET within the energy balance condition, [b]p<0.05 for comparison with medium ET within the energy balance condition. Linear mixed model with multiple contrast tests.

Figures

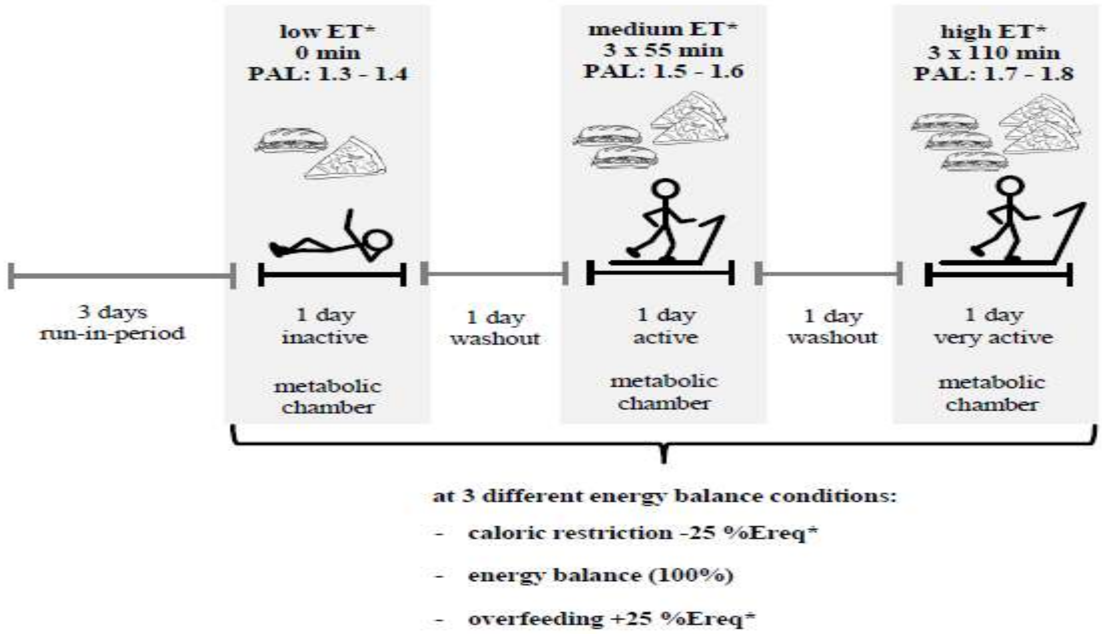

Figure 1 Outline of the study protocol of a randomized crossover trial with 24-h interventions in a metabolic chamber with 3 different levels of ET: low, medium, and high; each at energy balance, caloric restriction and overfeeding (100%, 75% or 125% of individual energy requirement). Different levels of ET were accomplished by walking on a treadmill with 4 km/h for various time periods (0, 3 x 55 min, 3 x 110 min). A 3-day run-in period with a controlled diet preceded the intervention phase and ET level interventions were separated by one washout-day. *Randomly assigned. ET, energy turnover; PAL, physical activity level; Ereq, energy requirement

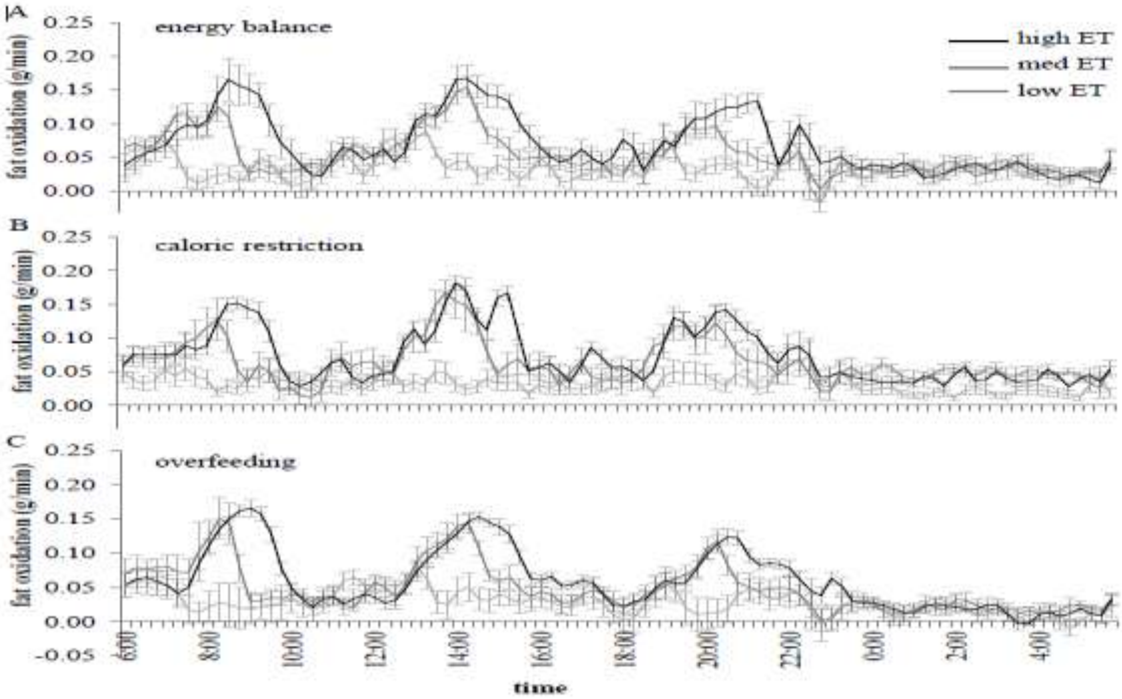

Figure 2 Comparison of fat oxidation between the 3 ET levels at energy balance (A) caloric restriction (B) and overfeeding (C) (n = 9). Mean values are shown for 15 min intervals and SEs only at every 30 min for clarity. Differences in the corresponding 24-h fat oxidation are reported in Table 3. ET, energy turnover