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Appetite control is improved by high energy turnover at different levels of energy balance

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ABSTRACT

Background: Weight control is hypothesized to be improved when physical activity and energy intake are both high (high energy turnover, ET).

Objective: The impact of three levels of ET on short-term appetite control is therefore investigated at fixed levels of energy balance.

Design: In a randomized cross-over trial, 16 healthy adults (25.1 ±3.9 y; BMI 24.0 ±3.2 kg/m^2) spent 4 x 3 daylong protocols in a metabolic chamber. Four conditions of energy balance (ad libitum energy intake, zero energy balance, -25% caloric restriction and +25% overfeeding) were each performed at three levels of ET (PAL 1.3 low, 1.6 medium and 1.8 high ET; by walking on a treadmill). Levels of appetite hormones ghrelin, GLP-1 and insulin (tAUC) were measured over 14 hours. Subjective appetite ratings were assessed by Visual Analogue Scales.

Results: Compared to high ET, low ET led to decreased GLP-1 (at all energy balance conditions: $p<0.001$) and increased ghrelin concentrations (caloric restriction and overfeeding: $p<0.001$) which was consistent with higher feelings of hunger (zero energy balance: $p<0.001$) and desire to eat (all energy balance conditions: $p<0.05$) and a positive energy balance during ad libitum intake (+17.5%; $p<0.001$).

Conclusion: Appetite is regulated more effectively at a high level of energy turnover, whereas overeating and consequently weight gain is likely to occur at low levels of energy turnover. In contrast to the prevailing concept of body weight control, the positive impact of physical activity is independent from burning up more calories and is explained by improved appetite sensations.

PRÉCIS

The concept of energy turnover was used to show that a physical activity level of 1.76 is required for prevention of weight gain under the condition of an energy dense western diet.

INTRODUCTION

Energy turnover (ET) is defined as the level of energy balance, i.e. a high ET is achieved at a high level of energy expenditure (very physically active) and a corresponding high energy intake. The same energy balance can be achieved with a low ET at a low level of energy expenditure and corresponding low energy intake (see **Figure 1**).

From a teleological point of view energy expenditure should directly impact the control of appetite and energy intake (1). A historical study led to the hypothesis that appetite is homeostatically controlled when the physical activity level is high but this control is lost at lower energy expenditure (2). This paradigm of an asymmetric control of energy balance with a "regulated zone" at high energy expenditure and an "unregulated zone" at low energy expenditure (3) is incompletely verified because it requires a fixed energy balance at different levels of energy expenditure – a condition that can be obtained in the complex setting of a metabolic chamber. Studies under free-living conditions provide only indirect evidence for this paradigm because energy balance is not fixed.

Data from Stubbs et al. suggest that there is no compensatory increase in ad libitum food intake in response to 9 days of medium or high exercise level leading to a negative energy balance compared to inactive control under free-living conditions (4). On the other hand, inactivity has been shown to promote excess energy intake and weight gain (5), sedentary time (physical inactivity) is positively associated with adipose tissue mass (6) and exercise is known to facilitate weight control. Time spent in moderate-to-vigorous physical activity was inversely

46 associated with prospective weight gain in young adults (7) and weight regain after weight loss
47 (8). In addition, a high level of structured aerobic exercise has been shown to reduce body fat
48 mass by increasing energy expenditure which is not compensated by an increase in energy
49 intake (9).
50 Hunger and satiety are responses to neural and hormonal information reporting the current state
51 of energy balance to key brain regions via signals like appetite hormones (10). A dramatic
52 reduction in energy expenditure during 1-day of sitting was not accompanied by reduced
53 appetite signals (11). Changes in appetite-regulating hormones following acute exercise might
54 be intensity-dependent, with increasing intensity leading to a greater suppression of orexigenic
55 signals and greater stimulation of anorexigenic signals (12). Hunger and appetite regulatory
56 hormones were found to be insensitive to low-intensity bouts of physical activity suggesting
57 beneficial implications on body weight control (13). The effect of energy turnover modulated
58 by different durations of low-intensity physical activity on appetite control remains unclear.
59 The aim of the present study was to investigate the effect of different levels of energy turnover
60 obtained by varying amounts of low-intensity physical activity (brisk walking) on short-term
61 appetite control during different degrees of energy balance under highly controlled conditions
62 in a metabolic chamber. We hypothesize that appetite is better controlled at high energy
63 turnover independent of energy balance.

SUBJECTS AND METHODS

Study population

Sixteen healthy adults (3 women, 13 men) aged between 20 and 40 years were recruited from December 2016 to January 2018 by notice board postings at the Universities of Hohenheim and Stuttgart, Germany and from social networks. Exclusion criteria were chronic diseases, regular use of medication or supplements, alternative eating habits, food allergies or intolerances, claustrophobia and smoking. The study protocol was approved by the ethics committee of the State Medical Council of Baden-Württemberg, Germany (F-2016-099) in accordance with the Declaration of Helsinki. This trial was registered at clinicaltrials.gov as NCT03361566. All subjects provided written informed consent before participation.

Study protocol

The randomized cross-over trial comprised a highly controlled nutritional intervention for 4 weeks in every subject for which 3 days per week were spent in a metabolic chamber at the Institute of Nutritional Medicine, University of Hohenheim in Stuttgart, Germany. An outline of the study protocol is given in **Figure 2.** Three different levels of daylong energy turnover (ET; low: physical activity level (PAL) 1.3; medium: PAL 1.6; high: PAL 1.8) were obtained by controlled walking on a treadmill in the metabolic chamber and correspondingly matched energy intake. Energy requirement at different levels of physical activity was determined during ad libitum energy intake (ad libitum EI). In the following, energy balance on intervention days was changed between zero energy balance (EB), 25% caloric restriction (CR) and 25% overfeeding (OF). The order of ET-levels within each energy balance condition was randomized as well as the order of CR and OF as second and fourth condition. Block-randomization was conducted using computer-generated random numbers. Each energy balance condition started with a 3-day run-in period with controlled diet and fixed macronutrient composition in order to adapt macronutrient oxidation to macronutrient intake

(14) and to ensure equal baseline conditions. Subjects entered the metabolic chamber the evening before each 24h-intervention in order to adapt to the environment. Daily routine was strictly controlled with wakeup at 6:00, breakfast at 7:00, lunch at 13:00, dinner at 19:00 and bedtime at 22:30. The three energy turnover days were separated by one washout day each and participants were allowed to leave the institute for 12 h on these days.

Diet composition

Macronutrient composition was 50% carbohydrates, 35% fat and 15% protein for all days and for each meal using a western style diet. During ad libitum EI, subjects were asked to complete all meals within 30 minutes and eat until they feel comfortably full. Leftovers were weighed and the energy intake calculated. Individual energy requirement (Erq) for the three levels of physical activity was measured in a metabolic chamber under ad libitum EI condition. On the following intervention days, participants were fed 100 %Erq at EB, 75 %Erq at CR and 125 %Erq at OF. Leftovers were not allowed during these periods and all meals were consumed within 30 minutes. During the different energy balance conditions, the diet consisted of the same food items on chamber days, washout days and during run-in periods, respectively. Every food item was weighted to the nearest 0.1 g for each participant according to their individual energy requirement using a digital scale. Individual diet composition and actual energy and macronutrient intake were calculated using Prodi®6 software (Wissenschaftliche Verlagsgesellschaft Stuttgart, Germany). Food was provided by the Institute for Nutritional Medicine, University of Hohenheim and subjects were instructed to only consume the allocated foods, water and unsweetened fruit or herbal tea and to abstain from any exercise during the whole study period.

24h-energy expenditure

The metabolic chambers are two 9 m² rooms and have a total volume of 21,000 liters each (D & S Consulting Services Inc, New York, NY). Equipment and methodology are described in

detail elsewhere (15). Room temperature and flow rate were set to 24.5°C and 120 L/min, respectively. Response time correction of the metabolic chamber data was performed using z-Transformation (16). Total energy expenditure over 24h (TEE) was continuously measured (from 6:00-6:00) by rates of oxygen consumption and carbon dioxide production using the Promethion integrated whole room indirect calorimeter system (Sable Systems International, Las Vegas, USA) and by using the Weir equation (17). 24h-urea excretion was assessed to calculate nitrogen excretion on intervention days at EB, CR and OF in order to correct TEE for protein oxidation (for details see (15)). Due to technical problems during data collection, full TEE-data for the whole intervention period are available for 11 participants only, TEE-data for ad libitum EI are available for all 16 participants.

Appetite control

Objective and subjective appetite control were assessed. For the objective assessment concentrations of appetite regulating hormones ghrelin, GLP-1 and insulin were measured in blood samples on intervention days during EB, CR and OF. Using energy intake and TEE-data, individual energy balances for different ET levels were calculated as Δenergy balance [%] = (EI / TEE x 100) – 100.

For subjective assessment of appetite participants reported their sensations of hunger, fullness and desire to eat using visual analogue scales (VAS) every 2 hours during the day and at 0, 30, 60 and 120 minutes postprandial on intervention days during EB, CR and OF. A subsample of 8 subjects additionally completed VAS on desire for something sweet, salty and fatty at the same time points. VAS consisted of a 100 mm horizontal line with “not at all” anchored at 0 mm and “extremely” at 100 mm (18). Higher ratings indicate greater experienced sensations. Total area under the curve (tAUC) was calculated for appetite hormones for 14h (7:00 – 21:00) and for VAS for 15h (7:00 – 22:00) using trapezoidal rule (19). Since energy requirement for CR and OF was calculated as -25% and +25% of the energy intake at EB, absolute values for

energy intake differed between ET-conditions (e.g. –600 kcal at CR low ET and –832 kcal at CR high ET). Results on appetite hormones and VAS were therefore adjusted for differences in absolute intake of calories.

Blood sampling and analytical methods

Blood samples were taken every 2 hours during the day from 7:00-21:00 at EB, CR and OF and 0, 30, 60 and 120 minutes postprandially. Plasma samples for the measurement of appetite hormones were collected in BD™ P800 tubes (Becton Dickinson Inc., Franklin Lakes, USA) containing a mixture of protease, esterase and DPP-IV inhibitors to prevent hormonal degradation. Samples were stored on ice after collection and then centrifuged immediately. Total ghrelin, total GLP-1 and insulin were measured using a Bio-Plex Pro™ human Diabetes 3-Plex Kit (Bio-Rad, Hercules, USA) containing antibody conjugated microspheres. Analysis was performed on a Bio-Plex® 200 suspension array reader (Bio-Rad, Hercules, USA) according to manufacturers' instructions. Standard curve optimization and data analysis were performed with Bio-Plex Manager™ Software 6.1. 24h-urea excretion was determined using photometry (Beckman Coulter, Brea, USA; AU5800). Aliquots of all samples were stored at -80°C until analysis.

Body composition

Height was measured using a stadiometer (seca 274, seca GmbH & Co.KG, Hamburg, Germany) and body composition was assessed using Air Displacement Plethysmography (ADP) by the BodPod™ Body Composition System (COSMED Rome, Italy) at baseline. Fat mass index (FMI) was calculated as fat mass divided by height squared (kg/m^2). Weight was assessed by an electronic scale coupled to the BodPod™ system. Body weight was also measured at the beginning and end of each energy balance condition using a calibrated electronic scale (seca mBCA 515, seca GmbH & Co.KG, Hamburg, Germany). All measurements were conducted in the fasting state and in underwear.

164 Physical activity

165 Prior to study start, it was tested which walking time and speed were needed to reach the
166 predetermined PALs. Medium and high ET were achieved through different treadmill
167 protocols. On medium ET days, participants walked 3 times for 55 minutes (11 km) on a
168 treadmill (Kettler Track 9 with software World Tours 2.0, Kettler GmbH, Ense-Parsit,
169 Germany) at a speed of 4 km/h starting 10 minutes after meal termination. On high ET days,
170 time on the treadmill was doubled to 3 times 110 minutes (22 km) at the same speed. Besides
171 the prescribed time on the treadmill participants remained sedentary (sitting and lying) but
172 awake till bedtime. On the low ET days participants remained sedentary all the time. PAL was
173 calculated as PAL = TEE / REE (REE, resting energy expenditure) with
174 REE = SEE + 0.5 x SEE (20) (SEE, sleeping energy expenditure) using TEE- and SEE-data
175 from metabolic chambers. SEE was measured as reported by Schrauwen et al. as the lowest
176 energy expenditure value of three consecutive hours during sleep between 24:00-06:00 (21).
177 Physical activity was continuously measured via step count using the triaxial activity monitor
178 ActivPAL^{TM} 3C, (Paltechnologies Ltd., Glasgow, UK). The ActivPAL^{TM} uses dynamic triaxial
179 acceleration and inclination logging technology and is a valid and reliable device (22). The
180 ActivPAL^{TM} was fixed in the middle of the upper thigh with waterproof patches and was worn
181 permanently during the whole study period and subjects were requested to refrain from exercise
182 during this time. Data were analyzed with ActivPAL^{TM} Professional v7.2.32 software. Due to
183 technical failure, one subject had no valid data of physical activity for the whole intervention
184 and 2 participants had missing data for one energy balance condition each. For washout days
185 activPAL-data are available for n=7-12 participants only.

186 Statistical analyses

187 Primary outcome variables for the present analysis are ghrelin, GLP-1 and insulin
188 concentrations. Secondary outcomes are subjective appetite sensations (VAS) and energy

189 balance during ad libitum energy intake. Calculation of the required sample size was conducted
190 using G*Power 3.1.9.2 software (written by Faul F., University of Kiel, Germany) for ghrelin
191 concentration as the primary outcome variable. King et al. observed 29.9% lower ghrelin
192 concentrations after exercise compared to inactivity at the same energy balance in a randomized
193 cross-over intervention (23). Based on these results, 13 participants are required to detect this
194 difference in ghrelin concentration between low ET and high ET at the same energy balance
195 (assuming a two-sided power of 95% and an alpha level of 5%). The statistical software R
196 (2018) was used to analyze the data. Data evaluation started with the definition of an appropriate
197 statistical mixed model (24,25). The data were assumed to be normally distributed and to be
198 heteroscedastic with respect to the different levels of energy balance and energy turnover. These
199 assumptions were based on a graphical residual analysis. The statistical model included energy
200 balance (ad libitum EI, EB, CR, OF) and energy turnover (low, medium, high), as well as their
201 interaction term as fixed factors. The subject identity was regarded as random factor. In
202 addition, the correlations of the measurement values due to the several intervention days were
203 taken into account (auto-correlation). Based on this model, a Pseudo R^2 was calculated (26) and
204 an analysis of variance (ANOVA) was conducted, followed by multiple contrast tests (e.g., see
205 (27,28)) in order to compare the three levels of ET within each energy balance condition.
206 Deviations of Δenergy balance (%) during ad libitum EI from an equal energy balance were
207 tested versus the fixed value (0%) by multiple contrast tests also. Taking this model into
208 account, Pearson correlations were calculated for all levels of ET combined comparing daylong
209 insulin and ghrelin Data are presented as mean ±SD and a two-sided p-value < 0.05 was
210 considered to be statistically significant.

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RESULTS

Basal characteristics of the study population

Sixteen adults (3 women and 13 men) aged 20 – 32 years (25.1 ±3.9 years) with a BMI between 19.6 – 31.2 kg/m^2 (24.0 ±3.2 kg/m^2) and a FMI between 1.8 – 14.1 kg/m^2 (5.3 ±3.2 kg/m^2) participated in the trial. According to WHO criteria one women and four men were overweight and one women was obese. Taking body composition into account, two women and two men had a FMI above the age and sex adjusted 95th percentile (29). Body weight did not change during any of the four energy balance conditions (all $p>0.05$, data not shown).

Parameters of energy balance

Components of energy balance are shown in **Table 1**. Compared to low ET, TEE was 24% higher with medium and 40% higher with high ET during EB. Energy intake and TEE differed between all ET levels (all $p<0.01$). PAL and steps per day were similar between same ET levels (all $p>0.05$) except for a lower PAL at low ET during CR compared to OF ($p<0.01$). As intended by study protocol, energy balance was negative during CR, positive during OF (all $p<0.001$) and equal during EB. Steps per day were similar on all washout days following the interventions (all $p>0.05$, data not shown).

Appetite hormones

Results for daylong concentrations of appetite hormones are shown in **Figure 3**. Ghrelin concentrations were higher with lower ET during CR and OF (all $p<0.05$, **Figure 3A**) except for a non-significantly lower ghrelin with high compared to medium ET during CR. GLP-1 concentrations were lower with lower ET during all energy balance conditions (all $p<0.01$, **Figure 3B**) except for non-significant differences between low and medium ET during CR and between medium and high ET during OF. Insulin concentrations were higher with lower ET during OF (low vs. high ET and medium vs. high ET, both $p<0.01$) and EB (medium vs. high ET, $p<0.05$) whereas no differences were found between insulin concentrations at three levels

of ET during CR despite a tendency of higher concentrations with low compared to high ET ($p = 0.058$; **Figure 3C**). Daylong insulin and ghrelin concentrations were negatively correlated with $r = -0.34$ ($p<0.001$).

Subjective appetite ratings

Results for subjective appetite ratings are shown in **Table 2**. Hunger was higher with lower ET during EB (all $p<0.01$), whereas no differences in hunger were found between levels of ET during CR and OF. Fullness was lower with lower ET during all energy balance conditions (all $p<0.05$) except for non-significant differences between medium and high ET during EB and OF. Desire to eat was higher with lower ET during all energy balance conditions (all $p<0.05$) except for non-significant differences between low and medium ET for CR and OF and between medium and high ET for EB and OF. Desire for something sweet, salty or fatty was assessed in a subsample of n=8 but showed no consistent results (data not shown).

Energy balance during ad libitum EI

In line with the findings for appetite hormones and subjective appetite ratings, ad libitum EI led to a more positive energy balance at a lower ET (low vs. medium and low vs. high ET, both $p<0.01$, **Figure 4**). During low and medium ET, energy balance was positive (+17.5%, $p<0.001$ and +7.0%, $p<0.05$, respectively), but with high ET energy balance was equal ($p>0.05$).

DISCUSSION

The present study confirms the hypothesis that appetite control is improved at higher levels of ET achieved by low-intensity physical activity. The results are independent of energy balance and are verified by changes in endocrine signals, subjective appetite ratings and ad libitum energy intake: Ghrelin concentrations, hunger and desire to eat were higher, GLP-1 concentrations were lower and energy balance during ad libitum EI was positive with low ET compared to high ET.

In a prospective study by Hume et al., gain in fat mass within a 2-3 year period was predicted by low ET but not by energy surfeit at baseline using state of the art methods for assessment of total energy expenditure (30). However, the study was criticized for not measuring real ET but simply examining baseline energy expenditure of weight stable persons against long-term changes in body fat (31). Nevertheless, the results by Hume et al. are in line with the present randomized intervention study that carefully measured ET at different levels of energy balance in a whole room calorimeter and which has demonstrated improved appetite control with increased ET.

Ghrelin concentrations are known to rise in the fasted state until meal initiation and to fall afterwards proportional to the ingested energy content (32). Reduced ghrelin concentrations with increasing ET could therefore be due to higher energy content of meals with increasing ET. It was shown that stomach distention does not directly affect declining ghrelin concentrations (33), indicating that the size or volume per se has no effect on postprandial ghrelin suppression. Because insulin is essential for postprandial ghrelin suppression (34) the inverse correlation between insulin and ghrelin concentrations in the present trial supports an indirect anorexigenic effect of insulin via ghrelin suppression. Since daylong insulin secretion decreased with higher ET (Figure 3C) the direct anorexigenic effect of insulin maybe of minor importance. The anorexigenic effect implied by high GLP-1 (Figure 3B) and the suppressed orexigenic effect of ghrelin (Figure 3A) with a higher ET both seem to be sufficient to promote

satiety and decrease desire to eat (Table 2). These effects were also found with caloric restriction and overfeeding (Figure 3A and B), therefore the impact of ET on appetite control seems to be independent of energy balance.

Beyond ET, the type of energy deficit might also impact appetite control. Equal energy deficits (-1100 kcal/d) induced by 90 min of exercise at 70% VO_{2max} or caloric restriction differently affected appetite control and food intake in healthy males with only caloric restriction leading to elevated ghrelin concentrations and a compensation of the energy deficit (23). A similar effect in women was already reported earlier (35). The present study confirms these findings despite a lower intensity of physical activity because a high ET achieved by brisk walking led to lower appetite and ad libitum energy intake compared with low ET. Similarly, no compensatory increases in acylated ghrelin, appetite or energy intake were observed after an acute brisk walking session (36). This corresponds to the idea of "exercise-induced anorexia" after an acute bout of moderate-to-vigorous exercise which is characterized by a short-lived reduction in hunger and a delayed onset of energy intake but without a full compensation of the energy deficit (37). It is discussed that "exercise-induced anorexia" is mediated by lowered concentrations of acylated ghrelin and elevated concentrations of GLP-1 and PYY (for review see (38)). The results of the present trial confirm these findings since ghrelin was reduced and GLP-1 increased with high ET.

At a low ET, the macronutrient intake may have been too small and therefore not sufficient to stimulate GLP-1 secretion and ghrelin suppression. A threshold effect could therefore explain the lack in satiety (Table 2) and energy overconsumption during ad libitum EI (Figure 4). This assumption is supported by the finding of higher prospective weight gain with snacking behavior (frequent consumption of small amounts of food) under free-living conditions (39).

In line with our findings, Stubbs et al. showed in a metabolic chamber trial that ad libitum EI does not adequately adapt to a reduction in physical activity (PAL 1.8 to 1.4) and thus led to a positive energy balance (40). In the present study, during ad libitum EI energy balance was

17.5% positive with low ET. Assuming 13.1 kcal/g for energy content and energy cost of fat mass synthesis (41) an exclusive gain in fat mass would resemble about +32 g for one day of inactivity. Therefore, an inactive lifestyle facilitates caloric overconsumption and thus weight gain.

Low-intensity physical activity was chosen to increase the PAL simulating daily activities without effects of vigorous exercise on metabolism and avoiding fatigue or pain in persons with very low fitness. Furthermore, low-intensity physical activity is considered to play an important role in promoting health and may be implemented more easily in public health strategies than high intensity exercise (42). In the present study, a PAL of 1.76 was required to achieve the "regulated zone" of energy expenditure, where appetite is matched to actual energy demand. The setting of a 9 m^2 metabolic chamber only allows physical activity on a treadmill and not manifold physical activities (like housekeeping or taking the stairs) as compared with free-living conditions. Therefore, the amount of steps that is necessary to achieve a high ET was ≥34,500 steps/d. Under free-living conditions, the appropriate amount of steps per day would therefore be much lower. In line with our findings, the WHO in 2001 recommended a PAL target of ≥1.75 that represents an individual whose occupation involves regular physical activity (i.e. regular exercise) while a PAL of 1.4 represents a sedentary individual (43). Because the difference between a PAL of 1.4 and 1.75 equates to an additional energy expenditure of 490 kcal/d (with an REE of 1400 kcal/d), a public health recommendation of only 150 - 300 min/week moderate intensity physical activity from 2010 (44) is unrealistically low (42,45,46). As an outcome of the IASO 1st Stock Conference, a consensus statement was published that confirmed the necessary PAL of 1.7 or 45-60 min/d moderate intensity physical activity per day to prevent unhealthy weight gain (45). Accordingly, another study demonstrated that a habitual PAL of ≥1.62 was associated with improved appetite control compared to a PAL of ≤1.49 (47).

The role that exercise can play in energy expenditure is commonly exaggerated (48). Our findings show that the positive impact of physical activity on weight control is partially independent of burning up more calories and is additionally explained by an improvement in appetite sensations. Furthermore, increasing physical activity may be easier for most people than eating less (for review see (49)). Therefore, maintaining an equal energy balance due to achieving a high ET seems to be easier compared to obtain a low ET.

The extensive study protocol in randomized cross-over design and the highly standardized metabolic ward conditions are a strength of our study. Nevertheless, there are also some limitations that should be addressed. We measured total ghrelin concentrations which may mask relevant changes in acylated ghrelin that seems to be essential for appetite control (36). However, it was found that the ratio of acylated and total ghrelin is relatively robust rendering total ghrelin an appropriate surrogate (32). Since only acute effects of changes in ET were investigated in the present trial, the results cannot be transferred to long-term habitual high or low ET. In daily life, ET is highly variable from day to day. For example, brief periods where energy intake far exceeds energy expenditure last from one meal to several days and regularly occur over the weekend (50). In line with our results, others have found that individuals with higher habitual physical activity levels better adjust energy intake in response to energy balance perturbations compared to habitually inactive individuals (47,51). Because only 3 women were investigated in the present study, sex differences in appetite control could not be analyzed. The impact of sex on appetite control remains unclear with some studies showing sex-differences (for review see (38)) whereas others did not (51).

In conclusion, a high energy turnover improves appetite control, reflected by changes in hormonal biomarkers including increased GLP-1 and decreased ghrelin and insulin concentrations independent of energy balance. These results indicate an asymmetric control of appetite where reduced energy expenditure was not compensated by an appropriate adaptation in energy intake. In contrast to the prevailing concept of body weight control, the positive

359 impact of physical activity is not completely explained by burning up more calories but – at
360 certain volumes – also involves improving appetite control. Importantly, for prevention of
361 weight gain a PAL of 1.76 does not require vigorous physical activity or exercise but can be
362 obtained by low-intensity physical activity (e.g. reached by occupational activities like postmen
363 or nursing professions).
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LEGENDS FOR FIGURES AND TABLES

Table 1: Energy balance parameters for interventions differing in energy turnover and energy balance[1]

Table 2: Comparison of daylong subjective appetite feelings from visual analogue scales (all tAUC) between interventions with differing energy turnover and differing energy balance[1]

Figure 1: Graphic depiction of the energy turnover concept

Figure 2: Study protocol of the randomized cross-over intervention at three different levels of energy turnover under four different energy balance conditions. Different levels of ET were achieved by walking on a treadmill at the speed of 4 km/h for either 0 min (low ET), 3 x 55 min (medium ET) or 3 x 110 min (high ET). Energy balance conditions were ad libitum energy intake, zero energy balance, caloric restriction (-25%) and overfeeding (+25%). Levels of ET during each energy balance condition were randomized as well as order of caloric restriction and overfeeding. Intervention days were preceded of 3 days of run-in at every energy balance condition and separated by one washout day each. During the entire study protocol subjects received a controlled diet with constant macronutrient relation (50% carbohydrates, 35% fat, 15% protein). EI, energy intake; ET, energy turnover; PAL, physical activity level.

Figure 3: Comparison of daylong appetite hormone responses for ghrelin (A), GLP-1 (B) and insulin (C) as tAUC for 14 hours between interventions with differing energy turnover and differing energy balance. *$p<0.05$; **$p<0.01$; ***$p<0.001$; linear mixed model with multiple contrast tests; mean ±SD; n = 16; tAUC, total area under the curve.

Figure 4: Comparison of energy balance (%) between interventions with differing energy turnover during ad libitum energy intake. *$p<0.05$; ***$p<0.001$; linear mixed model with multiple contrast tests; mean ±SD; n = 16; ET, energy turnover.

Table 1:

	ad libitum energy intake			**caloric restriction**			**zero energy balance**			**overfeeding**		
	Low ET	Med ET	High ET	Low ET	Med ET	High ET	Low ET	Med ET	High ET	Low ET	Med ET	High ET
TEE, kcal/d	2,380 ±99	2,873 ±134	3,320 ±277	2,258 ±107	2,653 ±117	3,098 ±189	2,296 ±99	2,847 ±37	3,210 ±157	2,340 ±86	2,846 ±91	3,271 ±172
steps/d	399 ±967	17,599 ±299	34,603 ±1,257	543 ±894	17,609 ±483	34,536 ±1,884	429 ±910	17,703 ±327	34,747 ±1,110	443 ±845	17,788 ±370	34,765 ±1,593
PAL	1.31 ±0.04	1.55 ±0.06	1.76 ±0.09	1.27 ±0.05	1.53 ±0.08	1.75 ±0.08	1.30 ±0.03	1.57 ±0.04	1.76 ±0.07	1.34 ±0.05	1.55 ±0.05	1.74 ±0.09
EI, kcal/d	2,789 ±182	3,060 ±254	3,308 ±405	1,794 ±182	2,158 ±254	2,486 ±405	2,390 ±182	2,874 ±254	3,325 ±405	2,978 ±182	3,586 ±254	4,161 ±405
EB, %	17.5	7.0	-0.3	-20.5	-19.0	-21.0	3.4	0.0	1.6	27.6	25.4	24.2

[1]Values are means ±SDs; n = 16; steps/d n = 13-15; PAL n = 11; TEE and EB for zero energy balance, caloric restriction and overfeeding n = 11. Parameters differed between interventions according to study protocol. EB, energy balance; EI, energy intake; ET, energy turnover; Med, medium; PAL, physical activity level; TEE, total energy expenditure.

Table 2:

	caloric restriction			equal energy balance			overfeeding		
	Low ET	Medium ET	High ET	Low ET	Medium ET	High ET	Low ET	Medium ET	High ET
hunger, mm x 15 h	660 ±146	578 ±139	542 ±201	559 ±74	466 ±71***	409 ±46***,††	412 ±347	348 ±30	303 ±92
fullness, mm x 15 h	470 ±79	537 ±82*	630 ±85***,††	535 ±72	636 ±114**	710 ±178***	709 ±120	813 ±123*	879 ±145***
desire to eat, mm x 15 h	678 ±151	630 ±200	502 ±77***,†	574 ±112	437 ±71***	391 ±68***	381 ±148	330 ±82	277 ±107*

[1]Values are means ±SDs, n = 16. Linear mixed model with multiple contrast tests, *p<0.05, **p<0.01, ***p<0.001, significantly different from low ET. †p<0.05, ††p<0.01, †††p<0.001, significantly different from medium ET. ET, energy turnover; tAUC, total area under the curve.

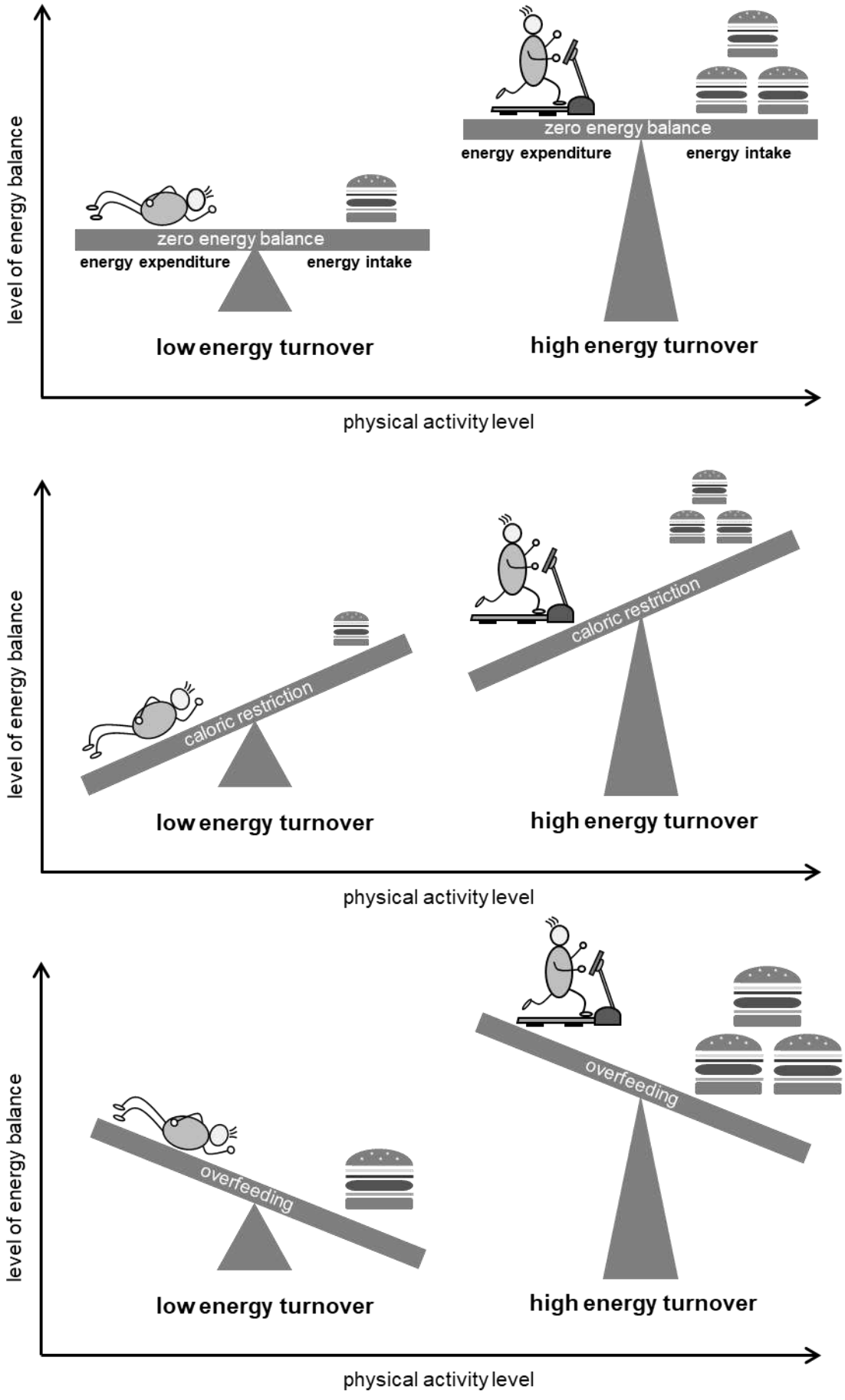
level of energy balance
zero energy balance
energy expenditure
energy intake
low energy turnover
zero energy balance
energy expenditure
energy intake
high energy turnover
physical activity level
level of energy balance
caloric restriction
low energy turnover
caloric restriction
high energy turnover
physical activity level
level of energy balance
overfeeding
low energy turnover
overfeeding
high energy turnover
physical activity level

Figure 1

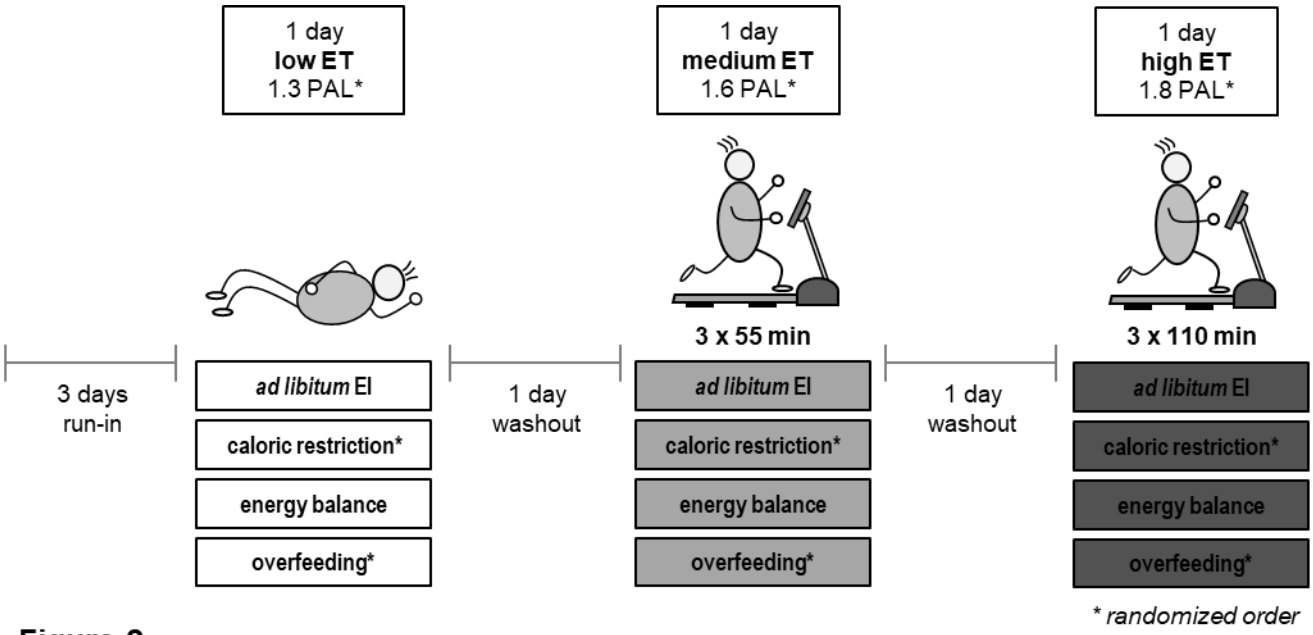
1 day
low ET
1.3 PAL*
1 day
medium ET
1.6 PAL*
1 day
high ET
1.8 PAL*
3 x 55 min
3 x 110 min
3 days
run-in
ad libitum EI
caloric restriction*
energy balance
overfeeding*
1 day
washout
ad libitum EI
caloric restriction*
energy balance
overfeeding*
1 day
washout
ad libitum EI
caloric restriction*
energy balance
overfeeding*
* randomized order

Figure 2

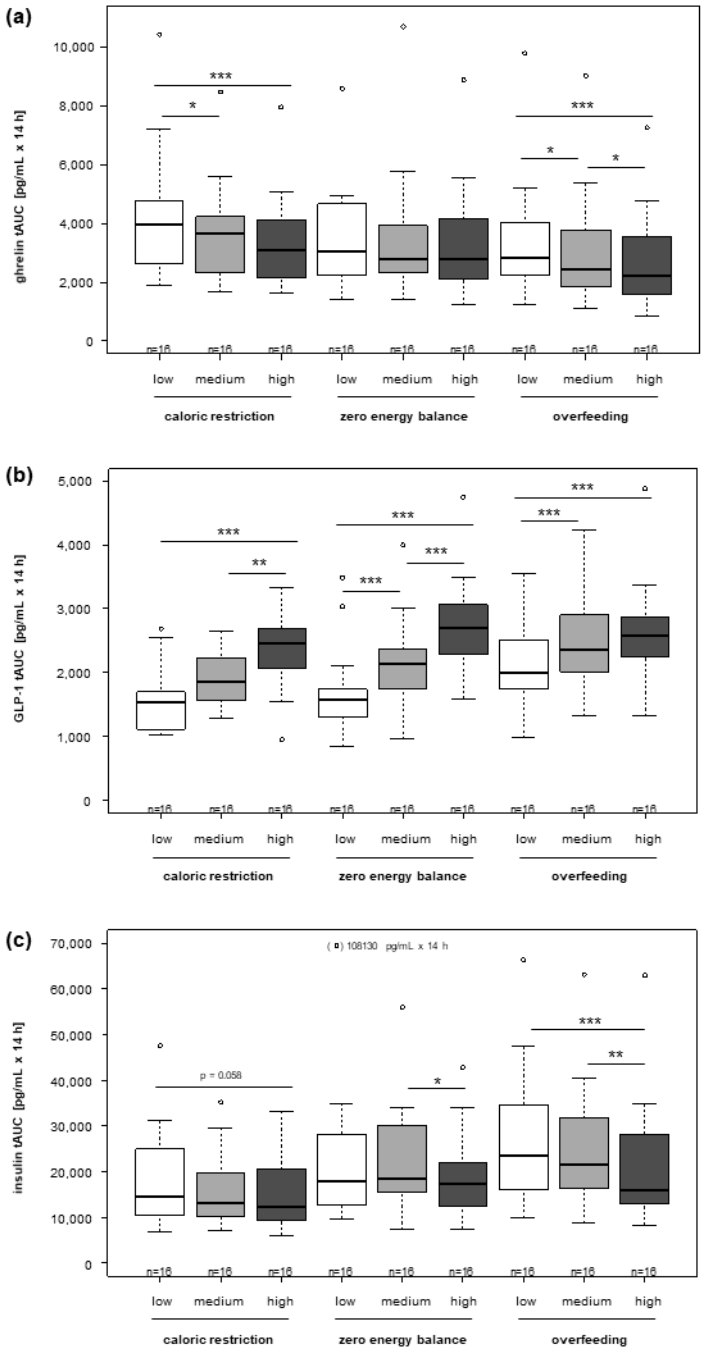
(a)
ghrelin tAUC [pg/mL x 14 h]
10,000
8,000
6,000
4,000
2,000
0
***
*
***
*
*
n=16
low
medium
high
caloric restriction
zero energy balance
overfeeding
(b)
GLP-1 tAUC [pg/mL x 14 h]
5,000
4,000
3,000
2,000
1,000
0
***
**
***
***
***
***
***
(c)
insulin tAUC [pg/mL x 14 h]
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
(▫) 108130 pg/mL x 14 h
p = 0.058
*
***
**

Figure 3

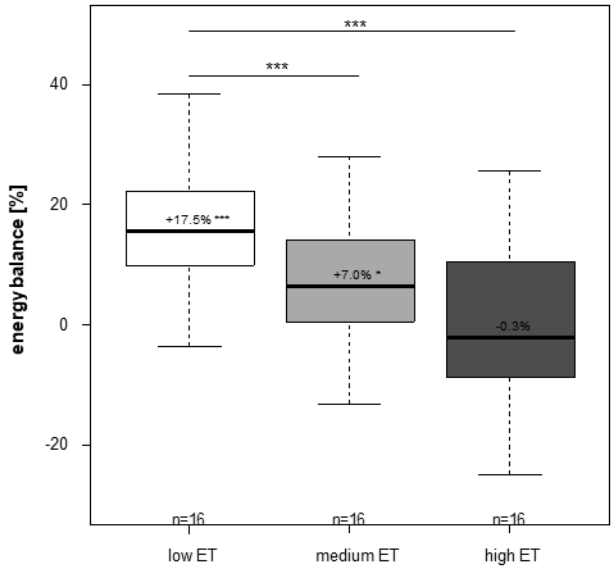
***
***
40
20
0
-20
energy balance [%]
+17.5% ***
+7.0% *
-0.3%
n=16
n=16
n=16
low ET
medium ET
high ET

Figure 4