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Ability to Adjust Nocturnal Fat Oxidation in Response to Overfeeding Predicts 5-year Weight Gain in Adults

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Abstract

Objective—To determine whether metabolic responses to short-term overfeeding predict longitudinal changes in body weight.

Methods—24h energy expenditure (EE) and substrate utilization were measured at baseline in a room calorimeter following 3d of eucaloric and hypercaloric feeding (40% excess) in a sample of lean adults (n: 34; age: 28±2 years; body mass index: 22±3 kg/m^2). Body mass and fat mass (DXA) were measured annually for 5yrs. Regression analyses examined whether changes in EE and fuel use with overfeeding predicted body weight and composition changes over 5yrs.

Results—Overfeeding increased EE and reduced fat oxidation when examined over the 24h, waking, and nocturnal periods. Absolute change in body mass over 5y was 3.0±0.6 kg (average rate of change = 0.7±0.1 kg per year, $p<0.001$). Lower nocturnal (but not 24h or waking) fat oxidation ($r = -0.42$, $p=0.01$) and EE ($r=-0.33$, $p =0.05$) with overfeeding were the strongest predictors of 5y weight gain. When adjusted for covariates, changes in nocturnal fat oxidation and EE with overfeeding predicted 41% of the variance weight change ($p=0.02$).

Conclusions—Failure to maintain fat oxidation at night following a period of overfeeding appears to be associated with a metabolic phenotype favoring weight gain.

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Author contributions: CAR analyzed and interpreted the data and wrote the first draft of the manuscript. CAR and AB assisted in the interpretation of the data and writing. EK carried out the experiments. DB conceived and carried out the experiments, interpreted the data, and edited the final draft of the manuscript. All authors were involved in writing the paper and had final approval of the submitted and published versions.

Keywords

room calorimetry; substrate oxidation; obesity

INTRODUCTION

Obesity affects millions of adults in the United States and the prevention of weight gain and problem of weight maintenance have become a serious public health challenge (1). Although two-thirds of the US population is overweight or obese (2), some people appear to be more resistant to weight gain than others despite living in a similar obesogenic environment (3, 4). Individuals can be susceptible to weight gain because of factors such as a low metabolic rate, reduced activity thermogenesis, decreased fat oxidation, insulin resistance, low leptin, and reduced sympathetic nervous system activity (5–8). In addition, behavioral and genetic factors also play an important role in the susceptibility to weight gain (6). A better understanding of the factors that predispose to or protect from weight gain will be useful in developing strategies to prevent obesity.

Our group has focused on the variability in substrate oxidation as a potential mechanism underlying predisposition to weight gain (3, 4). Specifically, our Energy Adaptations over Time Study (EATS) study explored the idea that exposing persons to a short period of positive energy balance (EB) might reveal metabolic differences in the propensity for weight gain. EATS enrolled subjects from families where obesity was a problem (obesity prone; OP) and who self-identified themselves as prone to weight gain. Others were from families where obesity was not a problem and self-identified themselves as constitutively thin or resistant to weight gain (obesity resistant; OR). A surprising finding from EATS was that short-term overfeeding (compared to a eucaloric control condition) had the most pronounced influence on nocturnal, not 24h or daytime substrate utilization (3). The results showed that the nocturnal non-protein respiratory quotient (npRQ) did not change with overfeeding in the OR subjects, but increased significantly in OP subjects. The results further suggested that fat oxidation during the night was downregulated in the OP subjects following a brief period of overfeeding, whereas the obesity resistant subjects appeared to maintain their usual rate of fat oxidation.

Our previous cross-sectional study was limited by the fact that our subjects were selected as OP or OR on clinical features. We did not know at the time whether they would be predisposed to weight gain or not. To address this limitation, we present results of our 5 year follow up on these subjects. To our knowledge no previous studies have tested whether the ability to maintain fat oxidation at night when faced with brief periods of overeating (e.g. 1–3 days) predicts weight maintenance or weight gain. This is an important question because transient periods of overfeeding are common in modern society with vacations, holidays, and celebrations (9). Here, we wondered whether the changes in nocturnal energy metabolism with overfeeding observed in our previous studies of OP and OR predict weight or adipose tissue gain over 5 y. For the present analysis, we combined OP and OR subjects because we hypothesize that the two groups originally selected are simply enriched in individuals likely or not to gain weight over time.

METHODS

Participants

Participants were a sub-set (n=34) of men and women aged between 25 and 35 years that participated in our previous studies examining the effects of overfeeding on nutrient oxidation (3, 10). Additional details on the participants are provided in the Supporting Information. Participants provided written informed consent prior to enrolling and received a stipend upon completing the study, per the principles expressed in the Declaration of Helsinki. The study was approved by the Colorado Multiple Institutional Review Board.

Preliminary Assessments

At baseline, subjects underwent a physical examination; had body composition measured by DXA (Hologic Discovery-W, Bedford, MA); resting energy expenditure (EE) measured by hood indirect calorimetry (ParvoMedics Model: TrueOne 2400, Sandy, UT); and a 24-hour EE measurement by whole-room calorimetry (3, 10). Physical activity was assessed for 1 week using a pedometer (Digi-Walker, New-Lifestyles, Inc. Lee's Summit, MO).

Design

Subjects were studied on the Clinical Translational Research Center (CTRC) at the University of Colorado - Anschutz Medical Campus on two occasions separated by at least 1 month. Two days prior to CTRC study (free-living conditions), subjects consumed either a controlled eucaloric or hypercaloric diet (1.4 times estimated baseline energy needs) in a randomized order. On the third day of each controlled diet period, subjects spent 23-hr in the whole-room calorimeter to measure substrate oxidation and EE(3). Additional details on the study design are in the Supporting Information.

Whole room calorimeter and study day

Participants entered the calorimeter at 0800h after a standardized breakfast (25% of daily energy). Lunch and dinner were given at 1200h and 1700h (both contained 30% of daily energy), with a snack (15% of daily energy) given at 2000h. The subjects went to bed at 2200h and subjects exited the chamber at 0700 the following morning (3). Oxygen (O_2) consumption and carbon dioxide (CO_2) production were determined from the air flow rates and differences in gas concentrations between air entering and air exiting the calorimeter, as previously described (12). 23-hr urine output was collected to measure nitrogen excretion. EE and substrate oxidation were calculated from O_2 consumption, RQ, and urinary nitrogen excretion based on published equations (13; and Supporting Information).

Body composition measurements at baseline and follow-up visits

Whole-body fat mass (FM), fat free mass (FFM), and body weight were measured at baseline by dual-energy X-ray absorptiometry (DXA, Hologic Discovery-W, Bedford, MA). Subjects reported back to the University of Colorado Hospital every year for 5 years to have repeat DXA measurements. Only subjects completing ≥ 3yrs of follow-up were included in the present analysis.

Statistical Analysis

The primary goal of the analysis was to determine the degree to which nocturnal responses to 3 days of overfeeding a mixed diet predicted the rate of change in outcomes of body weight and FM measured over a 5-yr follow-up period. Candidate predictor variables included changes in 24h, daytime, and nocturnal EE, npRQ, fat oxidation, and carbohydrate oxidation in response to the feeding conditions measured during the baseline chamber stay (overfed minus eucaloric). The daytime period began at ~0800h and ended at ~2200h (lights out) and the nocturnal period ended at ~0700h. Fat and carbohydrate oxidation values were expressed as a percentage of energy expended over 24h, or over the day and night segments. The exact duration of the day and night (nocturnal) periods were individually determined and normalized to 16h and 8h, respectively, as described in the Supporting Information. Given that sleep was not specifically measured, we also report data from a 90-min time segment between 0300h and 0530h that is representative of sleeping metabolic rate (labeled sleep in results).

The rate of change in body mass and FM were calculated as the difference between the last follow-up time point minus baseline divided by the number of follow-up years. Only subjects with ≥ 3yrs of follow-up were included in the present analyses. A flow diagram showing study numbers from the parent study (3) and reasons for exclusion from the present statistical data analysis are shown in Figure 1. Associations of candidate variables with the body composition outcomes were first examined individually with Pearson correlations. Significant correlates were placed in multivariate linear regression analyses along with age, sex, baseline FFM, and baseline FM to adjust for these characteristics.

Repeated measures mixed models adjusted for sex, FM, and FFM were used to compare the room calorimeter data between feeding conditions at baseline. Paired t-tests were used to test for significant changes in the body composition outcomes. A p-value ≤ 0.05 was considered statistically significant. Subject characteristics are presented as mean ± SD. Metabolic and body composition parameters are presented as mean ± SEM. Data were analyzed using SPSS version 23.0 (IBM, SAS, Cary, NC).

RESULTS

The present analysis includes 34 subjects (n=15M/19F) who completed annual body composition follow-up visits for ≥3yrs years after the initial overfeeding study (mean follow-up time: 4.6 ± 0.6yrs). Baseline characteristics are shown in Table 1. Body weight was not significantly different between the baseline eucaloric and overfed study periods ($p>0.05$).

Changes in energy intake, expenditure and balance in response to overfeeding

Table 2 reports energy intake (EI), balance, and expenditure for all subjects during the two diet phases. By design net EI was increased by 40% in the overfeeding condition resulting in an average 24h positive EB of 523.5 ± 45.6 kcal ($p<0.001$). Short-term overfeeding led to a significant increase in 24h EE (5% increase, $p=0.003$) and nocturnal EE (6% increase, $p=0.05$). EE measures calculated between 0330h – 0500h (the time where EE was at its

lowest level of the 24 hrs) were significantly lower than those calculated during the entire sleep opportunity for both feeding conditions ($p<0.001$). However, EE averaged over 0330h – 0500h did not significantly change with overfeeding ($p=0.19$).

Changes in non-protein respiratory quotient in response to overfeeding

Table 2 also displays changes in the npRQ for the two diet phases. Short term overfeeding led to significant increase in the 24h npRQ indicating higher carbohydrate oxidation (5% increase, $p=0.003$). Nocturnal npRQ tended to increase by 4% with overfeeding, but this change did not reach significance ($p=0.06$). The npRQ between 0330h – 0500h was not different between eucaloric and overfed conditions ($p=0.19$).

Changes in nutrient balance and oxidation in response to overfeeding

Table 3 presents the macronutrient intake, balance, and oxidation data over 24h and for the nocturnal and sleep segments. Overfeeding resulted in positive nutrient balance for all three macronutrients ($p<0.001$). Twenty-four-hour carbohydrate and protein oxidation increased and fat oxidation significantly decreased with overfeeding. Nocturnal and sleep responses mirrored the 24h changes, indicating that more carbohydrate, more protein, and less fat were oxidized at night when subjects were overfed compared to the eucaloric condition. We note high variability in the nocturnal fat oxidation responses with some individuals showing an ability to match (or increase) nocturnal fat oxidation to increased fat intake and others showing a large decrease in nocturnal fat oxidation with overfeeding (range = +21% increase to − 64% decrease in nocturnal fat oxidation).

Five-year body composition changes

Follow-up body composition data (absolute and rate of change) are presented in Table 1. Over the follow-up period, the absolute change in body mass was 3.0 ± 0.6 kg (range −4.1 to 11.8 kg) with an average rate of change of 0.7 ± 0.1 kg per year ($p<0.001$). The absolute change in fat mass was 2.1 ± 0.5 kg (range − 2.3 to 10.9 kg) with an average rate of change of 0.5 ± 0.1 kg/yr ($p<0.001$). The rate of change in body mass and FM was similar between sexes ($p>0.05$).

Relationship between metabolic adaptations to short term overfeeding and 5yr body composition changes

Changes in 24h npRQ, EE, fat oxidation, and carbohydrate oxidation with overfeeding were not significantly correlated with the changes in body composition (r-values ranged from −0.19 to 0.19, p-values from 0.27 to 0.96). Similar non-significant relationships were observed when correlating the changes in daytime chamber measurements to the body composition changes (r-values ranged from −0.24 to 0.16, with p-values from 0.17 to 0.99). Only when examined over the nocturnal period was the rate of change in body weight significantly related to baseline changes in npRQ ($r=0.48$, $p=0.004$, Figure 2A), fat oxidation ($r=-0.42$, $p=0.01$, Figure 2B), and EE ($r=-0.33$, $p=0.056$, Figure 2C). Change in fat mass was not related to the change in the npRQ ($r=0.26$, $p=0.14$, Figure 2D), but were correlated with changes in fat oxidation expressed as a percentage of nocturnal EE ($r=-0.34$, $p=0.05$, Figure 2E). Change in fat mass tended to be correlated with the baseline change in nocturnal

EE (r=−0.32, p=0.07 for body; Figure 2F). Together, in a multivariate model including age, sex, FFM (baseline), and FM (baseline), changes in npRQ and EE at night explained 41% of the variance in the rate of weight change (Table 4). After adjustment for baseline covariates (age, sex, FFM, and FM), changes in the nocturnal npRQ independently predicted 18% of the variance in 5yr weight change such that a 0.1-unit increase in nocturnal npRQ (increased carbohydrate oxidation) in response to a period of short-term overfeeding predicted a 0.28 kg/yr increase in body weight. Multivariate models predicting the change in fat mass from the baseline changes in npRQ and EE did not reach statistical significance (Table 4). Although, the change in EE tended to remain as a significant predictor of the change in fat mass (Table 4).

DISCUSSION

We correlated the metabolic responses to short term overfeeding with measured changes in weight over 5yrs of follow-up in a group of subjects who varied in their predisposition to weight gain. Our working hypothesis based on findings of the original cross sectional study (3, 10) was that the ability of some individuals to maintain fat oxidation in the face of overfeeding would correlate with resistance to weight gain over time. The novel observation is that individual differences in nocturnal but not 24h or waking fat oxidation in response to overfeeding predicted 5yr weight gain in a sample of lean subjects.

Our group has previously examined fat metabolism in subjects who are prone to gaining weight as compared to those who are relatively protected from weight gain (3, 10). These studies were motivated by our previous work in animal models of obesity which led us to the idea that reductions in the oxidation of dietary fat following a period of passive overfeeding predispose to weight gain (4, 14). To determine whether these findings were relevant in humans, we measured 24-hr fat metabolism in situations where the systems regulating EB are challenged by various states of energy imbalance (e.g., overfeeding and exercise). The findings from these independent studies unexpectedly showed differences in nocturnal but not daytime fat oxidation following acute metabolic challenges (3, 15). The present study provides compelling evidence in support of the idea that failure to maintain or even increase fat oxidation at night following a period of overfeeding may be associated with a metabolic phenotype favoring fat storage. Specifically, we observed that a shift in the nocturnal npRQ in favor of decreased fat oxidation with overfeeding independently predicted 18% of the variance in 5yr weight change (0.3 kg/yr; range=−0.8 to +3.0 kg/yr). While the average rate of weight gain may appear modest, the magnitude of change agrees with other reports in the literature (16).

When examined over 24h, differences in fat oxidation under both eucaloric and overfed conditions at baseline did not predict 5y weight changes. This is in contrast with studies that suggest differences in 24h fat oxidation predispose to weight gain (5, 7, 8, 16–20). For example, Zurlo et al. reported in a sample of nondiabetic Pima Indians fed a weight-maintenance diet that individuals in the highest decile of 24h npRQ had a 2.5-fold increased risk of at least a 5 kg weight gain compared to those in the lowest decile (19). Also, elevated 24h npRQ under EB conditions was positively associated with ad libitum food intake (16, 18), and a higher waking (postprandial) npRQ is linked to weight gain (16). The reason that

changes in 24h npRQ with overfeeding did not predict weight gain in the present study may be related to the fact that subjects were lean at baseline and the overall change in weight was relatively small. It may be that 24h fat oxidation responses to overfeeding in relevant populations (e.g., persons with obesity, known increased genetic risk) will be significant predictors of future weight gain.

Fatty acids are the primary energy source during the overnight-fasted period (8, 21), and impaired nocturnal fatty acid oxidation has been observed in obese insulin-resistant and type 2 diabetic adults (22, 23). Here we provide evidence that the inability to increase the oxidation of fatty acids during an overnight fast when challenged with a positive EB may be related to a greater propensity for weight gain. Our data are particularly relevant in light of accumulating evidence showing that sleep plays an important role in the regulation of EB and metabolic health (24–27). Controlled in-laboratory sleep studies in normal weight subjects demonstrate rapid declines in insulin sensitivity, glucose tolerance, increased hunger, and food intake with short-term sleep restriction (25). Although many studies have related sleep and obesity, the functional significance of the sleep period in regards to metabolism and EB is unclear. The fact that changes in nocturnal fat oxidation under positive EB conditions could predict longitudinal weight change in a sample of lean subjects highlights a need to better understand the role of EB on the metabolic processes delegated to sleep.

It is becoming increasingly clear that a network of molecular circadian clocks regulate gene transcription-translation patterns in anticipation of the dynamic, oscillatory changes in energy status across the day (28). Animal and human studies have shown that lipid metabolism shows a daily rhythm that serves to anticipate changes in energy requirements across sleep-wake and fasting-feeding cycles (21, 29). Free fatty acids, display a clear nocturnal rise and gene expression patterns of several enzymes involved in lipolysis (e.g., Atgl, Lpl, Hsl) and beta-oxidation (e.g., Cpt-1) of fatty acids appear to be regulated in a circadian manner (30–32). It may be that differences in the availability and/or oxidation of nocturnal FFA (under control of sleep and circadian systems) play an important role in the regulation of EB. Studies on dogs suggest that overfeeding increases nocturnal but not daytime FFA availability (24). However, in human studies, nocturnal FFA availability appears to be reduced by short-term overfeeding (33). Indeed, in the present study, plasma FFA measured across waking hours and at one time point during the night (2am) was reduced with overfeeding (data not shown). In preliminary analyses, the change in the nocturnal FFA area under the curve (overfed – eucaloric) was only modestly related to the change in nocturnal fat oxidation and changes in FFA with overfeeding were not predictors of 5y weight change (data not shown). The lack of significance may relate to the fact that FFAs were only measured at one time point during the night. More detailed and controlled studies of nocturnal FFA profiles in response to energy imbalances are warranted.

Curiously the change in nocturnal fat oxidation with overfeeding was not strongly related to fat mass gain. Therefore, we cannot directly infer that the maintenance of fat oxidation at night is associated with fat storage. We hypothesize that variation in nocturnal npRQ was not more strongly related to fat mass gain because of the modest changes in both total body weight and fat mass in our sample. The prediction of fat mass from changes in nocturnal

physiology might become more prominent with age or in studies of relevant populations such as reduced obese.

Changes in EE with overfeeding were negatively correlated with the change in fat mass and tended to be negatively correlated with the rate of change in body mass. These findings corroborate with previous studies showing that reduced 24h EE and sleeping metabolic rate are predictors of weight gain (3, 7, 16, 20, 34–36). Previous studies have observed that the decrease in 24h EE with short term overfeeding might be related to changes in spontaneous physical activity (10). However, in a prior analysis of spontaneous physical activity from this same group of subjects, we found only modest changes in activity with overfeeding (10). Given that periods of overfeeding are episodic during periods of weight gain, it may be that changes in activity over a single episode of overfeeding are minimal, but significant when compounded over multiple overfeeding episodes.

A strength of the study is that we measured metabolic responses to overfeeding in non-obese individuals that had a propensity to gain weight or remain thin. A main limitation to the study is a lack of an objective measure of sleep quality, as we cannot rule out individual differences in sleep duration on the nocturnal responses used in the regression analysis to predict changes in body composition. Sleep restriction (extended wakefulness) increases 24h EE and we cannot rule out the possibility that some of the changes in EE were due to differences in sleep duration and not overfeeding. Finally, the increased npRQ in response to overfeeding may reflect individual differences in autonomic nervous system, hormonal activity, and/or cardiorespiratory fitness. Unfortunately, detailed nocturnal profiles of sympathetic nervous system activity and hormones (e.g., GH) were not measured. Other limitations include that we did not specifically measure night time protein oxidation and sleep was not specifically controlled because subjects were awakened for a blood draw and breath sample in the middle of the night.

Taken together, the present results suggest that metabolic responses to daytime energy imbalances manifest overnight while people sleep and this period may play an important role in the regulation of body weight. Sleep has been linked to metabolic health and obesity in several studies but it is still unclear what the normal function of sleep is for metabolism. It may be that sleep is a critical window when regulatory systems respond to periods of overfeeding in a manner that restores EB and these regulatory mechanisms are blunted in people with obesity or those who are at-risk of becoming obese.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What is already known about this subject?

- Decreased fat oxidation is a predictor of longitudinal weight gain
- Short-term overfeeding decreases fat oxidation and this primarily occurs at night
- There is a large degree of individual variability in nocturnal fat oxidation adaptations to brief periods of overeating

What does this study add?

- The ability to maintain fat oxidation at night when faced with brief periods of overeating is associated with a metabolic phenotype weight gain over 5-years
- Energy imbalances that manifest overnight while people sleep may play an important role in the regulation of body weight

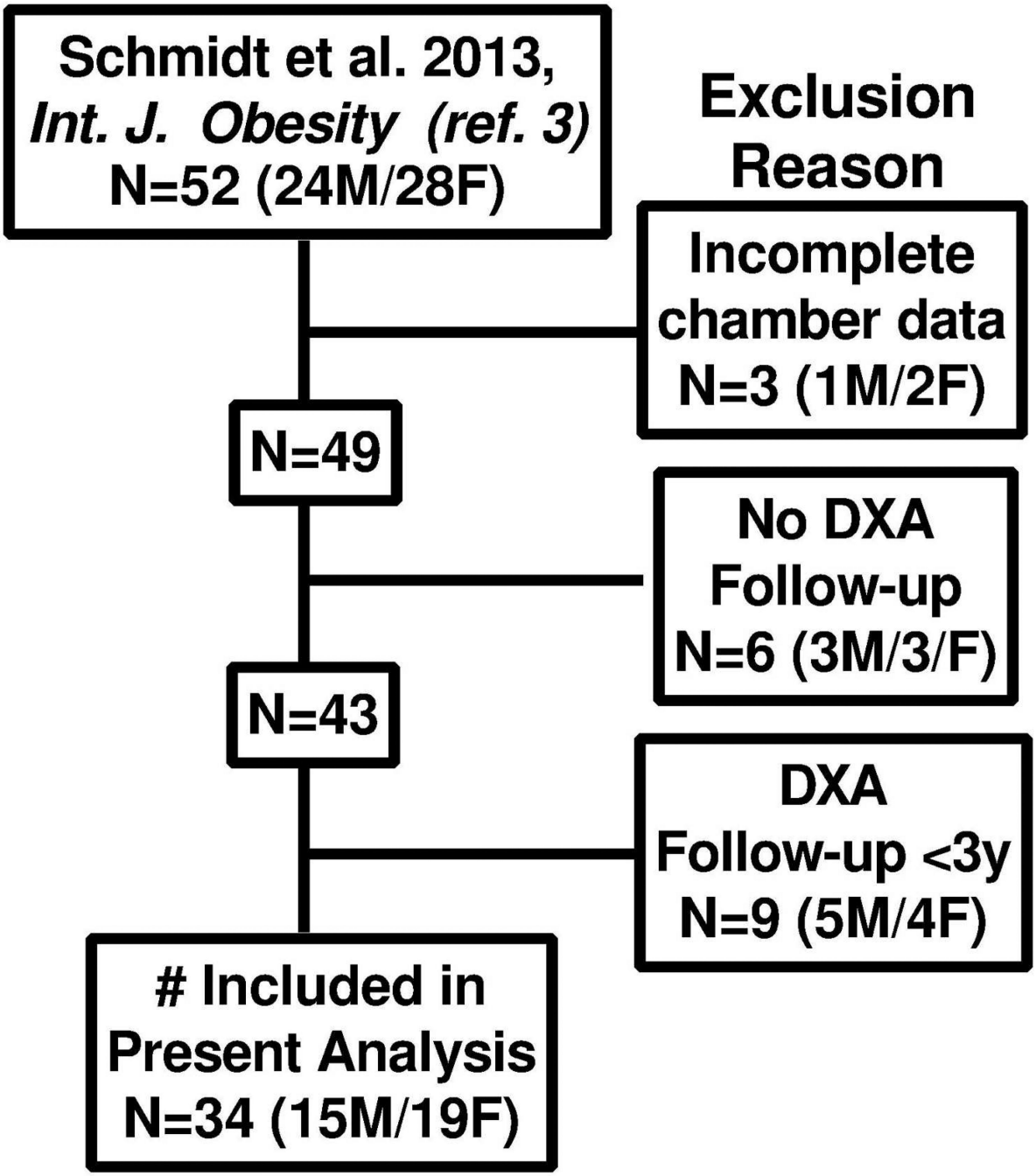

Figure 1.
Flow diagram showing study numbers and reasons for exclusion from the statistical data analysis.

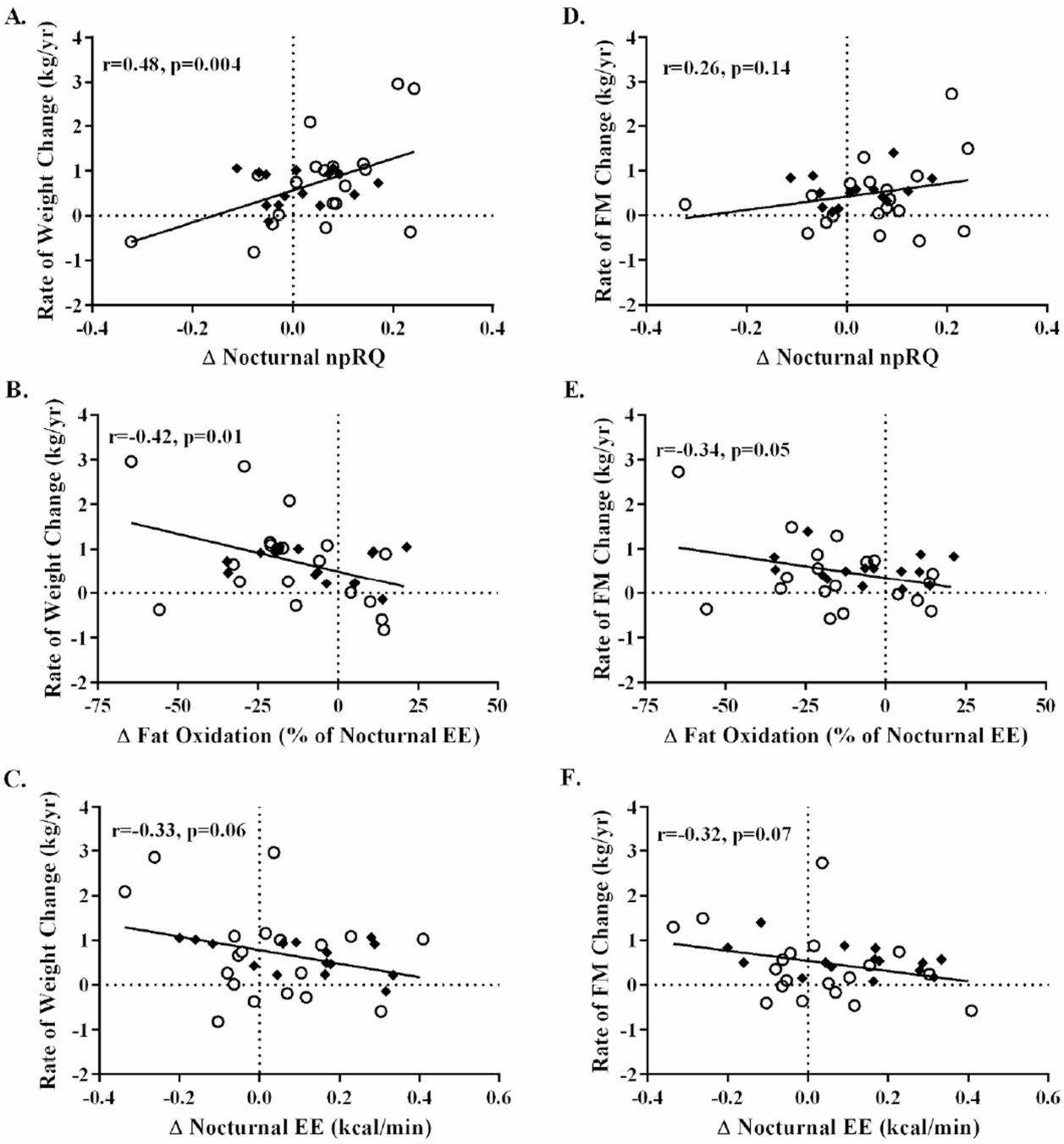

Figure 2.

Scatter plots showing the relationship between the changes in nocturnal non-protein respiratory quotient (npRQ), fat oxidation (as a percentage of nocturnal energy expenditure), and energy expenditure (EE) with overfeeding and longitudinal changes in body mass and fat mass (FM). The change in nocturnal npRQ, fat oxidation (as a percentage of nocturnal energy expenditure), and energy expenditure (EE) were calculated as the overfed minus

eucaloric condition. Pearson correlation coefficients and p-values displayed for the combined group; ♦ males, ○ females.

Table 1

Subject characteristics

	Combined	Males	Females
N	34	15	19
Baseline			
Age, yr	27.9 ± 2.4	27.8 ± 2.4	28.0 ± 2.4
Height, m	1.73 ± 0.1	1.8 ± 0.08	1.67 ± 0.07 *
Weight, kg	65.9 ± 11.7	73.8 ± 9.6	59.7 ± 9.4 *
BMI, kg/m^2	21.9 ± 2.9	22.6 ± 2.4	21.3 ± 3.1
Fat mass, kg	14.4 ± 6.0	11.7 ± 4.5	16.6 ± 6.3
Fat-free mass, kg	51.0 ± 11.1	61.6 ± 6.9	42.7 ± 5.0
Body Fat %	22.2 ± 8.1	15.6 ± 4.6	27.4 ± 6.3 *
Follow-up			
Years of follow-up	4.7 ± 0.6	4.7 ± 0.6	4.6 ± 0.7
Δ Body mass, kg	3.0 ± 3.4	3.0 ± 1.9	3.0 ± 4.4
Rate of weight change, kg/yr	0.70 ± 0.8	0.64 ± 0.4	0.73 ± 1.1
Δ Fat mass, kg	2.1 ± 2.7	2.6 ± 1.7	1.8 ± 3.3
Rate of fat mass change, kg/yr	0.47 ± 0.6	0.55 ± 0.3	0.41 ± 0.8
Δ Fat-free mass, kg	0.8 ± 2.3	0.22 ± 1.8	0.69 ± 1.5
Rate of fat-free mass change, kg/yr	0.11 ± 0.4	0.04 ± 0.4	0.16 ± 0.3

Demographic data are presented as mean ± SD; BMI, body mass index;

* significantly different from males $p<0.05$

Table 2

Energy metabolism measured in the room calorimeter after 3 days of a eucaloric diet and after 3 days of overfeeding.

	Eucaloric	Overfeeding	P-value
24h			
Energy Intake (kcal/d)	2097.5 ± 67.4	2945.6 ± 94.0	<0.001
Energy Balance (kcal/d)	−207.6 ± 51.3	523.5 ± 45.6	<0.001
EE adj (kcal/min)[a]	1.60 ± 0.03	1.68 ± 0.02	0.003
npRQ adj[a]	0.86 ± 0.01	0.90 ± 0.01	0.002
Nocturnal			
EE adj (kcal/min)[a]	0.94 ± 0.03	1.0 ± 0.02	0.05
npRQ adj[a]	0.82 ± 0.02	0.85 ± 0.02	0.06
Sleep (0330 – 0500)			
EE adj[a] **(kcal/min)**	0.86 ± 0.02	0.90 ± 0.03	0.19
npRQ adj[a]	0.82 ± 0.02	0.85 ± 0.02	0.19

Variables are means ± SEM;

EE = total (24h) energy expenditure; npRQ, non-protein respiratory quotient.

[a]Adjusted for sex, fat-free mass, and fat mass

Table 3

Substrate utilization measured in the room calorimeter after 3 days of a eucaloric diet and after 3 days of overfeeding.

	Eucaloric	Overfeeding	P-value
24h Nutrient Intake and Balance			
Fat Intake, g (kcal)	81.5 ± 2.6 (733 ± 23)	113.8 ± 3.6 (1024 ± 33)	<0.001
Carbohydrate Intake, g (kcal)	250.1 ± 8.2 (932 ± 32)	347.1 ± 32.8 (1317 ± 44)	<0.001
Protein Intake, g (kcal)	108 ± 3.4 (432 ± 14)	151.2 ± 4.8 (605 ± 19)	<0.001
Fat Balance, g (kcal)	−16.1 ± 8.0 (−152 ± 75)	41.3 ± 7.0 (390 ± 67)	<0.001
Carbohydrate Balance, g (kcal)	−24.5 ± 15.3 (−102 ± 64)	−5.5 ± 16.9 (−23 ± 71)	<0.001
Protein Balance, g (kcal)	14.4 ± 3.9 (65 ± 17)	37.7 ± 4.7 (169 ± 21)	<0.001
24h Substrate Oxidation			
Fat oxidation adj, g/min (kcal/min)	0.07 ± 0.005 (0.66 ± 0.05)	0.05 ± 0.005 (0.47 ± 0.05)	0.003
Fat oxidation, %TEE	37.6 ± 2.8	25.8 ± 2.4	<0.001
Carbohydrate oxidation adj, g/min (kcal/min)	0.18 ± 0.01 (0.75 ± 0.04)	0.23 ± 0.01 (0.96 ± 0.04)	<0.001
Carbohydrate oxidation, %TEE	44.5 ± 2.7	54.5 ± 2.6	0.002
Protein oxidation adj, g/min (kcal/min)	0.07 ± 0.002 (0.31 ± 0.009)	0.08 ± 0.003 (0.36 ± 0.01)	<0.001
Protein oxidation, %TEE	17.8 ± 1.0	19.6 ± 0.8	0.02
Nocturnal Substrate Oxidation			
Fat oxidation adj, g/min (kcal/min)	0.05 ± 0.004 (0.47 ± 0.04)	0.04 ± 0.003 (0.38 ± 0.03)	0.03
Fat oxidation, % nocturnal EE	43.7 ± 3.7	32.2 ± 3.2	0.003
Carbohydrate oxidation adj, g/min (kcal/min)	0.06 ± 0.009 (0.25 ± 0.04)	0.08 ± 0.007 (0.33 ± 0.03)	0.03
Carbohydrate oxidation, % nocturnal EE	24.7 ± 3.6	33.4 ± 3.4	0.02
Protein oxidation adj, g/min (kcal/min)	0.07 ± 0.002 (0.31 ± 0.009)	0.08 ± 0.003 (0.36 ± 0.01)	<0.001
Protein oxidation, % nocturnal EE	31.9 ± 2.1	34.4 ± 1.6	0.18
Sleep Substrate Oxidation (0330 – 0500)			
Fat oxidation adj, g/min (kcal/min)	0.04 ± 0.005 (0.38 ± 0.05)	0.03 ± 0.004 (0.28 ± 0.04)	0.05
Fat oxidation, % sleep EE	42.4 ± 4.2	30.8 ± 3.5	0.01
Carbohydrate oxidation adj, g/min (kcal/min)	0.05 ± 0.009 (0.21 ± 0.04)	0.07 ± 0.009 (0.29 ± 0.04)	0.08
Carbohydrate oxidation, % sleep EE	22.8 ± 3.7	31.5 ± 4.0	0.05
Protein oxidation adj, g/min (kcal/min)	0.07 ± 0.003 (0.31 ± 0.01)	0.08 ± 0.004 (0.36 ± 0.02)	<0.001
Protein oxidation, % sleep EE	34.8 ± 2.4	37.8 ± 1.6	0.16

Variables are means ± SEM;

g = grams; EE = total (24h) energy expenditure; adj, Adjusted for sex, fat-free mass, and fat mass.

The values are expressed in grams, grams/minute, and as a percentage of energy expenditure. Values in parentheses are expressed in kilocalories and kilocalories/minute.

Table 4

Multiple regression results predicting the rate of change in body mass and fat mass from baseline to follow-up.

Predictors	Change in Weight (kg/yr)	Change in Fat Mass (kg/yr)
Age	0.06 ± 0.05 (p=0.25)	0.04 ± 0.05 (p=0.43)
Sex (Female)	−0.61 ± 0.55 (p=0.28)	−0.51 ± 0.49 (p=0.60)
Baseline FM	0.04 ± 0.03 (p=0.12)	0.02 ± 0.02 (p=0.43)
Baseline FFM	−0.02 ± 0.02 (p=0.52)	−0.009 ± 0.02 (p=0.67)
Change in Nocturnal npRQ	0.28 ± 0.12 (p=0.02)	0.12 ± 0.10 (p=0.25)
Change in Nocturnal EE	−0.28 ± 0.15 (p=0.07)	−0.25 ± 0.13 (p=0.08)
Intercept	−0.56 ± 2.0	0.004 ± 1.8
Explained Variance R^2	0.41 (p=0.019)	0.23 (p=0.27)

EE, energy expenditure; FM, fat mass; FFM, fat-free mass; npRQ, non-protein respiratory quotient; β coefficients in each cell are reported as mean values with standard error (±SE) and significance (p-value).

Δ = 5y rate of change in body mass. Rate of weight change per 0.1 change in sleeping npRQ with overfeeding. Rate of weight change per 10 kcal change in sleeping EE