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# Cycling Efficiency during Incremental Cycle Ergometry after 24-hours of Overfeeding or Fasting

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## Abstract

**Objective—**To determine whether *net* cycling efficiency (NET) is altered by 24-hour fasting or overfeeding, and correlates with dietary-related energy expenditure (EE) and future weight change.

**Methods—**In a crossover design, healthy subjects fasted or were overfed for 24-hours while in a whole-room calorimeter using five diets with doubled energy needs: standard, high-carbohydrate (75%), high-fat (60%), high-protein (30%) and low-protein (3%) diets. Graded cycling exercise at low-power outputs (10–25–50W) was performed the day before and after each dietary intervention.

**Results—**NET did not change following any dietary intervention (all $p>0.05$ versus 0). Individual changes in NET did not correlate with EE responses to dietary interventions. However, the change in NET after low-protein overfeeding was inversely correlated with baseline body fat ($r=-0.60$, $p=0.01$), i.e., NET increased in lean but decreased in overweight subjects ($\Delta=0.010\pm0.010$ versus $-0.013\pm0.009$, $p=0.0003$). Increased NET following the low-protein diet was associated with weight gain after six months ($r=0.60$, $p=0.05$).

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**Conclusions—**Despite no substantial effect of acute overfeeding or fasting on NET, the change in NET following low-protein overfeeding depends on adiposity and may influence weight change, suggesting that increased efficiency in a setting of protein scarcity is an adaptive response that may ultimately lead to weight gain.

**Keywords**

exercise physiology; energy expenditure; obesity phenotypes

## Introduction

Obesity results from the imbalance between food intake and energy expenditure (EE). However, the interaction between food intake and EE involves complex mechanisms (1), hardly summarized by a simple static equation. For instance, fasting is known to decrease EE (2, 3) and overfeeding, even for a single-day, is known to increase EE in humans. The average increase in EE is only a small fraction (5–15%, depending on the degree and type of overfeeding) of the increased intake (3–5), but there is a substantial inter-individual variability in the EE response to overfeeding and fasting, which may be related to the individual predisposition to weight change (1). Our previous studies have shown that the individual EE responses to acute dietary perturbations exhibit a strong intra-individual component, which is consistent across overfeeding diets with differing macronutrients composition (6). These EE responses predict both weight loss in subjects with obesity undergoing caloric restriction (7) and free-living weight change (8), such that subjects with a smaller EE response when overeating 200% of their energy needs with a 3%-protein diet and higher EE response to a 75%-carbohydrate diet gain more weight after six months (8). The 24-h EE (24hEE) response to 2-fold increase in energy needs is mainly dependent on macronutrient content of the overfeeding diet (3, 6), such as the largest average increase in EE is observed during a 75%-carbohydrate 20%-protein diet while the lowest average increase is observed during a 3%-protein diet (3, 6, 8), but the different macronutrient content does not fully explain the large inter-individual variability of 24hEE response (3, 6). As chronic overfeeding may lead to weight gain over time, and 10%-weight gain results in a decrease in cycling efficiency above resting, i.e., *net* cycling efficiency (NET) (9, 10) as reflective of skeletal muscle work efficiency, we sought to investigate whether the change in NET following acute overfeeding explains part of the variability of the EE response to these dietary interventions which predict weight gain.

The EE of low-intensity exercise provides similar results to the EE of usual, non-intentional daily exercise activities, i.e., walking to and from work, cooking, strolling (9). When subjects are overfed for longer periods, NET decreases more than expected given body composition changes (9), and this effect happens primarily at low levels of physical activity (9–11). Change in NET (estimated by the change in $VO_2$ above rest, or calculated during specific grade of exercise) by cycling (10) or walking (12) is an intrinsic characteristic of one individual; however, some studies did not find a significant change in NET after overfeeding by walking (4) or cycling (13, 14). The reasons for inconsistencies among studies for changes in NET following overfeeding or underfeeding are not clear, but it may be due to different dietary macronutrient proportions. For instance, an animal study shows a reduction in mechanical efficiency with decreased content of dietary protein (15). It is unclear, however, if the changes in NET occur as early as after 24 hours of dietary intervention, or if these require a meaningful change in body weight. Further, it is unknown whether the individuals who maintain higher EE during fasting, i.e., metabolic *spendthrift* individuals, also have lower or higher NET when cycling after 24-h overfeeding or fasting, respectively, as compared to metabolic *thrifty* individuals (1). Therefore, our aims for this current study were: 1) to determine whether NET at low-power outputs changes in response to 24-hour fasting and 200%-overfeeding; 2) to evaluate whether the change in NET differs based on the macronutrient content of the overfeeding diet, and if it correlates with the 24hEE response to fasting and overfeeding; and 3) to assess whether the individual changes in NET after low-protein and high-carbohydrate overfeeding diets, whose 24hEE responses independently predicted weight change in our previous study (8), predict free-living weight change after 6 months.

## Methods

### Subjects

Fifty-seven adults, age 18 years or older from the Phoenix-Arizona area were screened between 2013 and 2015, as part of a larger ongoing study (clinicaltrials.gov: NCT00523627). Thirty-two subjects met initial inclusion criteria and were admitted to the Clinical Research Unit (Figure 1). All subjects had no history of recent weight loss and were considered healthy by history, physical, and fasting blood tests. On admission, subjects were placed on a standard weight-maintaining diet (WMD; 50% carbohydrate, 30% fat, and 20% protein), using unit specific equations based on weight and gender (16). The WMD was adjusted to maintain a constant (±1%) body weight measured daily by calibrated scale (mean inpatient weight change=0.3 kg) (8). Body composition was measured by dual-energy X-ray absorptiometry (DPX-1, Lunar Corp, Madison, Wisconsin, USA). All 32 subjects underwent a 75g oral glucose tolerance test after 3 days on the WMD, and only those with normal glucose regulation were eligible to continue in the study (n=23) (17). Subjects were asked to restrict their activities to those available on the research unit during their stay, except during the time of the cycle ergometry. Three subjects met all the inclusion criteria to complete the study, but data were lost because of equipment failures (n=2) and early discharge secondary to personal reasons (n=1). The remaining 20 participants completed at least two ergometry pairs (see below) and were included in the final data analysis. Fourteen subjects returned 6 months after discharge to determine free-living weight change. All participants provided written informed consent prior to beginning the study. The Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases approved this study.

### Intervention diets

The experimental protocol for acute dietary interventions was described previously (3). Briefly, following two assessments of 24hEE in energy balance, all subjects had, in random order and with a three days' washout period on the WMD, six 24-h sessions inside the whole-room indirect calorimeter with the following dietary interventions: fasting and 5 different overfeeding diets. The 24-h energy intake of each overfeeding diet was calculated by doubling the 24hEE from the second energy balance assessment. The overfeeding diets were: 1) standard (SOF), with 50% carbohydrate, 30% fat, 20% protein; 2) high-carbohydrate (CNP), with 75% carbohydrate, 5% fat, 20% protein; 3) high-fat (FNP), with 20% carbohydrate, 60% fat, 20% protein; 4) low-protein (LPF), with 51% carbohydrate, 46% fat, 3% protein; and 5) high-protein (HPF), with 26% carbohydrate, 44% fat, 30% protein. Non-completely eaten meals were returned to kitchen and measured for accurate determination of energy consumption. If less than 95% of food was eaten, the chamber session and the graded cycling efficiency (GCE) data were excluded from analysis (4 sessions excluded: 2 CNP, 1 LPF, and 1 HPF).

## Metabolic measurements

**Twenty-four-hour energy expenditure—**The experimental protocol for the assessment of 24hEE and substrate oxidation while in the whole-room indirect calorimeter was previously described (10). See supplement for brief description. Propane burn tests to determine the accuracy of the 24hEE measurement demonstrated mean recoveries of 0.97±0.02 and 0.98±0.02 for $O_2$ and $CO_2$, respectively (12). Room temperature averaged 23.9±0.7°C.

**Graded cycle ergometry (GCE)—**All subjects performed an incremental, computer-driven multistage cycle ergometry test on the same electromagnetically braked cycle ergometer (Monark Ergometric 839 E, Vansbro, Sweden) at a pedal cadence of approximately 60–70 rpm on the day before and on the day after every dietary session. All rides occurred approximately 2 hours after breakfast (details on supplement), including after 24-h fasting. Participants abstained from caffeine during the time preceding the test. Subjects were seated in an upright position after seat adjustment. During the test, subjects breathed only through a low resistance two-way non-rebreathing valve (2700; Hans Rudolph, Kansas City, Missouri, USA). Using a Parvomedics TrueOne 2400 Metabolic Measurement System (Parvomedics Inc., Salt Lake City, Utah, USA) (18, 19) with Beckman $O_2$ (OM −11) and $CO_2$ analyzers (LB-2; Beckman Instruments Inc., Fullerton, California, USA) and Hans Rudolph linear pneumotachometer with heater controller (Hans Rudolph Inc., Kansas City, Missouri, USA), oxygen consumption ($VO_2$), carbon dioxide production ($VCO_2$), and respiratory exchange ratio ($RER=VCO_2/VO_2$) were measured and calculated continuously (response for $VO_2$ analyzer: 200 milliseconds; $VCO_2$ analyzer: 100 milliseconds) and an average was reported every minute (20). EE during GCE was calculated using the equation of Lusk (21). The metabolic system was calibrated with a 3-Liter calibration syringe and medical gases of known concentrations (16.0% $O_2$, 1% $CO_2$, $N_2$-balance) before each session. Heart rate was monitored continuously by a Polar H1 Heart Rate Sensor (Polar Electro Inc., Lake Success, New York, USA) throughout the test. After 8 minutes of stabilization at rest and 2 minutes of unloaded pedaling, the work load was incrementally increased in consecutive 4-min intervals to 10, 25, and 50 Watts each, and each interval was separated by 2 minutes of unloaded pedaling. The final 3 minutes of measurements during resting and the final 2 minutes of each graded interval were considered steady-state values.

### Calculations

The percent change in EE (%EE) during dietary interventions was calculated as: ($24EE_{Diet}$ – $24hEE_{EnergyBalance}$)/$24hEE_{EnergyBalance}$*100 (8). Cycling efficiency was derived from calorimetry data (16) during the GCE procedure as: 1) *gross* efficiency, the ratio of power generated at power outputs of 10W, 25W, and 50W with respect to the concomitant EE (Grade/$EE_{Grade}$); and 2) *net* efficiency (NET), the ratio of power generated at power outputs of 10W, 25W, and 50W to the concomitant change in EE above resting EE [(Grade/($EE_{Grade}$–$EE_{rest}$)], which was the primary outcome.

### Statistical analysis

Power calculations performed *a priori* determined that a sample size of 16 would provide 80% power (2-sided alpha=0.05) to detect a 0.015±0.020 (mean±SD) change in NET at 10W after fasting or overfeeding by paired *t*-test. The expected change in NET was hypothesized to be half the average change in NET reported after 10%-weight loss in a previous study (9). The final sample size (n=20) achieved 89% power. The level of statistical significance for each test was prospectively set at 0.05 (2-sided) to maximize potential findings given the exploratory nature of the data.

Data are reported as mean±SD. For each dietary intervention, mixed models were used to evaluate post-intervention change in RER and $VO_2$ trajectories during the exercise test (Figures 2 and 3) accounting for repeated measures using a first-order autoregressive covariance structure. The reproducibility of pre-diet NET was quantified by the coefficient of variation (CV) and the intraclass correlation coefficient (ICC). Changes (Δ) in NET after each dietary intervention were analyzed by paired *t*-test. Associations were quantified by Pearson correlation. For each subject, the average change in NET over the three power outputs was calculated for only LPF and CNP and tested for association with weight change, as the change in 24hEE during these overfeeding diets predicted weight change in our previous study (8). Statistical analyses were performed in SAS (version 7.11; Cary, NC). Sensitivity analyses for the primary aim were done including only men and results were unchanged (data not shown).

## Results

### RER and $VO_2$ changes during cycle ergometry following 24-h fasting or overfeeding

Baseline characteristics of the studied subjects are presented in table 1. In the resting phase of the exercise test, RER decreased after 24-h fasting (Δ=−0.06±0.06, p<0.0001) and after HPF (Δ=−0.03±0.03, p=0.01), whereas RER increased after SOF (Δ=0.03±0.04, p=0.02), CNP (Δ=0.04±0.06, p=0.03) and LPF (Δ=0.03±0.05, p=0.04). There was no change in RER after FNP (Figure 2). During the resting phase, $VO_2$ increased only after FNP (Δ=0.03±0.03 L/min, p=0.003) (Figure 3).

During exercise at different power outputs, RER increased after the standard, CNP, and LPF diets (Figure 2) and decreased after fasting, while no changes were observed after FNP and HPF. In mixed models, overall RER increased after CNP and LPF overfeeding (Δ=0.029±0.011, p=0.01; and Δ=0.024±0.007, p=0.002, respectively), and decreased after fasting (Δ=−0.057±0.009, p<0.0001) (Figure 2). Except for changes in exercise $VO_2$ after FNP [overall Δ=0.03 (CI=0.01 to 0.06) L/min, p=0.009], no $VO_2$ changes were found following the other diets (Figure 3, Supplement Table S1).

### Cycling efficiency changes after 24-h fasting or overfeeding

The CVs and ICCs of pre-diet NET values were: 0.14, 0.12, 0.08 and 0.53 (p=0.01), 0.55 (p=0.01), 0.46 (p=0.02), for 10W, 25W, and 50W, respectively, indicating good precision and reproducibility of the GCE method. With the exception of LPF at 25W (Δ= −0.011±0.019, p=0.04), there was no substantial change in NET after 24-h overfeeding or fasting (all p>0.05, Table 2, Figure 4). Similar results were obtained for gross cycling efficiency (Supplement Table S2). No correlations between dietary protein content and changes in NET during any overfeeding diet were observed (all p>0.05).

LPF was the only diet with consistent associations between NET and body composition. Specifically, the average change in NET after LPF was negatively correlated with age (r= −0.53, p=0.03), BMI (r=−0.54, p=0.03, Figure 5A), body fat percentage (%fat; r=−0.60, p=0.01, Figure 5B), and FM (r=−0.66, p=0.005, figure 5C) but not with FFM (p=0.16, figure 5D). Results for %fat were driven by the change in NET at 25W (r=−0.55, p=0.03), and were similar after adjustment for age and sex (data not shown). After classifying subjects into lean, overweight and obesity categories (BMI<25 kg/$m^2$, ≥25 and <30kg/$m^2$, ≥30kg/$m^2$, respectively, Figure 5C), on average lean subjects increased (Δ=0.011±0.010, n=5), whereas subjects with overweight (Δ=−0.013±0.011, n=8) or obesity (Δ=−0.008±0.007, n=3) decreased their NET after LPF (p=0.004). Similar results were observed when classifying subjects by %fat (Figure 5D) (22), where lean (%fat <25 in men and <35% in women) individuals increased, while non-lean subjects decreased their NET after LPF (Δ=0.010±0.010, n=6 versus −0.013±0.009, n=10, respectively; p=0.0003).

### Correlation between net cycling efficiency changes and 24hEE responses to diet

There were no associations between the change in cycling efficiency indexes (*gross* and *net* efficiencies) and the 24hEE responses to dietary interventions (all p>0.05).

### Body weight change at 6 months

From the 20 initial participants, 14 (70%) returned for a follow-up visit after 6 months (median [interquartile range]: 7.1 [6.9, 7.3] months). The average weight change at this visit was 0.5±4.3 kg (p=0.70 versus zero). Of the 16 subjects who completed the GCE before and after LPF, 11 returned for follow-up. There was a positive association between the average change in NET across grades following LPF and weight change (r=0.60, p=0.05, Figure 6), such that a 1%-increase in NET after LPF was associated with 1.9 kg-weight gain at follow-up. However, if the subject of highest weight gain was excluded from the analysis, the association was no longer statistically significant (r=0.25, p=0.49). There was no association between the change in NET following CNP and weight change (p=0.92).

## Discussion

We evaluated the effects of 24-hour fasting and, separately, five overfeeding diets of different macronutrient composition on net cycling efficiency in healthy subjects during weight maintenance. We demonstrated that, despite changes in macronutrient utilization after these acute dietary interventions, there was no average change in NET following diets. However, the individual change in NET after LPF was inversely associated with body fat percentage, such that lean individuals were more efficient after this overfeeding diet, and an increase in NET following LPF was associated with weight gain after 6 months.

There was an increase in carbohydrate utilization during the GCE test after dietary interventions composed by 50% or more of energetic intake from carbohydrate, and an expected increase in lipid utilization during fasting, as demonstrated by the change in RER, an index of carbohydrate-to-lipid oxidation ratio, indicating an acute adaption to these extreme dietary interventions. Consistent with our results, RER increases following meals with rising caloric content as the meals progress from underfeeding to overfeeding in young men, despite a lack of change in efficiency (14). A previous study also found that resting RER rises after 24-h overfeeding versus 24-h fasting, and remains elevated even after a standard meal and exercise (23). Others found that RER increases after one overfeeding meal with an additional increase in RER with exercise (24). Our study is the first to include multiple overfeeding diets with differing macronutrient content given over 24 hours. Our results further illustrate how the body switches sources of energy, i.e. between carbohydrate and fat, when challenged with proportional differences in macronutrients.

It is recognized that 10%-weight gain as a result of long-term overfeeding decreases NET, and, conversely, that 10%-weight loss after underfeeding increases NET, with the change in NET being correlated to the change in non-resting EE (9). We did not observe substantial changes in NET after 24-hour fasting or overfeeding with different macronutrient contents (the only exception was a small decrease after LPF at 25W), nor did we find that the variability of NET was associated with the acute metabolic response to these extreme dietary interventions. Although we did not detect a significant change in NET overall, some subjects increased while others decreased their NET, indicating that the individual response to acute overfeeding and fasting has a wide inter-subject variability which may be indicative of the propensity of one individual to weight gain.

The changes in NET following dietary interventions were not associated with the concomitant changes in 24hEE. We found, however, a negative correlation between the change in NET after low-protein overfeeding and %fat. Thus, lean individuals were more efficient after doubling their caloric intake with a low-protein diet, which could be considered surprising because lean individuals are more likely to be spendthrift based on the EE response to fasting (25) and, therefore, they should be less efficient after overeating. However, it is also possible that differences in skeletal muscle fiber proportion between lean versus non-lean individuals, such as increased proportion of fiber IIb on females with obesity (26), may play a role on the NET response to overfeeding. Yet, from the evolutionary perspective, it might be reasonable for lean individuals to increase their NET after a low-protein diet to preserve muscle mass during physical activity when available protein sources are low, thereby increasing the chances of survival. Alternatively, for non-lean subjects, the decreased NET after low-protein overfeeding may be a mechanism to compensate for the excess energy, thus preventing further weight gain.

We showed that subjects who became more efficient while exercising at a level consistent with daily activities following a day of low-protein overeating gained more weight after 6 months. Some previous researches are in agreement with our current findings: 1) a previous report indicates that the highest cost of weight gain is observed with overfeeding diets containing 3% of protein (27); 2) an animal study shows decreased energetic efficiency as dietary protein content decreases (15); and 3) the 24hEE response to low-protein overfeeding diet independently identifies thrifty from spendthrift individuals, and predicts weight change (8). Protein is a key energy source to maintain lean body mass. The increased NET may be an adaptation to preserve relative lean body mass (e.g., by promoting weight stability or weight gain in leaner individuals, or weight and fat mass loss in more overweight individuals) to allow ongoing foraging for food.

There are some limitations to our study. First, most of the subjects were males; therefore, our results may not be generalizable to women. Furthermore, we did not assess the level of physical activity before admission, although our aim was to evaluate exercise loads at lower outputs that mimic sedentary daily living activities (walking, climbing a flight of stairs). Importantly, each volunteer was used as his own control in our paired analyses, so pre-admission individual fitness should have not biased our results as NET is not largely influenced by fitness (28–30). A larger sample size might have demonstrated a difference in NET after overfeeding or fasting; however, our paired design had adequate power to detect a clinically meaningful effect on NET due to such extreme dietary manipulations with at least 16 subjects, although some diets (e.g., SOF) had smaller sample size. All the measurements of cycling efficiency were conducted the day before and the day after each dietary intervention given inside the whole-room calorimeter, following an overnight fast and a regular breakfast given 2 hours prior to the cycling test. The consumption of the breakfast prior to the test may constitute a bias in interpreting the data, especially when evaluating the 24-h fasting period inside the calorimeter. Nonetheless, we still observed expected reduction in RER after 24-h fasting followed by the consumption of a regular breakfast, albeit the magnitude of this change in RER might have been lower than expected due to breakfast consumption. It is possible that instrumental errors on cycling efficiency estimate might have an effect on our results, although the pre-diet CVs were $<0.15$ as shown in other studies (31). Lastly, the association between the change in NET after low-protein overfeeding and weight gain was driven by one high-leverage observation as the sensitivity analysis removing this subject resulted in a non-significant correlation value, partly because of the loss of 1 degree of freedom. Therefore, this association should be validated in a larger cohort with higher statistical power.

In conclusion, despite changes in whole-body fuel utilization during light exercise following different 24-h dietary interventions, there was no substantial change in NET after 24-h overfeeding or fasting in a carefully controlled and well monitored inpatient study, implying that short-term overfeeding does not substantially alter cycling efficiency in humans. Nevertheless, there was a wide inter-individual variation in the change of NET following acute low-protein overfeeding, mostly driven by the degree of adiposity, where lean individuals increased while non-lean individuals decreased their NET, which was in turn associated with weight change at six months, such as a decrease in NET determined less weight gain. In the setting of low availability of dietary protein, preservation of body weight in lean individuals and predisposition to weight loss in overweight individuals might be favorable responses to allow ongoing search for nutrients, and thus may be an important metabolic adaptation to relative dietary scarcity.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **%EE** | Percent change in 24-h EE during the dietary intervention from 24-h EE during energy balance |
| **%fat** | Body fat percentage |
| **24hEE** | 24-h energy expenditure |
| **CNP** | High-carbohydrate overfeeding diet |
| **DIT** | Diet induced thermogenesis |
| **EE** | Energy expenditure |
| **FFM** | Fat free mass |
| **FM** | Fat mass |
| **FNP** | High-fat overfeeding diet |
| **GCE** | Graded cycling ergometry |
| **HPF** | High-protein overfeeding diet |
| **LPF** | Low-protein overfeeding diet |
| **NET** | Net cycling efficiency |
| **RER** | Respiratory exchange ratio |
| **SOF** | Standard overfeeding diet |
| **SPA** | Spontaneous physical activity |

| | |
|---|---|
| **TEF** | Thermic effect of food |
| **$VCO_2$** | Carbon dioxide production |
| **$VO_2$** | Oxygen consumption |
| **WMD** | Weight-maintaining diet |

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## Study importance questions

**What is already known about the subject?**

While cycling efficiency increases after 10%-weight loss and decreases after 10%-weight gain, there are controversies among studies regarding the short-term effect of 24-hour overfeeding on cycling efficiency.

The respiratory exchange ratio (RER) during exercise increases acutely after overfeeding diets (50% carbohydrate, 35% fat and 15% protein), but there is no clear information regarding RER response to overfeeding diets that have different macronutrient proportions.

**What does this study add?**

The respiratory exchange ratio during cycling only increases following overfeeding diets with 50% or greater carbohydrate content, but not with diets with a high fat content (>40%).

On average, there is no acute change in net cycling efficiency after 24 hours of overfeeding with five different diets, or fasting. Normal weight subjects increase their net cycling efficiency if provided with a 3% protein overfeeding diet, whereas, overweight and subjects with obesity decrease their net cycling efficiency.

Subjects able to respond to acute low-protein overfeeding with a decrease in net efficiency gain less weight over the next six months.

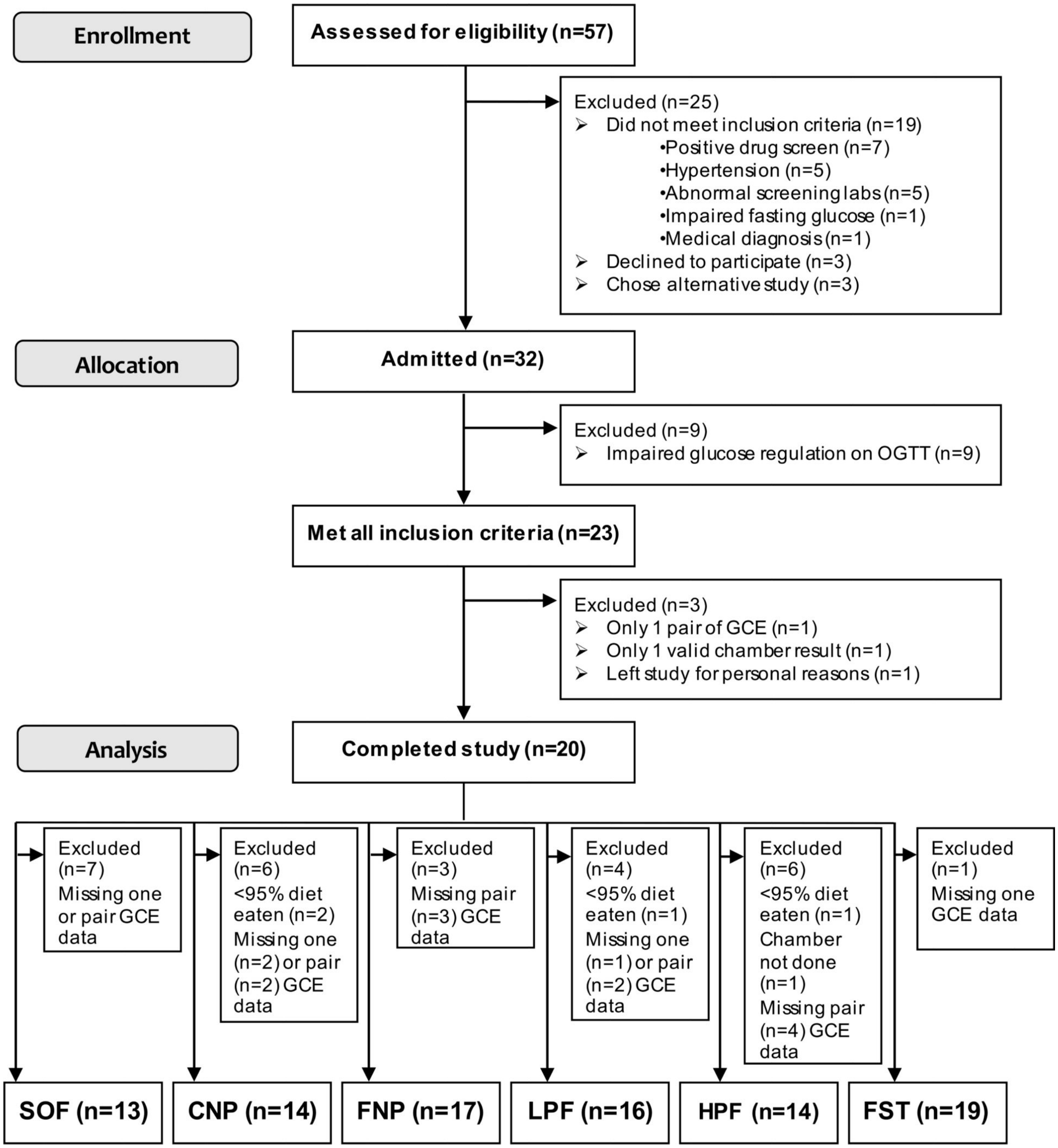


**Figure 1. Flow diagram of subjects' inclusion and participation on study**

Abbreviations: OGTT, oral glucose tolerance test; GCE; graded-cycling exercise; chamber, 24 hours whole-room indirect calorimeter; SOF, standard overfeeding; CNP, high-carbohydrate overfeeding; FNP, high-fat overfeeding; FST, 24-h fasting; HPF, high-protein overfeeding; LPF, low-protein overfeeding.

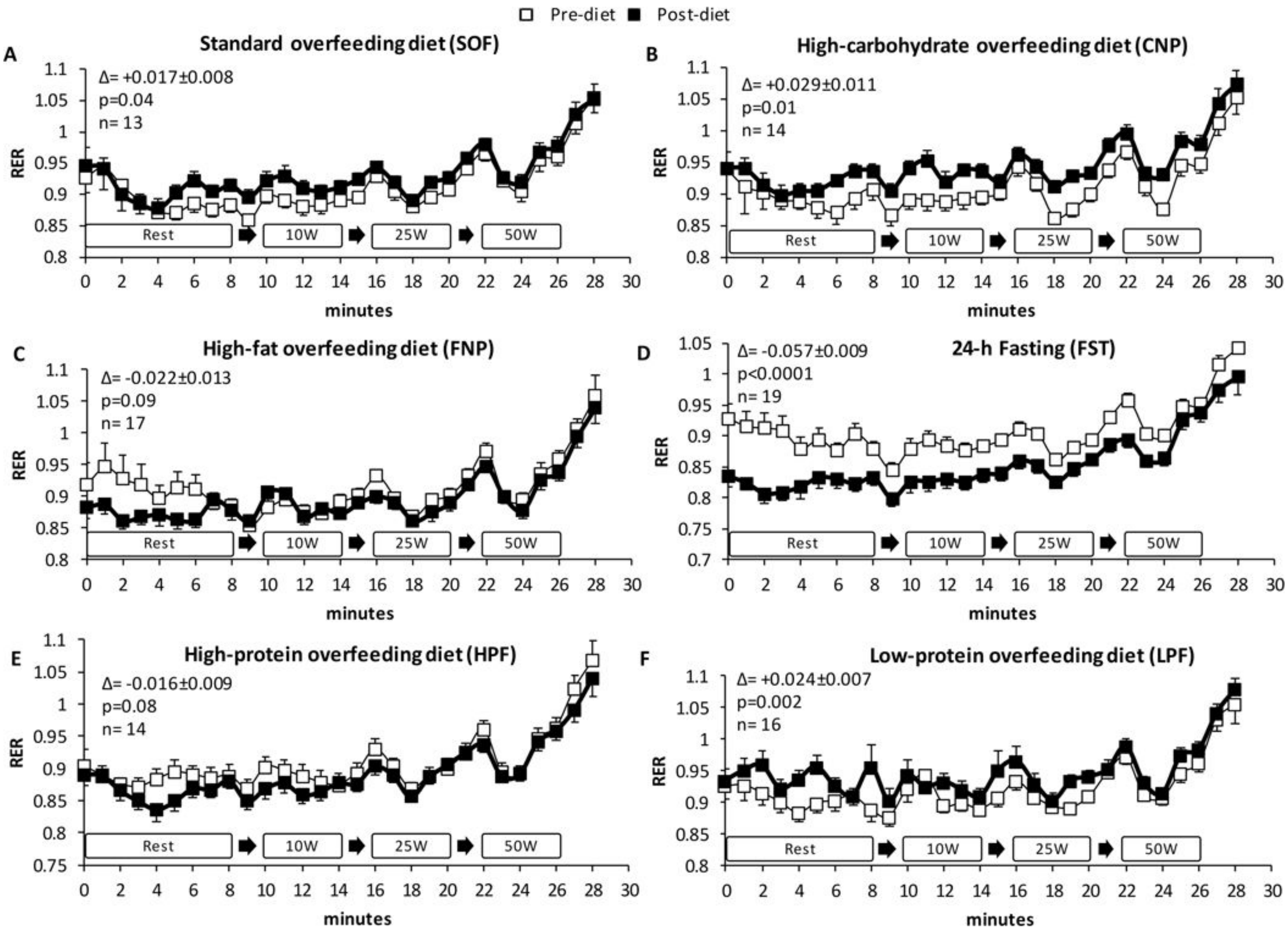


**Figure 2. Minute-by minute RER during GCE before and after each dietary intervention**

Minute-by-minute respiratory exchange ratio (RER) during graded cycling exercise (GCE) the day before (white square) and the day after (black square) dietary interventions. Bars represent standard error. Diets composition: standard overfeeding (SOF), 50% carbohydrate, 30% fat, 20% protein; high-carbohydrate overfeeding (CNP), 75% carbohydrate, 5% fat, 20% protein; high-fat overfeeding (FNP), 20% carbohydrate, 60% fat, 20% protein; high-protein overfeeding (HPF), 26% carbohydrate, 44% fat, 30% protein; low-protein overfeeding (LPF), 51% carbohydrate, 46% fat, 3% protein. Analysis done using mixed models with a first-order autoregressive covariance structure to account for repeated measurements. Delta (Δ) values are presented with standard error.

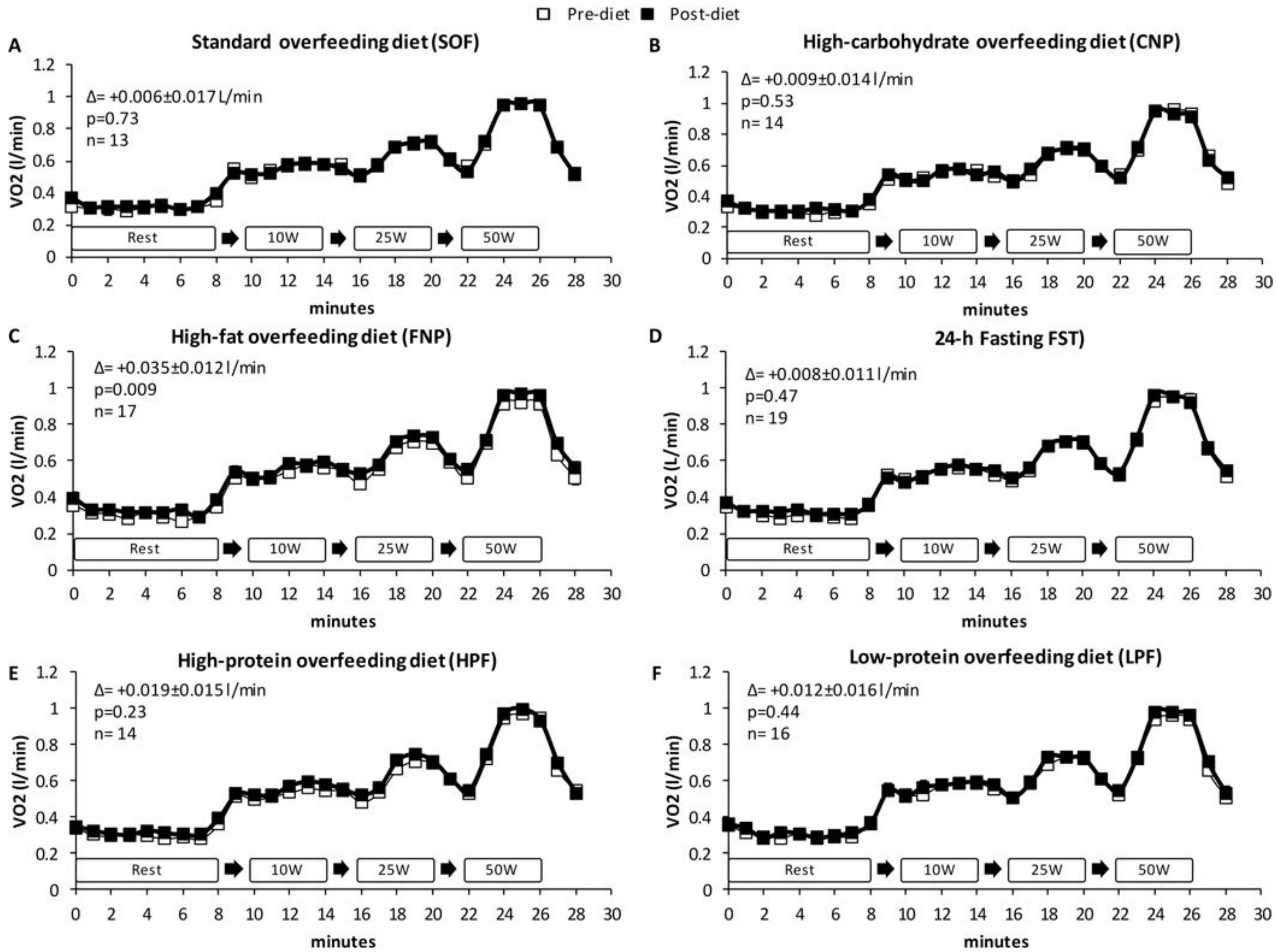


**Figure 3. Minute-by minute $VO_2$ during GCE before and after each dietary intervention**

Minute-by-minute VO2 in L/min during graded cycling exercise the day before (white square) and the day after (black square) dietary interventions. Bars represent standard error. Diets composition: standard overfeeding (SOF), 50% carbohydrate, 30% fat, 20% protein; high-carbohydrate overfeeding (CNP), 75% carbohydrate, 5% fat, 20% protein; high-fat overfeeding (FNP), 20% carbohydrate, 60% fat, 20% protein; high-protein overfeeding (HPF), 26% carbohydrate, 44% fat, 30% protein; low-protein overfeeding (LPF), 51% carbohydrate, 46% fat, 3% protein. Analysis done using mixed models with a first-order autoregressive covariance structure to account for repeated measurements. Delta (Δ) values are presented with standard error.

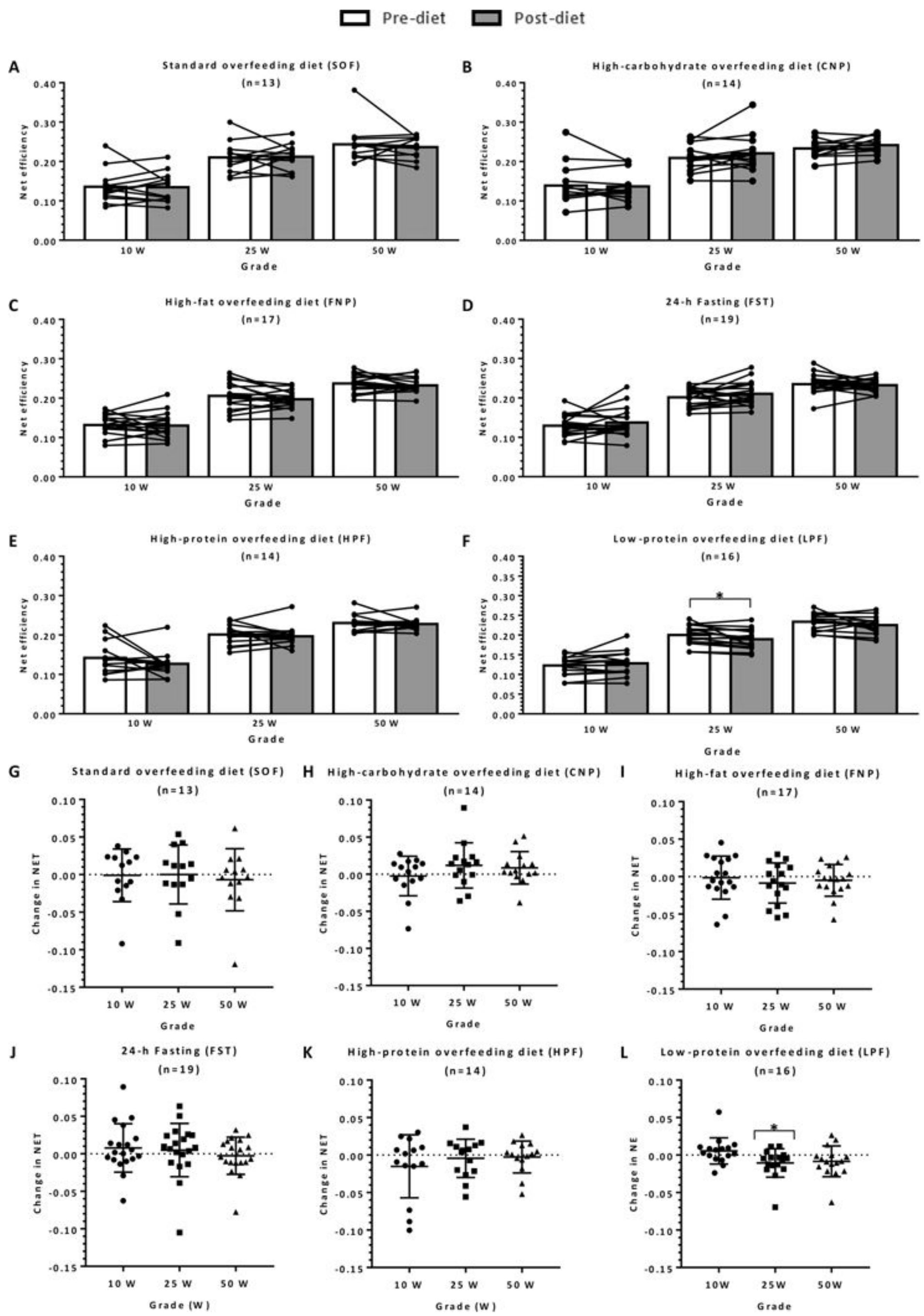


**Figure 4. Net Cycling Efficiency Before and After the Different Dietary Interventions**

Net cycling efficiency (NET) before (white bars) and after (grey bars) different dietary interventions given for 24 hours (A–F) by grade, where bars represent averages NET. Individual changes in NET after dietary interventions (G-L) by grade (post-diet NET minus pre-diet NET), where 10W are represented by circles, 25W by squares and 50W by triangles, and bars represent mean±SD: SOF, standard overfeeding (A, G); CNP, high-carbohydrate overfeeding (B, H); FNP, high-fat overfeeding (C, I); FST, 24-h fasting (D, J); HPF, high-protein overfeeding (E, K); LPF, low-protein overfeeding (F, L). Diets

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composition: SOF, 50% carbohydrate, 30% fat, 20% protein; CNP, 75% carbohydrate, 5% fat, 20% protein; FNP, 20% carbohydrate, 60% fat, 20% protein; HPF, 26% carbohydrate, 44% fat, 30% protein; LPF, 51% carbohydrate, 46% fat, 3% protein.

No statistical significance found by paired t-test, except for the change in NET at 25W after low-protein overfeeding diet (p=0.04, represented by *). An outlier value for the SOF pre-diet (NET=0.70) was substituted with the average pre-diet NET calculated over the other diets in the same individual, and results for SOF were still not significantly different from 0 (mean change= −0.001, 95% CI: −0.022 to 0.020, p=0.93 by paired t-test). Please see the flow diagram (figure 1) for actual number of subjects having valid data both before and after each dietary intervention. No sequence effect of previous dietary intervention on the change in NET was seen (for details, see supplement material, statistical analysis subsection).

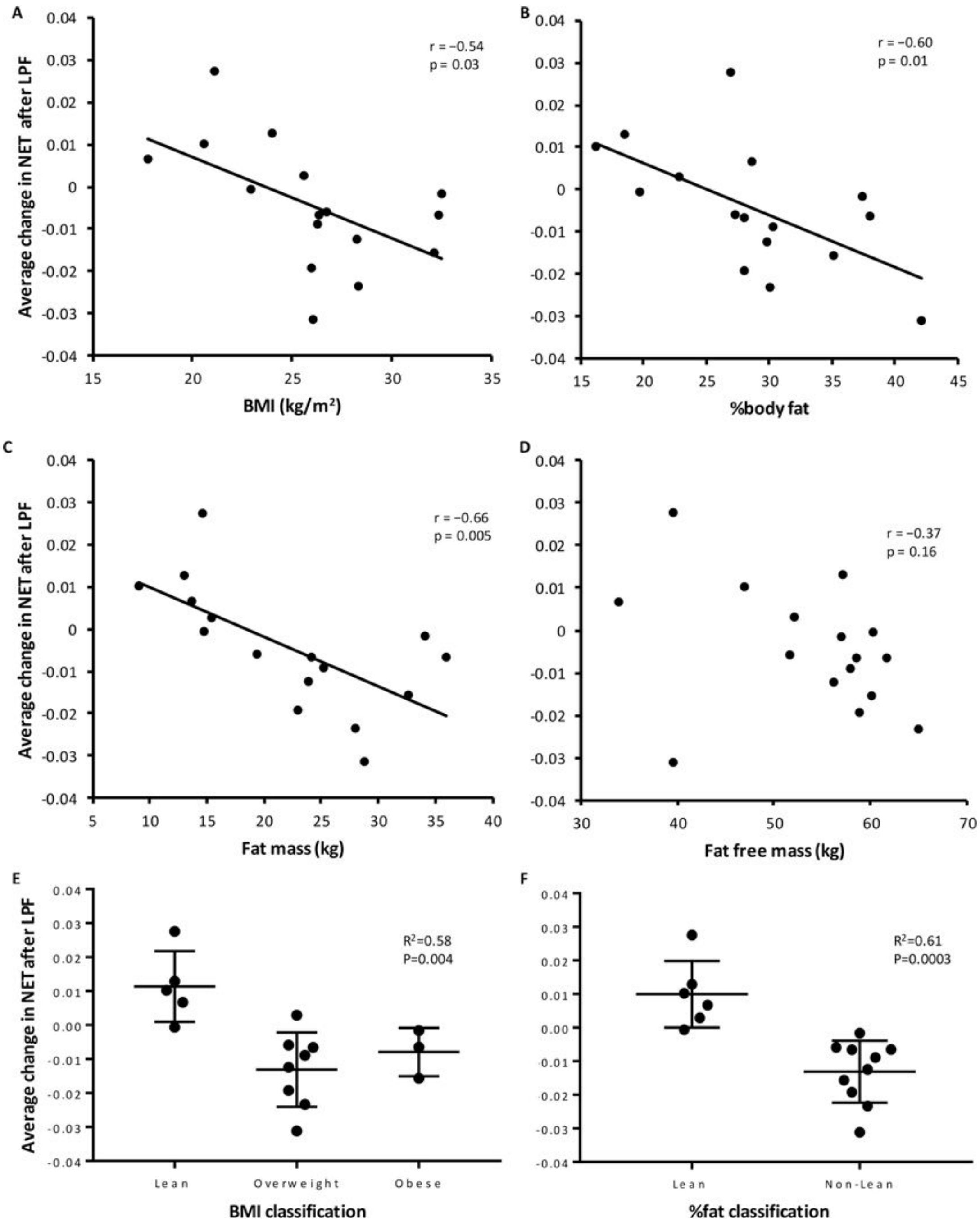


**Figure 5. Association between body composition measures and change in net cycling efficiency after low-protein overfeeding**

Pearson correlations between the average change in net cycling efficiency (NET) after low-protein overfeeding diet (LPF) and body mass index (BMI; A), percent body fat (%fat; B), fat mass (C), and fat free mass (D). Those results did not change after adjustment to age and sex or if using fat mass index (fat mass in Kg divided by height square in meters) in place of fat mass (r=−0.61, p=0.01) or fat free mass index in place of fat free mass (r=−0.26, p=0.34). Average change in net cycling efficiency by BMI (E) or %fat classification (F), by ANOVA. The subjects were classified as lean (BMI <25 kg/m$^2$), overweight (BMI ≥25 and <30 kg/m$^2$) and with obesity (BMI ≥30 kg/m$^2$). Lean and non-lean subjects were classified based on the cutoff values for %fat of 25% in men and of 35% in women. The change in net cycling efficiency after LPF was calculated as the difference between the average change in net cycling efficiency over the three grades after LPF minus the average change in net cycling efficiency over the three grades before LPF.

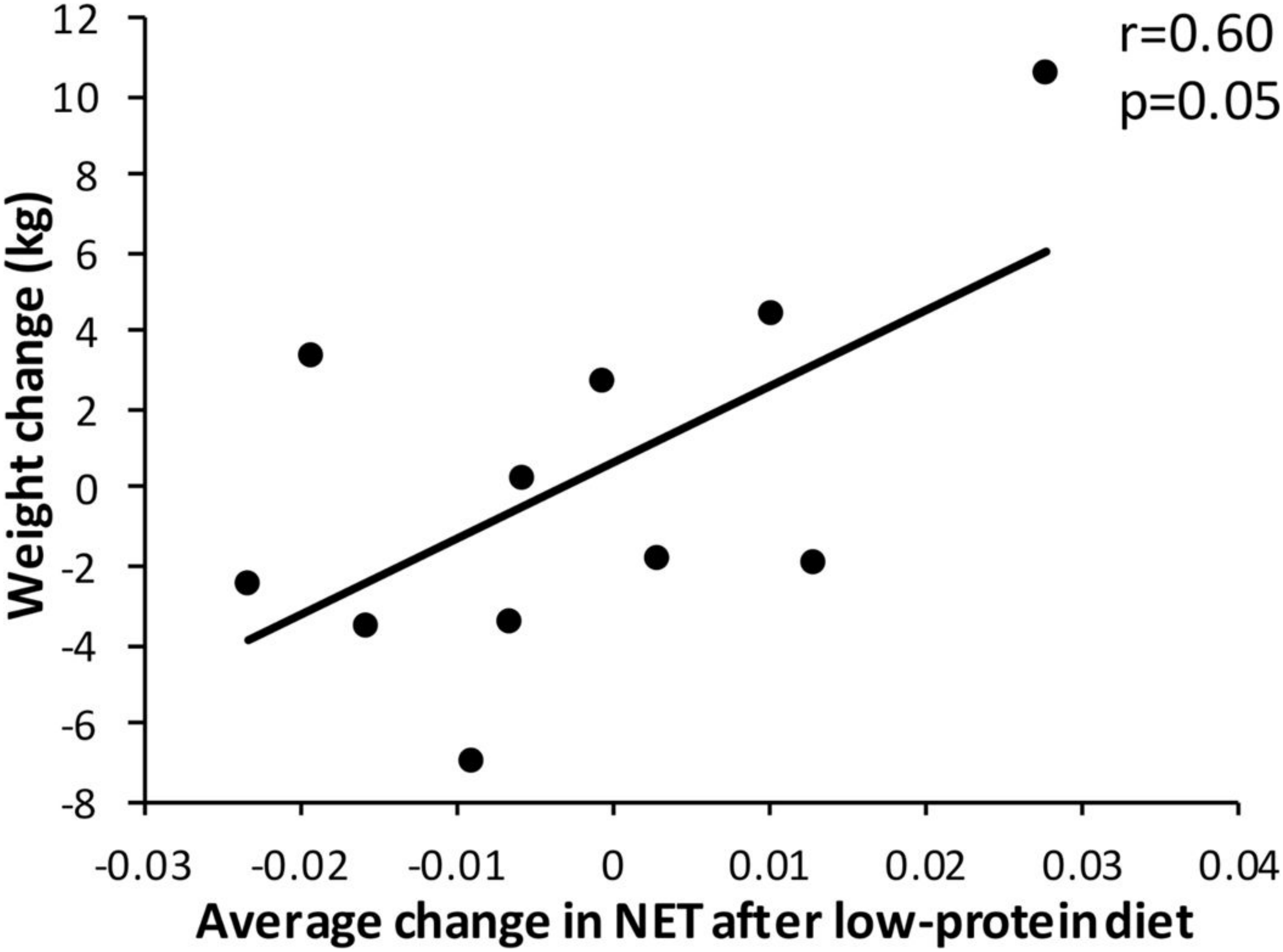


**Figure 6. Association between change in net cycling efficiency after low-protein overfeeding diet and weight change at 6 months**

The change in net cycling efficiency (NET) after low-protein overfeeding diet (LPF; x axis) was calculated as the difference between the average change in net cycling efficiency over the three grades after LPF minus the average change in net cycling efficiency over the three grades before LPF.

### Table 1

Baseline characteristics of the study group.

| Variable | All subjects (n=20) | Women (n=4) | Men (n=16) |
|---|---|---|---|
| Age (y) | 36.4±12.6 (18.2, 55.8) | 31.2±12.8 (20.4, 45.5) | 37.7±12.6 (18.2, 55.8) |
| Weight (kg) | 76.1±13.8 (47.5, 94.5) | 59.1±9.9 (47.5, 68.4) * | 80.4±11.2 (56, 94.5) |
| BMI (kg/m$^2$) | 25.9±3.8 (17.8, 32.5) | 22.6±3.9 (17.8, 26.1) * | 26.7±3.4 (20.6, 32.5) |
| %fat (%) | 27.6±7.8 (10.3, 42.1) | 32.6±6.8 (26.9, 42.1) | 26.3±7.7 (10.3, 38.1) |
| FM (kg) | 21.3±8.1 (7.2, 36.0) | 19.7±7.1 (13.6, 28.8) | 21.8±8.5 (7.2, 36) |
| FFM (kg) | 54.8±9.4 (33.9, 69.0) | 39.4±4.3 (33.9, 44.5) * | 58.7±5.4 (46.9, 69) |
| Fasting glucose (mg/dL) | 89±7 (74, 99) | 86±6 (79, 93) | 90±7 (74, 99) |
| 2h glucose (mg/dL) | 106±22 (72, 138) | 123±6 (119, 132) | 101±23 (72, 138) |
| 24-h EE (kcal/24h) | 1898±274 (1427, 2291) | 1570±168 (1427, 1794) * | 1980±232 (1573, 2291) |
| 24-h RQ (ratio) | 0.86±0.02 | 0.86±0.03 | 0.86±0.02 |
| DIT (kcal/24h) | 127±114 | 166±131 | 117±112 |
| TEF (%) | 6.35±5.68 | 10.17±7.51 | 5.39±5.11 |

Values are reported as mean ± SD (minimum, maximum). Abbreviations: BMI, body mass index; %fat, body fat percentage; FM, fat mass; FFM, fat free mass; EE, energy expenditure during energy balance; RQ, respiratory quotient during energy balance; DIT, diet-induced thermogenesis during energy balance; TEF; thermic effect of food during energy balance.

* $p<0.05$ versus males by Student's *t*-test.

## Table 2

Net cycling efficiency per grade before and after each dietary intervention.

| | SOF (n=13) | CNP (n=14) | FNP (n=16) | FST (n=19) | HPF (n=14) | LPF (n=16) |
|---|---|---|---|---|---|---|
| **10W** | | | | | | |
| ***Before diet*** | 0.173±0.167 | 0.139±0.051 | 0.132±0.027 | 0.130±0.026 | 0.142±0.041 | 0.123±0.024 |
| ***After diet*** | 0.135±0.038 | 0.137±0.036 | 0.130±0.032 | 0.137±0.034 | 0.127±0.031 | 0.128±0.030 |
| ***Change*** | −0.038±0.163 | −0.002±0.027 | −0.001±0.029 | 0.008±0.032 | −0.015±0.042 | 0.005±0.017 |
| **25W** | | | | | | |
| ***Before diet*** | 0.230±0.103 | 0.209±0.032 | 0.206±0.035 | 0.205±0.031 | 0.210±0.05 | 0.200±0.025 |
| ***After diet*** | 0.212±0.031 | 0.221±0.046 | 0.197±0.023 | 0.210±0.029 | 0.197±0.026 | 0.190±0.025 |
| ***Change*** | −0.018±0.098 | 0.012±0.030 | −0.009±0.027 | 0.005±0.036 | −0.013±0.05 | −0.011±0.019 * |
| **50W** | | | | | | |
| ***Before diet*** | 0.243±0.046 | 0.233±0.022 | 0.246±0.046 | 0.235±0.024 | 0.231±0.021 | 0.234±0.021 |
| ***After diet*** | 0.237±0.026 | 0.242±0.021 | 0.232±0.019 | 0.232±0.015 | 0.228±0.015 | 0.225±0.022 |
| ***Change*** | −0.007±0.041 | 0.009±0.022 | −0.013±0.043 | −0.003±0.025 | −0.003±0.021 | −0.008±0.021 |

Data are presented as mean±SD. Change=After–Before,

*: p≤0.05 by paired t-test.

Abbreviations: SOF, standard overfeeding diet; CNP, high-carbohydrate overfeeding diet; FNP, high-fat overfeeding diet; FST, 24-h fasting; HPF, high-protein overfeeding diet; LPF, low-protein overfeeding diet. Overfeeding diets macronutrient proportions: SOF, 50% carbohydrate, 30% fat and 20% protein; CNP, 75% carbohydrate, 5% fat, 20% protein; FNP, 20% carbohydrate, 60% fat, 20% protein; LPF, 51% carbohydrate, 46% fat, 3% protein; and HPF, 26% carbohydrate, 44% fat, 30% protein.