Impaired Metabolic Flexibility to High-fat Overfeeding Predicts Future Weight Gain in Healthy Adults

Brittany Begaye[1], Karyne L. Vinales[1,2], Tim Hollstein[1], Takafumi Ando[1], Mary Walter[3], Clifton Bogardus[1], Jonathan Krakoff[1], Paolo Piaggi[1,4]*

[1] *Obesity and Diabetes Clinical Research Section, Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Phoenix, AZ*

[2] *Endocrinology Division, Medicine Department, Phoenix VA Health Care System, Phoenix, AZ, USA*

[3] *Clinical Core Laboratory, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA*

[4] *Department of Information Engineering, University of Pisa, Pisa, Italy*

PubMed indexing: Begaye B, Vinales KL, Hollstein T, Ando T, Walter M, Bogardus C, Krakoff J, Piaggi P

Running title: Metabolic inflexibility to fats and weight gain

***Corresponding author:** Paolo Piaggi, Ph.D., Obesity and Diabetes Clinical Research Section, Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 4212 N 16th St., Phoenix, AZ 85016. Tel.: +1 (602) 200-5306. Fax: +1 (602) 200-5335.

E-mail: paolo.piaggi@gmail.com, paolo.piaggi@nih.gov

Manuscript word count: 3929

Number of tables and figures: 7

Clinical Trial Registration Number (from clinicaltrials.gov): NCT00523627

Diabetes Publish Ahead of Print, published online November 11, 2019

Funding: This study was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Keywords: respiratory quotient, energy expenditure, diet, dietary fat, fasting, fat oxidation, metabolic flexibility, lipid oxidation, overfeeding, substrate oxidation, weight change, weight gain.

Abbreviations

CARBOX: carbohydrate oxidation rate

CV: coefficient of variation

EBL: standard eucaloric feeding

EE: energy expenditure

FFM: fat free mass

FM: fat mass

FST: fasting

HCOF: high-carbohydrate overfeeding diet

HFOF: high-fat overfeeding diet

ICC: intraclass correlation coefficient

LIPOX: lipid oxidation rate

NEFA: non-esterified fatty acids

PROTOX: protein oxidation rate

RQ: respiratory quotient

STOF: standard overfeeding diet

Abstract

The ability to switch fuels for oxidation in response to changes in macronutrient composition of diet (metabolic flexibility) may be informative of the individual susceptibility to weight gain. Seventy-nine healthy, weight-stable participants underwent 24-h assessments of energy expenditure and respiratory quotient (RQ) in a whole-room calorimeter during energy balance (EBL; 50% carbohydrate, 30% fat) and then during 24-h fasting and three 200% overfeeding diets in a crossover design. Metabolic flexibility was defined as the change in 24-h RQ from EBL during fasting and standard (STOF: 50% carbohydrate, 30% fat), high-fat (HFOF: 60% fat, 20% carbohydrate), and high-carbohydrate (HCOF: 75% carbohydrate, 5% fat) overfeeding diets. Free-living weight change was assessed after 6 and 12 months. Compared to EBL, RQ decreased on average by 9% during fasting and by 4% during HFOF, while increasing by 4% during STOF and by 8% during HCOF. Smaller decrease in RQ, reflecting smaller increase in lipid oxidation rate, during HFOF but not during other diets, predicted greater weight gain at both 6 and 12 months. An impaired metabolic flexibility to acute, high-fat overfeeding can identify individuals prone to gain weight, indicating that the individual capacity to oxidize dietary fat is a metabolic determinant of weight change.

Clinical Trial Registration Number: NCT00523627

Introduction

Prolonged daily energy intake exceeding energy expenditure (EE) leads to an increase in body weight; however, even under highly controlled settings, some individuals are more prone to gain weight than others during sustained overfeeding (1; 2) or, conversely, some individuals are more resistant to weight loss during caloric restriction (3; 4) despite comparable dietary conditions among individuals. These results suggest that the individual metabolic response to over/under-feeding may partly determine the susceptibility to bodyweight change in conditions of persistent energy imbalance (5). Daily energy balance, which is the difference between 24-h energy intake and EE, is also reflective of macronutrient balances, i.e., proteins, fats, carbohydrates, which in turn play a major role in bodyweight regulation (6; 7). While carbohydrate and protein balances are usually met over 24 hours in conditions of total daily energy balance (8), there is a greater variability both in the time needed and in the extent to achieve fat balance, implying that fat balance may represent the strongest contributor to total daily energy balance (8). As positive fat balance leads to fat storage and, ultimately, to increase in body fat mass (9), a reduced fat oxidation that favors positive fat (thus, total) energy balance, may indicate a greater predisposition to weight gain over time (10).

The respiratory quotient (RQ) is a measured index of macronutrient preference for oxidation, which in turn influences macronutrient balances. In humans, the association of RQ measured during energy balance and future weight change is mixed with studies showing that a higher RQ is a determinant of future weight gain (11-15), and others studies showing no such association (16-18). Oxidation rates measured during energy balance and eucaloric feeding with a standard diet may not be as indicative as the individual's ability to switch fuel for oxidation in response to diets of altered macronutrient proportions (e.g., high-carbohydrate vs. high-fat diets). This fuel

switching, or "*metabolic flexibility*" (15-18), may be more informative of susceptibility to weight change (19; 20), particularly in settings of positive energy balance (i.e., overfeeding) leading to weight gain. Prior studies assessed metabolic flexibility using the hyperinsulinemic-euglycemic glucose clamp technique (21-24), which allows for precise measurement of metabolic flexibility specific to glucose. These studies showed that glucose disposal rate is a determinant of glucose inflexibility (25) and that greater metabolic flexibility to glucose during the clamp is associated with decreased metabolic flexibility to lipids during fasting (26). Although carefully conducted, studies assessing metabolic flexibility during the glucose clamp may not be physiologically reflective of daily energy intake patterns that include solid diets with a varying mixture of carbohydrates, fats, and proteins. Whether the magnitude of metabolic flexibility to 24-h fasting or overfeeding diets with varying macronutrient proportions in one individual predicts his/her predisposition to weight change is unknown.

The aim of the current study was to investigate metabolic flexibility to 24-h dietary interventions including fasting and three normal-protein overfeeding diets (standard, high-carbohydrate, and high-fat) in healthy participants with normal glucose regulation. We quantified the metabolic flexibility (ΔRQ) as the dietary-related change in 24-h RQ compared to the 24-h RQ as precisely measured during weight stability and energy balance. We hypothesized that an impaired metabolic flexibility during these extreme dietary interventions may increase the individual predisposition to weight gain over time.

Research Design and Methods

Study Participants

Study volunteers between the ages of 18-55 were recruited from 2008 through 2017 from the Phoenix, AZ metropolitan area to participate in an ongoing clinical trial whose primary aims were

to assess the metabolic responses to acute dietary interventions in relation to free-living weight change (ClinicalTrials.gov identifier: NCT00523627). Prior to admission to the clinical research unit, recruited participants had to be weight stable for 6 months and deemed healthy by medical history, physical examination, and basic laboratory measurements. Once admitted, participants were placed on a standard weight maintenance diet (WMD) calculated from previously derived equations based on sex and weight (27), consisting of 50% carbohydrate, 30% fat, and 20% protein. After 3 days on this WMD, an oral glucose tolerance test (OGTT) was performed and only participants with normal glucose regulation based on the ADA criteria (28) continued the study (Supplemental Figure 4). Plasma glucose concentrations were measured by the glucose oxidase method (Beckman Glucose Analyzer 2; Beckman Instruments, Brea, CA).

The WMD was consumed on all days when 24-h EE was not measured and volunteers' physical activity was limited to activities on the unit such as watching television, playing pool, etc. Body composition was measured by dual x-ray absorptiometry scan (DXA, Prodigy encore 2003 software version 7.53.002, GE Lunar Radiation Corp, Madison, Wisconsin). Fat mass (FM) and fat free mass (FFM) were calculated from the measured body fat percentage (PFAT) and weight as following: FM=weight×PFAT/100; FFM=weight–FM). Body weight was measured daily, on a precision scale every morning upon awakening after an overnight fast, to assure weight was within ±1% of the admission weight and the WMD was adjusted to maintain weight stability throughout the admission stay. The mean coefficient of variation (CV) for body weight during the entire admission was <1% (0.6±0.3%, mean±SD). All participants were fully informed of the nature of the study and provided written informed consent before participation. The Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases approved this experimental protocol.

Measurements of 24-h RQ and EE during Dietary Interventions

Twenty-four-hour EE and RQ were measured inside a whole-room indirect calorimeter (metabolic chamber) as previously described in detail (29; 30). Participants entered the metabolic chamber the day following the OGTT and one hour after consuming breakfast at 07:00 and subsequent meals were given inside the metabolic chamber at 11:00, 16:00, and 19:00. During the 24-h fasting, no food was provided following previous day dinner but participants were permitted to drink water and non-caloric, non-caffeinated beverages. Both O_2 consumption and CO_2 production during each 24-h EE assessment were measured every minute, averaged and extrapolated to 24 hours, and used to calculate the 24-h RQ, as an index of fat-to-carbohydrate oxidation (29). Quality control tests of the metabolic chamber were performed monthly during the period of this study by burning instrument grade propane with average recoveries of predicted O_2 consumption and CO_2 production equal to 98.8% (CV=3.6%) and 98.3% (CV=3.4%), respectively. Ambient temperature inside the metabolic chamber was set to 24°C, monitored every minute, and averaged 23.9±1.3˚C. Spontaneous physical activity (SPA) was measured by a radar system inside the metabolic chamber and expressed as a percentage of time when motion was detected. Urine was collected over the 24 hours to measure urea nitrogen excretion to calculate the non-protein RQ and substrate oxidation rates, i.e., lipid (LIPOX), carbohydrate (CARBOX), and protein oxidation rates, as previously described (8; 31). Fasting plasma samples were collected prior to entering the metabolic chamber and frozen to −70°C for later measurements. Non-esterified fatty acids (NEFA) were measured using the kit from Fujifilm-Wako Diagnostics (Mountain View, CA, USA) at the NIDDK clinical core laboratory in Bethesda, MD. Intra-assay CV was 4.4% and inter-assay CV was 5.8%.

To precisely attain metabolic measurements in conditions of energy balance inside the metabolic chamber, two sequential, eucaloric assessments of 24-h EE were performed. During the first eucaloric assessment, participants were fed the WMD reduced by 20% to account for reduced movement inside the metabolic chamber. The calculated 24-h EE from this initial eucaloric assessment was used as the prescribed 24-h energy intake for the second session. The 24-h EE and RQ measured in this second eucaloric session were considered the baseline measurements obtained in conditions close to perfect energy balance with standard eucaloric feeding (EBL, 50% carbohydrate, 30% fat, and 20% protein). The subsequent dietary interventions, fasting and the three overfeeding diets, were given in random order with 3-4 days on the WMD between each assessment. The value of 24-h EE obtained during EBL was doubled and used as the prescribed 24-h energy intake for the overfeeding sessions (i.e., 200% of energy requirements). All overfeeding diets contained 20% protein but varied in carbohydrate and fat contents as follows: 1) standard overfeeding (STOF) with 50% carbohydrate and 30% fat; 2) high-fat overfeeding (HFOF) with 60% fat and 20% carbohydrate; and, 3) high-carbohydrate overfeeding (HCOF) with 75% carbohydrate and 5% fat. The metabolic kitchen weighed any remaining food for each overfeeding session in the metabolic chamber to calculate the actual food intake consumed. Only overfeeding sessions where 95% of the food provided was actually eaten were included in the analysis of 24-h RQ.

Follow-up visits

Following completion of the inpatient study, study participants were discharged from the clinical research unit and invited back after six months (median follow-up time: 6.6 months, IQR: 6.0-6.9) and one year (median: 12.9 months, IQR: 12.1-13.5) for one-day outpatient visits to obtain

measures of free-living weight change. Participants were not provided with any counseling regarding lifestyle changes.

Statistical Analysis

Target sample sizes of 58 and 46 subjects with available data at 6-month and 1-year follow-up, respectively, were calculated before data analyses to provide 80% power (alpha=0.05) to detect correlation coefficients ≥0.35 between the changes in 24-h RQ during overfeeding or fasting at baseline and bodyweight change at follow-up (primary endpoint). Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Data are presented as mean±SD or mean with 95% confidence interval (CI). Differences by sex were evaluated by Student's unpaired *t*-test and comparisons between dietary interventions were performed by mixed models accounting for repeated measurements using a compound symmetry covariance structure to estimate the intraclass correlation coefficient (ICC). Paired *t*-tests were used to assess the changes in 24-h RQ from energy balance conditions (ΔRQ, metabolic flexibility) during the dietary interventions. The Pearson's correlation coefficient was used to quantify the relationships between ΔRQ and changes in body weight at each follow-up visit. Multivariable linear models were calculated to assess the effect of ΔRQ on weight change after adjusting for sex, age, and ethnicity. Similar analyses were done for CARBOX and LIPOX. Sensitivity analyses using changes in non-protein RQ in place of 24-h RQ, as well as accounting for baseline weight using the ANCOVA approach, provided similar results (data not shown).

Data and Resource. The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Results

The baseline characteristics of the study group are presented in Table 1. The two consecutive eucaloric assessments allowed for precise determination of 24-h RQ in conditions very close to perfect energy balance with an average 24-h deviation of 25±71 kcal/day (range: –6% to 9%). The CV of 24-h RQ measurements between the two consecutive EBL assessments was 1.6±1.2% with a high intra-individual consistency (ICC=0.76, $p<0.001$).

The average 24-h RQ during energy balance was 0.86, a value very close to the expected food quotient (FQ=0.87) (32). Despite being on average approximately equal to the FQ, the 24-h RQ showed a large inter-individual variability (SD=0.03) which was unrelated to sex (p=0.37), age (p=0.52), ethnicity (p=0.51), SPA inside the metabolic chamber (p=0.76), or any measures of body size or adiposity including BMI (p=0.66, Supplemental Figure 1A), or PFAT (p=0.73, Supplemental Figure 1B), FM (p=0.59), FFM (p=0.54), waist circumference (p=0.96), or waist-to-thigh ratio (p=0.36). Similarly, there were no associations between 24-h RQ and the deviation from 24-h energy balance inside the metabolic chamber (p=0.41, Supplemental Figure 1C) or the rate of body weight change during the first days of admission (p=0.12, Supplemental Figure 1D).

Metabolic Flexibility to 24-h Fasting and Overfeeding Diets

The time courses of the average 24-h RQ are shown in Figure 1, while the metabolic measurements during each dietary intervention are reported in Table 2 and Figure 2. Overall, RQ increased following meal times in the feeding interventions, remained elevated during the day while decreasing in all dietary interventions at night to varying degrees depending on the diet. The values of 24-h RQ across the dietary interventions showed a strong intra-individual consistency (ICC=0.66, $p<0.001$), such that a lower (or higher) 24-h RQ during fasting was associated with lower (or higher) 24-h RQ during feeding and overfeeding, respectively (Figure 3A and

Supplemental Figure 2). This is graphically shown in Figure 2A, where "carbohydrate oxidizer" individuals with the top-5 highest RQ values during energy balance (red circles) showed above-average values for 24-h RQ during all dietary interventions. Similarly, this was the case for "fat oxidizer" individuals with the bottom-5 lowest RQ during energy balance (blue circles) showing below-average values for 24-h RQ during other diets (Figure 2A). Although the macronutrient composition of the dietary interventions was the main determinant of 24-h RQ explaining approximately ⅔ of its variance (67%, $p<0.001$), after accounting for differences among diets, the intra-individual component of 24-h RQ explained an additional 1/5 of its variance (21%, $p<0.001$) (Figure 2B).

Compared to energy balance conditions, the 24-h RQ decreased in all participants during fasting by an average of 8.6% (CI: –9.3 to –7.9%, $\Delta RQ= –0.07\pm0.03$, $p<0.001$) (Figure 3A-3B) of the 24-h RQ during energy balance. Similarly, the average 24-h RQ decreased by 4% (CI: –5 to –3%, $\Delta RQ= –0.03\pm0.03$, $p<0.001$) during high-fat overfeeding, indicating a greater proportion of lipids were oxidized during these two dietary conditions (Table 2, Figure 3C). Conversely, during the overfeeding diets containing a higher carbohydrate content (>50%), on average 24-h RQ increased during standard overfeeding by 4% (CI: 3.0 to 4.3%, $\Delta RQ= 0.03\pm02$, $p<0.001$) and increased the highest during high-carbohydrate overfeeding by 8% (CI: 7 to 9%, $\Delta RQ= 0.07\pm0.03$, $p<0.001$), reflecting increased oxidation of carbohydrates during these two diets (Figure 3D).

The substrate oxidation rates (LIPOX and CARBOX) during each dietary intervention are shown in Table 2 and Figure 2C-2D. Compared to energy balance conditions, on average LIPOX increased by 66% (CI: 58 to 74%) during fasting and by 31% (CI: 29 to 69%) during high-fat overfeeding. Conversely, LIPOX decreased during standard overfeeding by 32% (CI: –39 to –25%) and more so during high-carbohydrate overfeeding by 69% (CI: –79% to –59%) (Figure

3C). Concordant with changes in 24-h RQ, CARBOX increased during standard overfeeding by 35% (CI: 30 to 40%) and during high-carbohydrate overfeeding by 72% (CI: 64 to 79%), whereas CARBOX decreased by 19% (CI: –25 to –12%) during high-fat overfeeding with the largest decrease (–55%, CI: –60 to –49%) observed during 24-h fasting (Figure 3D).

A higher fasting NEFA concentration was associated with lower 24-h RQ during energy balance ($r=-0.35$, $p=0.005$, Figure 4A) and high-fat overfeeding ($r=-0.43$, $p=0.001$, Figure 4B). Similarly, fasting NEFA concentrations were positively associated with LIPOX both during energy balance ($r=0.35$, $p=0.005$, Figure 4C) and high-fat overfeeding ($r=0.44$, $p=0.001$, Figure 4D). There was a weak inverse correlation between fasting NEFA and CARBOX during high-fat overfeeding ($r=-0.30$, $p=0.03$), whereas there was no association with CARBOX during energy balance ($p=0.18$).

Metabolic Flexibility and Future Weight Change

Follow-up data for free-living weight change after 6 months were available in 58 individuals and in 46 individuals after 1 year (Table 1). Compared to the whole cohort (n=79), the subgroups with follow-up data did not differ in their baseline characteristics including 24-h RQ (Supplemental Table 1). On average, participants were weight stable at six months (mean weight change=0.8 kg, $p=0.17$ versus zero), despite a large inter-individual variability (SD= 4.3 kg, range: –7.0 to 11.2 kg) unrelated to sex ($p=0.80$), age ($p=0.14$), ethnicity ($p=0.90$), or initial body weight ($p=0.32$). Similarly, there was a large variability in free-living weight change after one year (SD=5.3 kg, range: –9.3 to 11.0 kg) with participants remaining on average weight-stable (mean=0.4 kg, $p=0.63$ versus zero).

A smaller decrease in 24-h RQ (ΔRQ) during high-fat overfeeding, but not during the other dietary interventions (all p>0.15), was associated with weight gain both at six months (r=0.32, p=0.02, r^2=10%, Figure 5A) and at one year (r=0.39, p=0.01, r^2=15%, Figure 5C). After adjustment for age, sex, and ethnicity, the ΔRQ during high-fat overfeeding was an independent determinant of weight change at six months (β=2.1 kg per 0.05-change in 24-h RQ during HFOF, p=0.02, total r^2=19%) and at one year (β=2.6 kg, p=0.02, total r^2=46%). The change in 24-h EE during high-fat overfeeding did not predict weight change at any follow-up (both p>0.30) and the results for ΔRQ during high-fat overfeeding and weight change were still significant after adjustment for the concomitant change in 24-h EE (data not shown).

When examining the changes in macronutrient oxidation rates, a greater increase in LIPOX during high-fat overfeeding was associated with more weight loss at 6-month (r=–0.36, p=0.008, Figure 5B) and 1-year (r=–0.40, p=0.009, Figure 5D) follow-up. After adjustment for age, sex, and ethnicity, ΔLIPOX during high-fat overfeeding was still a determinant of weight loss after 6 months (β=–1.5 kg per 250 kcal/day increase in LIPOX during HFOF, p=0.02) and 1 year (β=–2.1 kg, p=0.02). Fat intake and fat balance (=fat intake−LIPOX) during 24-h high-fat overfeeding were not associated with weight change at any follow-up visit (both p>0.55).

There were no associations between ΔRQ and 6-month weight change during fasting (p=0.73, Supplemental Figure 3), standard (p=0.87) and high-carbohydrate (p=0.29) overfeeding. Similar results were observed at the 1-year follow-up, such that there were no associations between ΔRQ and weight change during 24-h fasting (p=0.91, Supplemental Figure 3), standard (p=0.17), and high-carbohydrate (p=0.20) overfeeding.

Discussion

In the present study, we evaluated metabolic flexibility (ΔRQ), defined as the change in 24-h RQ from energy balance conditions to extreme dietary interventions including 24-h fasting and 200% overfeeding with high-fat or a high-carbohydrate content, to assess whether the extent of ΔRQ is a metabolic determinant of free-living weight change. The 24-h RQ measurements obtained during these acute dietary interventions were predominantly dependent on macronutrient composition, which explained approximately ⅔ of 24-h RQ variance among diets. However, there was still a strong intra-individual reliance for fuel oxidation observed in each dietary condition, such that individuals more relying on a specific substrate for oxidation (e.g., carbohydrates or lipids) manifested this preference in any dietary conditions. We demonstrated that inter-individual variability in ΔRQ, specifically, a reduced metabolic flexibility to high-fat overfeeding, predicted future weight gain both at six months and one year, and this was due to an impairment in the ability to switch to lipid oxidation in a setting of surplus of dietary fats.

Cross-sectional studies have shown that a higher RQ during energy balance or fasting, reflecting a lower fat-to-carbohydrate oxidation, leads to future weight gain (11-14; 27), although other studies failed to find such association (17; 18). In free-living conditions, energy balance is likely transient, thus investigating change in fuel selection during acute over and underfeeding is important. Concordant with the observations made during energy balance that higher RQ, indicative of lower fat oxidation, predicts greater weight gain, we now show that during acute conditions of energy surplus the metabolic inflexibility to lipids is also a determinant of weight gain. Specifically, individuals who did not decrease their RQ as much in a setting of high-fat overfeeding, that is, those who were not able to increase their fat oxidation in a setting of dietary fat surplus, gained more weight at follow-up. Concordant with our current results, previous studies

reported that, in obesity-prone individuals, nighttime RQ is higher after 3 days of overfeeding (33) and measures of metabolic inflexibility predicts long-term weight gain (34). The individual ability to be metabolically flexible, which is the capacity to readily adjust substrate oxidation in response to fuel availability (21), may be postulated to be advantageous in the current obesogenic environment where food, specifically energy-dense high-fat food, is readily available.

The mechanism by which metabolic inflexibility to fats leads to greater weight gain could be through decreased adipocyte lipolysis and, ultimately, impaired capacity to increase LIPOX. In those individuals who are metabolically inflexible to dietary fats, lipolysis may increase to a smaller degree during a high-fat diet (35). We have previously demonstrated that lower rates of *in-vitro* lipolysis is associated with higher 24-h RQ and lower LIPOX during eucaloric feeding, and these individuals with reduced lipolysis gain more weight at follow-up due to an increase in fat mass (36). Supportive of a causal role for lipolysis in determining the degree of metabolic flexibility, we found that higher fasting concentrations of NEFA, a product of fat cell lipolysis (37) and regulators of LIPOX (38), were associated with lower 24-h RQ and greater LIPOX both during eucaloric feeding and high-fat overfeeding. Concordant with our current findings, lower nocturnal concentrations of plasma NEFA during high-fat overfeeding predict weight gain in obesity-prone individuals (34). Altogether, these results strongly point to a key role for lipolysis in obesity and bodyweight regulation (39; 40).

To obtain accurate measurements of metabolic flexibility during each diet, we utilized a carefully controlled measurement of 24-h RQ during energy balance and eucaloric, standard feeding. Prior to this baseline assessment of 24-h RQ, participants were on a weight-maintaining diet for five days and two metabolic measurements inside the metabolic chamber were employed to obtain a baseline RQ value in conditions of almost perfect energy balance and weight maintenance. We

evaluated the determinants of baseline RQ and found no associations with body size, body composition, deviations from 24-h energy balance in the metabolic chamber, or prior fluctuations in body weight, despite these variables being found to be determinants of RQ during energy balance in previous studies (11; 41). This was likely due to more controlled conditions characterized by sequential 24-h EE assessments that led to more accurate metabolic measurements within a 10%-range of expected 24-h energy balance (=intake−EE) as opposed to a wider range (±30%) that has been previously reported (11). More importantly, we only evaluated individuals with normal glucose regulation (28), therefore eliminating the confounding effect of insulin resistance which has previously been shown to be a determinant of the metabolic inflexibility to glucose (22; 25).

We used extremes of dietary interventions by precisely designing the overfeeding diets to provide twice the individual-specific daily energy needs, so that we could maximize the extent of metabolic flexibility for both RQ and substrate oxidation rates. During these 24-h dietary interventions, there was an expected increase in RQ (shift to carbohydrate oxidation) during standard and high-carbohydrate overfeeding, but we also demonstrated a decrease in RQ (greater lipid oxidation) during fasting and high-fat overfeeding. The rapid change in RQ in response to 24-h overfeeding observed in our current study is in contrast to a recent study showing no change in RQ after three days of overfeeding a diet with a composition similar to our standard overfeeding diet (34), although the degree of overfeeding in our current study (200% of energy needs) was much higher than that (140%) of this previous study (34). Interestingly, three days of eucaloric feeding with a high dietary fat content (50%) - similar to our high-fat overfeeding diet (60%) - induced a decrease in 24-h RQ down to an average value of 0.83 (42), which is exactly the same value obtained in our current study during high-fat overfeeding (Table 2). These results strongly support the use of short-

term (24 hours) but extreme (200% of eucaloric requirements) dietary interventions to obtain valid measures of metabolic flexibility that can be obtained in less extreme but prolonged dietary conditions typical of free-living settings. Importantly, as previously shown in a subset of 14 subjects undergoing repeat assessments of energy metabolism inside the whole-room calorimeter(43), measures of 24-h RQ and EE during fasting, eucaloric feeding, and balanced overfeeding were highly consistent within an individual (CV<5%), indicating high reproducibility of metabolic flexibility during these acute dietary interventions.

Although diet explained most of the variance in 24-h RQ among diets, we found a subject-specific reliance for macronutrient oxidation during these dietary interventions, which is independent of body habitus and macronutrient proportions in the diet as we have previously shown (44). Thus, the substantial variability in metabolic flexibility to acute overfeeding and fasting also has a strong intra-individual component, which is indicative of the propensity to future weight gain and it is independent of body size and the concomitant changes in 24-h EE during these dietary interventions. The extent of metabolic flexibility to change in diets is likely to be genetically determined given the significant heritability of 24-h RQ quantified in family studies of Caucasians (45) and American Indians of southwestern heritage (11).

Although our dietary interventions act to create short-term energy imbalance are not necessarily normal physiological or habitual conditions, we propose these interventions may constitute an important tool to quantify the propensity to weight gain by acute dietary challenges that can uncover informative metabolic responses. In our carefully controlled setting, we obtained 24-h measures of substrate oxidation in conditions of energy surplus (overfeeding) and energy deficit (fasting) to assess whether these metabolic changes are indicative of the propensity to weight gain to provide insight into the pathogenesis of obesity.

The major limitation of our study is the lack of formal assessments of free-living energy intake or physical activity in the follow-up period. Yet, participants were recruited to be weight stable for at least six months before baseline admission and, on average, were also weight stable at each follow-up visit, suggesting that there were no substantial changes in physical activity or diet in this time period that might have confounded our results. While the strength of the relationship between impaired metabolic flexibility to high-fat overfeeding and weight gain explained up to ~15% of the inter-individual variance in future weight change, this estimate can be considered a large effect size for a single metabolic parameter given that other metabolic determinants of weight change explain 5-10% of its variance (5).

In summary, we demonstrated that the 24-h RQ responses to different diets with varying macronutrient content are highly consistent within an individual, such that the individual capacity of oxidizing dietary fats is manifested under any dietary regimen, thus indicating that metabolic flexibility is an intrinsic metabolic characteristic of a given individual. Importantly, differences in the degree of metabolic flexibility to high-fat overfeeding across subjects is indicative of the individual propensity to future weight gain. In conclusion, in healthy individuals with normal glucose regulation, we identified a novel metabolic phenotype in which the impaired ability to switch fuels in response to an acute, high-fat overload is a determinant of greater weight gain. Specifically, individuals that are more metabolically inflexible to lipids may gain more weight over time than individuals who can readily adjust their macronutrient oxidation to favor lipid oxidation in a setting of fat surplus. Our data indicate that future interventions targeting fuel selection by making people more “metabolically flexible” to dietary fats may help prevent or treat individuals with obesity.

Acknowledgements

The authors thank the study volunteers for their participation and the clinical research staff for their care of the volunteers.

Prior presentation. This study was presented in abstract form at ObesityWeek 2018, The Obesity Society annual meeting, Nashville, Tennessee, November 11-15, 2018.

Funding. This research was supported by the Intramural Research Program of The National Institutes of Health, The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The work of P.P. was supported by Programma per Giovani Ricercatori "Rita Levi Montalcini" granted by Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR).

Author Contributions. B.B. (data collection, data analysis, data interpretation, literature review, and drafting the manuscript), K.L.V. (data collection, data interpretation), T. H. (data analysis and interpretation), T.A. (data interpretation), MW (NEFA measurements and data interpretation), C.B. (data interpretation), J.K. (study design, data interpretation), P.P. (study design, data analysis, data interpretation, literature review, and drafting of the manuscript). All authors read and approved the final manuscript. Dr. Paolo Piaggi is the guarantor of this work, and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure summary. The authors have nothing to disclose.

References

1. Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, Theriault G, Dussault J, Moorjani S, Pinault S, Fournier G: The response to long-term overfeeding in identical twins. The New England journal of medicine 1990;322:1477-1482

2. Diaz EO, Prentice AM, Goldberg GR, Murgatroyd PR, Coward WA: Metabolic response to experimental overfeeding in lean and overweight healthy volunteers. The American journal of clinical nutrition 1992;56:641-655
3. Reinhardt M, Thearle MS, Ibrahim M, Hohenadel MG, Bogardus C, Krakoff J, Votruba SB: A Human Thrifty Phenotype Associated With Less Weight Loss During Caloric Restriction. Diabetes 2015;64:2859-2867
4. Leibel RL, Rosenbaum M, Hirsch J: Changes in energy expenditure resulting from altered body weight. The New England journal of medicine 1995;332:621-628
5. Piaggi P: Metabolic Determinants of Weight Gain in Humans. Obesity 2019;27:691-699
6. Flatt JP: Importance of nutrient balance in body weight regulation. Diabetes/metabolism reviews 1988;4:571-581
7. Flatt JP: Dietary fat, carbohydrate balance, and weight maintenance. Annals of the New York Academy of Sciences 1993;683:122-140
8. Abbott WG, Howard BV, Christin L, Freymond D, Lillioja S, Boyce VL, Anderson TE, Bogardus C, Ravussin E: Short-term energy balance: relationship with protein, carbohydrate, and fat balances. The American journal of physiology 1988;255:E332-337
9. Galgani J, Ravussin E: Energy metabolism, fuel selection and body weight regulation. Int J Obes (Lond) 2008;32 Suppl 7:S109-119
10. Astrup A, Raben A, Buemann B, Toubro S: Fat metabolism in the predisposition to obesity. Annals of the New York Academy of Sciences 1997;827:417-430
11. Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF, Swinburn BA, Knowler WC, Bogardus C, Ravussin E: Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. The American journal of physiology 1990;259:E650-657
12. Marra M, Scalfi L, Covino A, Esposito-Del Puente A, Contaldo F: Fasting respiratory quotient as a predictor of weight changes in non-obese women. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 1998;22:601-603
13. Seidell JC, Muller DC, Sorkin JD, Andres R: Fasting respiratory exchange ratio and resting metabolic rate as predictors of weight gain: the Baltimore Longitudinal Study on Aging. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 1992;16:667-674
14. Piaggi P, Thearle MS, Bogardus C, Krakoff J: Lower energy expenditure predicts long-term increases in weight and fat mass. The Journal of clinical endocrinology and metabolism 2013;98:E703-707
15. Ellis AC, Hyatt TC, Hunter GR, Gower BA: Respiratory quotient predicts fat mass gain in premenopausal women. Obesity (Silver Spring) 2010;18:2255-2259
16. Katzmarzyk PT, Perusse L, Tremblay A, Bouchard C: No association between resting metabolic rate or respiratory exchange ratio and subsequent changes in body mass and fatness: 5-1/2 year follow-up of the Quebec family study. European journal of clinical nutrition 2000;54:610-614
17. Roberts SB: Abnormalities of energy expenditure and the development of obesity. Obesity research 1995;3 Suppl 2:155s-163s
18. Weinsier RL, Nelson KM, Hensrud DD, Darnell BE, Hunter GR, Schutz Y: Metabolic predictors of obesity. Contribution of resting energy expenditure, thermic effect of food, and fuel utilization to four-year weight gain of post-obese and never-obese women. The Journal of clinical investigation 1995;95:980-985

19. Astrup A: The relevance of increased fat oxidation for body-weight management: metabolic inflexibility in the predisposition to weight gain. Obesity reviews : an official journal of the International Association for the Study of Obesity 2011;12:859-865
20. Lam YY, Ravussin E: Indirect calorimetry: an indispensable tool to understand and predict obesity. Eur J Clin Nutr 2017;71:318-322
21. Kelley DE, Goodpaster B, Wing RR, Simoneau JA: Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 1999;277:E1130-1141
22. Kelley DE, Mandarino LJ: Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 2000;49:677-683
23. Galgani JE, Heilbronn LK, Azuma K, Kelley DE, Albu JB, Pi-Sunyer X, Smith SR, Ravussin E, Look AARG: Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. Diabetes 2008;57:841-845
24. Wohl P, Wohl P, Girman P, Pelikanova T: Inflexibility of energy substrate oxidation in type 1 diabetic patients. Metabolism: clinical and experimental 2004;53:655-659
25. Galgani JE, Moro C, Ravussin E: Metabolic flexibility and insulin resistance. American journal of physiology Endocrinology and metabolism 2008;295:E1009-1017
26. Galgani J.E. F-VR, Santos J.L., Olmos P.: High metabolic flexibility in a glucose clamp relates with low metabolic flexibiilty to fasting. In *ObesityWeek2018* Nashville, TN, 2018
27. Pannacciulli N, Salbe AD, Ortega E, Venti CA, Bogardus C, Krakoff J: The 24-h carbohydrate oxidation rate in a human respiratory chamber predicts ad libitum food intake. The American journal of clinical nutrition 2007;86:625-632
28. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37 Suppl 1:S81-90
29. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C: Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. The Journal of clinical investigation 1986;78:1568-1578
30. Schlogl M, Piaggi P, Pannacciuli N, Bonfiglio SM, Krakoff J, Thearle MS: Energy Expenditure Responses to Fasting and Overfeeding Identify Phenotypes Associated With Weight Change. Diabetes 2015;64:3680-3689
31. Jequier E, Acheson K, Schutz Y: Assessment of energy expenditure and fuel utilization in man. Annual review of nutrition 1987;7:187-208
32. Jequier E, Acheson K, Schutz Y: Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987;7:187-208
33. Schmidt SL, Kealey EH, Horton TJ, VonKaenel S, Bessesen DH: The effects of short-term overfeeding on energy expenditure and nutrient oxidation in obesity-prone and obesity-resistant individuals. International journal of obesity (2005) 2013;37:1192-1197
34. Rynders CA, Pereira RI, Bergouignan A, Kealey EH, Bessesen DH: Associations Among Dietary Fat Oxidation Responses to Overfeeding and Weight Gain in Obesity-Prone and Resistant Adults. Obesity (Silver Spring) 2018;26:1758-1766
35. Kather H, Wieland E, Scheurer A, Vogel G, Wildenberg U, Joost C: Influences of variation in total energy intake and dietary composition on regulation of fat cell lipolysis in ideal-weight subjects. J Clin Invest 1987;80:566-572
36. Frankl J, Piaggi P, Foley JE, Krakoff J, Votruba SB: In Vitro lipolysis is associated with whole-body lipid oxidation and weight gain in humans. Obesity 2017;25:207-214
37. Stich V, Berlan M: Physiological regulation of NEFA availability: lipolysis pathway. The Proceedings of the Nutrition Society 2004;63:369-374

38. Lillioja S, Bogardus C, Mott DM, Kennedy AL, Knowler WC, Howard BV: Relationship between insulin-mediated glucose disposal and lipid metabolism in man. Journal of Clinical Investigation 1985;75:1106
39. Arner P, Andersson DP, Backdahl J, Dahlman I, Ryden M: Weight Gain and Impaired Glucose Metabolism in Women Are Predicted by Inefficient Subcutaneous Fat Cell Lipolysis. Cell metabolism 2018;28:45-54 e43
40. Arner P: Human fat cell lipolysis: biochemistry, regulation and clinical role. Best Pract Res Clin Endocrinol Metab 2005;19:471-482
41. Weyer C, Snitker S, Rising R, Bogardus C, Ravussin E: Determinants of energy expenditure and fuel utilization in man: effects of body composition, age, sex, ethnicity and glucose tolerance in 916 subjects. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 1999;23:715-722
42. Galgani JE, de Jonge L, Most MM, Bray GA, Smith SR: Effect of a 3-day high-fat feeding period on carbohydrate balance and ad libitum energy intake in humans. International journal of obesity (2005) 2010;34:886-891
43. Thearle MS, Pannacciulli N, Bonfiglio S, Pacak K, Krakoff J: Extent and determinants of thermogenic responses to 24 hours of fasting, energy balance, and five different overfeeding diets in humans. The Journal of clinical endocrinology and metabolism 2013;98:2791-2799
44. Vinales KL, Schlogl M, Piaggi P, Hohenadel M, Graham A, Bonfiglio S, Krakoff J, Thearle MS: The Consistency in Macronutrient Oxidation and the Role for Epinephrine in the Response to Fasting and Overfeeding. The Journal of clinical endocrinology and metabolism 2017;102:279-289
45. Toubro S, Sorensen TI, Hindsberger C, Christensen NJ, Astrup A: Twenty-four-hour respiratory quotient: the role of diet and familial resemblance. The Journal of clinical endocrinology and metabolism 1998;83:2758-2764

Table 1. Demographic, anthropometric, and metabolic characteristics of the study group.

	Total (*n*=79)	Males (*n*=64)	Females (*n*=15)
Ethnicity	16BL/15HI/23NA/25WH	11BL/12HI/20NA/21WH	5BL/3HI/3NA/4WH
Age (y)	36.8±10.5 (18.2, 55.8)	37.3±10.8 (18.2, 55.8)	34.4±9.5 (20.6, 47.0)
Body weight (kg)	78.9±8.0 (52.8, 127.1)	80.6±13.1 (52.8, 127.1)	71.7±14.6 (54.1, 107.5)*
Height (cm)	173.0±8.0 (156.5, 196.4)	175.0±7.2 (156.5, 196.4)	164.2±4.8 (156.8, 170.0)*
BMI (kg/m^2)	26.3±4.2 (18.3. 44.0)	26.3±4.2 (18.3, 44.0)	26.5±4.8 (20.7, 39.2)
Body fat (%)	27.8±9.7 (6.9, 53.8)	25.3±8.2 (6.9, 42.6)	38.5±7.9 (24.2, 53.8)*
FM (kg)	22.5±10.2 (4.9, 54.3)	21.1±9.4 (4.9, 54.4)	28.5±11.7 (13.6, 54.3)*
FFM (kg)	56.4±9.4 (34.2, 79.4)	59.5±7.3 (43.4, 79.4)	43.1±4.1 (34.2, 53.2)*
Fasting glucose (mg/dL)	90.6±5.6 (77.0, 99.5)	90.7±5.7 (77.0, 99.5)	90.2±5.2 (79.5, 97.5)
2-h OGTT glucose (mg/dL)	105.1±19.1 (64, 138)	105.0±19.8 (64.0, 138.0)	105.7±16.7 (80.0, 132.0)
Fasting NEFA (mEq/L)	0.229±0.070 (0.128, 0.387)	0.229±0.071 (0.143, 0.387)	0.232±0.067 (0.128, 0.357)
24-h RQ (ratio)	0.86±0.03 (0.77, 0.93)	0.86±0.03 (0.77, 0.93)	0.86±0.03 (0.81, 0.91)
24-h EE (kcal/day)	2028±308 (1427, 2810)	2098±287 (1573, 2810)	1733±205 (1427, 2156)*
24-h energy intake (kcal/day)	2053±307 (1461, 2921)	2126±277 (1622, 2921)	1742±117 (1461, 2190)*
24-h energy balance (kcal/day)	25±71 (–159, 169)	28±71 (–159, 154)	10±67 (–81, 169)
24-h energy balance (%)	1.3±3.5 (–6.3, 8.8)	1.5±3.4 (–6.3, 7.5)	0.5±3.8 (–4.5, 8.8)
6 mo. weight change (kg) [a]	0.8±4.3 (–7.0, 11.2)	0.71±4.2 (–7.0, 11.2)	1.1±4.8 (–5.2, 10.7)
1 yr. weight change (kg) [b]	0.4±5.3 (–9.3, 11.0)	0.2±4.9 (–9.3, 10.3)	1.4±7.3 (–9.2, 11.0)

Data are presented as the mean±SD (minimum, maximum values). *P values for differences between males and females by Student's t test.

[a]: n = 58 with follow-up weight after 6 months. [b]: n = 46 with follow-up weight after 1 year.

Abbreviations: BL: Black; HI: Hispanic; NA: Native American; WH: White; BMI: body mass index; FM: fat mass; FFM: fat free mass; EE: energy expenditure; RQ: respiratory quotient.

Table 2. Measurements of 24-h RQ and substrate oxidation during each dietary intervention.

	Energy Balance (EBL) (*n*=79)	Fasting (FST) (*n*=75)	High-fat overfeeding (HFOF) (*n*=68)	Standard overfeeding (STOF) (*n*=64)	High-carbohydrate overfeeding (HCOF) (*n*=71)
24-h RQ (ratio) [a]	0.86±0.03 (0.77, 0.93)	0.79±0.03 (0.71, 0.90)	0.83±0.04 (0.75, 0.90)	0.89±0.04 (0.82, 0.97)	0.93±0.04 (0.82, 1.0)
Change in 24-h RQ (ratio) [a,b]	N/A	–0.07±0.03 (–0.14, 0.01)	–0.03±0.03 (–0.14, 0.03)	0.03±0.02 (–0.03, 0.07)	0.07±0.03 (–0.06, 0.12)
Change in 24-h RQ (%)	N/A	–8.1 (–9.3 to 7.9)	–3.6 (–4.5 to –2.7)	3.6 (3.0 to 4.3)	8.1 (7.4 to 9.2)
Daytime RQ (ratio) [a]	0.87±0.03 (0.76, 0.94)	0.79±0.03 (0.74, 0.89)	0.83±0.04 (0.74, 0.90)	0.90±0.03 (0.83, 0.99)	0.95±0.04 (0.86, 1.02)
Nighttime RQ (ratio) [a]	0.82±0.04 (0.71, 0.94)	0.76±0.04 (0.67, 0.88)	0.82±0.04 (0.70, 0.90)	0.88±0.04 (0.77, 0.97)	0.91±0.06 (0.76, 1.06)
Non-protein 24-h RQ (ratio) [a]	0.87±0.04 (0.77, 0.97)	0.78±0.04 (0.69, 0.92)	0.84±0.05 (0.73, 0.97)	0.92±0.05 (0.82, 1.0)	0.97±0.06 (0.81, 1.1)
Change in non-protein RQ (ratio) [a,b]	N/A	–0.09±0.04 (–0.22, –0.01)	–0.04±0.04 (–0.16, 0.05)	0.05±0.03 (–0.04, 0.12)	0.09±0.05 (–0.07, 0.21)

24-h EE (kcal/day)	2028±308 (1427, 2810)	1859±268 [c] (1287, 2655)	2150±315 [d] (1555, 3114)	2228±365 [c,d] (1481, 3251)	2322±351 [c,d] (1573, 3229)
Change in 24-h EE (%) [a,b]	N/A	–7.5±4.4 (–19.3, 4.6)	7.9±5.4 (–7.2, 18.8)	10.9±5.4 (–0.7, 23.8)	14.2±6.0 (–0.27, 31.1)
CARBOX (kcal/day) [a]	945±230 (425, 1466)	435±204 (— 105, 1121)	768±275 (124, 1338)	1274±312 (287, 1884)	1630±381 (884, 2379)
Change in CARBOX (kcal/day) [a,b]	N/A	–517±205 (–1509, –170)	–177±253 (–1124, 253)	330±191 (–80, 844)	676±296 (–326, 1348)
Change in CARBOX (%)	N/A	–55 (–60 to –49)	–19 (–25 to –12)	35 (30 to 40)	72 (64 to 79)
LIPOX (kcal/day) [a]	690±292 (106, 1587)	1127±227 (351, 1838)	882±349 (304, 1934)	444±302 (–109, 1130)	205±375 (–613, 1492)
Change in LIPOX (kcal/day) [a,b]	N/A	455±233 (–88, 1267)	217±256 (–281, 927)	–222±198 (–716, 311)	–479±289 (–926, 632)
Change in LIPOX (%)	N/A	66 (58 to 74)	31 (22 to 40)	–32 (–39 to –25)	–69 (–79 to –59)
PROTOX (kcal/day)	368±98 (15, 573)	275±73 (26, 415)	467±157 [c,d] (4, 767)	476±115 [c,d] (175, 732)	456±132 [c,d] (3, 735)

Data are presented as mean±SD (minimum, maximum values) or mean (95% CI).

[a]: p <0.05 for all pairwise differences between diets. [b]: p <0.05 versus zero. [c]: p <0.05 versus EBL. [d]: p <0.05 versus FST.

Abbreviations: CARBOX, carbohydrate oxidation rate; EE, energy expenditure; LIPOX, lipid oxidation rate; N/A: not applicable; PROTOX, protein oxidation rate; RQ, respiratory quotient.

Figure legends

Figure 1. Twenty-four-hour Time Courses of RQ during Dietary Interventions.

The average time course of respiratory quotient (RQ) over 24 hours is plotted for each dietary intervention: eucaloric standard diet in energy balance (EBL, 50% carbohydrate and 30% fat) shown in black; 24-h fasting (FST) shown in blue; the high-fat overfeeding diet (HFOF, 20% carbohydrate and 60% fat) shown in green; the standard overfeeding diet (STOF, 50% carbohydrate and 30% fat) shown in orange; and the high-carbohydrate overfeeding diet (HCOF, 75% carbohydrate and 5% fat) shown in red. The three meals provided inside the metabolic chamber were: lunch at 11:00, dinner at 16:00, and a snack at 19:00. The total caloric intake of overfeeding diets was equal to twice the 24-h EE value obtained during energy balance.

Figure 2. Measures of 24-h RQ and Substrate Oxidation Rates during Dietary Interventions.

Error bars represent the mean±SD in each dietary condition. The 24-h respiratory quotient (RQ, panel A) is shown during each dietary intervention, where the red circles indicate “carbohydrate oxidizers”, that are the five individuals with the highest 24-h RQ during energy balance and standard eucaloric feeding (EBL). The “fat oxidizers” are denoted in blue and are identified as the five individuals with the lowest 24-h RQ during EBL. These same two groups of individuals are subsequently highlighted during each intervention in all panels: 24-h fasting (FST), standard overfeeding (STOF), high-fat overfeeding (HFOF), and high-carbohydrate overfeeding (HCOF), where the “carbohydrate oxidizers” remain above the mean 24-h RQ during each intervention and the “fat oxidizers” remain below the mean 24-h RQ despite being challenged with overfeeding. The determinants of 24-h RQ (panel B) are shown where two thirds of the total

variance of RQ measurements is explained by diet, one-fifth of RQ is explained by intrinsic factors, and the remaining variance (12%) is explained by other unmeasured factors. The lipid oxidation (LIPOX) (panel C) and carbohydrate oxidation (CARBOX) (panel D) rates are shown during each dietary intervention, where the red dots signify carbohydrate oxidizers during each dietary intervention and these remain on the lower end during LIPOX and the upper end during CARBOX. Similarly, the fat oxidizers in blue remain on the upper end for LIPOX and are on the lower end of the spectrum during CARBOX.

Figure 3. Metabolic Flexibility (ΔRQ) and Changes in Substrate Oxidation Rates during Dietary Interventions.

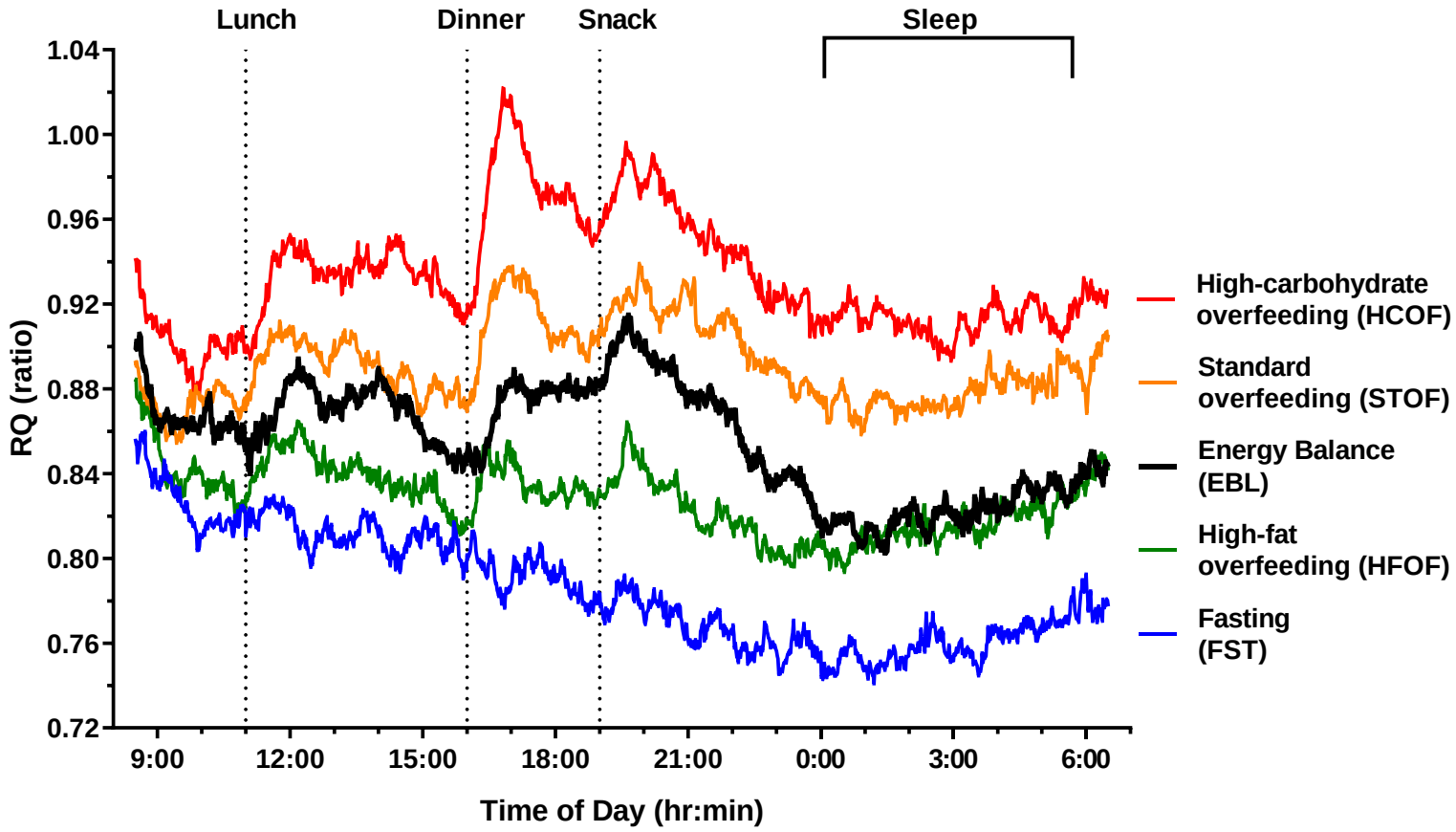
The relationships (±95% CI) between 24-h RQ during each diet and 24-h RQ during energy balance is shown in panel A, while the individual changes in 24-h RQ (ΔRQ, *metabolic flexibility*) from energy balance (EBL) are shown in panel B. The individual changes in lipid and carbohydrate oxidation rate are shown in panels C and D, respectively. Diet abbreviations: 24-h fasting (FST), high-fat overfeeding (HFOF), standard overfeeding (STOF), and high-carbohydrate overfeeding (HCOF).

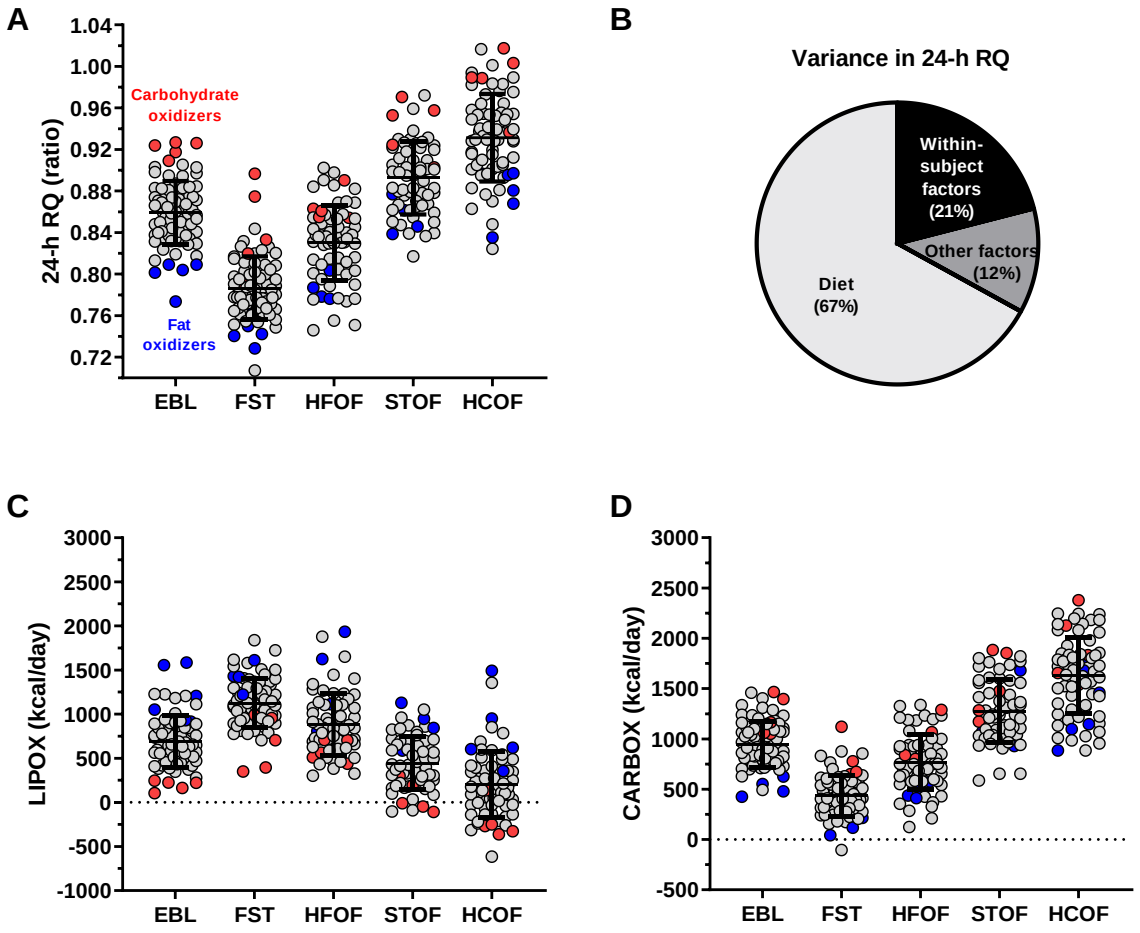
Figure 4. Relationships between fasting plasma NEFA concentrations and 24-h RQ and LIPOX.

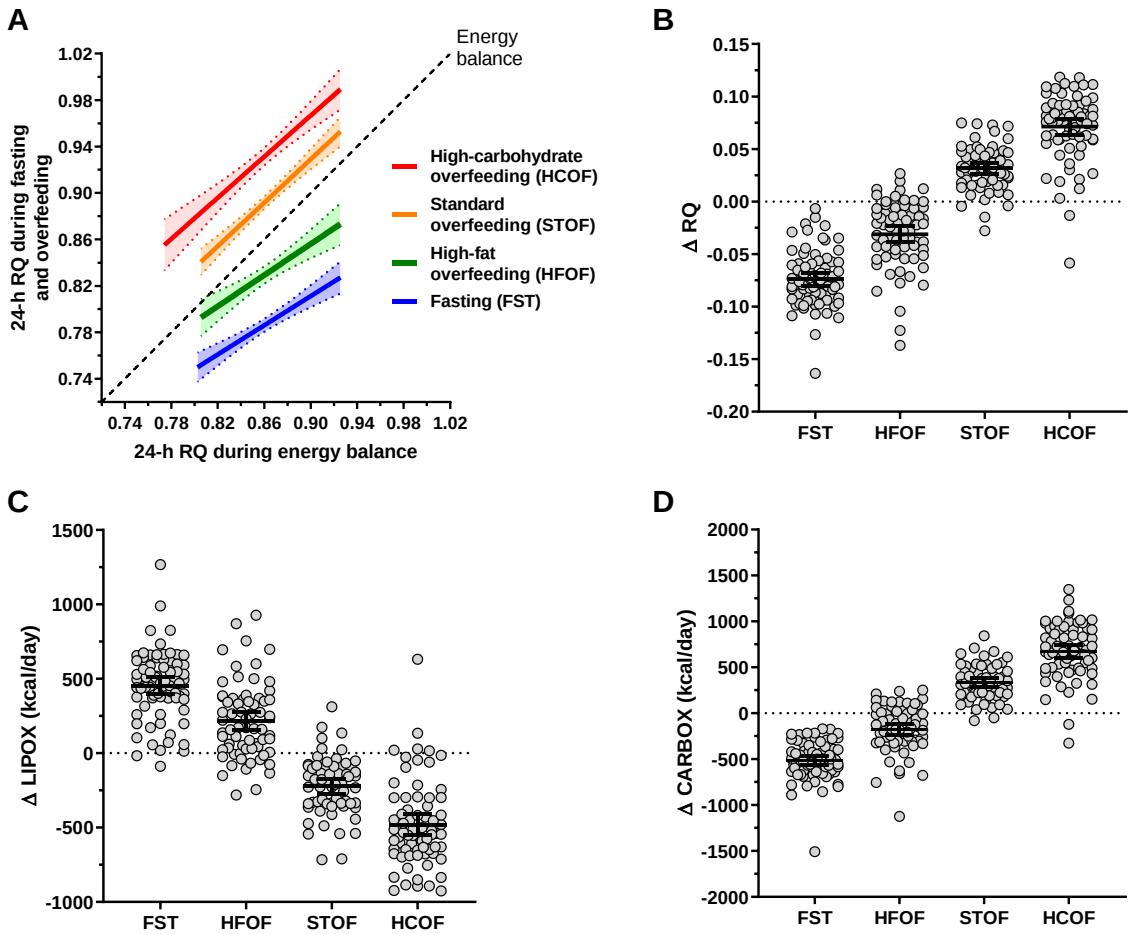
Inverse relationships between fasting plasma non-esterified fatty acids (NEFA) and 24-h respiratory quotient (RQ) during energy balance (panel A) and high-fat overfeeding (panel B). Direct relationships between fasting plasma NEFA and 24-h lipid oxidation rate (LIPOX) during energy balance (panel C) and high-fat overfeeding (panel D). Relationships were quantified by the Pearson correlation coefficient. Effect size estimates (β coefficient) were obtained via linear regression analysis.

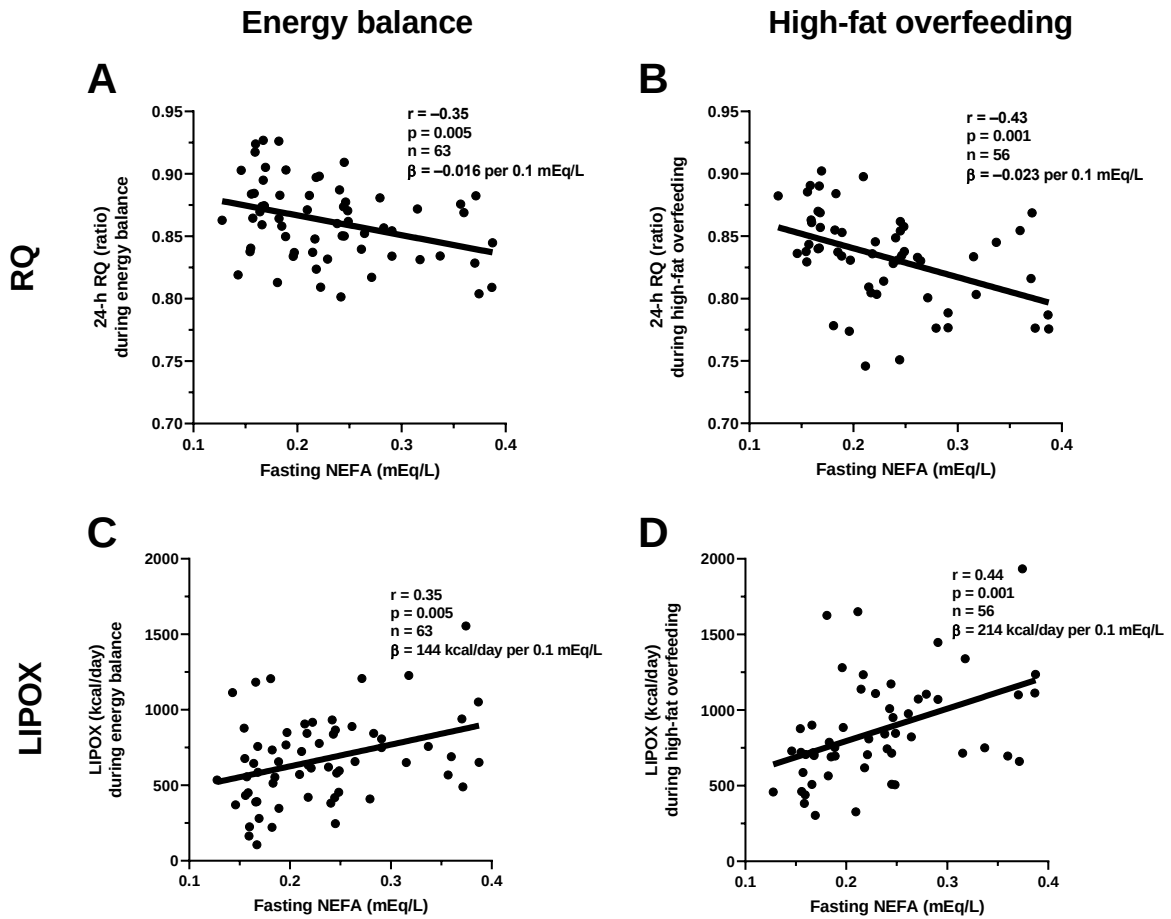
Figure 5. Metabolic Flexibility (ΔRQ) during High-fat Overfeeding Predicts Future Weight Change.

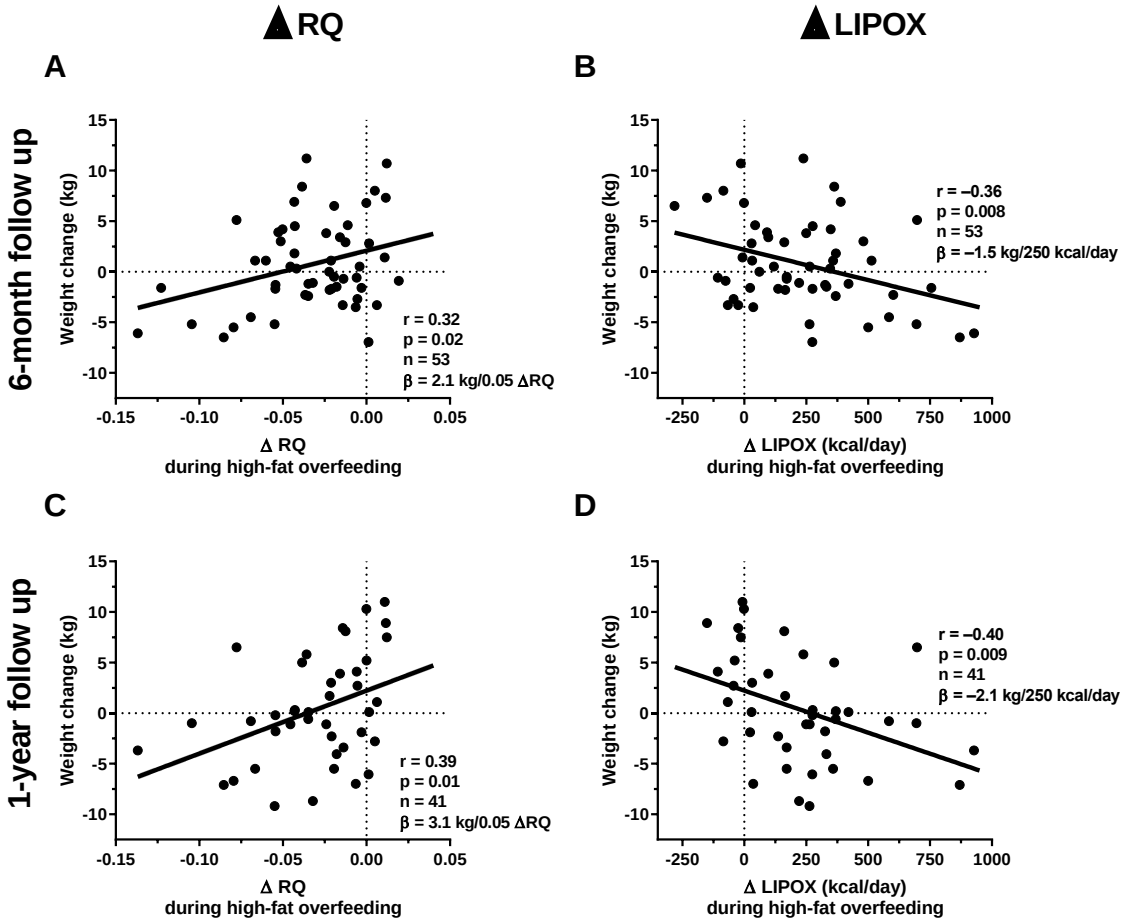
The change in RQ (ΔRQ, *metabolic flexibility*) from energy balance during high-fat overfeeding (HFOF) predicted future weight change at 6-months (panel A) and 1-year (panel C), that is a smaller (or lack of) decrease in RQ during HFOF was associated with greater weight gain. The change in 24-h lipid oxidation rate (LIPOX) from energy balance conditions was inversely associated with weight gain at 6 months (panel B) and 1 year (panel D), such that an impaired shift to LIPOX during high-fat overfeeding was associated with greater future weight gain. The vertical and horizontal dotted lines denote no changes in 24-h RQ, LIPOX, or bodyweight at follow-up visits compared to the baseline visit. Relationships were quantified by the Pearson correlation coefficient. Effect size estimates (β coefficient) were obtained via linear regression analysis.


Lunch
Dinner
Snack
Sleep
1.04
1.00
0.96
0.92
0.88
0.84
0.80
0.76
0.72
RQ (ratio)
9:00
12:00
15:00
18:00
21:00
0:00
3:00
6:00
Time of Day (hr:min)
High-carbohydrate overfeeding (HCOF)
Standard overfeeding (STOF)
Energy Balance (EBL)
High-fat overfeeding (HFOF)
Fasting (FST)


A
24-h RQ (ratio)
1.04
1.00
0.96
0.92
0.88
0.84
0.80
0.76
0.72
Carbohydrate oxidizers
Fat oxidizers
EBL
FST
HFOF
STOF
HCOF
B
Variance in 24-h RQ
Within-subject factors (21%)
Other factors (12%)
Diet (67%)
C
LIPOX (kcal/day)
3000
2500
2000
1500
1000
500
0
-500
-1000
EBL
FST
HFOF
STOF
HCOF
D
CARBOX (kcal/day)
3000
2500
2000
1500
1000
500
0
-500
EBL
FST
HFOF
STOF
HCOF

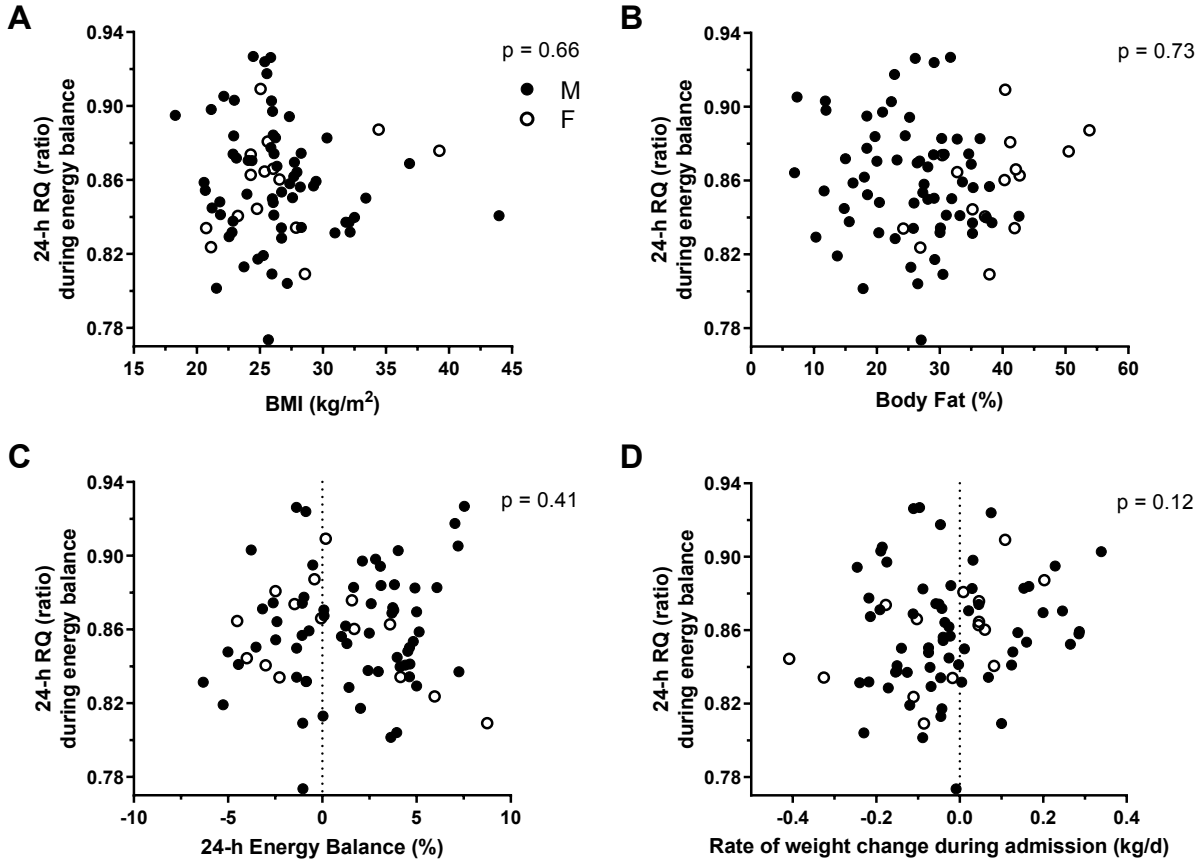

A
Energy balance
High-carbohydrate overfeeding (HCOF)
Standard overfeeding (STOF)
High-fat overfeeding (HFOF)
Fasting (FST)
24-h RQ during fasting and overfeeding
24-h RQ during energy balance
1.02
0.98
0.94
0.90
0.86
0.82
0.78
0.74
B
Δ RQ
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
FST
HFOF
STOF
HCOF
C
Δ LIPOX (kcal/day)
1500
1000
500
0
-500
-1000
D
Δ CARBOX (kcal/day)
2000
1500
1000
500
0
-500
-1000
-1500
-2000


Energy balance
High-fat overfeeding
A
B
C
D
RQ
LIPOX
24-h RQ (ratio) during energy balance
24-h RQ (ratio) during high-fat overfeeding
LIPOX (kcal/day) during energy balance
LIPOX (kcal/day) during high-fat overfeeding
Fasting NEFA (mEq/L)
r = –0.35
p = 0.005
n = 63
β = –0.016 per 0.1 mEq/L
r = –0.43
p = 0.001
n = 56
β = –0.023 per 0.1 mEq/L
r = 0.35
p = 0.005
n = 63
β = 144 kcal/day per 0.1 mEq/L
r = 0.44
p = 0.001
n = 56
β = 214 kcal/day per 0.1 mEq/L


RQ
LIPOX
A
B
C
D
6-month follow up
1-year follow up
Weight change (kg)
Δ RQ
during high-fat overfeeding
Δ LIPOX (kcal/day)
during high-fat overfeeding
r = 0.32
p = 0.02
n = 53
β = 2.1 kg/0.05 ΔRQ
r = –0.36
p = 0.008
n = 53
β = –1.5 kg/250 kcal/day
r = 0.39
p = 0.01
n = 41
β = 3.1 kg/0.05 ΔRQ
r = –0.40
p = 0.009
n = 41
β = –2.1 kg/250 kcal/day

Supplemental Data

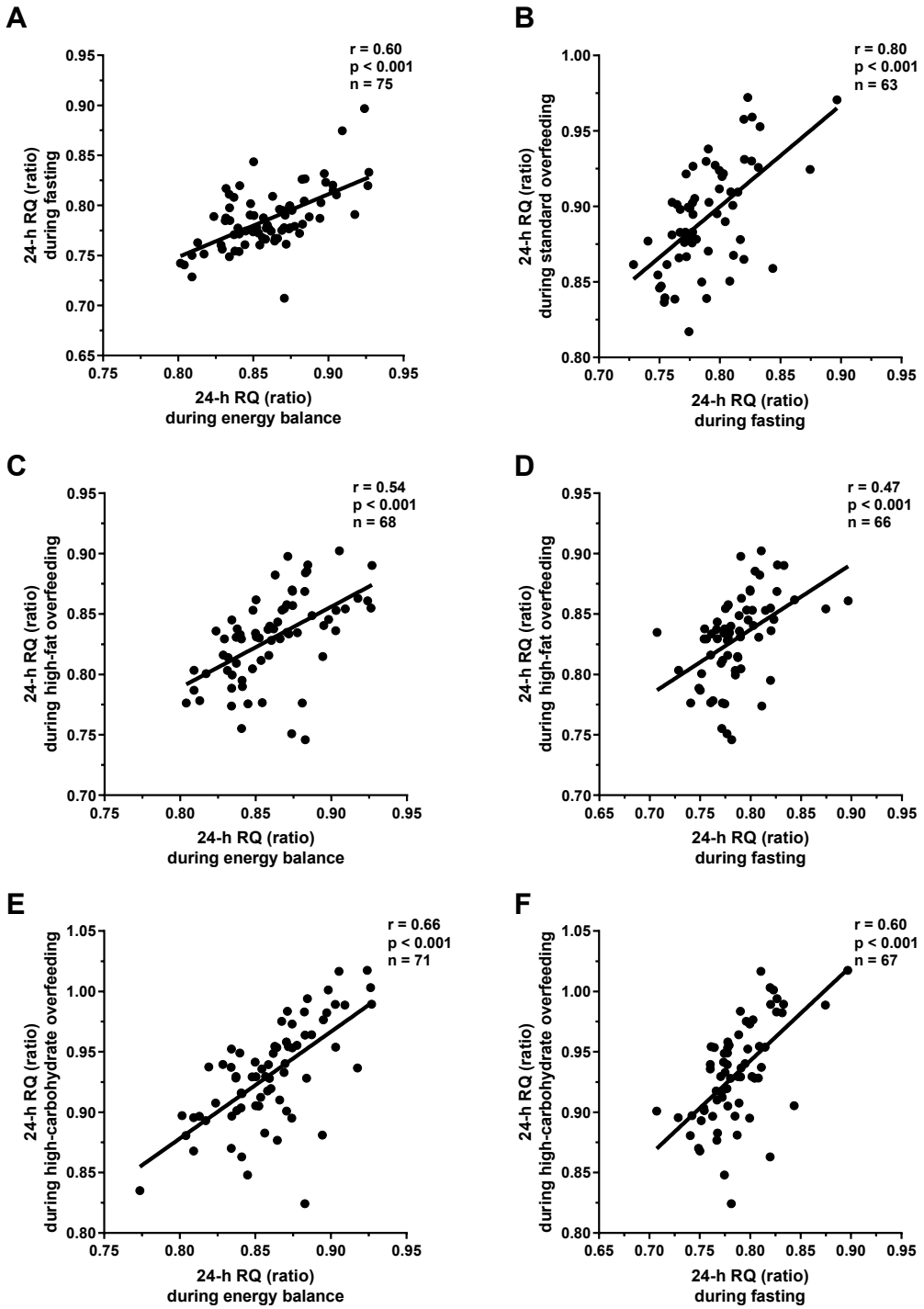
Supplemental Figure 1. Lack of relationships between 24-h RQ during energy balance and subjects' anthropometric characteristics or experimental conditions prior to and during metabolic assessments.



The 24-h respiratory quotient (RQ) measured during energy balance (EBL) was not associated with BMI (panel A) nor percentage body fat by DXA (panel B) at baseline. There were no associations between 24-h RQ and deviations from 24-h energy balance, calculated as the difference between 24-h energy intake and 24-h energy expenditure inside the calorimeter, (panel C), and the rate of weight change during the first week of admission prior to RQ assessment (panel D). Closed black circles represent males and open circles represent females.

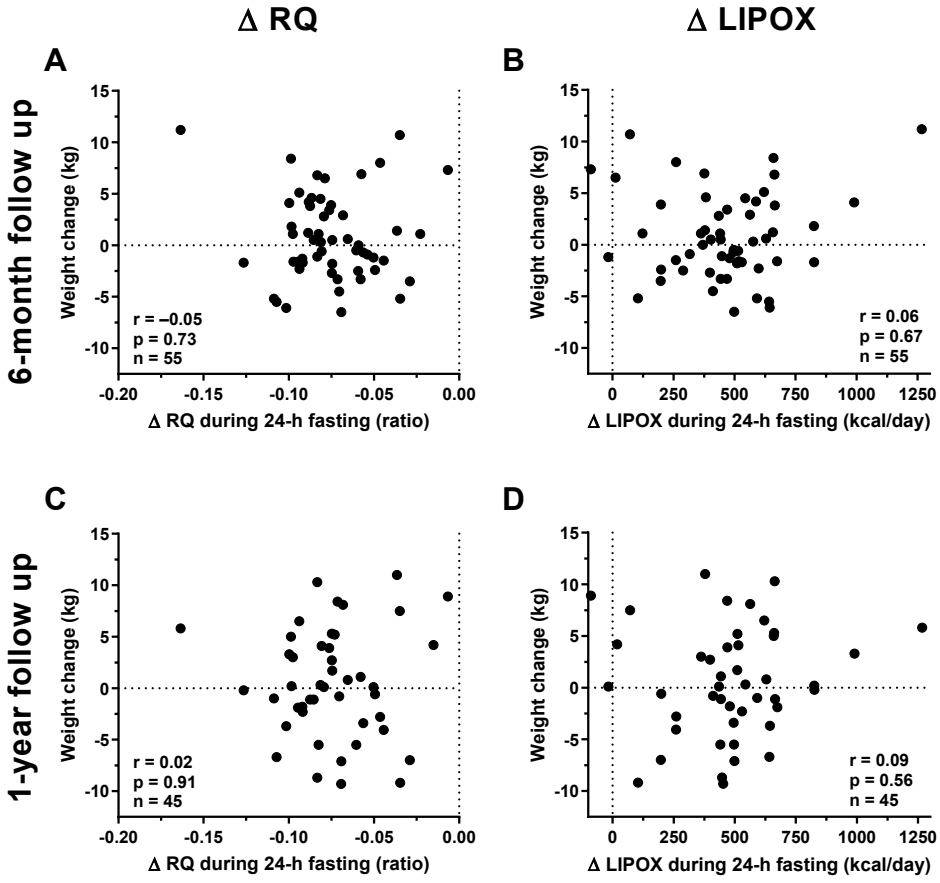
There were still no associations when analyses were stratified by gender. Relationships were quantified by the Pearson correlation coefficient.

Supplemental Figure 2. Relationships between 24-h RQ during energy balance and 24-h RQ during fasting and overfeeding.



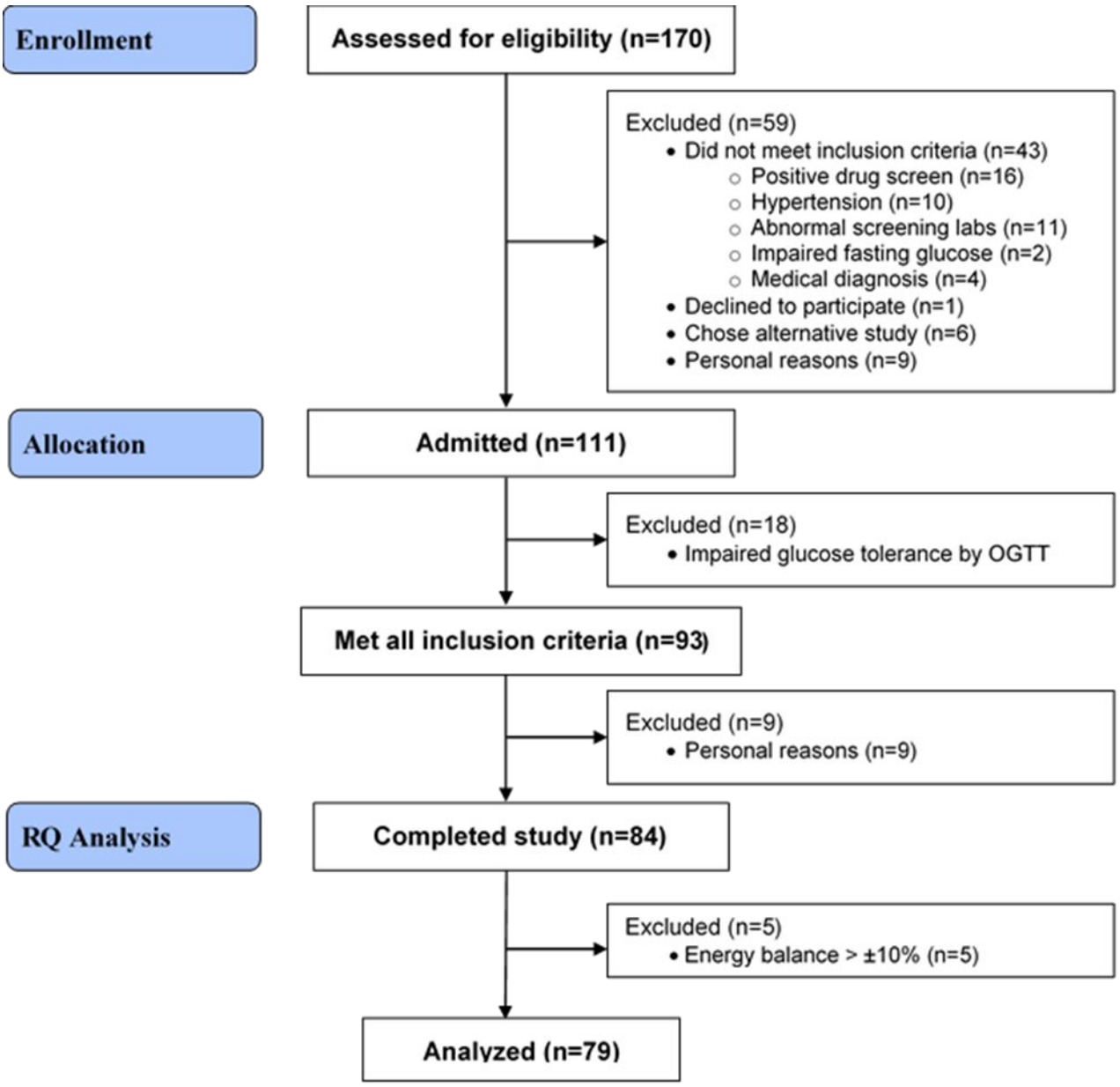
Relationships were quantified by the Pearson correlation coefficient.

Supplemental Figure 3. Lack of relationships between changes in 24-h RQ and LIPOX during acute fasting and future weight change.



Relationships were quantified by the Pearson correlation coefficient.

Supplemental Figure 4. CONSORT flow diagram.



Supplemental Table 1. Baseline characteristics of entire cohort vs. follow-up cohorts.

	Baseline cohort (*n*=79)	**Follow up at 6 mo (*n*=58)**	**Follow up at 1 yr (*n*=46)**
Sex (F/M)	15F/64M	12F/46M	8F/38M
Ethnicity	16BL/15HI/23NA/25WH	12BL/11HI/14NA/21WH	10BL/8HI/10NA/18WH
Age (y)	36.8±10.5 (18.2, 55.8)	36.9±10.6 (18.2, 55.8)	37.7±10.7 (18.2, 55.8)
Body weight (kg)	78.9±8.0 (52.8, 127.1)	77.3±13.3 (52.8, 127.1)	80.1±13.4 (54.1, 127.1)
Height (cm)	173.0±8.0 (156.5, 196.4)	173.1±7.9 (156.5, 188.1)	174.2±6.9 (158.3, 186.5)
BMI (kg/m^2)	26.3±4.2 (18.3. 44.0)	25.7±4. 0(18.3, 44.0)	26.4±4.3 (18.3, 44.0)
Body fat (%)	27.8±9.7 (6.9, 53.8)	27.0±9.6 (6.9, 53.8)	26.6±10.0 (6.9, 53.8)
FM (kg)	22.5±10.2 (4.9, 54.3)	21.3±9.7 (4.9, 54.1)	22.0±10.7 (4.9, 54.1)
FFM (kg)	56.4±9.4 (34.2, 79.4)	55.9±9.6 (34.2, 79.4)	58.1±8.9 (39.5, 74.9)
24-h RQ (ratio)	0.86±0.03 (0.77, 0.93)	0.86±0.03 (0.77, 0.93)	0.86±0.03 (0.81, 0.92)
24-h EE (kcal/day)	2028±308 (1427, 2810)	1956±282 (1427, 2625)	2031±258 (1427, 2732)

Data are presented as the mean±SD (minimum, maximum values).

Abbreviations: BL: Black; HI: Hispanic; NA: Native American; WH: White; BMI: body mass index; FM: fat mass; FFM: fat free mass; EE: energy expenditure; RQ: respiratory quotient.