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] and Paolo Piaggi[1,4]

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The ability to switch fuels for oxidation in response to changes in macronutrient composition of diet (metabolic flexibility) may be informative of individuals' 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 overfeeding (STOF) (50% carbohydrate, 30% fat), high-fat overfeeding (HFOF) (60% fat, 20% carbohydrate), and high-carbohydrate overfeeding (HCOF) (75% carbohydrate, 5% fat) diets. Free-living weight change was assessed after 6 and 12 months. Compared with EBL, RQ decreased on average by 9% during fasting and by 4% during HFOF but increased by 4% during STOF and by 8% during HCOF. A smaller decrease in RQ, reflecting a smaller increase in lipid oxidation rate, during HFOF but not during the other diets predicted greater weight gain at both 6 and 12 months. An impaired metabolic flexibility to acute HFOF can identify individuals prone to weight gain, indicating that an individual's capacity to oxidize dietary fat is a metabolic determinant of weight change.

Prolonged daily energy intake exceeding energy expenditure (EE) leads to an increase in body weight; however, even in 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 overfeeding/underfeeding may partly determine the susceptibility to body weight change in conditions of persistent energy imbalance (5). Daily energy balance (EBL), 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 body weight regulation (6,7). While carbohydrate and protein balances are usually met over 24 h in conditions of total daily EBL (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 EBL (8). Because positive fat balance leads to fat storage and, ultimately, to increased body fat mass (FM) (9), a reduced fat oxidation that favors positive fat (and thus total) EBL 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 EBL and future weight change is mixed, with studies showing that a higher RQ is a determinant of future weight gain (11–15) and others showing no such association (16–18). Oxidation rates measured during EBL and eucaloric feeding with a standard

[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
[3]Clinical Core Laboratory, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD
[4]Department of Information Engineering, University of Pisa, Pisa, Italy

Corresponding author: Paolo Piaggi, paolo.piaggi@gmail.com, paolo.piaggi@nih.gov

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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 EBL (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 R_d 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 or 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 with the 24-h RQ as precisely measured during weight stability and EBL. We hypothesized that an impaired metabolic flexibility during these extreme dietary interventions may increase individual predisposition to weight gain over time.

RESEARCH DESIGN AND METHODS

Study Participants

Study volunteers between the ages of 18 and 55 years were recruited from 2008 through 2017 from the Phoenix, Arizona, metropolitan area to participate in an ongoing clinical trial. The primary aim was to assess metabolic responses to acute dietary interventions in relation to free-living weight change. Before 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 on the basis of sex and weight (27). The WMD consisted of 50% carbohydrates, 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 on the basis of American Diabetes Association criteria (28) continued in the study (Supplementary Fig. 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 (watching television, playing pool, etc.). Body composition was measured by DXA scan (Prodigy enCORE 2003, version 7.53.002, software; GE Lunar Corporation, Madison, WI). FM and fat-free mass (FFM) were calculated from the measured body fat percentage (PFAT) and weight as follows: FM = weight × PFAT / 100 and FFM = weight – FM. Body weight was measured daily on a precision scale every morning upon awakening after an overnight fast to ensure that 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% (mean ± SD 0.6 ± 0.3%). 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

The 24-h 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 after the OGTT and 1 h after consuming breakfast at 0700 h, and subsequent meals were given inside the metabolic chamber at 1100, 1600, and 1900 h. During the 24-h fast, no food was provided after the previous day's dinner, but participants were permitted to drink water and noncaloric, noncaffeinated beverages. Both O_2 consumption and CO_2 production during each 24-h EE assessment were measured every minute, averaged and extrapolated to 24 h, 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, was monitored every minute, and averaged 23.9 ± 1.3°C. Spontaneous physical activity 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 h to measure urea nitrogen excretion to calculate the nonprotein 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 before entering the metabolic chamber and frozen at −70°C for later measurements. Nonesterified fatty acids (NEFAs) were measured using the kit from FUJIFILM Wako Diagnostics (Mountain View, CA) at the National Institute of Diabetes and Digestive and Kidney Diseases

clinical core laboratory in Bethesda, Maryland. Intra-assay CV was 4.4%, and interassay CV was 5.8%.

To precisely attain metabolic measurements in conditions of EBL 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 EBL with standard eucaloric feeding (50% carbohydrate, 30% fat, and 20% protein). The subsequent dietary interventions—fasting and 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 content 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

After completion of the inpatient study, participants were discharged from the clinical research unit and invited back after 6 months (median follow-up time 6.6 months, interquartile range 6.0–6.9) and 1 year (median 12.9 months, interquartile range 12.1–13.5) for 1-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 participants with available data at the 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 body weight change at follow-up (primary end point). Statistical analyses were performed using SAS 9.2 software (SAS Institute, Cary, NC). Data are presented as mean $\pm$ SD or mean with 95% CI. Differences by sex were evaluated by Student 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 EBL conditions (ΔRQ, metabolic flexibility) during the dietary interventions. The Pearson 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 nonprotein 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 Availability

The data sets analyzed in 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 EBL, with an average 24-h deviation of 25 $\pm$ 71 kcal/day (range –6% to 9%). The CV of 24-h RQ measurements between the two consecutive EBL assessments was 1.6 $\pm$ 1.2% with high intraindividual consistency (ICC 0.76; $P < 0.001$).

The average 24-h RQ during EBL was 0.86, a value very close to the expected food quotient (FQ) of 0.87 (31). Despite being approximately equal to the FQ on average, the 24-h RQ showed a large interindividual variability (SD 0.03), which was unrelated to sex ($P = 0.37$), age ($P = 0.52$), ethnicity ($P = 0.51$), spontaneous physical activity inside the metabolic chamber ($P = 0.76$), or any measures of body size or adiposity, including BMI ($P = 0.66$) (Supplementary Fig. 1*A*), PFAT ($P = 0.73$) (Supplementary Fig. 1*B*), FM ($P = 0.59$), FFM ($P = 0.54$), waist circumference ($P = 0.96$), and waist-to-thigh ratio ($P = 0.36$). Similarly, there were no associations between 24-h RQ and the deviation from 24-h EBL inside the metabolic chamber ($P = 0.41$) (Supplementary Fig. 1*C*) or the rate of body weight change during the first days of admission ($P = 0.12$) (Supplementary Fig. 1*D*).

Metabolic Flexibility to 24-h Fasting and Overfeeding Diets

The time courses of the average 24-h RQ are shown in Fig. 1, while the metabolic measurements during each dietary intervention are reported in Table 2 and Fig. 2. Overall, RQ increased after meal times in the feeding interventions, remained elevated during the day, and decreased 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 intraindividual 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 (Fig. 3*A* and Supplementary Fig. 2). This is graphically shown in Fig. 2*A*, where "carbohydrate oxidizer" individuals with the

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

	Total (n = 79)	Males (n = 64)	Females (n = 15)
Ethnicity, *n*			
Black	16	11	5
Hispanic	15	12	3
Native American	23	20	3
White	25	21	4
Age (years)	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)	2,028 ± 308 (1,427, 2,810)	2,098 ± 287 (1,573, 2,810)	1,733 ± 205 (1,427, 2,156)*
24-h energy intake (kcal/day)	2,053 ± 307 (1,461, 2,921)	2,126 ± 277 (1,622, 2,921)	1,742 ± 117 (1,461, 2,190)*
24-h EBL (kcal/day)	25 ± 71 (–159, 169)	28 ± 71 (–159, 154)	10 ± 67 (–81, 169)
24-h EBL (%)	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-month 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-year 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 mean ± SD (minimum, maximum) unless otherwise indicated. *P values for differences between males and females by Student t test. [a]n = 58 with follow-up weight after 6 months. [b]n = 46 with follow-up weight after 1 year.

top five highest RQ values during EBL showed above-average values for 24-h RQ during all dietary interventions. Similarly, this was the case for "fat oxidizer" individuals with the bottom five lowest RQ values during EBL showing below-average values for 24-h RQ during other diets. Although the macronutrient composition of the dietary interventions was the main determinant of 24-h RQ, explaining approximately two-thirds of its variance (67%; $P < 0.001$), after accounting for differences among diets, the intraindividual component of 24-h RQ explained an additional one-fifth of its variance (21%; $P < 0.001$) (Fig. 2*B*).

Compared with EBL conditions, the 24-h RQ decreased in all participants during fasting by an average of 8.6% (95% CI –9.3 to –7.9%; ΔRQ = –0.07 ± 0.03; $P < 0.001$) (Fig. 3*A* and *B*) of the 24-h RQ during EBL. Similarly, the average 24-h RQ decreased by 4% (–5 to –3%; ΔRQ = –0.03 ± 0.03; $P < 0.001$) during HFOF, indicating that a greater proportion of lipids were oxidized during these two dietary conditions (Table 2 and Fig. 3*C*). Conversely, during the overfeeding diets containing a higher carbohydrate content (>50%), 24-h RQ increased on average during STOF by 4% (3.0–4.3%; ΔRQ = 0.03 ± 02; $P < 0.001$) and increased the highest during HCOF by 8% (7–9%; ΔRQ = 0.07 ± 0.03; $P < 0.001$), reflecting increased oxidation of carbohydrates during these two diets (Fig. 3*D*).

The substrate oxidation rates (LIPOX and CARBOX) during each dietary intervention are shown in Table 2 and Fig. 2*C* and *D*. Compared with EBL conditions, LIPOX increased on average by 66% (95% CI 58–74%) during fasting and by 31% (29–69%) during HFOF. Conversely, LIPOX decreased during STOF by 32% (–39 to –25%) and more so during HCOF by 69% (–79 to –59%) (Fig. 3*C*). Concordant with changes in 24-h RQ, CARBOX increased during STOF by 35% (30–40%) and during HCOF by 72% (64–79%), whereas CARBOX decreased by 19% (–25 to –12%) during HFOF, with the largest decrease (–55% [–60 to –49%]) observed during 24-h fasting (Fig. 3*D*).

A higher fasting NEFA concentration was associated with lower 24-h RQ during EBL ($r = -0.35$; $P = 0.005$) (Fig. 4*A*) and HFOF ($r = -0.43$; $P = 0.001$) (Fig. 4*B*). Similarly, fasting NEFA concentrations were positively associated with LIPOX during both EBL ($r = 0.35$; $P = 0.005$) (Fig. 4*C*) and HFOF ($r = 0.44$; $P = 0.001$) (Fig. 4*D*). There was a weak inverse correlation between fasting NEFA and CARBOX during HFOF ($r = -0.30$; $P = 0.03$), whereas there was no association with CARBOX during EBL ($P = 0.18$).

Metabolic Flexibility and Future Weight Change

Follow-up data for free-living weight change after 6 months were available in 58 individuals and after

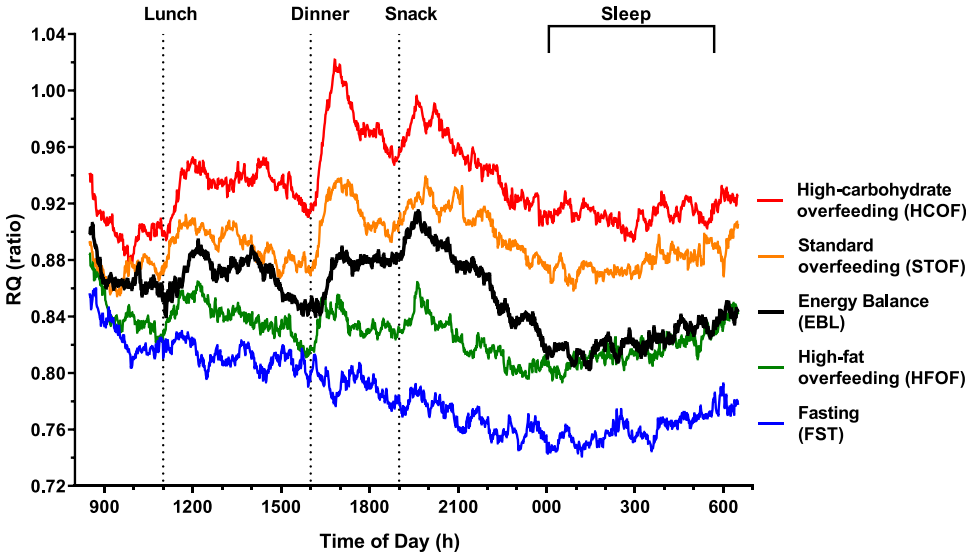

Figure 1—Twenty-four-hour time courses of RQ during dietary interventions. The average time course of RQ over 24 h is plotted for each dietary intervention: eucaloric standard diet in EBL (50% carbohydrate and 30% fat), 24-h fasting (FST), HFOF (20% carbohydrate and 60% fat), STOF (50% carbohydrate and 30% fat), and HCOF (75% carbohydrate and 5% fat). The three meals provided inside the metabolic chamber were lunch at 1100 h, dinner at 1600 h, and snack at 1900 h. The total caloric intake of the overfeeding diets was equal to twice the 24-h EE value obtained during EBL.

1 year in 46 individuals (Table 1). Compared with the whole cohort (n = 79), the subgroups with follow-up data did not differ in their baseline characteristics, including 24-h RQ (Supplementary Table 1). On average, participants were weight stable at 6 months (mean weight change 0.8 kg; P = 0.17 vs. 0), despite a large interindividual variability (SD 4.3 kg, range −7.0 to 11.2) 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 1 year (SD 5.3 kg, range −9.3 to 11.0), with participants remaining on average weight stable (mean 0.4 kg; P = 0.63 vs. 0).

A smaller decrease in 24-h RQ (ΔRQ) during HFOF, but not during the other dietary interventions (all $P > 0.15$), was associated with weight gain both at 6 months (r = 0.32; P = 0.02; r^2 = 10%) (Fig. 5*A*) and at 1 year (r = 0.39; P = 0.01; r^2 = 15%) (Fig. 5*C*). After adjustment for age, sex, and ethnicity, the ΔRQ during HFOF was an independent determinant of weight change at 6 months (β = 2.1 kg per 0.05 change in 24-h RQ during HFOF; P = 0.02; total r^2 = 19%) and at 1 year (β = 2.6 kg; P = 0.02; total r^2 = 46%). The change in 24-h EE during HFOF did not predict weight change at any follow-up (both $P > 0.30$), and the results for ΔRQ during HFOF 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 HFOF was associated with more weight loss at 6 months (r = −0.36; P = 0.008) (Fig. 5*B*) and 1 year (r = −0.40; P = 0.009) (Fig. 5*D*). After adjustment for age, sex, and ethnicity, ΔLIPOX during HFOF 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 HFOF 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) (Supplementary Fig. 3), STOF (P = 0.87), and HCOF (P = 0.29). 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) (Supplementary Fig. 3), STOF (P = 0.17), and HCOF (P = 0.20).

DISCUSSION

In the current study, we evaluated metabolic flexibility (ΔRQ), which is defined as the change in 24-h RQ from EBL conditions to extreme dietary interventions, including 24-h fasting and 200% overfeeding with a high-fat or 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 predominantly depended on macronutrient composition, which explained approximately two-thirds of the 24-h RQ variance among diets. However, there was still a strong intraindividual reliance for fuel oxidation observed in each dietary condition, such that individuals relying more on a specific substrate for oxidation (e.g., carbohydrates, lipids) manifested this preference in any dietary conditions. We demonstrated that interindividual variability in ΔRQ, specifically, a reduced metabolic flexibility to HFOF, predicted future weight gain

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

	EBL (n = 79)	Fasting (n = 75)	HFOF (n = 68)	STOF (n = 64)	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]	NA	–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 (%)	NA	–8.1 (–9.3 to 7.9)	–3.6 (–4.5 to –2.7)	3.6 (3.0–4.3)	8.1 (7.4–9.2)
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)
Nonprotein 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 nonprotein RQ (ratio)[a,b]	NA	–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)	2,028 ± 308 (1,427, 2,810)	1,859 ± 268 (1,287, 2,655)[c]	2,150 ± 315 (1,555, 3,114)[d]	2,228 ± 365 (1,481, 3,251)[c,d]	2,322 ± 351 (1,573, 3,229)[c,d]
Change in 24-h EE (%)[a,b]	NA	–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, 1,466)	435 ± 204 (–105, 1,121)	768 ± 275 (124, 1,338)	1,274 ± 312 (287, 1,884)	1,630 ± 381 (884, 2,379)
Change in CARBOX (kcal/day)[a,b]	NA	–517 ± 205 (–1,509, –170)	–177 ± 253 (–1,124, 253)	330 ± 191 (–80, 844)	676 ± 296 (–326, 1,348)
Change in CARBOX (%)	NA	–55 (–60 to –49)	–19 (–25 to –12)	35 (30–40)	72 (64–79)
LIPOX (kcal/day)[a]	690 ± 292 (106, 1,587)	1,127 ± 227 (351, 1,838)	882 ± 349 (304, 1,934)	444 ± 302 (–109, 1,130)	205 ± 375 (–613, 1,492)
Change in LIPOX (kcal/day)[a,b]	NA	455 ± 233 (–88, 1,267)	217 ± 256 (–281, 927)	–222 ± 198 (–716, 311)	–479 ± 289 (–926, 632)
Change in LIPOX (%)	NA	66 (58–74)	31 (22–40)	–32 (–39 to –25)	–69 (–79 to –59)
PROTOX (kcal/day)	368 ± 98 (15, 573)	275 ± 73 (26, 415)	467 ± 157 (4, 767)[c,d]	476 ± 115 (175, 732)[c,d]	456 ± 132 (3, 735)[c,d]

Data are mean ± SD (minimum, maximum) or mean (95% CI). NA, not applicable; PROTOX, protein oxidation rate. [a]$P < 0.05$ for all pairwise differences between diets. [b]$P < 0.05$ vs. 0. [c]$P < 0.05$ vs. EBL. [d]$P < 0.05$ vs. fasting.

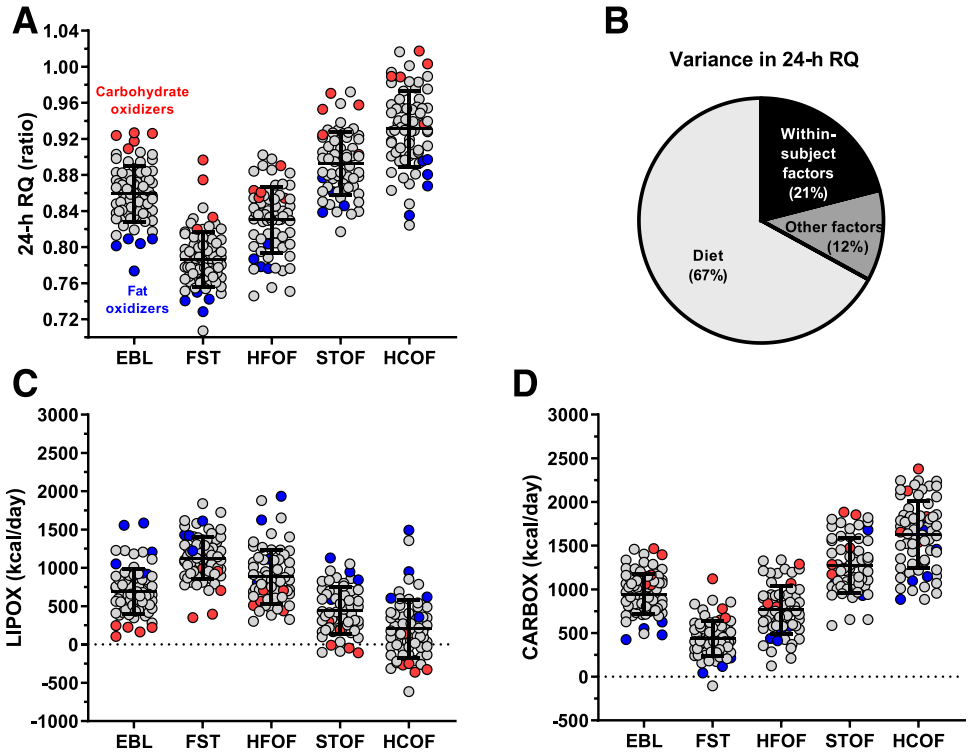

Figure 2—Measures of 24-h RQ and substrate oxidation rates during dietary interventions. Error bars represent the mean ± SD in each dietary condition. The 24-h RQ (*A*) is shown during each dietary intervention. Red circles indicate "carbohydrate oxidizers": the five individuals with the highest 24-h RQ during EBL and standard eucaloric feeding. Blue circles indicate "fat oxidizers": the five individuals with the lowest 24-h RQ during EBL. These same two groups of individuals are subsequently highlighted during each intervention: 24-h fasting (FST), STOF, HFOF, and HCOF, where the carbohydrate oxidizers remained above the mean 24-h RQ during each intervention and the fat oxidizers remained below the mean 24-h RQ despite being challenged with overfeeding. The determinants of 24-h RQ (*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 substrate oxidation rates LIPOX (*C*) and CARBOX (*D*) are shown during each dietary intervention, where the red dots signify carbohydrate oxidizers during each dietary intervention, and these remained on the lower end during LIPOX and the upper end during CARBOX. Similarly, the fat oxidizers in blue remained on the upper end for LIPOX and were on the lower end of the spectrum during CARBOX.

both at 6 months and at 1 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 EBL 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, EBL is likely transient; thus, investigating change in fuel selection during acute over- and underfeeding is important. Concordant with the observations made during EBL 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 HFOF, 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 (32) and that measures of metabolic inflexibility predict long-term weight gain (33). The individual's 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 individuals who are metabolically inflexible to dietary fats, lipolysis may increase to a smaller degree during a high-fat diet (34). We have previously demonstrated that lower rates of in vitro lipolysis are associated with higher 24-h RQ and lower LIPOX during eucaloric feeding, and these individuals with reduced lipolysis gained more weight at follow-up as a result of an increase in FM (35). 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 (36), and regulators of LIPOX

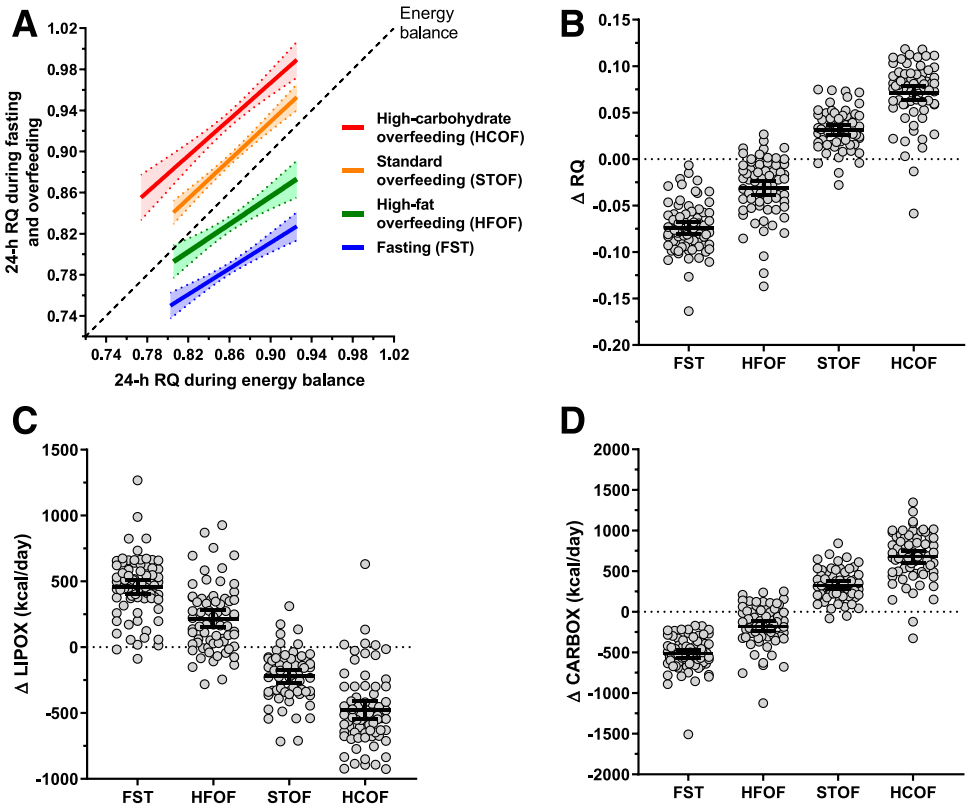

Figure 3—Metabolic flexibility (ΔRQ) and changes in substrate oxidation rates during dietary interventions. Relationships (±95% CI) between 24-h RQ during each diet and 24-h RQ during EBL (*A*) and individual changes in 24-h RQ (ΔRQ, metabolic flexibility) from EBL (*B*) are shown. Individual changes in LIPOX (*C*) and CARBOX (*D*) rates also are shown.

(37), were associated with lower 24-h RQ and greater LIPOX during both eucaloric feeding and HFOF. Concordant with our current findings, lower nocturnal concentrations of plasma NEFAs during HFOF predict weight gain in obesity-prone individuals (33). Altogether, these results strongly point to a key role for lipolysis in obesity and body weight regulation (38,39).

To obtain accurate measurements of metabolic flexibility during each diet, we used a carefully controlled measurement of 24-h RQ during EBL and eucaloric, standard feeding. Prior to this baseline assessment of 24-h RQ, participants were on an WMD for 5 days, and two metabolic measurements inside the metabolic chamber were used to obtain a baseline RQ value in conditions of almost perfect EBL and weight maintenance. We evaluated the determinants of baseline RQ and found no associations with body size, body composition, deviations from 24-h EBL in the metabolic chamber, or prior fluctuations in body weight, despite these variables being found to be determinants of RQ during EBL in previous studies (11,40). 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 EBL (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 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 STOF and HCOF, but we also demonstrated a decrease in RQ (greater lipid oxidation) during fasting and HFOF. The rapid change in RQ in response to 24-h overfeeding observed in the current study is in contrast to a recent study that showed no change in RQ after 3 days of an overfeeding diet with a composition similar to our STOF diet (33), although the degree of overfeeding in the current study (200% of energy needs) was much higher than that (140%) of the previous study. Of note, 3 days of eucaloric feeding with a high dietary fat content (50%)—similar to our HFOF diet (60%)—induced a decrease in 24-h RQ to an average value of 0.83 (41), which is exactly the same value obtained in the current study during HFOF (Table 2). These results strongly support

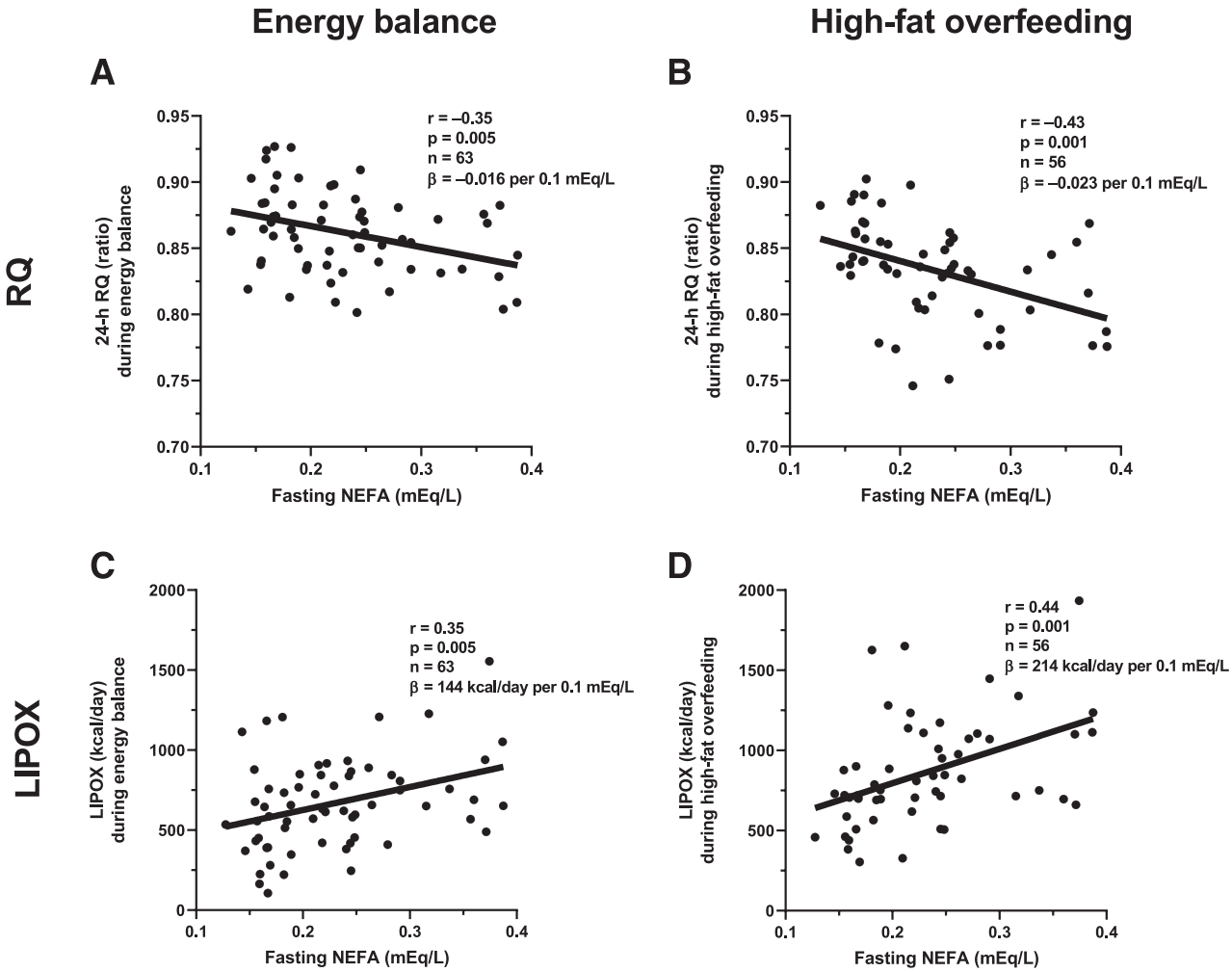

Figure 4—Relationships between fasting plasma NEFA concentrations and 24-h RQ and LIPOX. Inverse relationships between fasting plasma NEFAs and 24-h RQ during EBL (*A*) and HFOF (*B*) and direct relationships between fasting plasma NEFAs and 24-h LIPOX during EBL (*C*) and HFOF (*D*) are shown. Relationships were quantified by the Pearson correlation coefficient. Effect size estimates (β-coefficient) were obtained through linear regression analysis.

the use of short-term (24-h) 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 who underwent repeat assessments of energy metabolism inside a whole-room calorimeter (42), 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 participant-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 (43). Thus, the substantial variability in metabolic flexibility to acute overfeeding and fasting also has a strong intraindividual component, which is indicative of the propensity for future weight gain and 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 (44) and American Indians of southwestern heritage (11).

Although our dietary interventions to create short-term energy imbalance are not necessarily normal physiological or habitual conditions, we propose that these interventions may constitute an important tool to quantify the propensity for 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 for weight gain to provide insight into the pathogenesis of obesity.

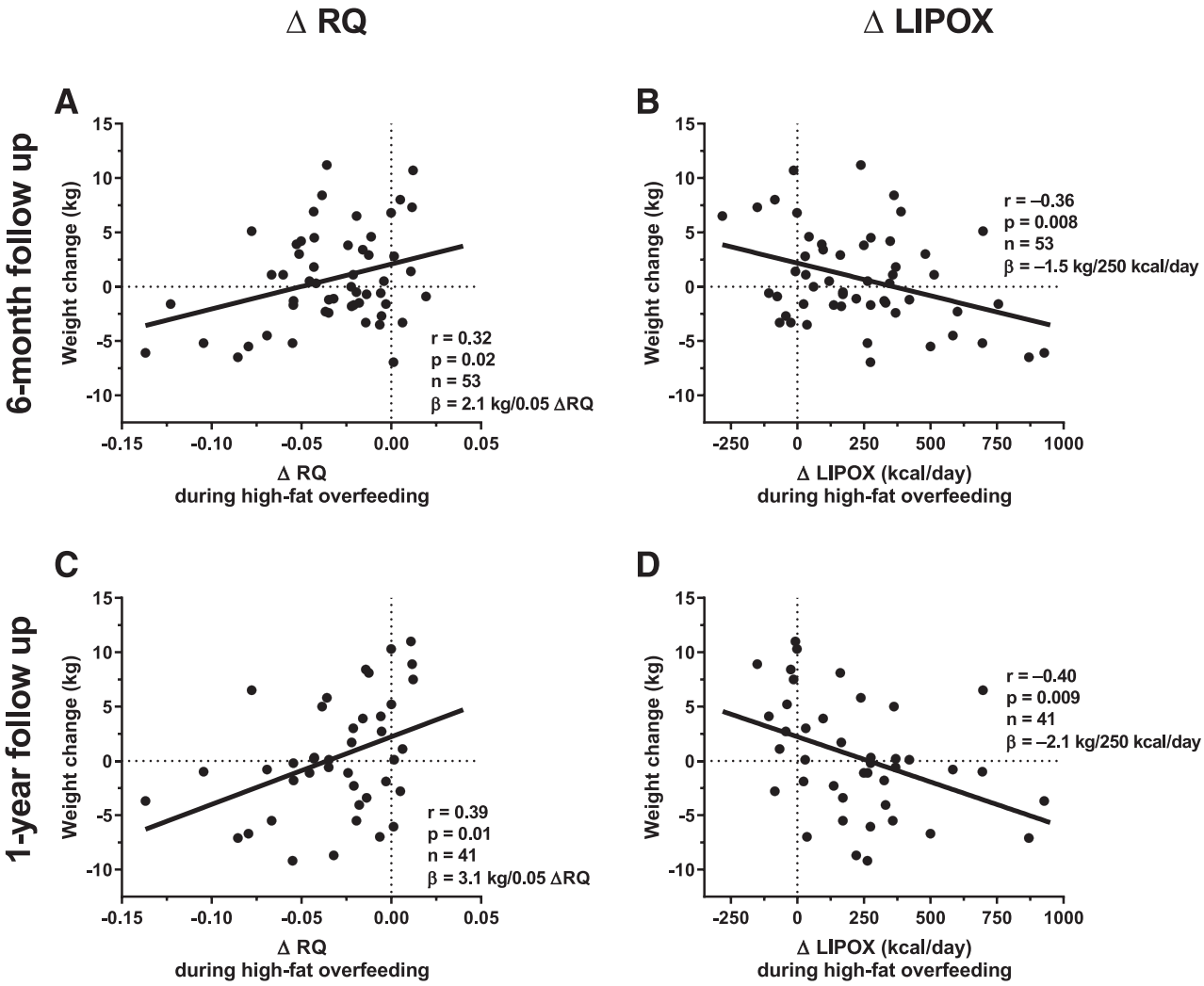

Figure 5—Metabolic flexibility (ΔRQ) during HFOF predicts future weight change. The ΔRQ (metabolic flexibility) from EBL during HFOF predicted future weight change at 6 months (*A*) and 1 year (*C*); that is, a smaller (or lack of) decrease in RQ during HFOF was associated with greater weight gain. The change in 24-h LIPOX from EBL conditions was inversely associated with weight gain at 6 months (*B*) and 1 year (*D*), such that an impaired shift to LIPOX during HFOF was associated with greater future weight gain. The dotted lines denote no changes in 24-h RQ, LIPOX, or body weight at follow-up visits compared with the baseline visit. Relationships were quantified by the Pearson correlation coefficient. Effect size estimates (β-coefficient) were obtained via linear regression analysis.

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 6 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 HFOF and weight gain explained up to ∼15% of the interindividual 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 to oxidize 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 HFOF across participants are indicative of the individual propensity for 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 who 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 individuals more "metabolically flexible" to dietary fats may help to prevent or treat obesity.

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