

Respiratory Quotient Predicts Fat Mass Gain in Premenopausal Women

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High respiratory quotient (RQ) has been associated with fat mass (FM) gain in some, but not all studies. Variability among results may reflect differences in the RQ variable measured (fasting vs. 24-h) or may be due to differences in control for factors that affect RQ, such as diet, energy balance, circulating insulin, and insulin sensitivity. The objective of this study was to determine whether different RQ values (fasting, sleeping, nonsleeping, and 24-h) would predict change in FM over 2 years in obesity-prone women, controlling for diet and adjusting for energy balance, circulating insulin, and insulin sensitivity. Participants were 33 previously overweight premenopausal women. Fasting, sleeping, nonsleeping, and 24-h RQ values were measured during controlled-diet conditions by respiratory chamber calorimetry. Intravenous glucose tolerance tests were also performed to adjust for fasting insulin, acute insulin response to glucose, and insulin sensitivity. Over the following 2 years, changes in FM were tracked annually by dual-energy X-ray absorptiometry. High nonsleeping RQ (NSRQ) predicted 2-year change in FM independently of energy balance, circulating insulin, and insulin sensitivity. This observation suggests that low postprandial fat oxidation may uniquely predispose obesity-prone individuals to accrual of adipose tissue.

Obesity (2010) **18**, 2255–2259. doi:10.1038/oby.2010.96

INTRODUCTION

More than two-thirds of American adults are classified as overweight or obese (1). Although various diet interventions can facilitate intentional weight loss, most dieters regain weight within 3–5 years (2). Identifying inherent metabolic characteristics that predispose obesity-prone individuals to weight regain presents an important public health challenge.

Inherent variability in substrate oxidation may be one such mechanism that affects weight regain and fat storage. Substrate oxidation can be assessed clinically by measuring respiratory quotient (RQ), the ratio of carbon dioxide expired to oxygen consumed during indirect calorimetry. High RQ values are indicative of low fat oxidation and high carbohydrate oxidation (3). Previous studies relating RQ to weight gain have yielded inconsistent results. Although some investigators have associated high RQ with weight gain (4–9), other studies demonstrated no association (10–12).

Equivocal reports relating RQ and fat mass (FM) may be due to population differences and variable control for dietary confounders in different study designs. For example, differences in macronutrient intake between individuals may influence RQ measurements, and fasting RQ will be elevated if an individual is in positive energy balance during the days preceding the measurement (3). Individual differences in circulating insulin or insulin sensitivity may also confound reported associations between RQ and FM gain. Insulin is an anabolic hormone

that acts to decrease fat oxidation and increase lipid storage (13). Accordingly, fasting serum insulin has been positively correlated with weight (10) and RQ (14). Moreover, high tissue sensitivity to insulin has also been associated with fat accumulation (15–17). Thus, when studying the relationship between RQ and change in FM, it is important to carefully control for energy balance and macronutrient intake and to consider the influence of circulating insulin and insulin sensitivity.

Discrepant results may also suggest that RQ values measured across specific periods of the day differentially predict FM gain. Most studies to date have focused exclusively on either fasting or 24-h RQ. However, these values may not reflect postprandial fat oxidation. Rather, RQ measured during fasting or sleeping would reflect periods of high reliance on endogenous free fatty acids for fuel. Alternatively, nonsleeping RQ (NSRQ) measured during waking hours in the respiratory chamber would specifically correspond to an individual's ability to oxidize lipid and carbohydrate across typical physiological postprandial periods of the day.

In a previous analysis of weight-reduced African-American and white women, we reported that fasting, sleeping, and 24-h RQ were not predictive of long-term weight change (12). In this study, we examined the potential for NSRQ to predict change in FM among this cohort of previously overweight premenopausal women, controlling for diet and adjusting for circulating insulin, insulin sensitivity, and energy balance.

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Received 9 September 2009; accepted 30 March 2010; published online 6 May 2010. doi:10.1038/oby.2010.96

The specific aim of this study was to determine whether RQ measured during specific time periods (fasting, sleeping, non-sleeping, and 24-h) would uniquely predict change in FM 2 years following weight loss.

METHODS AND PROCEDURES

Participants

Participants were 33 healthy, premenopausal women evaluated after a dietary intervention for weight loss to BMI <25 kg/m² (ref. 11). A total of 49 women were originally recruited for evaluation, but only those participants with all data available for RQ, insulin measures, and 2-year body composition were included in this analysis. The final study cohort included 20 African Americans and 13 whites with normal menstrual cycles and normal glucose tolerance verified by oral glucose tolerance tests. Participants were nonsmokers and sedentary (self-report of exercising less than one time per week over the previous year), and none were taking any medications known to affect energy expenditure or substrate oxidation. All subjects reported a family history of overweight or obesity (BMI >27 kg/m²) in a first degree relative. Participants provided written informed consent, and the study protocol was approved by the institutional review board at the University of Alabama at Birmingham.

Study design

Baseline measurements were obtained after successful completion of a structured dietary weight-loss intervention (11) during which time the women lost an average of 12.1 ± 3.5 kg. For 4 weeks before testing, food was provided to maintain each participant in a weight-stable state, and weight stability of <1% variation was documented. After 4 weeks of the weight-maintenance diet, participants were admitted to the General Clinical Research Center for 4 days, during the follicular phase of the menstrual cycle. Baseline testing included respiratory chamber calorimetry for fasting RQ, 24-h RQ, sleeping RQ, and NSRQ and intravenous glucose tolerance tests for fasting insulin, acute insulin response to glucose, and insulin sensitivity. Body composition testing by dual-energy X-ray absorptiometry (DXA) was performed at baseline and annually over the following 2 years. During this time, the controlled diet protocol was repeated with 4 weeks of weight maintenance on the diet followed by body composition testing.

Outcome measures

RQ values were measured by indirect calorimetry as previously described (18). Briefly, subjects were transported from the General Clinical Research Center to the respiratory chamber of the Energy Metabolism Research Unit at 0700 hours, after a 12-h fast. The chamber had a volume of 17,500 liters (3.4 m long, 2.1 m wide, and 2.6 m high). Oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were continuously measured over 24 h by an O₂ analyzer (Magnos 4G) and a CO₂ analyzer (Uras 3G; Hartmann & Braun, Frankfurt, Germany). Substrate oxidation was calculated from CO₂ production and O₂ consumption rates, and this ratio was expressed as RQ. In addition to 24-h RQ, RQ values were quantified for sleeping and nonsleeping periods in the chamber. Sleeping RQ was averaged from RQ values measured during the sleeping period. The sleeping period began when lights were turned off (between 9:30 and 11:00 PM) and ended when each participant was awakened at 6:30 AM. Radar motion sensors were used to ensure that participants were inactive during the sleeping period. NSRQ was calculated for the period of the day when participants were awake. NSRQ encompassed the portion of the day when each participant was in a postprandial state, beginning with breakfast and continuing through the period following dinner. Fasting RQ was also measured and averaged over 30 min for each participant in the chamber upon waking at 6:30 AM.

Energy balance was calculated as total energy expenditure in the respiratory chamber subtracted from total energy intake of food consumed

during that time. Although in the chamber, participants consumed three scheduled meals with a macronutrient distribution of 20–23% energy from fat, 16–23% as protein, and 55–64% as carbohydrate. Total kilocalories were calculated from the Harris–Benedict equation (19) multiplied by an activity factor of 1.2, and participants were required to consume all food provided.

Fasting insulin, acute insulin response to glucose (AIR_g), and insulin sensitivity were assessed by a tolbutamide-modified, frequently sampled intravenous glucose tolerance test with minimal modeling (MINMOD version 3.0; Richard N Bergman) (20). Details of this test have been previously described (21). Briefly, intravenous catheters were placed in both arms. Three blood samples were drawn over 20 min for fasting glucose and insulin measurements. At time_{0min}, a bolus of 50% dextrose was administered intravenously at a dose of 11.4 g/m². A series of 29 blood samples were obtained over the following 3 h for measurements of serum glucose and insulin. At time_{20min}, a bolus of tolbutamide (125 mg/m²) was injected intravenously. Insulin sensitivity is expressed as the insulin sensitivity index (S_I) derived from the model. AIR_g represents the integrated incremental area under the curve for insulin during the first 10 min of the test. Glucose and insulin were analyzed in the Core Laboratory of UAB's General Clinical Research Center and Clinical Nutrition Research Center. Glucose was measured in 10 µl sera using an Ektachem DT II System (Johnson and Johnson Clinical Diagnostics, Rochester, NY). In the Core Laboratory, this analysis has a mean intra-assay coefficient of variation of 0.61%, and a mean interassay coefficient of variation of 1.45%. Insulin was assayed in duplicates of 200 µl aliquots with Diagnostic Products (Los Angeles, CA) "Coat-A-Count" kits. This assay has a sensitivity of 1.9 µIU/ml, a mean intra-assay coefficient of variation of 5%, and a mean interassay coefficient of variation of 6%. Commercial quality control sera of low, medium, and high insulin concentration (Lyphochek; Bio-Rad, Anaheim, CA) were included in every assay to monitor variation over time.

FM and fat-free mass were determined by dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI), using ADULT software, version 1.33 (Lunar Radiation). Change in FM (ΔFM) was calculated as the difference in FM between the baseline and the annual follow-up evaluations. Height (stretch stature to the nearest 0.1 cm) was measured by a stadiometer, and body weight (to the nearest 5 g) was recorded on a digital scale following an overnight fast. BMI (kg/m²) was calculated from weight and height.

Statistical analysis

Descriptive statistics were calculated for all variables of interest. Results are presented as means ± s.d. Fasting insulin, AIR_g, and S_I were log₁₀ transformed for normality before statistical analyses. Pearson correlations were used to examine associations between each RQ measure and ΔFM. Because NSRQ was the only RQ value correlated with ΔFM, multiple linear regression analysis was used to further examine the relationship between baseline NSRQ and subsequent ΔFM over the following 2 years. To ensure that the relationship between NSRQ and ΔFM was independent of individual differences in insulin action, regression models were adjusted for energy balance and either circulating insulin (fasting insulin or AIR_g) or S_I. All statistical tests were two-sided with a type 1 error rate of 0.05 and were performed using SPSS software, version 15.0 (Chicago, IL).

RESULTS

Participant characteristics are displayed in Table 1. By study design, all participants were normal weight (BMI <25 kg/m²) at the baseline evaluation. With the exception of one woman, all participants gained weight and FM over the 2-year follow-up period. No significant differences between ethnic groups were observed for any variable (data not shown). Of all RQ values examined, only NSRQ correlated with ΔFM ($r = 0.388$, $P = 0.026$ at 1 year). Multiple linear regression analysis revealed

Table 1 Subject characteristics (mean \pm s.d. and range)

Baseline characteristics ^a	
Age (years)	37.1 \pm 5.6 (22.9 to 47.3)
African Americans/whites	20/13
Body weight (kg)	65.3 \pm 6.9 (50.5 to 78.0)
BMI (kg/m ²)	24.1 \pm 1.1 (21.6 to 25.8)
Fat mass (kg)	21.3 \pm 4.2 (15.4 to 29.7)
Fat-free mass (kg)	40.6 \pm 4.4 (30.9 to 49.4)
Fasting Insulin (μ U/ml)	7.3 \pm 2.4 (3.0 to 14.0)
AI _{R_g} (μ U/ml) \times 10 min)	570.9 \pm 362.0 (95.0 to 1689.5)
S _I ($\times 10^{-4}$ min ⁻¹ /(μ U/ml))	6.5 \pm 3.3 (2.22 to 16.80)
24-h Energy expenditure (kcal)	1620.9 \pm 207.1 (1138.3 to 2038.5)
Energy balance (kcal)	-200.7 \pm 171.6 (-571.0 to 163.3)
24-h RQ	0.88 \pm 0.03 (0.81 to 0.94)
Fasting RQ	0.86 \pm 0.04 (0.77 to 0.94)
Sleeping RQ	0.84 \pm 0.04 (0.75 to 0.92)
NSRQ	0.89 \pm 0.03 (0.82 to 0.96)
Measurements at 1 year	
Δ Weight (kg)	+7.2 \pm 4.5 (-3.2 to 14.2)
Δ Fat mass (kg)	+6.9 \pm 3.8 (-1.8 to 12.8)
% Weight Δ	+11.1 \pm 7.3 (-4.0 to 24.0)
% Fat mass Δ	+6.5 \pm 3.2 (-0.1 to 12.1)
Measurements from 2 years	
Δ Weight (kg)	+11.1 \pm 6.1 (-3.6 to 19.4)
% Weight Δ	+17.2 \pm 9.7 (-5.0 to 33.0)
Δ Fat mass (kg)	+10.6 \pm 5.4 (-1.6 to 19.7)
% Fat mass Δ	+9.2 \pm 4.4 (-0.8 to 17.1)

AI_{R_g}, acute insulin response to glucose; NSRQ, nonsleeping RQ; RQ, respiratory quotient; S_I, insulin sensitivity index.

^aRecorded after 4 weeks of a controlled diet for weight maintenance following successful completion of a weight-loss intervention.

that NSRQ predicted Δ FM at both 1 and 2 years after adjusting for energy balance and either circulating insulin or insulin sensitivity. Final models that explained the most variance in Δ FM included NSRQ, energy balance, and S_I (Table 2 and Figure 1). Similar trends were observed when statistical analyses were performed with Δ weight (β for NSRQ = 63.99 \pm 34.87, P = 0.08) and percent Δ FM (β for NSRQ = 43.18 \pm 23.02, P = 0.07) as dependent variables in place of Δ FM.

DISCUSSION

Inherent individual variability in substrate oxidation may predispose to obesity. In this study, we found that among premenopausal obesity-prone women, high NSRQ predicted Δ FM independent of energy balance, circulating insulin, and insulin sensitivity. No association was observed between fat accumulation and other RQ values, including fasting, sleeping, and 24-h RQ. These results suggest that low postprandial fat oxidation and/or elevated postprandial carbohydrate oxidation may predispose certain women to weight gain and excess adiposity.

Table 2 Multiple linear regression models

Independent variable	Parameter estimate \pm s.e.e.	P	Model R^2
$y = \Delta$ FM at 1 years ($F = 1.83$, $P = 0.16$)			
Intercept	-38.953 \pm 19.767	0.058	0.159
NSRQ	50.160 \pm 21.454	0.026	
Energy balance	<-0.001 \pm 0.004	0.985	
Log ₁₀ S _I	1.708 \pm 3.627	0.641	
$y = \Delta$ FM at 2 years ($F = 2.90$, $P = 0.05$)			
Intercept	-54.055 \pm 26.951	0.054	0.231
NSRQ	64.018 \pm 29.251	0.037	
Energy balance	-0.003 \pm 0.006	0.554	
Log ₁₀ S _I	9.255 \pm 4.945	0.071	

The boldface values signify the significance levels.

FM, fat mass; NSRQ, nonsleeping RQ; RQ, respiratory quotient; S_I, insulin sensitivity index.

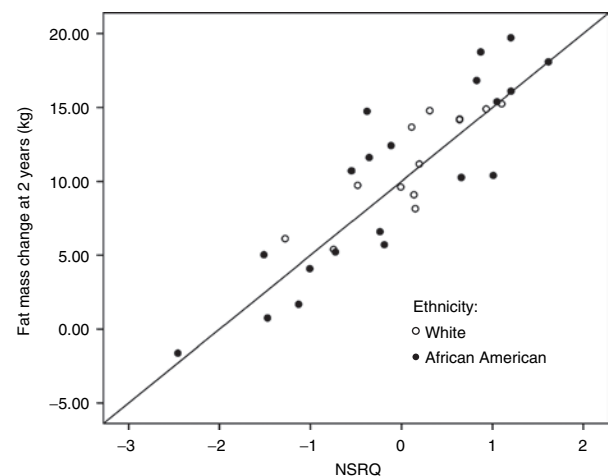


Figure 1 Standardized residual plot of NSRQ vs. Δ FM adjusted for energy balance and S_I. Δ FM, change in fat mass; NSRQ, nonsleeping respiratory quotient; S_I, insulin sensitivity index.

Results of this study advance existing evidence about the relationship between RQ and weight gain. Although some previous studies have reported positive associations between weight gain and either fasting or 24-h RQ (4–8,22), we have reported no correlation (10–12). Incongruent reports may reflect population-specific differences in energy metabolism. Notably, several studies supporting an association between weight gain and high 24-h RQ were conducted among Pima Indians (5,6,22), and recent genetic studies have identified single-nucleotide polymorphisms specific to this population that may underlie these findings (23).

Discrepancies in the literature are also likely due to variable control for dietary factors known to acutely influence RQ. For example, it is well-established that either positive energy balance or high carbohydrate intake can transiently elevate RQ (3). However, few studies relating RQ to weight gain have accounted for differences in macronutrient intake and/or energy balance between subjects. In this study, all participants

were provided with food for 4 weeks before RQ measurement in order to ensure uniform macronutrient intake and stable body weight. During their stay in the respiratory chamber, diet was controlled to provide a standard macronutrient profile and kilocalories for energy balance. Additionally, we used statistical methods to adjust for energy balance inside the respiratory chamber.

Individual differences in circulating insulin and/or insulin sensitivity may also confound reported relationships between weight gain and RQ. Circulating insulin suppresses lipid oxidation and facilitates fat storage (13). Among a cohort of 293 white women, Nagy *et al.* demonstrated that fasting insulin was inversely associated with fat oxidation (14). Similarly, Weinsier *et al.* reported that fasting insulin predicted 4-year weight gain among white, postmenopausal, normoglycemic women (10). Insulin sensitivity has been associated with fat accumulation in some (15–17), but not all (24) studies. Consistent with these findings, S_i among our sample showed a trend toward positive association with ΔFM after 2 years ($r = 0.316$, $P = 0.073$). By statistically adjusting for fasting insulin, AIR_g , and S_i , we assured that the observed relationship between ΔFM and NSRQ was not influenced by confounding relationships between insulin and RQ or FM. The final multiple linear regression model that included NSRQ, energy balance, and S_i explained ~23% of the variance in ΔFM after 2 years.

Our previous analyses among African-American and white women with tight dietary control also identified no relationship between weight gain and other RQ measures, including fasting, 24-h, or sleeping RQ (10–12). These three RQ measures encompass periods of the day when RQ should signify fat oxidation of endogenous free fatty acids. NSRQ, however, is measured specifically during the nonsleeping period in the respiratory chamber and would correspond to the typical, physiological postprandial period. Sources of variability in NSRQ may include endocrine factors such as concentrations of estrogen (25) or thyroid function (26). In his study, NSRQ distinctively and independently predicted FM accrual. High NSRQ may represent suppressed oxidation of dietary lipid and/or an enhanced ability to switch to carbohydrate oxidation in response to a mixed substrate meal. If lipid oxidation is suppressed, these individuals may be less able to increase fat oxidation after dietary fat intake, and they may therefore be prone to store fat as adipose leading to a partitioning of energy toward FM at the expense of lean mass. Moreover, previous studies have suggested that greater carbohydrate oxidation and lesser fat oxidation may predispose certain individuals to store less glycogen and therefore experience more hunger (22,27). This increased hunger may lead to chronic positive energy imbalance and gain in body fat.

The study was strengthened by robust measures for all variables, including intravenous glucose tolerance test for insulin sensitivity, chamber calorimetry for RQ assessments, and dual-energy X-ray absorptiometry for body composition. Strengths of this study also included tight control of dietary intake before and during RQ measurements. Despite attempts to achieve zero energy balance within the

chamber, variability in energy balance may present a potential confounder. Therefore, all models included statistical adjustment for energy balance. Efforts were made to minimize confounders by the homogeneity of the cohort. However, because we examined a very specific subject population of normal glucose tolerant, premenopausal women of only African-American and European-American ethnicity, it is impossible to extrapolate these findings to other ethnicities, men, older women, never-overweight individuals, or those with impaired glucose tolerance. Given the limited sample size and overall significance of the models, results should be interpreted with caution and repeated in larger samples.

In conclusion, this study revealed that among obesity-prone premenopausal women, NSRQ uniquely predicted ΔFM independent of energy balance and insulin sensitivity. Because all of the women in our cohort reported a family history of overweight or obesity in a first degree relative, high NSRQ may be a marker for nonmodifiable genetic factors affecting their capacity to oxidize lipid and carbohydrate. Studies are needed to determine whether these women may experience more success with weight maintenance by modifying their food choices to reduce fat intake or by modifying their physical activity to enhance fat oxidation.

ACKNOWLEDGMENTS

This work was supported by R01DK49779, M01-RR-00032, and P30-DK56336. Stouffer's Lean Cuisine provided food used before metabolic testing. David Bryan and Robert Petri provided technical assistance; Maryellen Williams and Kangmei Ren conducted laboratory analyses; Susan Davies and Paul Zuckerman served as project coordinators.

DISCLOSURE

The authors declared no conflict of interest.

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