

Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ

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ZURLO, FRANCESCO, STEPHEN LILLIOJA, ANTONELLA ESPOSITO-DEL PUENTE, BULANGU L. NYOMBA, ITAMAR RAZ, MOHAMMED F. SAAD, BOYD A. SWINBURN, WILLIAM C. KNOWLER, CLIFTON BOGARDUS, AND ERIC RAVUSSIN. *Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ*. Am. J. Physiol. 259 (Endocrinol. Metab. 22): E650-E657, 1990.—Reduced oxidation of fat leading to a positive fat balance could be a factor in the development of obesity. Twenty-four-hour respiratory quotient (RQ) was measured in 152 nondiabetic Pima Indians fed a weight-maintenance diet [87 males and 65 females; 27 ± 6 yr (mean \pm SD); 93.9 ± 22.9 kg; $32 \pm 9\%$ fat]. Twenty-four-hour RQ varied from 0.799 to 0.903. Prior change in body weight, 24-h energy balance, sex, and percent body fat explained 18% of the variance in 24-h RQ ($P < 0.001$). In a subgroup of 66 siblings from 28 families, family membership explained 28% of the remaining variance in 24-h RQ ($P < 0.05$). In 111 subjects for whom follow-up data (25 ± 11 mo) were available, 24-h RQ was correlated with subsequent changes in body weight and fat mass ($r = 0.27$, $P < 0.01$ and $r = 0.19$, $P < 0.05$, respectively). Subjects with higher 24-h RQ (90th percentile) independent of 24-h energy expenditure were at 2.5 times higher risk of gaining ≥ 5 kg body weight than those with lower 24-h RQ (10th percentile). We conclude that in Pima Indians fed a standard diet 1) family membership is the principal determinant of the ratio of fat to carbohydrate oxidation, and 2) a low ratio of fat to carbohydrate oxidation is associated with subsequent weight gain independent of low energy expenditure and may contribute to the familial aggregation of obesity.

obesity; indirect calorimetry; fat oxidation; body composition; respiratory quotient; Pima Indians

OBESITY IS A FAMILIAL DISORDER that may be genetically determined (23, 29). The familial occurrence of obesity could result from a similarity among siblings in either excessive caloric intake or low energy expenditure (26), or both. High fat intake per se may play a role in the etiology of obesity (5, 31). However, reduced rates of fat utilization could also play a role in the development of obesity (32). Indeed, studies in postobese subjects have suggested that reduced fat oxidation may be related to subsequent body weight gain (12, 18). The present study was conducted to explore the determinants of energy substrate utilization (respiratory quotient) and to test

the hypothesis that a reduced fat-to-carbohydrate oxidation ratio is a predictor of body fat gain.

The simultaneous measurements of gaseous exchange and urinary nitrogen excretion can be used to calculate the rate of fuel oxidation by the body and the type of fuel being oxidized (11, 20). The respiratory quotient, i.e., the ratio between carbon dioxide production and oxygen consumption, reflects the ratio of carbohydrate to fat oxidation and varies under different conditions. In subjects consuming a balanced diet that meets energy requirements for body weight maintenance, the main oxidative substrate after an overnight fast is fat and the respiratory quotient is typically ~ 0.80 (2, 21). After a meal rich in carbohydrate, glucose is the principal substrate oxidized, and the respiratory quotient increases to values close to 1.00 (2, 10). Changes in the 24-h respiratory quotient are also associated with qualitative variations of the diet, so that the proportion of carbohydrate and fat utilized tends to match the diet composition under conditions of energy balance (7, 17, 18). However, even when individuals are on the same diet, the respiratory quotient after an overnight fast varies more between individuals than within a single individual measured on separate days (21). This suggests that factors other than diet may influence the ratio of fat to carbohydrate oxidized. Any such differences between the nutrients being utilized as fuel and the proportions of those nutrients in the diet imply ongoing changes in body composition even under conditions of energy balance.

We determined the relationship between the daily respiratory quotient and a collection of variables comprising family membership (siblings), age, sex, prior change in body weight, 24-h energy balance, body fat, spontaneous physical activity, and fasting plasma concentrations of glucose, insulin, and free fatty acids. The respiratory quotient was measured using a human respiratory chamber. One hundred fifty-two Pima Indians receiving a nutritionally balanced diet were studied, and in 111 of these follow-up data were available to permit an analysis of the impact of daily fuel utilization on subsequent changes in body weight and body composition.

METHODS

Subjects. Since 1985, Pima Indian subjects have been admitted to the metabolic ward of the Clinical Diabetes and Nutrition Section of the National Institutes of Health in Phoenix, AZ, for longitudinal studies that have included measurements of 24-h energy expenditure, using a human respiratory chamber (25, 26). On admission, all subjects were determined to be in good health by means of medical history, physical examination, electrocardiogram, and blood and urine screening tests. None of the subjects was dieting, taking any medicine, or had clinical evidence of illness apart from obesity. Written informed consent was obtained. The subjects received a nutritionally balanced diet that met energy requirements for body weight maintenance and, after at least 2 days on the metabolic ward, were given an oral glucose tolerance test (24), during which plasma glucose (glucose oxidase method) and insulin (15) were measured. Only those subjects with fasting glucose concentrations <110 mg/dl, not diabetic according to the National Diabetes Data Group criteria (24), and studied in the respiratory chamber 2 full days after admission were included in the analysis. A total of 152 subjects met these criteria. In 109 subjects, fasting plasma free fatty acids were also measured (22). The percent body fat of each subject was determined by underwater weighing with simultaneous measurement of residual lung volume (13). Subject characteristics are listed in Table 1.

Diet. After admission to the metabolic ward, the subjects consumed a weight-maintenance, solid-food diet that supplied 50% of the calories as carbohydrate, 30% as fat, and 20% as protein; the calculated food respiratory quotient was 0.866 (8). Meals were freshly and individually prepared in the metabolic kitchen from standardized recipes with weighed and/or measured ingredients. The caloric content of the weight-maintenance diet was initially estimated on the basis of body weight and then adjusted on the basis of changes in daily body weight to maintain a constant weight ($\pm 1\%$). Body weight was measured before breakfast with the subjects lightly dressed, and the weight of clothes was subtracted. An index of short-term energy balance was assessed as the rate of change in body weight from the 1st day after the admission to the day of the test in the respiratory chamber. During the 24 h spent in the respiratory chamber, the subjects were fed a diet calculated as previously reported, which provided $\sim 80\%$ of the calories received on the metabolic ward to allow for reduced physical activity in the respiratory chamber (1). Returned food

was weighed, and corrections were made in the calculation of energy and nutrient intakes. Acute energy balance, i.e., 24-h energy balance during the test in the respiratory chamber, was calculated as the percent of 24-h energy expenditure measured in the respiratory chamber as follows: $(24\text{-h energy intake} - 24\text{-h energy expenditure}) / (24\text{-h energy expenditure}) \times 100$.

Respiratory chamber. After at least 2 full days on the metabolic ward (7 ± 4 days, mean \pm SD, range 2–36, excluding the day of admission), the subjects spent 24 h in the respiratory chamber, an open-circuit, indirect calorimeter in which energy expenditure and respiratory quotient were measured (25). The respiratory chamber is an air-tight room with a net volume of 19,000 liters maintained at a constant temperature of $24 \pm 0.5^\circ\text{C}$. Fresh atmospheric air is continuously drawn through the chamber, and after determinations of flow, barometric pressure, and humidity, a fraction of the outflowing air is dried and then analyzed for oxygen and carbon dioxide concentrations (Hartman and Braun analyzers, Frankfurt, FRG). Spontaneous physical activity is assessed in the respiratory chamber using two microwave radars (MICD 930; Honeywell, Minneapolis, MN). The description and calibration of the respiratory chamber have been reported previously in detail (25). The 24-h respiratory quotient is the ratio between 24-h carbon dioxide production and 24-h oxygen consumption.

The reproducibility of the respiratory quotient measurements in the respiratory chamber was tested in 15 subjects, in whom 24-h respiratory quotient was measured on at least two different admissions (12 subjects had 2 measurements, 2 subjects had 3, and 1 subject had 4) with a body weight change $<5\%$, and in 9 subjects who spent 7 consecutive days in the respiratory chamber. Interindividual variability of 24-h respiratory quotient in the 152 subjects was compared with the intraindividual variability.

Calculations and statistical analyses. Statistical analyses were performed using the procedures of the SAS Institute, Cary, NC (27). Correlations are Pearson product-moment correlations. The logarithm of insulin concentration was used in the statistical analyses to normalize the distribution. The residual unexplained respiratory quotient in each individual was calculated by the general linear model procedure using the following four covariates: the rate of body weight change during the time in the metabolic ward preceding the test (an index of short-term energy balance), acute energy balance (energy balance during the 24 h of the test), percent body fat, and sex (see RESULTS). For each subject, an adjusted

TABLE 1. Physical characteristics and fasting plasma glucose, insulin, and free fatty acid concentrations of 152 nondiabetic Pima Indians

	Men (n = 87)		Women (n = 65)	
Age, yr	27 \pm 6	(18–42)	26 \pm 6	(18–42)
Height, cm	171 \pm 5	(160–183)	159 \pm 5	(148–170)
Weight, kg	94.6 \pm 23.5	(50.6–159.0)	93.0 \pm 22.3	(49.0–141.6)
Body fat, %	27 \pm 8	(5–43)	39 \pm 6	(20–50)
Fasting glucose, mg/dl	94 \pm 6	(83–109)	96 \pm 7	(82–109)
Fasting insulin, $\mu\text{U/ml}^*$	34 \pm 19	(6–101)	45 \pm 21	(16–106)
Fasting free fatty acids, $\mu\text{mol/l}^\dagger$	316 \pm 86	(132–528)	410 \pm 97	(197–592)

Values are means \pm SD; ranges are in parentheses. * n = 135 (76 men, 59 women); † n = 109 (60 men, 49 women).

24-h respiratory quotient was calculated by adding the residual respiratory quotient to the mean 24-h respiratory quotient for the whole group.

The familial aggregation of the adjusted 24-h respiratory quotient was determined for subjects who had at least one other sibling measured. Each member of a family (i.e., brothers and sisters) was assigned the same value of an indicator (class) variable to denote family membership, as previously described in detail (3). The statistical significance of the familial effect was determined by comparing the variability among families with the variability within families after the effects of body weight change prior to testing, acute energy balance, percent body fat, and sex had been accounted for. The intraclass correlation coefficient (r_1) measures the proportion of total variance explained among families; values close to 1.00 suggest that members of a family are similar, whereas 0 denotes no resemblance within families (28).

To determine whether the 24-h respiratory quotient and the adjusted 24-h respiratory quotient predicted subsequent weight gain, we selected the 111 subjects (62 males and 49 females) in whom body composition was remeasured at a follow-up visit (mean \pm SD follow-up duration 25 ± 11 mo, range 5–48 mo). Sixty-one of the 111 subjects with follow-up data have been included in a study on the relationship between metabolic rate and subsequent weight change (26). The 111 subjects had a total of 260 follow-up admissions to the metabolic ward. Follow-up data were analyzed in two different ways. First, the relationships of 24-h respiratory quotient, both adjusted and not, with body weight and fat mass changes were assessed by simple correlation. The first and last admissions were considered. The relationships between adjusted 24-h respiratory quotient and changes in body weight and composition were also assessed by multiple linear regressions with 24-h energy expenditure (adjusted for fat-free mass and fat mass) as an additional covariate. This was to test whether the adjusted 24-h respiratory quotient was a predictor of weight and body composition change, independent of 24-h energy expenditure. We expressed the change of body weight over time as a rate of change (weight change/years of follow-up) and as a rate of percent change (percent of initial weight/years of follow-up). Similarly, we expressed the change of body fat mass over time as a rate of change and as a rate of percent change (percent of initial body fat mass/years of follow-up). Similar analyses were also performed for the 24-h nonprotein respiratory quotient. Second, the predictive value of adjusted 24-h respiratory quotient on body weight and body fat changes was assessed by survival analysis using Cox's proportional-hazards function analysis (4, 14). The survival analysis takes varying periods of follow-up into account, and all 260 follow-up visits were included. "Failure" was defined by different values of body weight gain (≥ 2.5 , ≥ 5 , or ≥ 10 kg). All variables included in the different models satisfied the proportional-hazards assumption.

RESULTS

Variability of respiratory quotient measurement. The mean 24-h respiratory quotient for the 152 subjects was

0.847 with a standard deviation (SD) of 0.022 (interindividual SD). After adjustment of the 24-h respiratory quotient for prior change in body weight (during admission), acute energy balance, percent body fat, and sex, the interindividual SD was 0.020. Nine patients had a 24-h respiratory quotient measured on 7 consecutive days and 15 on at least 2 nonconsecutive days. The mean adjusted respiratory quotient was 0.847 for both groups. An SD of 24-h adjusted respiratory quotient could be calculated for each of these individuals; the mean SD value for the 9 subjects was 0.002 and for the 15 subjects was 0.006, both values significantly lower than the interindividual variation ($P < 0.001$) (Fig. 1). Part of the observed variability within a subject was related to the error of the measurements previously reported (25).

Respiratory gas exchanges. The subjects spent on average 7 ± 4 days (range 2–36 days, excluding the day of admission) on the metabolic ward before measurement in the respiratory chamber. Despite efforts to achieve weight maintenance, body weights varied from -2.4 to $+1.6$ kg (means \pm SD -0.2 ± 0.7 kg, $P < 0.01$ for comparison with 0) from admission to the measurement in the respiratory chamber. Also, the rate of body weight change was different from zero (-0.04 ± 0.15 kg/day, $P < 0.01$). The mean 24-h energy expenditure in the chamber was $2,424 \pm 367$ kcal/day for men and $2,081 \pm 321$ kcal/day for women, whereas the mean 24-h energy intake in the respiratory chamber was $2,368 \pm 262$ and $2,152 \pm 331$ kcal/day, respectively. Energy balance of the day spent in the respiratory chamber expressed as a percentage of the measured energy expenditure varied in men from -23 to 26% (mean \pm SD $-2 \pm 8\%$, $P = 0.06$ for comparison with 0) and from -27 to $+31\%$ (mean \pm SD $4 \pm 12\%$, $P < 0.01$ for comparison with 0) in women. The variability of the acute energy balance was a consequence of differences in spontaneous physical activity, uneaten food, and incorrect estimation of energy require-

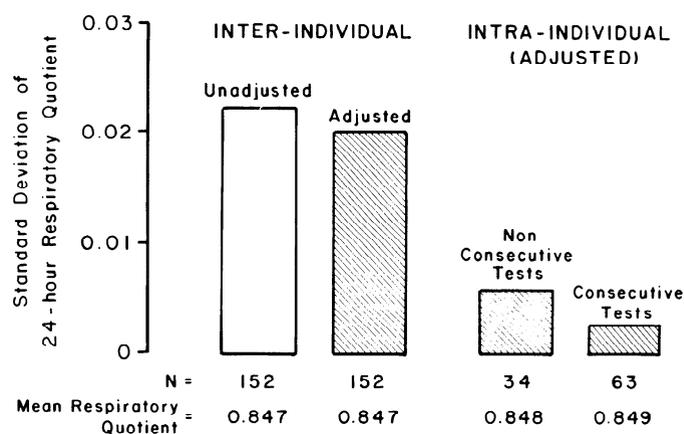


FIG. 1. SD in 24-h respiratory quotient (RQ) among 152 subjects of study (interindividual variance), compared with intraindividual SD in 24-h RQ in 34 nonconsecutive repeated measurements in 15 subjects (12 subjects had 2, 2 subjects had 3, and 1 subject had 4), and in 63 repeated measurements in 9 subjects who had measurements on 7 consecutive days. The 24-h RQ was adjusted for rate of body weight change on metabolic ward, current 24-h energy balance, sex, and percent body fat (hatched bars). Intraindividual variability was significantly lower than interindividual variability ($P < 0.001$). Because of the particular design of these tests, RQ for consecutive measurements were not adjusted for rate of body weight change before testing.

TABLE 2. Relationships between 24-h respiratory quotient and physical and metabolic parameters by Pearson product-moment correlation

	All Subjects		Males		Females	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Prior change in body weight*	0.32	<0.001	0.25	0.02	0.39	<0.01
Acute energy balance	0.25	<0.01	0.27	0.01	0.20	0.11
Age	0.11	0.16	0.12	0.26	0.12	0.35
Fasting plasma glucose	-0.03	0.73	-0.04	0.72	-0.06	0.65
Fasting plasma insulin	-0.17	0.04	-0.30	0.01	-0.18	0.18
Percent body fat	-0.08	0.30	-0.22	0.04	-0.23	0.06
Fasting plasma free fatty acids	-0.07	0.47	-0.14	0.28	-0.26	0.08
Spontaneous physical activity	0.01	0.92	0.07	0.54	-0.05	0.72

Correlation performed with 152 nondiabetic Pima Indians (87 men and 65 women). Acute energy balance, 24-h energy balance (%) during test [(intake - expenditure)/(expenditure) × 100]. Fasting insulin, natural logarithm of fasting plasma insulin concentration to normalize distribution. *n* = 135 (76 males, 59 females). Fasting free fatty acids, *n* = 109 (60 males, 49 females). * Rate of body weight change (kg/day) during time in metabolic ward preceding test.

ments in the respiratory chamber. The 24-h respiratory quotient varied from 0.799 to 0.903 and was similar in men and women (0.845 ± 0.021 and 0.850 ± 0.024 , respectively, $P = 0.13$), even though the women were fatter ($39 \pm 6\%$ body fat vs. $27 \pm 8\%$ in men, $P < 0.001$).

Determinants of 24-h respiratory quotient. There was no relationship between the 24-h respiratory quotient and the duration of the weight-maintenance diet before the measurement ($r = -0.04$, $P = 0.66$). However, by simple correlation analysis, 24-h respiratory quotient was best correlated with the rate of body weight change during the days spent in the metabolic ward before respiratory exchange measurement ($r = 0.32$, $P < 0.001$, Table 2, Fig. 2). The twenty-four-hour respiratory quotient also correlated with the acute energy balance [(food intake - 24-h energy expenditure)/24-h energy expenditure]; $r = 0.25$, $P < 0.01$]. The 24-h respiratory quotient had a negative correlation with percent body fat when men and women were considered separately ($r = -0.22$, $P = 0.04$ and $r = -0.23$, $P = 0.06$, respectively). Fasting plasma insulin concentration correlated negatively with 24-h respiratory quotient ($r = -0.17$, $P = 0.04$ in all subjects; $r = -0.30$, $P = 0.01$ in men; and $r = -0.18$, $P = 0.18$ in women), but fasting plasma glucose was not correlated with 24-h respiratory quotient. Fasting plasma free fatty acid concentration and 24-h respiratory quotient were weakly correlated in women ($r = -0.25$, $P = 0.08$) but not in men, and there was no correlation between the 24-h respiratory quotient and the spontaneous physical activity measured in the chamber ($r = 0.01$, $P = 0.92$). In multiple linear regression analysis, prior change in body weight, acute energy balance, percent body fat, and sex together explained 18% of the variance in 24-h respiratory quotient ($P < 0.001$). No correlation was found between the adjusted 24-h respiratory quotient and either 24-h energy expenditure or 24-h energy expenditure adjusted for fat-free mass and fat mass ($r = 0.04$, $P = 0.67$ and $r = 0.09$, $P = 0.25$, respectively). Similar results (not presented) were found using 24-h nonprotein respiratory quotient.

Familial effect on 24-h respiratory quotient. Sixty-six siblings (37 males and 29 females) from 28 different families were studied: 21 of the families had 2 siblings, 4 had 3 siblings, and 3 had 4 siblings. The adjusted 24-h respiratory quotient showed a familial dependence ($P <$

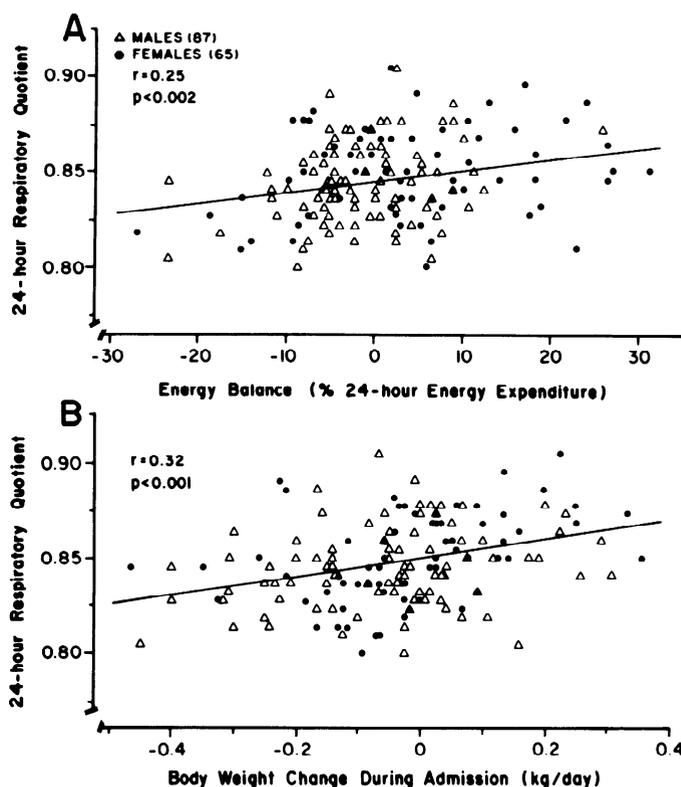


FIG. 2. A: relationship between mean 24-h respiratory quotient (RQ) and acute energy balance expressed as percentage of energy expenditure [(24-h energy intake - 24-h energy expenditure)/(24-h energy expenditure) × 100] during day of RQ measurement. B: relationship between 24-h RQ and rate of body weight change (kg/day) during time spent on metabolic ward preceding 24-h RQ measurement in 152 nondiabetic Pima Indians.

0.05) (Fig. 3). The intraclass correlation coefficient (r_i) for the effect of family membership on the adjusted 24-h respiratory quotient was 0.28 ($P < 0.05$). This means that 28% of the variance in 24-h respiratory quotient not explained by prior body weight change, acute energy balance, percent body fat, and sex was explained by family membership. The intraclass correlation coefficient for the effect of family membership on the adjusted 24-h nonprotein respiratory quotient was 0.26 ($P = 0.05$).

Twenty-four-hour respiratory quotient and change in body weight. Follow-up measurements were available in

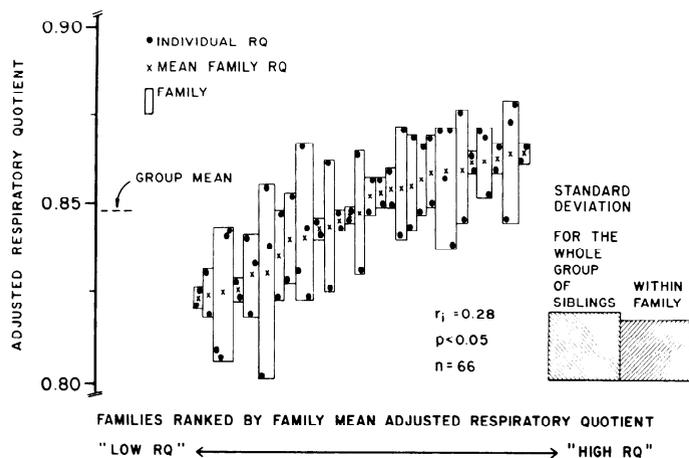


FIG. 3. Familial aggregation of 24-h respiratory quotient (RQ) in 66 nondiabetic Pima Indian siblings from 28 different families. ●, Individual 24-h RQ adjusted for rate of body weight change on metabolic ward, current 24-h energy balance, sex, and percent body fat. x, mean values for different families. Each bar represents 1 family, and families have been ranked according to increasing adjusted 24-h RQ. SD among all siblings and within families are depicted by hatched bars on right. Family membership accounts for 28% of variance in 24-h RQ not explained by covariates energy balance, body fat, and sex.

TABLE 3. Relationships between 24-h RQ and subsequent body weight and body fat changes by Pearson product-moment correlation in 111 nondiabetic Pima Indians

	24-h RQ		Adjusted 24-h RQ	
	r	P	r	P
Body weight gain, kg	0.27	<0.01	0.24	0.01
Rate of body weight gain, kg/yr	0.24	0.01	0.24	0.01
Rate of body weight gain, %initial weight/yr	0.28	<0.01	0.24	0.01
Body fat mass gain, kg	0.19	0.04	0.17	0.08
Rate of fat mass gain, kg/yr	0.17	0.08	0.15	0.11
Rate of fat mass gain, %initial fat mass/yr	0.22	0.02	0.17	0.07

Values for 62 males and 49 females. RQ, respiratory quotient. 24-h RQ adjusted for rate of body weight change on metabolic ward preceding test, acute energy balance during test, sex, and percent body fat.

111 subjects (62 males, 49 females). The mean follow-up duration was 25 ± 11 mo.

By simple correlation analysis (Table 3), the 24-h respiratory quotient and the adjusted 24-h respiratory quotient were correlated with subsequent body weight change in kilograms ($r = 0.27$, $P < 0.01$ and $r = 0.24$, $P = 0.01$, respectively) and body fat mass change in kilograms ($r = 0.19$, $P = 0.04$ and $r = 0.17$, $P = 0.08$, respectively). Both the 24-h respiratory quotient and the adjusted 24-h respiratory quotient also correlated with the rate of body weight change (kg/yr; $r = 0.24$, $P = 0.01$ for both) and the rate of body weight change as a percent of the initial weight ($r = 0.28$, $P < 0.01$ and $r = 0.24$, $P = 0.01$, respectively).

Using multiple linear regression, the adjusted 24-h respiratory quotient was an independent predictor of both rate of body weight gain ($P < 0.01$) and rate of body fat mass gain ($P = 0.04$), independent of adjusted 24-h energy expenditure. As previously reported (26), a low

rate of adjusted 24-h energy expenditure predicts both rate of body weight and fat mass gain ($P = 0.03$ and $P < 0.01$, respectively). There was no correlation between adjusted 24-h respiratory quotient and adjusted 24-h energy expenditure ($r = -0.03$, $P = 0.72$). Similar results were found using 24-h nonprotein respiratory quotient (data not shown).

In the proportional-hazards analysis, the adjusted 24-h respiratory quotient was a significant predictor of body weight gain using different criteria such as 2.5, 5, and 10 kg body weight gain, even when controlled for adjusted 24-h energy expenditure (Table 4). This effect was also independent of the body weight and body composition at the first study. As an example, the rate at which subjects attained a weight gain ≥ 5 kg was 2.5 times as great (95% confidence interval, 1.3–4.9) for a subject with an adjusted 24-h respiratory quotient (RQ) at the 90th percentile (RQ = 0.877) as for a subject at the 10th percentile (RQ = 0.822; Fig. 4). Adding the 24-h energy expenditure

TABLE 4. Predictive effect of adjusted 24-h RQ on body weight gain by proportional-hazards linear model in 111 nondiabetic Pima Indians

Definition of Body Weight Gain, kg	n	Effect of Adjusted 24-h RQ		Effect of Adjusted 24-h RQ Independent of 24-h Energy Expenditure	
		RR	P	RR	P
>2.5	65	1.8 (1.0–3.2)	0.048	1.8 (1.0–3.3)	0.036
≥ 5	44	2.4 (1.2–4.8)	0.012	2.5 (1.3–4.9)	0.009
≥ 10	17	3.7 (1.2–11.2)	0.023	3.8 (1.3–11.1)	0.017

RQ, respiratory quotient. n, no. of subjects gaining specified amounts of weight. RR, rate ratio (95% confidence interval) for person in 90th percentile compared with person in 10th percentile of adjusted RQ. 24-h RQ adjusted for rate of body weight change on metabolic ward preceding test, acute energy balance, sex, and percent body fat. 24-h Energy expenditure adjusted for fat-free mass and fat mass.

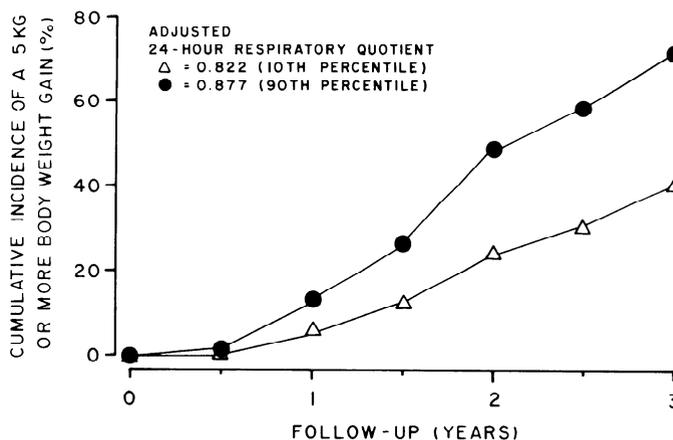


FIG. 4. Cumulative incidence of 5 kg body wt gain or more at 10th and 90th percentile of adjusted 24-h respiratory quotient (RQ; 0.822 and 0.877, respectively) measured in 111 subjects on whom follow-up measurements were available. No. of subjects studied at each time interval was 109 after 6 mo, 95 after 1 yr, 79 after 1.5 yr, 57 after 2 yr, 43 after 2.5 yr, and 18 after 3 yr. Cumulative incidence was calculated by proportional-hazards model adjusting 24-h RQ for differences in rate of body wt change on metabolic ward, acute energy balance, percent body fat, and sex and controlling for energy expenditure adjusted for fat-free mass and fat mass. With outcome defined as a weight gain of 5 kg, ratio of hazard rates for a person at 90th percentile of adjusted RQ compared with one at 10th percentile was 2.5 (95% confidence interval 1.3–4.9).

adjusted for fat-free mass and fat mass to the model did not substantially change this estimate.

Twenty-four-hour respiratory quotient measurements were repeated on 20 subjects at follow-up admission. The change in 24-h respiratory quotient correlated with both the change in body weight ($r = -0.42$, $P = 0.07$) and the change in plasma free fatty acid concentration ($n = 16$, $r = -0.53$, $P < 0.05$).

DISCUSSION

In Pima Indians, higher 24-h respiratory quotients, reflecting low rates of fat oxidation relative to carbohydrate oxidation, are associated with a higher rate of subsequent weight gain. In addition, in Pima Indians fed a standard diet, ~18% of the variation in 24-h respiratory quotient is associated with the set of variables including prior change in body weight, current 24-h energy balance, the proportion of body weight as fat, and the sex of the subject. The 24-h respiratory quotient also showed familial aggregation, which explained another 28% of its variance.

Determinants of 24-h respiratory quotient. It is known that the composition of the diet can greatly influence the respiratory quotient (7, 17, 18). However, the first aim of this study was to measure the effect of other possible determinants of the respiratory quotient. Some potentially important factors that could not be measured but that could have affected the 24-h respiratory quotient as well include major changes in body weight, physical activity, or composition of the diet in the weeks or months before admission. However, the lack of correlation between the duration of the time spent on the ward preceding the test and the 24-h respiratory quotient suggests that the impact of the change from free-living conditions to the metabolic ward was limited. Despite our efforts, stable weight on the metabolic ward was not always maintained. The rate of body weight change before the 24-h calorimetry was related to the 24-h respiratory quotient (Fig. 2) and explained part of its large variability. Similarly, the acute energy balance during the 24-h test was related to the 24-h respiratory quotient with lower respiratory quotient values found in subjects in negative energy balance. These observations emphasize the effect of the short-term changes in body weight before testing and of the acute energy balance during the measurement on the mixture of fuels oxidized and therefore stress the importance of the pretest conditions when assessing fuel utilization.

Of the variables measured in this study, family membership was the greatest determinant of 24-h respiratory quotient. However, other determinants such as body composition, fasting plasma insulin levels, fasting plasma free fatty acids, and sex had some impact on the 24-h respiratory quotient. The 24-h respiratory quotient and insulin concentration were negatively correlated. High fasting insulin concentrations reflecting insulin resistance were associated with lower carbohydrate oxidation and higher fat oxidation, as might be expected if both glucose uptake and suppression of free fatty acid release were resistant to insulin (19).

On average, females were fatter than males, and only

when sexes were considered separately did the degree of obesity positively correlate with fat oxidation. Moreover, we found a significant and independent sex effect on the respiratory quotient, with a generally lower ratio of fat to carbohydrate oxidation (i.e., higher respiratory quotient) in females than males. Therefore these observations suggest the existence of different patterns of fat utilization and/or storage between sexes that might help to explain the higher relative fat mass in women. The lack of a significant effect of age on the respiratory quotient could be merely a consequence of the narrow range of age in the subjects studied. The absence of correlation between spontaneous physical activity in the chamber and respiratory quotient suggests that this limited level of activity does not influence the composition of the fuel mix oxidation.

Despite potential errors (20), the measurement of the 24-h respiratory quotient was reproducible. The interindividual SD of the adjusted 24-h respiratory quotient was 3–10 times greater than that observed within individuals. This strongly indicates that there are large differences in the mixture of fuels oxidized by individuals consuming a standard diet.

Risk factor for body weight gain. Despite the fact that the subjects were already considerably obese at the beginning of the study, the amounts of weight gained during the follow-up study were not trivial. Overall, 40% of the 111 subjects gained at least 5 kg and 15% at least 10 kg. The predictive value of respiratory quotient on subsequent weight change was found using two different analyses of longitudinal data, 1) by a simple correlation between respiratory quotient and subsequent rate of body weight change and 2) by survival analyses. The results of both analyses showed a significant impact of 24-h respiratory quotient on subsequent body weight change.

The relationship of respiratory quotient to body weight gain is not very strong. However, the simple correlation between 24-h respiratory quotient and rate of body weight change ($r = 0.24$) was comparable with the correlation reported in the Framingham study (16) between degree of obesity [body mass index (BMI)] and systolic blood pressure ($r = 0.2$ – 0.4), BMI and cholesterol ($r = 0.10$ – 0.15), and BMI and blood sugar ($r = \sim 0.1$). Furthermore, in the Framingham study, men with the highest index of obesity (5th quintile of BMI) compared with those with the lowest index of obesity (1st quintile of BMI) experienced 1.9 and 1.4 times the risk of coronary heart disease and cerebral vascular accident, respectively (16), i.e., a lower risk ratio than that observed in our study for a body weight gain ≥ 5 kg. Thus the magnitude of the association of low relative fat oxidation and weight gain is at least as great as that of obesity and coronary heart disease, the importance of which is widely accepted.

Recently, Wade et al. (32) in a cross-sectional study have suggested that small but persistent reductions in the oxidation of fatty acids by the skeletal muscle might be an etiological factor for obesity. Longitudinal studies also suggest an important relationship between the ratio of energy substrate oxidation (respiratory quotient) and weight gain. Lean and James (18) reported higher 24-h respiratory quotients in postobese subjects compared with obese subjects and compared with weight- and body-

composition-matched, nonobese controls. Froidevaux et al. (12) reported that subjects failing to maintain a body weight reduction had higher 24-h respiratory quotients than those succeeding in keeping their weight down. Taken together, these observations suggest that in postobese subjects the ratio of fat to carbohydrate oxidation is reduced and may favor positive fat balance and weight gain.

Recent evidence suggests that fat balance, unlike carbohydrate balance, is not tightly regulated (1). Because the amount of carbohydrate stores (expressed in energy units) are <0.05 the amount of fat stores, regulatory mechanisms probably allow the organism to give priority to the maintenance of carbohydrate balance (8, 9). Positive carbohydrate balance presumably is a signal for either an increased adaptive thermogenesis (30), a reduction of food intake (7), or both. None of these mechanisms favors weight gain. Conversely, if the 24-h fat oxidation is low relative to carbohydrate oxidation, the excess in energy intake is stored mainly as fat with a reduced thermogenic effect and without the negative feedback regulation on the food intake, both of which favor weight gain.

Whether the reduced fat oxidation in some people is the consequence of accelerated fat storage, reduced fat mobilization, or impaired fat oxidation could not be determined in our study. Higher lipoprotein lipase activity in the adipose tissue, as suggested by studies in postobese patients, might explain differences in fat storage (6). Also, fatty acids are oxidized in proportion to their availability, i.e., plasma concentrations (19). Hence low lipid oxidation rates could be due to relatively low availability of free fatty acids. If triglyceride is stored in adipose tissue but is not readily released, then the respiratory quotient will be high. Among the 20 subjects in whom measurements of 24-h respiratory quotient were repeated, changes in body weight were negatively correlated with changes in 24-h respiratory quotient but positively correlated with changes in fasting free fatty acids; i.e., the largest weight gains were associated with the greatest decreases in 24-h respiratory quotient and the largest increases in free fatty acids. This suggests that expansion of fat stores tends to promote fat mobilization and consequently fat oxidation and therefore represents a compensatory mechanism for low rates of fat oxidation. This is in agreement with the model of nutrient balance proposed by Flatt (9), in which substantial changes in the size of the adipose tissue mass could increase fat oxidation and spare carbohydrate oxidation.

In conclusion, in Pima Indians fed a standard weight-maintenance diet, 1) the ratio of fat to carbohydrate oxidation aggregates in families independently of body composition; 2) women have a higher respiratory quotient, i.e., lower ratio of fat to carbohydrate oxidation, which might explain their higher body fat content; 3) a low ratio of fat to carbohydrate oxidation is associated with a higher risk of subsequent body weight gain independent of low energy expenditure; and 4) by increasing free fatty acid release and fat oxidation, weight gain could compensate for low fat oxidation rates. We conclude that the familial dependence of energy substrate oxidation may reflect underlying mechanisms that con-

tribute to the familial aggregation of human obesity.

We are indebted to Carol Lamkin and the nursing staff of the clinical research unit, to Vicky Boyce and the dietary staff, Susan Elson for help in preparation of the manuscript, Dr. Jean-Pierre Flatt for helpful comments and advice, Robert Baird for assistance in the statistical analysis, Karen Larson and Thomas E. Anderson for help in conducting the study, Dr. David M. Mott and the laboratory staff for their technical assistance, and to all of the residents and leaders of the Gila River Indian Community who have made this study possible.

The study was supported in part by Grant 204.2618 to F. Zurlo from the Consiglio Nazionale Delle Ricerche, Rome, Italy, and by Grant 09210903784 to E. Ravussin from the Flinn Foundation.

Part of this work has appeared previously in abstract form (F. Zurlo, C. Bogardus, D. Freymond, S. Lillioja, V. Boyce, and E. Ravussin. *Clin. Res.* 36: 360a, 1988.)

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Received 26 December 1989; accepted in final form 25 June 1990.

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