

# Twenty-Four-Hour Respiratory Quotient: The Role of Diet and Familial Resemblance\*

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## ABSTRACT

Body weight and obesity show familial resemblance that could be the result of familial correlation of fat oxidation, low levels of which have been implicated in the etiology of weight gain and obesity. We studied the familial correlation of both 24-h respiratory quotient (RQ), an index of the ratio of fat to carbohydrate oxidation, and the possible influence of dietary macronutrient composition expressed by the food quotient (FQ), *i.e.* the theoretical RQ produced by the diet. We measured the habitual FQ of the 7 days diet by weighed food records, followed by measurement of 24-h RQ in respiration chambers in 71 healthy Caucasian siblings from 31 families.

After adjustment for age, gender, and 24-h energy balance, 24-h RQ correlated in families as indicated by an intraclass correlation coefficient ( $r_i$ ) of 0.31 ( $P = 0.03$ ). FQ, adjusted for age and gender, was also a familial trait for the two days immediately preceding diet ( $r_i = 0.32$ ,  $P < 0.01$ ). The familial effect on 24-h RQ, adjusted for age, gender, and

24-h energy balance, remained after adjustment for the FQ of the two days preceding diet ( $r_i = 0.27$ ,  $P < 0.05$ ) and was reduced but not abolished after further adjustment for fasting plasma insulin plus free fatty acids ( $r_i = 0.24$ ,  $P < 0.09$ ). By a correlation analysis aimed at separating familial and individual nonfamilial factors influencing both 24-h RQ and FQ, we found a great but insignificant familial ( $\eta^F = 0.49$ ,  $P < 0.18$ ) and a somewhat lower, but significant individual nonfamilial correlation ( $\eta^{NF} = 0.35$ ,  $P < 0.03$ ).

We conclude that substrate oxidation rates measured by RQ exhibit familial correlation after proper adjustment for confounders such as energy balance, gender, and age, and that this effect could not be fully explained by preceding diet composition, fasting plasma insulin, and free fatty acids. Further RQ and the habitual dietary composition shared familial and nonfamilial factors. (*J Clin Endocrinol Metab* 83: 2758–2764, 1998)

IT IS generally accepted that obesity is a familial disorder that is caused by a combination of a genetic predisposition and certain environmental factors (1). The familial influences may be mediated by low resting energy expenditure in combination with high-fat diets and low physical activity (2). Propensity to gain weight on a high-fat diet may be a familial trait (3) and may be based on a low-fat oxidation capacity (4).

The simultaneous measurements of gaseous exchange and urinary nitrogen excretion can be used to calculate the rate of fuel oxidation and the type of fuel being oxidized by the body (5). The respiratory quotient (RQ), *i.e.* the ratio between carbon dioxide production and oxygen consumption, reflects the ratio of fat to carbohydrate oxidation.

Zurlo *et al.* (6) suggest that there are genetic influences on oxidation rates, based on their measurement of 24-h RQ in 66 obese nondiabetic siblings from 28 Pima Indian families on a controlled diet ensuring fixed macronutrient composition and weight maintenance (6). After adjustment for earlier changes in body weight, 24-h energy balance, gender, and percent body fat, family membership explained 28% of the

remaining variance in 24-h RQ. Those with a high 24-h RQ were more likely to gain weight.

A family component in the macronutrient composition of the diet expressed in percentage of total energy intake has been reported in monozygotic (MZ), dizygotic (DZ) twin, and family studies (7, 8). As the macronutrient oxidation rate is highly influenced by the preceding diet, this finding raises the possibility that the familial resemblance of 24-h RQ may be the result of familial correlation of the dietary macronutrient composition. Based on the same measurement, we have recently determined that energy expenditure was not a familial trait, as differences in body composition, thyroid and androgen hormones, and sympathetic activity explain the familial correlation (9). The present study was performed to assess familial effects on 24-h RQ and the possible influence of the habitual dietary macronutrient composition expressed by the food quotient (FQ), where FQ is the theoretical RQ produced by the diet. Furthermore, we performed an analysis to distinguish between the nonfamilial, individual, and familial influence on 24-h RQ and the FQ preceding the chamber stay. We also evaluated the possible mediating role of fasting plasma insulin and free fatty acids in the familial resemblance of RQ.

## Methods

### Subjects

Probands from two ongoing Danish population surveys ("Glostrup Population Surveys" and "Sund Valby") were invited to participate in

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the study if they: 1) were willing to participate; 2) had at least one full sibling eligible for inclusion; 3) proved to be in good health by means of medical history and biomedical screening; 4) were not dieting; 5) received no regular medication other than oral contraceptives. Twenty subjects were included from the "Glostrup Population Survey" and 12 subjects from the "Sund Valby" survey. A total of 32 probands and their 39 full siblings entered the study. The full sibling relationship was ascertained by interview and questionnaire.

By design the probands in the "Glostrup Population Survey" were 30 yr old, whereas the "Sund Valby" probands were on average 32.3 yr (range 21–39). The gender ratios (male/female) from the two surveys were 0.56 and 0.64. The study group consisted of 27 families of 2 members, 3 families of 3 members, 2 families of 4 members. None of the family members were actually sharing the same household. Anthropometric data and other characteristics of all participants completing the study are given in Table 1.

The study included: 1) measurement of body weight and composition; 2) one 24-h stay in a respiration chamber with determination of energy expenditure (EE) and RQ (failed in two subjects, caused by technical problems); 3) fasting blood samples taken the morning after the respiration chamber stay (sampling failed in one subject).

### Body weight

Body weight was measured on a decimal scale (Seca model 707, Copenhagen, Denmark). All the anthropometric variables stated in this article originate from fasting measurements performed the morning after the chamber stay. FFM and fat-mass (FM) were based on the bioimpedance equations given by Heitmann (10).

### Respiration chamber and diet

Twenty-four-hour EE was measured in two open-circuit respiration chambers, which have been described in detail by Toubro and Astrup (9, 11).

The diet during the chamber stay (24-h EI) provided FFM (kg)  $\times$  36.8 kcal/kg + 279 kcal, which is designed to be isoenergetic on the basis of previous 24-h EE measurements performed on nonobese subjects. The diet provided 48% energy from carbohydrate, 37% from fat, and 15% from protein, resulting in an FQ (see below) of 0.853. Bomb calorimetry revealed that the total energy content of the diet exceeded the estimated value by less than 1.6%. Food left by the subjects was reweighed, item for item, and subtracted in the calculation of actual energy intake.

### EE and RQ

The gas exchange of the subjects was calculated from measurements of oxygen and carbon dioxide concentrations (Urea 3 G, Hartman and Braun analyzers, Frankfurt, Germany) at the outlet of the chamber and of air flow through the chambers. The air flow was 72,000 L/24 h. The room temperature was maintained constant at 24 C in the daytime, and at 18 C at night. EE calculations were performed using the constants of Brouwer (12).

### Laboratory analyses

Immediately after the chamber stay blood was sampled without stasis in ice-cooled syringes and centrifuged at 4 C. Blood for catecholamines was collected in tubes containing reduced glutathione and ethylene-glycol-bis(aminoethyl-ether)tetra-acetate. The tubes were centrifuged

**TABLE 1.** Characteristics and anthropometric measures of 71 healthy siblings from 32 families

Variable		
Male/female ratio	27/44 = 0.61	
Age (yr)	29.1 $\pm$ 4.5	(19–40)
Height (cm)	173 $\pm$ 8.9	(159–198)
Body weight (kg)	71.4 $\pm$ 15	(44.5–121.3)
BMI (kg/m <sup>2</sup> )	23.7 $\pm$ 4.1	(16.9–38.8)
Body fat (%)	23.2 $\pm$ 8.2	(8.3–42.3)
Fat-free mass (kg)	54.3 $\pm$ 9.9	(39.0–90.0)

immediately and the plasma was stored at  $-80$  C, until determination of catecholamines by radio enzymatic method (13); the intra-assay coefficient of variation for norepinephrine and epinephrine in samples containing normal basal values were 6% and 8%, respectively (n = 10) (14). All plasma samples were coded and analyzed in random order to avoid any systematic error attributable to the order of analysis.

Free fatty acid (FFA) in the plasma was immediately extracted with a chloroform-heptane-methanol mixture containing activated silicic acid, and part of the solution was shaken with an alkaline copper nitrate-triethanolamine solution saturated with NaCl. An aliquot was mixed with dipenylcarbazine and measured spectrophotometrically (15). Immunoreactive insulin concentrations were measured in plasma with radioimmunoassay kits purchased from Novo (Copenhagen, Denmark). Plasma free triiodothyronine (T<sub>3</sub>) was also assessed enzymatically, using kits from Serono Diagnostics SA, Basel, Switzerland. Also plasma triglycerides were determined by enzymatic methods (Boehringer Mannheim, Mannheim, Germany). All plasma samples were coded and analyzed in random order to avoid any systematic error attributable to the order of analysis.

### Food Quotient (FQ) of the habitual diet

The habitual diet macronutrient composition was estimated by weighed food records collected during the seven days previous to the chamber stay. After the first contact had been made each subject was invited to the institute to have the respiratory chambers presented and to be instructed by a trained dietitian in filling out the diary forms. To measure the amount of food intake, each subject received a weighing balance (Soehnle, Montlingen, Switzerland), measuring cup, and spoons. All subjects were advised to complete the food records as carefully as possible without changing their normal dietary habits. Before the chamber stay each diary was checked by the dietitian to ensure completeness. Dietary energy content and composition were calculated for each of the seven days by Dankost dietary assessment software (Søborg, Denmark). With the macronutrient composition expressed in percentage we used the following equation:

$$FQ = \frac{0.207 \times \text{carbohydrate}(\%) + 0.159 \times \text{fat}(\%) + 0.193 \times \text{protein}(\%) + 0.137 \times \text{alcohol}(\%)}{0.207 \times \text{carbohydrate}(\%) + 0.226 \times \text{fat}(\%) + 0.243 \times \text{protein}(\%) + 0.206 \times \text{alcohol}(\%)}$$

We defined FQ<sub>12</sub> and FQ<sub>37</sub>, *i.e.* the FQ based on the food intake in the two days immediately before the chamber stay and on the remaining days respectively.

The study was approved by the Ethical Committee of Copenhagen and Frederiksberg Municipalities, and the subjects gave informed consent according to the Declaration of Helsinki II.

## Statistical Analysis

### Strategy of analysis

As a measure of familial resemblance of 24-h RQ, the intraclass correlation coefficient was used. This is the proportion of variance attributable to genetic and/or environmental factors shared by full siblings relative to the total variance in the population. We allowed the mean of 24-h RQ to depend on the siblings' age and gender in addition to energy balance.

Because it is reasonable to assume that the FQ immediately before the chamber stay has a larger effect on RQ than the FQ seven days earlier, we calculated FQ<sub>1</sub> through FQ<sub>7</sub> (*i.e.* the FQ for each of the seven days), with FQ<sub>1</sub> being the day just before the chamber stay. The mean of 24-h RQ was allowed to additionally depend on these covariates, and it was successively tested whether there was a significant effect of FQ<sub>7</sub>, FQ<sub>6</sub> and so forth.

As a more powerful approach, we also used FQ<sub>12</sub> and FQ<sub>37</sub>, *i.e.* the FQ based on the food intake in the two days immediately before the chamber stay and on the remaining days respectively.

Additionally, we tested whether various plasma metabolites were predictors of 24-h RQ. To investigate if the familial factors in 24-h RQ is related to familial factors in FQ or plasma metabolites, we estimated  $r_i$  for these variables and analyzed the degree to which the familial factors affecting 24-h RQ and the covariates were mutual.

### Methods of analysis

The distribution of the continuous variables was described by means, standard deviations (SD), and ranges.

A "mixed-model" was used to estimate the  $r_i$  of the variables with simultaneous estimation of effects of covariates (see the *Statistical Appendix*). Because only positive  $r_i$  were considered as a realistic alternative to a zero correlation, a one-sided test was performed for this hypothesis.

We investigated the degree to which the familial factors affecting two variables such as 24-h RQ and FQ were mutual by two methods explained in detail in the *Statistical Appendix*. First, FQ was included as a covariate in the mixed model used to describe 24-h RQ, then it was tested to determine if the intraclass correlation of 24-h RQ was different from zero.

The second method estimated the correlation between the familial factor of 24-h RQ and the familial factor of FQ ( $\eta^F$ ) as well as the correlation between the individual nonfamilial factors of 24-h RQ and FQ ( $\eta^{NF}$ ) respectively. A correlation between the familial factors close to one indicate that the familial factors affecting RQ and FQ are similar, whereas a correlation close to zero must be interpreted to mean that the familial factors affecting RQ are different from the familial factors affecting FQ. Likewise a correlation between the individual nonfamilial factors close to one indicates that the factors affecting FQ and RQ in the individual are similar, and a correlation close to zero means that the individual nonfamilial factors affecting FQ are different from those affecting RQ.

This model can also be used to calculate the cross-trait correlation between siblings, defined as the correlation between RQ for one sibling in a family and FQ for another sibling. However, the cross-trait correlation is always lower than the correlation between the familial factors, because it also depends on the individual nonfamilial factors. Even in the case where the correlation of familial factors is one, the cross-trait correlation will be smaller than one.

### Results

The habitual daily energy intake and, during the chamber stay, 24-h EE, 24-hour EI, and energy balance, together with FQ<sub>17</sub> and 24-h RQ are presented in Table 2. There was a good match between the average FQ<sub>17</sub> and the average 24-h RQ. The daily energy intake registered during free living conditions was 10.2% higher than the measured 24-h EE ( $P < 0.01$ ), and the between-subjects correlation for these variables was 0.58.

The unadjusted 24-h RQ intraclass correlation coefficient ( $r_i$ ) was 0.27 ( $P < 0.06$ ), which increased ( $r_i = 0.31$ ,  $P < 0.03$ ) after adjustment for the covariates age, gender, and 24-h energy balance.

The familial resemblance in the macronutrient composition of the habitual diet preceding the 24-h RQ measurement was analyzed by calculating the intraclass correlation coefficient ( $r_i$ ) for each of the seven days separately and for consecutive days accumulated (*i.e.* 2, 3, 4, 5, 6, and 7 days),

**TABLE 2.** Energy expenditure, energy intake, and related parameters

Variable	Mean $\pm$ SD	Range
7-days Habitual energy intake (kJ/d)	10,508 $\pm$ 2,557	(6,009–18,704)
7-days Food quotient <sup>1</sup>	0.838 $\pm$ 0.018	(0.794–0.875)
Chamber food quotient	0.853	
24-h Energy expenditure (kJ/d)	9,536 $\pm$ 1,440	(7,262–14,186)
24-h Energy intake (kJ/d)	9,604 $\pm$ 1,557	(7,000–15,200)
24-h Energy balance (kJ/d)	68 $\pm$ 662	(–1,207–1,806)
24-h Respiratory quotient	0.840 $\pm$ 0.016	(0.788–0.883)

<sup>1</sup> The food quotient of the habitual dietary intake registered by 7-day food records is the theoretically calculated RQ.

both after adjustment for confounders (age and gender) (Table 3). For the days separately,  $r_i$  values between 0 and 0.32 were observed, reflecting the fluctuation in daily macronutrient composition producing some day-to-day variation in the estimate of the familial correlation in FQ. When more days were taken together, more stable values were found in the range 0.32–0.46. No significant increase in the FQ  $r_i$  was achieved by exceeding 3 days of record.

The familial correlation of fasting FFA and insulin adjusted for age and gender was significant ( $P < 0.01$ ), whereas norepinephrine was borderline ( $P < 0.06$ ), and no familial correlation could be found for fasting triglycerides and free T<sub>3</sub> (Table 4).

The correlation between 24-h RQ adjusted for age, gender, and energy balance and FQ was tested by including FQ for all the 7 days separately, *i.e.* FQ<sub>1</sub>, . . . FQ<sub>7</sub>. We found that only FQ<sub>1</sub> and FQ<sub>2</sub> significantly affected the model. By introducing the accumulated early effect of FQ<sub>1</sub> and FQ<sub>2</sub> as FQ<sub>12</sub> and the accumulated late effect as FQ<sub>37</sub> in the model for 24-h RQ, only FQ<sub>12</sub> was significant.

Fasting plasma insulin concentration measured after the chamber stay was the most important determinant of 24-h RQ adjusted for age, gender, and energy balance, explaining 15% of the remaining variation. Body fat mass (FM) did not correlate with 24-h RQ or 24-h RQ adjusted for the three covariates age, gender, and energy balance. In a mixed model with 24-h RQ adjusted for age, gender, and energy balance, 33% ( $P < 0.01$ ) of the remaining variation between subjects was explained by insulin, FQ<sub>12</sub>, and FFA (Table 5). There was a correlation ( $r = 0.38$ ,  $P < 0.01$ ) between FM and insulin, but FM did not enter a forward stepwise analysis including the three covariates. Fasting plasma triglycerides, norepinephrine, and free T<sub>3</sub> were all nonsignificant and did not enter the mixed model.

Subsequently, the familial resemblance in 24-h RQ adjusted for the confounders age, gender, and energy balance was analyzed. When introducing FQ<sub>12</sub> to the model, the  $r_i$  of 24-h RQ was reduced from 0.31 to 0.27, but remained significant ( $P < 0.05$ ). If all six significant variables (*i.e.* age, gender, energy balance, FQ<sub>12</sub>, FFA, and insulin) were included in the mixed model the familial correlation in 24-h RQ was reduced but not abolished ( $P < 0.09$ ) (Table 6).

In a two-dimensional correlation model for 24-h RQ and FQ<sub>12</sub>, a significant correlation was found for the nonfamilial or individual factors ( $\eta^{NF} = 0.35$ ,  $P < 0.03$ ), whereas the mutual effect of familial factors were greater ( $\eta^F = 0.49$ ), but not significant. Using the same analysis (Table 7), 24-h RQ

**TABLE 3.** Daily and accumulated intraclass correlation ( $r_i$ ) of the food quotient (FQ) of habitual diet adjusted for age and gender

FQ <sub>daily</sub>	$r_i$	$P <$	FQ <sub>accumulated</sub>	$r_i$	$P <$
Day 1	0.20	0.10			
Day 2	0.32	0.01	Day <sub>12</sub>	0.32	0.01
Day 3	0.16	0.15	Day <sub>13</sub>	0.36	0.01
Day 4	0.24	0.06	Day <sub>14</sub>	0.43	0.01
Day 5	0.18	0.08	Day <sub>15</sub>	0.46	0.001
Day 6	0.23	0.05	Day <sub>16</sub>	0.42	0.001
Day 7	0 <sup>a</sup>		Day <sub>17</sub>	0.35	0.001

$P$  is based on a one-sided test. Day 1 is the last day before the chamber stay.

<sup>a</sup> The estimate was negative but not significantly different from 0.

**TABLE 4.** Fasting plasma concentrations and intraclass correlation coefficients ( $r_i$ ) after adjustment for age and gender of various plasma metabolites

Variable	Mean $\pm$ SD	Range	$r_i$	$P <$
Free fatty acids ( $\mu\text{mol/L}$ )	745 $\pm$ 235	(250–1,365)	0.46	0.001
Triglycerides (mmol/L)	1.16 $\pm$ 0.65	(0.4–4.7)	0.02	NS
Insulin (pmol/L)	90 $\pm$ 47	(7–195)	0.36	0.01
Norepinephrine (pmol/L)	0.16 $\pm$ 0.06	(0.05–0.32)	0.23	0.06
Free T <sub>3</sub> (pmol/L)	3.5 $\pm$ 1.6	(1.1–12.2)	0.14	NS

NS, not significant.

**TABLE 5.** Determinants of 24-h RQ in 69 siblings by mixed model analysis

Variable	Slope	$r^2$	$P <$
Intercept	0.693		
Age (yr)	-0.000568		0.07
Gender (male = 1, female = 0)	-0.00566		0.07
Energy balance (kJ/day)	$6.57 \times 10^{-6}$		0.01
Insulin (pmol/L)	$9.93 \times 10^{-5}$	0.15	0.01
FQ <sub>12</sub>	0.200	0.15	0.01
Free fatty acids ( $\mu\text{mol/L}$ )	$-1.37 \times 10^{-5}$	0.08	0.03
Total		0.33 <sup>a</sup>	0.01

$r^2$  is the proportion of variance additionally explained by inclusion of the covariates.

<sup>a</sup> All together insulin, FQ<sub>12</sub>, and free fatty acids explain 33% of the variation in 24-h RQ adjusted for age, gender, and energy balance.

**TABLE 6.** Twenty-four-hour RQ intraclass correlation coefficient ( $r_i$ ) after adjustment for various factors

	$r_i$	$P <$
24-h RQ	0.27	0.06
24-h RQ <sub>age,sex,EB</sub>	0.31	0.03
24-h RQ <sub>age,sex,EB,FQ12</sub>	0.27	0.05
24-h RQ <sub>age,sex,EB,FQ12,FFA,INS</sub>	0.24	0.09

$P$  is based on a one-sided test. EB is 24-h energy balance. FQ<sub>12</sub> is the theoretical RQ of the habitual food intake 48 h before the chamber stay. FFA is plasma fasting free fatty acids. INS is plasma fasting insulin.

**TABLE 7.** Correlations between familial ( $\eta^F$ ) and nonfamilial ( $\eta^{NF}$ ) factors for various pairs of variables

Variables	$\eta^F$	$P <$	$\eta^{NF}$	$P <$
24-h RQ and FQ <sub>12</sub>	0.49	0.18	0.35	0.03
24-h RQ and FFA	-0.47	0.15	-0.19	0.25
24-h RQ and insulin	0.47	0.20	0.34	0.05

Twenty-four-hour RQ is adjusted for age, gender, and energy balance. FQ<sub>12</sub> is the theoretical RQ of 2 days antecedent diet. FFA is free fatty acids. Both FFA and insulin are adjusted for age and gender.

and insulin shared some individual factors ( $\eta^{NF} = 0.34$ ,  $P < 0.05$ ) and showed higher ( $\eta^F = 0.47$ ) but nonsignificant familial correlation.

## Discussion

We found that a familial resemblance in habitual dietary macronutrient composition existed for nearly all 7 days and became more robust when accumulated. Further, the familial resemblance in 24-h RQ was not eliminated after adjustment for covariates such as age, gender, and energy balance, plus FQ<sub>12</sub> together with the plasma variables FFA and insulin.

When separating the nonfamilial and familial factors it was found that, as expected, 24-h RQ and FQ<sub>12</sub> shared individual factors. However, the correlation of the familial factors was high but nonsignificant possibly due to the relative low number of families in the study.

A number of observations suggest that differences in RQ are important for the propensity to weight gain and obesity (6, 16, 17). Because of increased energy requirements and fat stores, obesity brings about an increased proportion of fat utilized as fuel both in the fasting state and on 24-h basis (18). Formerly obese subjects, when challenged with high-fat foods and diets, failed to decrease RQ appropriately, which caused a positive fat balance (4, 20). The preferential storage of fat among the formerly obese on the high-fat diet was caused by a failure to increase fat oxidation sufficiently to match the consumed amount. This is supposed to increase subsequent appetite and energy intake.

Accurate estimation of nutrient intake is a difficult task, but the habitual macronutrient intake of our siblings was assessed by a 7-day weight and dietary record, which is regarded to produce reliable results (21). It is unknown how many days of food records are required to obtain a representative macronutrient composition for a reliable estimate of the intraclass correlation. It appears, however, that the  $r_i$  calculated from single days of the food record varies considerably (Table 3), whereas the estimate increases to 0.32–0.46 when at least two consecutive days are used. By contrast, no significant increase in  $r_i$  is achieved by using more than three days food record (Table 3). This supports previous findings that nutrient intakes obtained from a 3-day record, including one weekend day, provide estimates comparable to those achieved from 7-day food record (22).

Our finding of  $r_i$  of FQ based on 7-day food record was 0.35 ( $P < 0.001$ ) indicates that the dietary macronutrient composition is a familial trait. This is in accordance with Pérusse *et al.* (8) who also found intraclass correlations between siblings for dietary fat, carbohydrate, and protein expressed as percent of total energy to be 0.36–0.38. In a sibling study it is impossible to separate the genetic effect from shared environmental factors, and it may be difficult in twin studies too, where the nutrient intake is influenced by how frequently the twins see each other (23). However, in the study by Pérusse *et al.* they found significant correlations between macronutrient intakes of spouses, but were able to distinguish between home environmental effects and biological inheritance ( $h^2$ ), which was estimated to be 11–20% (8). It remains to be resolved whether this genetic effect is determining a certain set-point of fat intake or is a matter of preferences determining the selection of food items (24). This may have im-

portance for the energy compensation observed when fat-reduced food items covertly replace the usual high-fat foods.

It is obvious that the dietary macronutrient composition in the long run must be reflected in subsequent oxidative pattern, but hormonal and metabolic factors may also exert a substantial effect on the oxidation rates (16). The rate of adjustment between FQ and RQ may also be influenced by difference in habitual FQ (7-day FQ) and FQ of the diet served during the chamber stay. In our study the means of these variables were close (0.838 and 0.853, respectively). A familial resemblance in RQ may therefore be caused by shared dietary preferences leading to familial selection of dietary macronutrients, or by a combination of familial resemblance in the diet selection and a metabolically determined oxidation pattern. One of the major findings was that both 24-h RQ, measured on a standardized diet without control of preceding food composition, and the preceding dietary macronutrient composition, FQ, exhibited similar familial correlation as indicated by significant intraclass correlation coefficients (Table 3 and 6). To determine if the familial effect on 24-h RQ was accounted for by shared familial dietary habits, we adjusted 24-h RQ for FQ of the preceding diet. By this procedure we found the intraclass correlation coefficient only slightly reduced from 0.31 to 0.27, which indicated that a significant familial effect on RQ remained, and that this familial resemblance could not entirely be attributed to shared familial dietary habits. It cannot be ruled out that other shared environmental factors were responsible for the nondietary family effect, but a genetic influence is likely.

Twin studies also support heritability of RQ. Bouchard *et al.* (25) found greater similarity in RQ during exercise among monozygotic twins than among dizygotic twins (25). Moreover, in the Quebec Family Study (26) involving 300 individuals from 75 nuclear families, the genetic heritability of RQ was estimated at about 20%. It cannot be ruled out, however, that the genetic effect is indirect (*i.e.* mediated through food preferences) (24), which in turn is reflected in RQ. Against this possibility, other studies have been described where diet composition and energy intake have been rigorously controlled, such as in a 100-day controlled overfeeding study, where significant within-pair resemblance was found for changes in RQ, both in the postabsorptive and postprandial state (26).

Also, 24-h RQ studies suggest that the oxidation pattern is under a genetic influence independently of dietary macronutrient selection. Zurlo *et al.* (6) measured 24-h RQ in calorimeters on Pima Indian siblings fed a controlled weight maintenance diet (*i.e.* fixed FQ for at least 3 days) while staying at a metabolic ward (6). After adjustments for earlier change in body weight, 24-h energy balance, gender, and body fat, 24-h RQ was a familial trait where family membership explained 28% of the variation between individuals, which is in accordance with our present estimate of nondietary familial effect on 24-h RQ of  $r_i = 0.27$ .

The genetically determined differences in RQ may be found in a number of different pathways in fat and glucose metabolism. Direct evidence for a genetic influence on RQ has been delivered by Dériaz *et al.* (27), who studied the relationship between DNA variation at the genes coding for

the Na,K-ATPase peptides, RQ, and body fat. Postabsorptive RQ was found to be associated with the  $\alpha_2$  gene and linked with the  $\beta$  gene of the Na,K-ATPase, which suggests that these or neighbouring genes influence RQ. Also, differences in the neurohormonal and local tissue factors may influence or control adipose tissue lipolysis and skeletal muscle fat oxidation. Differences in the activity of the rate-limiting muscle endothelial enzyme lipoprotein lipase may play a role. In a cross-sectional study, Ferraro *et al.* (28) found skeletal muscle LPL activity to be inversely correlated with 24-h RQ ( $r = -0.57$ ) in subjects who had been on a controlled diet (*i.e.* fixed FQ for at least 3 days). Similarly, skeletal muscle insulin sensitivity has been demonstrated to be an important determinant of the local partitioning of fat and glucose substrates (29).

In the present study we found that 24-h RQ adjusted for age, gender, 24-h energy balance, and immediately preceding diet (FQ<sub>12</sub>), also correlated positively with plasma insulin concentration and negatively with plasma FFA (Table 5). This has been shown previously, and both fasting plasma insulin and FFA are regarded as determinants of fat and glucose oxidation (30). After adjustment for age and gender, both plasma insulin and free fatty acid concentrations as intermediary factors were found to be familial traits (Table 4). Further, part of the possible genetic influence on RQ may be exerted through insulin and free fatty acids, but  $r_i$  of 24-h RQ was reduced only from 0.27 to 0.24 after adjustment for differences in insulin and free fatty acids (Table 6).

Both familial and nonfamilial factors influenced FQ and RQ. Both familial and nonfamilial factors influenced FQ and RQ, but only the individual nonfamilial ( $n = 69$ ) were significant, probably caused by lack of power for the familial factors ( $n = 30$ ). One should keep in mind that dietary habits determining FQ may be genetically influenced, nongenetically shared familial habits, or personal preferences. Also 24-h RQ may be subject to the same influences—for example physical fitness is an important determinant of RQ—and fitness level may again be influenced by both familial habits and individual interests and preferences.

In conclusion, we found that a substantial part of the interindividual variation in 24-h RQ could be explained by the following six covariates: age, gender, energy balance, 2 days immediately preceding diet composition, and the fasting plasma variables insulin and FFA. Further, there was a strong familial resemblance in both the self-recorded habitual diet composition and the measured oxidation pattern after proper adjustment for confounders. Finally, both diet composition and RQ shared both individual and familial factors.

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## Statistical Appendix

In a study of siblings from  $N$  families the following “mixed model” can be used to estimate the intraclass correlation of a quantitative variable  $Y$ . Let  $Y_{ij}$  represent the value of  $Y$  for the  $i$ ’th sibling in the  $j$ ’th family and let  $n_j$  represent the number of siblings in the  $j$ ’th family. Assume that

$$Y_{ij} = \mu_{ij}^y + u_j^y + e_{ij}^y,$$

where  $u_j^y$  is a random factor belonging to the  $j$ ’th family and represents effects of shared genes and shared family environment, whereas  $e_{ij}^y$  is a random factor representing effects of nonshared genes and nonshared environmental factors. Assume that  $u_1^y, \dots, u_{n_j}^y, e_{11}^y, \dots, e_{n_j n_j}^y$  are independent, normally distributed with mean zero and that the variance of  $u_j^y$  equals  $\omega_y^2$  where  $\omega_y^2 \geq 0$ , whereas the variance of  $e_{ij}^y$  is  $\sigma_y^2$  where  $\sigma_y^2 > 0$ . This means that  $Y_{ij}$  is normally distributed with mean  $\mu_{ij}^y$  and variance  $\omega_y^2 + \sigma_y^2$  and that the intraclass correlation between two siblings equals

$$r_y = \frac{\omega_y^2}{\omega_y^2 + \sigma_y^2}.$$

The mean  $\mu_{ij}^y$  can for example depend on the siblings’ age and gender as well as other important covariates.

The trait  $Y$  is uncorrelated within families if  $\omega_y^2$  can be assumed to be zero. This hypothesis can be tested by a likelihood ratio test. Because  $r_y$  is considered as being greater than or equal to zero, a one sided test is used.

If it is documented in this way that  $Y$  is correlated within families, a natural step is to determine the cause of this correlation. Assume that  $Z$  is another quantitative family trait known to be correlated with  $Y$ . A central question to ask is to which degree  $Y$  and  $Z$  are affected by the same familial factors. In order to investigate this we employed the following two methods in our analysis.

## Method 1

The first method utilizes a one-dimensional approach where an effect of the covariate  $Z$  is simply included in the mixed model written above:

$$Y_{ij} = \mu_{ij}^y + \beta \cdot Z_{ij} + u_j^y + e_{ij}^y.$$

The mean of  $Y_{ij}$  now additionally depends on  $Z_{ij}$ . If the intraclass correlation between siblings can be assumed to be zero after this inclusion of  $Z_{ij}$  in the model, it is interpreted to mean that the familial component in  $Y$  was simply induced by the familial component in  $Z$  and the correlation between  $Y$  and  $Z$ .

## Method 2

The second method utilizes the following two-dimensional approach. Assume that

$$Y_{ij} = \mu_{ij}^y + u_j^y + e_{ij}^y$$

$$Z_{ij} = \mu_{ij}^z + u_j^z + e_{ij}^z,$$

where  $u_j^y$  and  $u_j^z$  are random factors describing effects of shared genes and shared environment on  $Y$  and  $Z$  respectively. Accordingly  $e_{ij}^y$  and  $e_{ij}^z$  are random factors describing effects of nonshared genes and nonshared environment on  $Y$  and  $Z$ .

Assume that  $(u_j^y, u_j^z)$  is normally distributed with mean zero and that the variance of  $u_j^y$  is  $\omega_y^2$ , the variance of  $u_j^z$  is  $\omega_z^2$ , whereas the correlation between  $u_j^y$  and  $u_j^z$  is

$$\eta_{yz}^F = \frac{\omega_{yz}}{\sqrt{\omega_y^2} \sqrt{\omega_z^2}}.$$

Assume accordingly that  $(\epsilon_{ij}^y, \epsilon_{ij}^z)$  is normally distributed with mean zero and that the variance of  $\epsilon_{ij}^y$  is  $\sigma_y^2$ , the variance of  $\epsilon_{ij}^z$  is  $\sigma_z^2$ , whereas the correlation between  $\epsilon_{ij}^y$  and  $\epsilon_{ij}^z$  is

$$\eta_{yz}^{NF} = \frac{\sigma_{yz}}{\sqrt{\sigma_y^2} \sqrt{\sigma_z^2}}$$

The correlation  $\eta_{yz}^F$  is termed *the correlation between the familial factors* and describes to which degree  $Y$  and  $Z$  are affected by the same shared environment and/or genes. If this correlation may be assumed to be zero, it is interpreted to mean that the familial factors affecting  $Y$  are different from the familial factors affecting  $Z$ . This hypothesis can be tested by a likelihood ratio test with one degree of freedom. Accordingly, the correlation  $\eta_{yz}^{NF}$  is termed *the correlation between the nonfamilial factors*

and describes to which degree  $Y$  and  $Z$  are affected by the same non-shared environmental and/or genetic factors.

This model can also be used to calculate what is known as *the cross-trait correlation* between siblings. This is defined as the correlation between  $Y_{lj}$  for the  $l$ 'th sibling in the  $j$ 'th family and  $Z_{ij}$  for the  $i$ 'th sibling, and is easily seen to yield:

$$\frac{\omega_{yz}}{\sqrt{\omega_y^2 + \sigma_y^2} \sqrt{\omega_z^2 + \sigma_z^2}}$$

The cross-trait correlation is a well-known measure of the resemblance of the familial factors affecting the traits  $Y$  and  $Z$ . It is apparent that the cross-trait correlation is smaller than the correlation between the familial factors.