Whole body fat oxidation is related to in situ adipose tissue lipolytic response to isoproterenol in males

S. Snitker, J. Hellmér, M. Boschmann, M. B. Monroe, and E. Ravussin. Whole body fat oxidation is related to in situ adipose tissue lipolytic response to isoproterenol in males. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E400–E404, 1998.—A high 24-h respiratory quotient (RQ), i.e., low fat oxidation, predicts weight gain. To determine whether impaired fat mobilization (lipolysis) may contribute to weight gain, we studied the relationship between lipolytic response to nonselective β-adrenergic stimulation and RQ measured in a respiratory chamber in 21 males (11 Caucasians, 10 Pima Indians; age 32 ± 5 yr, weight 93 ± 24 kg, body fat 30 ± 8%; means ± SD) and 23 females (10 Caucasians, 13 Pima Indians; age 32 ± 9 yr, weight 95 ± 26 kg, body fat 44 ± 8%). Lipolytic response was assessed as the relative increase in dialysate glycerol concentration (% above baseline) when isoproterenol (1 µmol/l) was added to the perfusate of a microdialysis probe inserted in the abdominal subcutaneous adipose tissue. In males, but not in females, basal RQ measured during sleep from 0500 to 0630 and adjusted for waist circumference was negatively correlated to lipolytic response (r = −0.66, P = 0.001). The results suggest that in males, impaired β-adrenergic-mediated lipolysis may contribute to low rates of fat oxidation, a condition known to predispose to weight gain.

indirect calorimetry; microdialysis

The relative amounts of the macronutrients oxidized by an individual are reflected in the respiratory quotient (RQ), i.e., the molar ratio between CO₂ produced and O₂ consumed. A high RQ, indicating a relatively low fat oxidation, predisposes to weight gain in Pima Indians (27) and Caucasians (22). There is evidence that an individual’s RQ is influenced by diet composition, energy balance, body composition, and genetic factors (27), but the physiological mechanisms underlying this interindividual variability in RQ are largely unknown. However, a number of findings indicate that the sympathetic nervous system is an important regulator of fat oxidation at rest and during exercise. In one study, 2-wk administration of the β-adrenergic agonist terbutaline increased fat oxidation, whereas the β-adrenergic antagonist propranolol decreased fat oxidation (1). Among the effects of the sympathetic nervous system is its ability to stimulate adipocyte lipolysis, the hydrolysis of stored fat into nonesterified fatty acids (NEFAs) and glycerol. This effect is mediated through β-adrenoceptors by norepinephrine and epinephrine, the only endogenous hormones with rapid and pronounced stimulatory effects on lipolysis in adult humans (2). That lipolytic rate is a determinant of whole body fat oxidation is suggested by the effect of plasma NEFA concentration on whole body fat oxidation (3, 9). Evidence indicates that the lipolytic response to β-adrenergic stimulation is decreased in obese men (7) and that this decrease persists after weight loss (8), suggesting that a low lipolytic response may be causally involved in the development of obesity.

The purpose of the present study was to test whether a low rate of fat oxidation is associated with a low lipolytic response to β-adrenergic stimulation. Lipolytic response to locally administered isoproterenol (a nonselective β-adrenergic agonist) was measured in situ by microdialysis, and whole body fat oxidation was measured by indirect calorimetry.

METHODS

Subjects. Twenty-one males (11 Caucasians, 10 Pima Indians) and 23 females (10 Caucasians, 13 Pima Indians) were studied (Table 1). All subjects were healthy, as determined by medical history, physical examination, and routine blood and urine tests, and none took medications or smoked. None had clinical or biochemical signs of thyroid or other metabolic disease. The study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and the Tribal Council of the Gila River Indian Community, and subjects gave written, informed consent. The subjects were residing in our metabolic research unit for 5–7 days and were fed a weight-maintenance diet (20% protein, 30% fat, 50% carbohydrate). Waist circumference was measured at the level of the umbilicus while the subject was in the supine position. Thigh circumference was measured at the gluteal fold while the subject was standing. Body composition was determined by dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation, Madison, WI) (24). After at least 3 days on the weight-maintenance diet, a 75-g oral glucose tolerance test (26) was performed to exclude subjects with diabetes mellitus or impaired glucose tolerance. Microdialysis. The microdialysis procedure has been described in detail elsewhere (12, 23). The procedure was done after 4 days on the weight-maintenance diet. At 0700, after an overnight fast, with the subject in the supine position, the microdialysis probe was inserted into the subcutaneous adipose tissue of the abdomen ~5 cm lateral of the umbilicus. The probe had a 30 × 1.2-mm shaft with a 10-mm-long 20-kDa cutoff membrane (CMA/20; CMA/Microdialysis, Solna, Sweden). No local anesthesia was used. The probe was perfused with Ringer solution (Abbott, North Chicago, IL) by a precision pump (CMA/102) at a rate of 5 µl/min. In situ recovery of glycerol at 5 µl/min, determined in separate experiments by dialysate glycerol concentrations at various flow rates (5), was similar in five lean and five obese subjects.
RESULTS

Microdialysis and indirect calorimetry results are reported by gender in Table 2.

RQ vs. lipolytic response in males. Basal RQ and lipolytic response to isoproterenol tended to be negatively correlated in all males (r = -0.40, P = 0.07) and were negatively correlated (r = -0.64, P = 0.004) (Fig. 1, top) when one outlier for waist circumference was deleted (waist circumference 165 cm, i.e., >5 SD from the mean of the remaining males). Basal RQ was also negatively correlated to waist circumference whether or not the outlier was included (r = -0.60, P = 0.004 in all males). By multiple linear regression, basal RQ was found to be inversely associated with lipolytic response (P = 0.001) independent of waist circumference. Lipolytic response and waist circumference were the only significant determinants of basal RQ, both being negatively associated with basal RQ and explaining 66% of its variability. There was thus no significant additional effect of percent body fat, waist-to-thigh ratio, fasting plasma insulin concentration, age, race, or 24-h energy balance on basal RQ. To illustrate the contribution of lipolytic response to the variability in basal RQ, waist-adjusted basal RQ against lipolytic response is shown in Fig. 1, middle (r = -0.66, P = 0.001). Basal RQ was not correlated to baseline dialysate concentration before or after adjustment for covariates. Twenty-four-hour RQ was not correlated to lipolytic response or baseline glycerol concentration before or after adjustment for covariates.

RQ vs. lipolytic response in females. In females, neither basal RQ nor 24-h RQ was correlated to lipolytic response or baseline glycerol concentration before or after adjustment for covariates. Figure 1, bottom, shows the relationship between basal RQ and lipolytic response in females.

Blood flow. In response to isoproterenol, ethanol outflow-to-inflow ratio decreased in both sexes (Table 2), suggestive of an increase in blood flow, but this decrease did not reach statistical significance. The

Table 1. Characteristics of subjects

<table>
<thead>
<tr>
<th>Males (n = 21)</th>
<th>Females (n = 23)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>32 ± 5 (24–41)</td>
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<tr>
<td>Weight, kg</td>
<td>93 ± 24 (57–167)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 6 (161–189)</td>
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<tr>
<td>%Body fat</td>
<td>30 ± 8 (13–50)</td>
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<tr>
<td>Waist circumfer-</td>
<td>103 ± 19 (79–165)</td>
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<tr>
<td>ence, cm</td>
<td>109 ± 19 (76–142)</td>
</tr>
<tr>
<td>Waist-to-thigh ratio</td>
<td>1.63 ± 0.24 (1.41–2.50)</td>
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<td>Fasting plasma glucose, mg/dl</td>
<td>85 ± 10 (71–110)</td>
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<tr>
<td>Fasting plasma insulin, µU/ml</td>
<td>17 ± 20 (2–90)</td>
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Values are means ± SD; range is in parentheses. *P < 0.005 vs. males.

(11.1 ± 2.0 and 10.3 ± 4.4%, respectively). For the determination of regional blood flow, ethanol was added to the Ringer solution for a final concentration of 50 mmol/l (11). The dialysate collected during the first 30 min after the insertion of the probes was discarded. Thereafter, dialysate was collected in 10-min fractions during a 30-min baseline and during 40 min while isoproterenol (Abbott) was added to the perfusate for a final concentration of 10⁻⁶ mol/l. The dialysate samples were aliquoted into two portions, one stored at -70°C for glycerol analysis and the other stored at +5°C for ethanol analysis within 24 h.

Indirect calorimetry. After ≥4 days on the weight-maintenance diet, the subject spent 23 h in a respiratory chamber (18). No exercise was allowed in the chamber, and spontaneous physical activity was monitored by a radar system. Twenty-four hour energy expenditure and 24-h RQ were calculated as previously described (18, 27). Basal RQ was calculated as the ratio between CO₂ produced and O₂ consumed for the 90-min period from 0500 to 0630. Twenty-four-hour energy balance was calculated by subtracting 24-h energy expenditure from energy intake during the stay and was expressed as a percentage of energy expenditure.

Analyses. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA) and plasma insulin concentrations by radioimmunoassay (Concept 4; ICN, Horsham, PA). Plasma insulin concentrations were log₁₀ transformed to approximate a normal distribution. Dialysate glycerol concentrations were determined by a luminometric assay (13, 17). Basal RQ and EE, energy expenditure; SMR, sleeping metabolic rate; RQ, respiratory quotient.

Table 2. Microdialysis and indirect calorimetry results

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tr>
<td>Baseline dialysate glycerol, µmol/l</td>
<td>30 ± 11</td>
<td>28 ± 12</td>
</tr>
<tr>
<td>Isoproterenol-stimulated dialysate glycerol, µmol/l</td>
<td>56 ± 20</td>
<td>45 ± 17</td>
</tr>
<tr>
<td>Lipolytic response to isoproterenol, % above baseline</td>
<td>88 ± 39</td>
<td>67 ± 36</td>
</tr>
<tr>
<td>Baseline ethanol outflow-to-inflow ratio</td>
<td>0.86 ± 0.21</td>
<td>0.91 ± 0.12</td>
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<tr>
<td>Isoproterenol-stimulated ethanol outflow-to-inflow ratio</td>
<td>0.83 ± 0.09</td>
<td>0.88 ± 0.18</td>
</tr>
<tr>
<td>24-h EE, kcal/day</td>
<td>2,390 ± 340</td>
<td>2,160 ± 280</td>
</tr>
<tr>
<td>24-h Spontaneous physical activity, % of time active</td>
<td>5.8 ± 1.6</td>
<td>5.9 ± 1.2</td>
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<tr>
<td>Physical activity level, 24-h EE/SMR</td>
<td>1.44 ± 0.12</td>
<td>1.42 ± 0.10</td>
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<tr>
<td>Basal RQ (measured during sleep)</td>
<td>0.84 ± 0.06</td>
<td>0.83 ± 0.07</td>
</tr>
<tr>
<td>24-h RQ</td>
<td>0.85 ± 0.02</td>
<td>0.85 ± 0.02</td>
</tr>
<tr>
<td>Relative energy balance, %</td>
<td>1 ± 1</td>
<td>-4 ± 10</td>
</tr>
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Values are means ± SD. EE, energy expenditure; SMR, sleeping metabolic rate; RQ, respiratory quotient.
relationship between basal RQ and lipolytic response was independent of the magnitude of the isoproterenol-induced change in blood flow.

**DISCUSSION**

The purpose of the present study was to test whether whole body RQ, reflecting the ratio of carbohydrate to lipid oxidation, is related to in situ β-adrenergically mediated lipolytic response. The results indicate that in males, but not in females, a low lipolytic response to β-adrenergic stimulation is associated with a high RQ, i.e., a low rate of fat oxidation.

A high RQ predisposes to weight gain (27, 22). In agreement with these findings, weight-reduced subjects (postobese) have a decreased capacity for fat oxidation compared with never-obese subjects (4, 16). The factors determining an individual's fat oxidation are not well established. Pharmacological studies suggest that effects of the sympathetic nervous system mediated by β-adrenoceptors play an important role in regulating fat oxidation (1). One possible mechanism by which β-adrenergic stimulation could promote fat oxidation is by its stimulatory effect on lipolysis (2), since plasma NEFA availability appears to be a determinant of whole body fat oxidation (3, 9). Obesity appears to be associated with low lipolytic sensitivity to β-adrenergic agonists, both in vitro (20, 21) and in vivo (7, 8, 10, 15, 25).

The present study was designed to test the hypothesis that lipolytic sensitivity to β-adrenergic stimulation is related to whole body fat oxidation. Lipolysis was assessed in situ by glycerol concentration in microdialysis dialysate samples. Measurements were made at baseline and in response to local administration of the nonspecific β-adrenergic agonist isoproterenol for 40 min. Isoproterenol was infused at a relatively high concentration (10^{-6} mol/l), which has been shown to cause maximal or near-maximal rates of lipolysis (5). On a separate day, RQ was measured during sleep in the postabsorptive state (basal RQ). The main finding of the study is that basal RQ adjusted for waist circumference was negatively related to lipolytic response in males. Basal RQ and lipolytic response were also negatively related in males without any adjustments when one extremely obese outlier was excluded. RQ was not related to lipolytic response in females. This lack of relationship may be due to the fact that most of the females were very obese (on average 44% body fat) and/or due to effects of the menstrual cycle and menopausal status. It is also possible that the abdominal subcutaneous fat depot is less representative of whole body lipolytic response to β-adrenergic stimulation in females than it is in males. Unlike basal RQ, 24-h RQ was not correlated with lipolytic response. This was not entirely surprising, since 24-h RQ is

![Fig. 1. Relation between basal respiratory quotient (RQ) and lipolytic response. Top: basal RQ against lipolytic response in males (1 outlier deleted). Middle: basal RQ adjusted for waist circumference against lipolytic response in males. Bottom: basal RQ against lipolytic response in females. NS, not significant.](image-url)
influenced mostly by the diet (composition and amount) and physical activity, whereas basal RQ is more dependent on genetic factors (27).

The reviewed literature suggests that the association in males between low fat oxidation and low lipolytic response to β-adrenergic stimulation is explained by reduced plasma NEFA availability. However, another mechanism, which does not exclude an effect on plasma NEFAs, may also contribute to the association. If low lipolytic response is caused by a generalized impairment in β-adrenergic receptor function, this impairment could contribute to lower fat oxidation by affecting β-adrenergically stimulated NEFA uptake in skeletal muscle. Concomitant impairments in β-adrenergically stimulated lipolysis and NEFA uptake in muscle have previously been demonstrated in reduced-obese subjects (8).

The use of microdialysate concentrations of glycerol as a measure of lipolysis has certain limitations, i.e., incomplete recovery (lower glycerol concentration in the dialysate than in the extracellular fluid) and changes in local blood flow in response to the interventions. Recovery was not determined in each subject and may have varied from one subject to another. However, our methodological studies showed small interindividual differences in recovery and no relation with percent body fat, making differences in recovery an unlikely explanation for the observed correlation in males. As demonstrated by others (5), the ethanol outflow/inflow data suggest an increase in adipose tissue blood flow during the isoproterenol infusion. Because local blood flow transports glycerol away from the tissue and thus competes with the microdialysis probe for available glycerol, putative individual differences in vascular response to isoproterenol could have contributed to the findings. However, because the relationship between basal RQ and lipolytic response was independent of individual isoproterenol-induced changes in blood flow, hemodynamic factors appear unlikely to explain the findings.

Fat cell size was not determined in the present study. In general, fat cell size increases with increasing degree of fatness, and larger fat cells have higher maximally isoproterenol-stimulated lipolysis rates in vitro (19). On the other hand, RQ is decreased with increasing degree of fatness (27). It could therefore be argued that the negative relationship between RQ and lipolytic response may be due to the common dependence of both variables on percent body fat. This argument appears unlikely for two reasons. First, the analysis showed that the relationship between basal RQ and lipolytic response was independent of percent body fat. Second, when measured in vivo, lipolytic response appears decreased in obese individuals (7, 10, 15, 25).

In conclusion, the present study indicates that in males, a low lipolytic response to isoproterenol in abdominal subcutaneous adipose tissue is associated with a low rate of fat oxidation, a known risk factor for body weight gain, suggesting that a low lipolytic response to β-adrenergic stimulation may contribute to weight gain.

The Clinical Diabetes and Nutrition Section is indebted to the members of the Gila River Indian Community, who for more than 30 yr have contributed to studies of the development of non-insulin-dependent diabetes mellitus. We thank the individuals, Pimas and non-Pimas, who volunteered for this study; J. Or Truesdale for technical assistance; and the technical, nursing, and dietary staffs of the Clinical Diabetes and Nutrition Section.

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