Basal and insulin-regulated free fatty acid and glucose metabolism in humans

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Shadid S, Kanaley JA, Sheehan MT, Jensen MD. Basal and insulin-regulated free fatty acid and glucose metabolism in humans. Am J Physiol Endocrinol Metab 292: E1770–E1774, 2007. First published February 13, 2007; doi:10.1152/ajpendo.00655.2006.—These studies were done to examine the effects of body composition, resting energy expenditure (REE), sex, and fitness on basal and insulin-regulated FFA and glucose metabolism. We performed 137 experiments in 101 nondiabetic, premenopausal women and men, ranging from low normal weight to class III obese (BMI 18.0–40.5 kg/m²). Glucose flux was measured using [6-3H]glucose and FFA kinetics with [9,10-3H]oleate during either basal (74 experiments) or euglycemic hyperinsulinemic (1.0 mU·kg FFM⁻¹·min⁻¹) clamp conditions (63 experiments). Consistent with our previous findings, REE and sex independently predicted basal FFA flux, whereas fat-free mass was the best predictor of basal glucose flux; in addition, percent body fat was independently and positively associated with basal glucose flux (total r² = 0.52, P < 0.0001). Insulin-suppressed lipolysis remained significantly associated with REE (r = 0.25, P < 0.05), but percent body fat also contributed (total adjusted r² = 0.36, P < 0.0001), whereas sex was not significantly related to insulin-suppressed FFA flux. Glucose disposal during hyperinsulinemia was independently associated with peak VO₂, percent body fat, and FFA concentrations (total r² = 0.63, P < 0.0001) but not with sex. We conclude that basal glucose production is independently related to both FFM and body fatness. In addition, hyperinsulinemia obscures the sex differences in FFA release relative to REE, but brings out the effects of fatness on lipolysis.

ABNORMALITIES OF ADIPOSE TISSUE free fatty acid (FFA) release have been directly and causally proved to mediate some of the metabolic abnormalities seen in obesity (8). The increasing prevalence of obesity and its detrimental influence on health requires that we better understand adipose tissue metabolism as it relates to FFA release. We (18) have previously examined the relationship among body composition, resting energy expenditure (REE), and basal FFA as well as glucose production rates. We found that systemic FFA flux was related to REE but not to fat free mass (FFM), whereas basal glucose production rates were related to FFM but not REE (18). In addition, FFA flux was ~40% greater in women than in men at comparable FFA concentrations, whereas glucose flux was not different between men and women relative to FFM.

Unfortunately, we do not have a similar level of understanding as to the major factors relating to adipose tissue FFA release during hyperinsulinemia. It has been reported that glucose disposal during hyperinsulinemia is related to FFM (11), indexes of fatness (10, 11), plasma FFA concentrations (12), and fitness (6), but we could not find evidence that similar influences on insulin-suppressed FFA release have been studied. We previously reported (13) that lipolysis is less readily suppressed by hyperinsulinemia in upper-body obese women than in nonobese or lower-body obese women and that obese men are resistant to insulin with respect to the suppression of lipolysis (19). In those studies, we did not include enough participants in those studies to test the effects of sex, fitness, and fat distribution vs. fatness on lipolysis.

To address this limitation, we studied a large number of normal-weight and obese volunteers under overnight postabsorptive and/or insulin clamp conditions. We studied plasma insulin concentrations routinely seen after meal ingestion to be able to relate the findings to postprandial regulation of lipolysis. Consistent with our previous findings, women had greater rates of basal lipolysis than men relative to REE (18). We also found that the factors known from other studies to affect glucose disposal during hyperinsulinemia were important correlates in our population. To our surprise, we found that body fatness was positively related to basal endogenous glucose production rates. We also found that moderate hyperinsulinemia abolishes the sex difference in FFA metabolism.

METHODS

Subjects. These studies were approved by the Mayo Clinic Institutional Review Board. Written, informed consent was obtained from 101 nondiabetic, premenopausal women and men, ranging from low normal weight (BMI 18 kg/m²) to Class III obese (BMI 40.5 kg/m²). The activity levels of the volunteers ranged from sedentary (no regular, vigorous activity) to almost daily, vigorous exercise. Thirty-six of the participants were studied under both euglycemic hyperinsulinemic clamp conditions and under saline control, leading to 137 different total experiments. The subjects were weight stable at least 3 mo before entering the research protocols, which were designed primarily to study intramuscular triglyceride metabolism (unpublished data). The volunteers underwent muscle biopsies as part of these studies; however, the analysis of the muscle samples is not yet complete. The FFA flux and related data for 20 of the obese men and 19 of the women presented herein have been previously published as the preintervention data for a study comparing diet/exercise to pioglitazone (20). The characteristics of the participants are given in Table 1.

Study protocol. Assessment of body composition was done with dual-energy X-ray absorptiometry (DEXA) and a single-slice CT scan at the level of L2–3 within 2 wk of the studies using previously described approaches (15). On or about the same day, oxygen consumption (VO₂ peak) and maximum heart rate were determined with a graded exercise test performed on a Quinton (Seattle, WA) motor-driven treadmill using a modified Bruce protocol (7). Heart rate and rhythms were monitored continuously via a 10-lead electrocardiogram. The volunteers consumed an isocaloric diet for 5 days prior to the study (55% carbohydrates, 30% fat, and 15% protein), prepared by our General Nutrition Center dietitian.

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Clinical Research Center (GCRC). The evening of the last day, the participants were admitted to the GCRC and remained fasted after the evening meal. Prior to the beginning of the tracer infusions, baseline blood samples were collected for measurement of background plasma FFA specific activity (SA) and glucose enrichment. An intravenous catheter was placed in a forearm vein and kept patent with a continuous infusion of 0.45% NaCl. At 0600, a primed continuous infusion of [6-2H2]glucose (0.06 Ci/min) was started to trace glucose kinetics. For the study of basal FFA kinetics, a continuous infusion of [9,10-3H]oleate (0.8 Ci/min) for the following 24 h. The same protocol was employed for those undergoing the insulin clamp study, except that at 0800 a euglycemic hyperinsulinemic (1.0 mU/min/m2) insulin clamp was started using [6-2H2]glucose-labeled 50% dextrose and continued for 6 h to assure steady-state glucose disposal and indirect calorimetry results. Blood was sampled every 10 min to allow adjustment of the glucose infusion rate to maintain glucose concentrations stable. Between 1330 and 1400, REE was measured, and blood samples were collected at 10-min intervals for measuring glucose enrichment, and FFA SA were collected.

Assessment of lipolysis using oleate flux. FFA flux was calculated as micromoles per minute using steady-state formulas because concentration and SA were stable over the sampling interval. Under steady-state conditions, the amount of FFA released (FFA Ra) from adipose tissue equals the amount taken up (FFA Rd). The average oleate SA and glucose enrichments were used to calculate steady-state rates of appearance (Ra) and disappearance (Rd) of oleate and glucose. Oleate flux was converted to FFA flux by use of the relationship between plasma oleate concentrations and total FFA concentrations (measured by HPLC) for the basal and insulin clamp conditions. The following assays were performed: for glucose, a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA); for glucose enrichment, gas chromatography-mass spectrometry (16); for insulin, C-peptide, and growth hormone concentrations, chemiluminescent sandwich assays (Sanofi Diagnostics, Chaska, MN); and for catecholamines, HPLC with electrochemical detection (4). Plasma oleate concentrations and specific activity (SA), as well as total FFA concentrations, were measured by HPLC (17). Plasma triglyceride concentrations were measured using a centrifugal autoanalyzer (14).

Statistical analysis. Values are expressed as means ± SD (range). Statistical analyses were done using the JMP 5.1.1 statistical package (SAS Institute, Cary, NC). Statistical comparisons between men and women were performed using nonpaired t-tests. Univariate regression analysis was performed to test for simple relationships (such as the relationship between FFA concentration and FFA flux), whereas multivariate regression analysis was used to test the relationship between basal and insulin regulated FFA and indirect calorimetry, fitness (VO2 peak) or body composition variables. The same approach was used to test for relationships between glucose flux and the aforementioned variables. For the analysis of insulin-stimulated glucose disposal, we initially included visceral fat area in the analysis, and although it contributed significantly and produced a greater r2, issues of multicolinearity of the data caused us to drop visceral fat area from the final model. Likewise, because VO2 peak was a significant predictor of insulin stimulated glucose disposal and is expressed as milliliters per kilogram FFM per minute, we did not include FFM independently in the model. We did not test models that expressed glucose or FFA turnover as micromoles per kilogram FFM per minute. This is because we previously found that for both of these variables the intercept of the relationship is not 0 and the slope is not 1 (18), which would be a prerequisite to expressing these kinetic relationships in such a ratio-standard manner (21).

RESULTS

Subject characteristics. The characteristics of the participants in the basal and insulin clamp studies are presented in Table 1. On average, the men were 3–6 yr younger than the women. Parenthetically, age was included in all of the analyses of FFA and glucose metabolism and did not contribute significantly. The differences between men and women in body weight, FFM, percent body fat, waist-to-hip ratio (WHR), and visceral fat were as expected on the basis of previous observations. The men and women were not different with respect to BMI, abdominal subcutaneous fat area, VO2 peak, and fasting plasma glucose concentrations, nor were there differences between the basal and insulin clamp groups.

Overnight postabsorptive results. Basal plasma insulin concentrations were not different between men and women. Plasma FFA concentrations were 17% lower (P = 0.03) in men than in women (Table 2). Plasma FFA flux (µmol/min) was also somewhat lower in men than in women (P = 0.12). Glucose flux (µmol/min) and REE were significantly greater in men than in women, and the basal respiratory exchange ratio (RER) was not different between men and women.

Basal plasma FFA concentrations were highly correlated with plasma FFA flux (r = 0.51, P < 0.0001). Consistent with our previous observations (18), plasma FFA flux was related to both REE (P < 0.0001) and sex (P < 0.0001). This relation-
ship is depicted in Fig. 1A. Together, these two factors could account for 26% of the variance of basal plasma FFA flux. Other factors, including WHR, percent body fat, visceral fat area, abdominal subcutaneous fat area, FFM, and VO2 peak were not significant contributors to basal FFA flux after REE and sex were taken into account.

As we previously observed (18), basal glucose flux was significantly related to FFM (adjusted \( r^2 = 0.58, P < 0.0001 \)), and REE did not add significantly to predicting glucose flux. Interestingly, the combination of FFM and percent body fat substantially improved the ability to predict basal glucose flux (adjusted \( r^2 = 0.52, P < 0.0001 \)). The relationship between FFM and basal glucose flux residuals is depicted in Fig. 2, and the relationship between basal glucose flux residuals and percent body fat is depicted in Fig. 3. None of the other variables, including basal plasma glucose or FFA concentrations, contributed significantly to the ability to predict basal glucose flux.

The basal RER was not significantly related to any of the body composition variables, but we found that basal FFA concentrations were inversely correlated with basal RER values (\( r = -0.28, P = 0.007 \)).

The plasma insulin and FFA concentrations observed during insulin clamp in men and women are also provided in Table 2. As expected, plasma FFA concentration and flux were suppressed as a result of hyperinsulinemia and plasma FFA concentrations and FFA flux were highly correlated with each other (\( r = 0.68, P < 0.0001 \)). Under the conditions of hyperinsulinemia, the relationship between plasma FFA flux (\( \mu mol/min \)) and REE was maintained (\( r = 0.22, P < 0.05 \); Fig. 1B); however, sex was no longer significantly related to FFA flux. The combination of REE and percent body fat predicted a significantly larger portion of the variance in insulin suppressed FFA flux (adjusted \( r^2 = 0.36, P < 0.0001 \)) than REE alone. None of the other variables significantly improved the ability to predict insulin suppressed FFA flux.

Glucose disposal (\( \mu mol/min \)) values during the clamp are provided in Table 2. Although no one factor accounted for the majority of interindividual variation in glucose disposal, a number of parameters combined could predict a large portion of the variance in glucose disposal (adjusted \( r^2 = 0.63, P < 0.0001 \)). These factors and the associated \( P \) values are provided in Table 3. VO2 peak was positively related to total glucose disposal, whereas plasma FFA concentrations and percent body fat were independently and negatively related to glucose disposal.

The insulin clamp RER values were not different between men and women (Table 2) and were negatively and independently correlated with plasma FFA concentrations and visceral fat (both \( P = 0.01 \)). Together, FFA and visceral fat could account for 25% of the variance in RER.

**DISCUSSION**

Our group (18) has previously examined the relationship between overnight postabsorptive FFA and glucose flux in order to understand the roles of energy expenditure, sex, and body composition in determining substrate metabolism. We found that FFA flux is more strongly related to REE than to body composition and that women have higher rates of lipolysis than men when adjusted for REE. This was not the case for basal glucose flux, which was similar in men and women and more strongly correlated with FFM than with REE. The goal of

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**Table 2. Insulin, FFA, glucose, and indirect calorimetry results**

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<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Insulin Clamp</th>
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<tbody>
<tr>
<td></td>
<td>Women (n = 40)</td>
<td>Men (n = 34)</td>
</tr>
<tr>
<td>Insulin, ( \mu U/ml )</td>
<td>5.9±5.1 (1.1–30.0)</td>
<td>5.9±5.3 (0.7–26.1)</td>
</tr>
<tr>
<td>FFA, ( \mu mol/l )</td>
<td>455±127 (183–653)</td>
<td>376±169 (138–1,042)*</td>
</tr>
<tr>
<td>FFA flux, ( \mu mol/min )</td>
<td>442±167 (181–843)</td>
<td>383±150 (60–854)</td>
</tr>
<tr>
<td>Glucose flux, ( \mu mol/min )</td>
<td>638±155 (396–1,027)</td>
<td>786±198 (492–1,190)†</td>
</tr>
<tr>
<td>Study interval REE, kcal/day</td>
<td>1,524±200 (1,155–1,935)</td>
<td>1,961±243 (1,586–2,482)†</td>
</tr>
<tr>
<td>Study interval RER</td>
<td>0.81±0.05 (0.73–0.91)</td>
<td>0.81±0.03 (0.74–0.90)</td>
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Values are means ± SD (range). FFA, free fatty acid; REE, resting energy expenditure; RER, respiratory exchange ratio. *Difference (\( P < 0.05 \)) between men and women. †Difference (\( P < 0.005 \)) between men and women. Comparisons between basal and insulin clamp values were not made.
this study was to examine, in a larger cohort, the additional effects of fitness level on basal lipolysis and glucose flux, as well as to understand whether hyperinsulinemia disrupts these relationships. We confirmed that basal FFA flux is best related to REE and sex but that during hyperinsulinemia body fatness rather than female sex is associated with greater FFA release, independent of REE. To our surprise, both body fat and FFM were positively related to basal glucose flux, whereas a number of factors independently influenced insulin-stimulated glucose disposal.

Consistent with our previous findings (18), basal FFA flux was not significantly related to body fat after adjustment for sex and REE. In this study, we were also able to test whether fitness, as measured by \( V'_O2 \) peak, is related to basal or insulin-suppressed lipolysis; we found that \( V'_O2 \) peak was not related to FFA flux under either condition. This suggests that to the extent that physical fitness improves metabolic function it does not do so via effects on basal or insulin-suppressed lipolysis. We also found that suppression of lipolysis by physiological insulin concentrations did not disrupt the relationship between FFA flux and REE. Of interest, body fat, rather than sex, accounted for a significant portion of the intraindividual variability in FFA flux under insulin clamp conditions. We conclude that whatever factors mediate greater FFA availability in overnight postabsorptive women are readily overcome by moderate hyperinsulinemia. In addition, increasing body fat content

![Image](80x570 to 278x722)

is a significant modulator of lipolysis under insulin-suppressed conditions, independent of sex.

The finding that FFM is most strongly correlated with basal glucose flux (9, 11, 18) does not inherently imply that dividing glucose flux rates by FFM is an optimal means of data expression (21). This is especially a problem when groups differ in FFM, because glucose flux and FFM are not in a ratio-standard relationship (a slope of 1 and an intercept of 0). A corollary to this issue is that it may be more difficult to detect the influence of other factors on glucose flux if a ratio-standard approach means of data expression is used. When we tested for the effects of fitness, body fat, and sex on basal glucose turnover, we were surprised to detect a significant, positive effect of percent body fat (Fig. 3). Thus, even after adjusting for FFM, adults with more body fat have greater overnight postabsorptive glucose production/uptake rates at the same blood glucose concentration. This effect was independent of plasma FFA concentrations. Although it has not been previously reported, we note that indexes of fatness have been associated with basal gluconeogenesis, but not endogenous glucose production, in smaller groups of nondiabetic subjects (9, 11) and in type 2 diabetes (10). We speculate that, by studying a much larger group of volunteers with a wide range of body fat and FFM, we were able to detect this effect of fatness on endogenous glucose production.

An implication of this finding is that investigators may need to be cautious in comparing glucose flux, especially if expressed relative to FFM, among persons with widely different amounts of body fat. For example, leaner men and more overweight women could have similar amounts of FFM but widely differing amounts of body fat. If basal endogenous glucose production were greater in the women, this would not be proof of a sex effect per se, but rather an effect of greater body fat. For example, for the seven men and eight women in this study with FFM between 50 and 60 kg (average of 53 kg for women and 56 kg for men), glucose flux was 10.7 ± 0.5 and 13.7 ± 0.9 \( \mu \)mol·kg FFM \(^{-1}\)·min \(^{-1}\), respectively (\( P = 0.01, \) men vs. \( \) women), despite having the same mean fasting glucose concentrations (90 vs. 91 mg/dl). From these studies it is not possible to determine whether the slightly greater production in those with more body fat is being driven by a factor produced by adipose tissue or whether greater glucose utilization is associated with greater amounts of adipose tissue. In theory, the latter would require greater glucose production to maintain plasma glucose concentrations.

The factors that we found to be predictive of insulin stimulated glucose disposal are those that would be predicted from the literature (1–3, 5). \( V'_O2 \) peak was positively associated with glucose uptake, whereas percent body fat and insulin-sup-
pressed FFA concentrations were independently negatively associated with glucose disposal. The amount of variance explained by these factors was quite high by most standards. We take this as evidence that our results are largely in keeping with the literature, which is reassuring given that some of our findings were unexpected.

The relationship between plasma FFA concentrations and RER deserves brief comment. It is difficult to attain true energy balance with 5 days of GCRC feeding. Thus, even though all volunteers received meals with the same food quotient, some probably were slightly underfed whereas others were slightly overfed. Because energy deficits are largely handled by mobilizing and oxidizing more FFA, it is possible that the association that we observed between postabsorptive RER and plasma FFA concentrations is an “energy deficit” effect. In contrast, it is doubtful that this same phenomenon would hold at the end of a 6-h euglycemic hyperinsulinemic clamp. Thus, it may be that adipose tissue resistance to the ability of insulin to suppress lipolysis, resulting in greater FFA concentrations and greater fat oxidation, is the etiology of the association of the lower RER in those with greater plasma FFA.

Some limitations to this study must be acknowledged. In this study, we had only a single measure of basal FFA flux and glucose flux, whereas we previously had quadruplicate measures of FFA flux and duplicate measures of glucose flux (18). Thus, although we have a larger group of participants, we do not have an equally precise measure of each individual’s “true” average basal rate of lipolysis or glucose production. Because of this, we may have missed some associations that were not as strong as those with REE, sex, and body fat. Another limitation is that we cannot determine whether the increase in endogenous glucose production that we observed as a function of body fat is related to effects on glycolysis or gluconeogenesis (9).

In summary, we report that FFA availability remains positively related to REE under conditions of moderate hyperinsulinemia but that it is additionally affected by percent body fat. Fitness, sex, and body fat distribution are not associated with insulin-suppressed lipolysis. We also found that percent body fat was significantly related to basal glucose turnover after adjustment for FFM. These new findings are in the context of data that confirm our previous work (18) regarding basal lipolysis and the work of others finding are in the context of data that confirm our previous work (18) regarding basal lipolysis and the work of others

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REFERENCES