# Relationship between postabsorptive respiratory exchange ratio and plasma free fatty acid concentrations

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**Abstract The relationship between overnight postabsorptive (fasting) respiratory exchange ratio (RER) and plasma FFA concentrations was addressed using data from three separate protocols, each of which involved careful control of the antecedent diet. Protocol 1 examined the relationship between fasting RER and the previous daytime RER. In Protocol 2 fasting, RER and plasma palmitate concentrations were measured in 29 women and 31 men (body mass index <30 kg·m$^{-2}$). Protocol 3 analyzed data from Nielsen et al. (Nielsen, S., Z. K. Guo, J. B. Albu, S. Klein, P. C. O'Brien, M. D. Jensen. 2003. Energy expenditure, sex and endogenous fuel availability in humans. J. Clin. Invest. 111: 981-988.) to understand how fasting RER and palmitate concentrations relate within individuals during four consecutive measurements. The results were as follows: *1*) Fasting RER was correlated ($r = 0.74$, $P < 0.001$) with the previous day's average RER, and less so with RER variability. *2*) Fasting RER was correlated ($r = -0.39$, $P = 0.007$) with fasting plasma palmitate concentrations. *3*) The pattern of the RER/palmitate relationship was similar within individuals and between individuals; a negative slope was observed significantly more often than a positive slope ($\chi^2$ test; $P < 0.001$). Our findings suggest that, despite a fixed food quotient, the slight departures from energy equilibrium in a controlled General Clinical Research Center environment can effect plasma FFA concentrations. We suggest that including indirect calorimetry as part of FFA metabolism studies may aid in data interpretation.**—Jensen, M. D., J. Bajnárek, S. Y. Lee, S. Nielsen, and C. Koutsari. **Relationship between postabsorptive respiratory exchange ratio and plasma free fatty acid concentrations. *J. Lipid Res.* 2009.** 50: **1863–1869.**

**Supplementary key words** energy balance • indirect calorimetry • palmitate • food quotient • lipolysis

Whole-body substrate oxidation can be measured using indirect calorimetry combined with urinary nitrogen excretion rates (1). Factors known to rapidly affect substrate oxidation include diet composition (2) and energy balance. Energy deficits typically result in mobilization and oxidation of body fat (3), which is reflected by a lower respiratory exchange ratio (RER). This is relevant because even in the absence of intentional weight loss efforts, using indirect calorimetry to assess interindividual differences in substrate oxidation is complicated by difficulties in achieving energy balance (4, 5). Despite careful attention to detail by controlling diet and making prestudy measures of metabolic rate, it is not uncommon to find 300 kcal differences between energy intake and expenditure as measured by room calorimetry (4, 5). We have attempted to minimize these issues by feeding volunteers participating in our studies of fatty acid metabolism a diet with fixed macronutrient distribution for 3–10 days prior to research studies (6, 7). In general, the metabolic kitchen in the Mayo General Clinical Research Center (GCRC) has been able to closely match energy intake to energy expenditure measured using double-labeled water (8).

Given these efforts, we hoped that volunteers participating in our protocols, having consumed a diet with the same food quotient (FQ), would have similar daytime RER values, because if energy balance is achieved, FQ should equal the 24 h respiratory quotient. Unfortunately, we still observe significant interindividual variability in overnight postabsorptive RER, which could be driven by constitutional factors (increased constitutive lipolysis driving fat oxidation) or environmental factors, such as energy balance. We believe that understanding the relative contribution of the environment to this measurement is important for our studies of lipolysis because we found a significant association between RER and overnight postabsorptive FFA concentrations (9) and because FFA concentrations are highly predictive of FFA flux (6).

The purpose of this report is to share our experience with factors that relate to inter- and intraindividual vari-

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Abbreviations: AEE, activity energy expenditure; BMI, body mass index; FQ, food quotient; GCRC, General Clinical Research Center; REE, resting energy expenditure; RER, respiratory exchange ratio.

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ability in postabsorptive RER and FFA concentrations. We also provide data on the relationship between these two metabolic variables in the hope that this will aid future investigations of FFA metabolism.

## METHODS

### Subjects

Data from a number of research projects, both published (6, 7) and unpublished, were used to address our hypotheses. All protocols were approved by the Mayo Clinic Institutional Review Board, and all participants provided informed written consent. These data are independent of our previous finding of a negative relationship between overnight postabsorptive RER and plasma FFA concentrations (9). Table 1 provides the age, body mass index (BMI), and percentage of body fat data for volunteers from each protocol.

For Protocol 1, data from 51 volunteers (25 women and 26 men) participating in studies of dietary fatty acid metabolism were used. The general approach to the studies is as previously outlined (7). The participants were all healthy, nonobese (BMI range 18.4–26.0 $kg \cdot m^{-2}$) adults aged 18–45.

For Protocol 2, 29 women and 31 men (BMI range 19.6–29.3 $kg \cdot m^{-2}$) underwent studies of overnight postabsorptive FFA metabolism to test for sex differences in nonoxidative FFA disposal. The RER and palmitate concentration data have not been published.

For Protocol 3, 50 volunteers (25 women and 25 men) participated in a study examining how body composition, hormone concentrations, and resting energy expenditure relate to FFA and glucose flux (2). The average FFA data from four experiments have been published. Participants of this study had a wide range of BMI (18.9–36.0 $kg \cdot m^{-2}$).

No smokers or persons taking medications known to influence carbohydrate or lipid metabolism were included. In order to participate, the volunteers were required to be weight stable for at least 2 months prior to the study. Hematological indices and liver and renal function studies were documented to be normal for each participant.

### Study design

Only procedures and circumstances influencing the variables assessed in our article are described in this section. For entire design of original studies, refer to Refs. 6 and 7.

*Protocol 1.* We examined whether overnight postabsorptive RER is related to characteristics of the previous day's RER, including the responsiveness of RER to the meals [metabolic flexibility (10)] and/or the average RER. The latter should in theory be identical for all volunteers consuming an energy neutral diet with the same FQ. To be included in the data set, the volunteers needed to have overnight, postabsorptive indirect calorimetry measurements on two consecutive days. In addition, we required that data be available from at least 8 of 10 possible indirect calorimetry measures performed on the first inpatient study day (the day prior to the second overnight postabsorptive indirect calorimetry). All subjects consumed a weight-maintaining diet (50% carbohydrate, 35% fat, and 15% protein) provided by the GCRC for 7 days before the study. Individual energy needs for weight maintenance were calculated multiplying the resting energy expenditure (REE) by an activity factor determined for each individual. REE was estimated using the Harris-Benedict equation (11). The activity factor reflected the individual habitual activity assessed by the interview. The volunteers were weighed and interviewed daily. If the individual's weight decreased by $\geq$1.0 kg, the amount of food provided was increased by 250 kcal/day in attempts to compensate, with continuing adjustments as needed if weight continued to decrease. If the volunteers reported feeling more hungry than usual, the dietitians would take this into account and increase food intake even if the weight loss was <1.0 kg. If body weight increased by $\geq$1.0 kg and/or if the volunteers were unable to consume all food provided, the amount was decreased by 250 kcal/day. For the studies with $\geq$5 days of prestudy diet control, the experiments were only carried out if we are able to maintain weight within these parameters.

The volunteers were admitted to the GCRC the evening prior to the study day and consumed their evening meal at the same, standardized time (1800 h). Meals were provided during the study day at 0800, 1300, and 1800 h. The breakfast was a liquid meal (Ensure Plus; Ross Laboratories) that provided 40% of resting energy needs based on indirect calorimetry performed that morning. Carbohydrates, fat, and protein represented 57, 27, and 15% of the energy content of breakfast, respectively. The volunteers were given solid meals from the GCRC metabolic kitchen at 1300 and 1800 h that provided 50, 35, and 15% of energy as carbohydrates, fat, and protein, respectively. The total energy content of these meals was designed to meet each individual's remaining daily energy needs based on the weight maintenance diet for the preceding 7 days. During the study day, however, the volunteers were at bed rest for almost the entire day so that blood and breath samples could be collected regularly. Indirect calorimetry was performed hly from 0700 h for 9 h, at 1700 h and the next morning at 0700 h. These studies were primarily designed to measure the storage of meal fat into subcutaneous adipose tissue using a radiolabeled triolein tracer incorporated into the breakfast meal (7).

*Protocol 2.* These data were used to examine the cross-sectional relationship between RER and plasma FFA (palmitate) concentrations in nonobese adult men and women. All volunteers consumed a diet providing 55% carbohydrate, 15% protein, and 30% fat prepared by the Mayo Clinic GCRC metabolic kitchen for 3 days preceding the study. The energy needs were calculated as described in Protocol 1. After 3 days of this diet, the volunteers were admitted to the GCRC at $\sim$1600 h and remained fasted after a standard 10 kcal/kg meal (55% carbohydrate, 15% protein, and 30% fat) meal administered at 1800 h. No caffeine was allowed during the study. The following morning, before the participants arose from bed, REE was measured and arterialized blood was sampled at 10 min intervals for 30 min for measurement of plasma insulin, growth hormone, epinephrine, and palmitate concentrations.

*Protocol 3.* We used these data to examine whether the relationship between single measures of RER and FFA in a group of individuals (Protocol 2) is also seen when multiple measures are made in the same individual. Adults with inherently high rates of adipose tissue lipolysis may oxidize more fat after an overnight fast, or those with constitutionally high fat oxidation tendencies may mobilize FFA more readily to meet this demand. If either of these explanations was correct, we would anticipate that individuals would have consistent high or low overnight postabsorptive RER values even on a fixed FQ diet. Alternatively, mild variations in individual energy balance could be due to imprecision of energy needs assessment and/or daily variations in energy expenditure. The individual daily data from Protocol 3 was analyzed to help address this question.

The study was 14 days in duration. The volunteers were provided all meals (40% carbohydrates, 40% fat, and 20% protein) by the Mayo Clinic GCRC metabolic kitchen for 14 days. The diet control protocol was the same as that described for Protocol 1. At some time during the first 10 days, the volunteers underwent

body composition testing. On the 10th study day, participants were admitted to the GCRC for four consecutive overnight stays. While at GCRC, the volunteers consumed their evening meal at 1800 h and a snack at 2100 h. During each of the last four study days, REE was measured before arising from bed in the morning, and arterialized venous blood was sampled for the measurement of plasma insulin, growth hormone, epinephrine, and palmitate concentrations as described for Protocol 2. The volunteers were then given breakfast and a carry-out lunch but allowed to go about their daily activities/work. They returned to the GCRC each day for their evening meal. The results for the average FFA and glucose flux have been published (6).

### Analytical techniques

Plasma palmitate concentrations were measured using HPLC (12, 13) or GC\Combustion\Isotope Ratio Mass Spectrometry (14). Plasma palmitate concentrations for the volunteers reported for Protocols 2 and 3 were consistently 25–30% of total FFA concentrations. REE was measured by indirect calorimetry (DeltaTrac Metabolic Cart, Yorba Linda, CA). The metabolic cart was calibrated each morning prior to the study, and additional quality control for the carts included monthly pressure calibrations and gas calibrations together with biannual calibrations of the metabolic carts using an alcohol burn test. The test-retest difference is <3% for duplicate measures of $VO_2$ and $VCO_2$ for adults in the same environment on sequential days using our instruments. We take the following precautions for our measurements: *1*) each day before beginning to use the instrument, we check the calibration with a known gas mixture and if the variance from the known is >1.25% the instrument is reset; *2*) if the ambient room $CO_2$ concentrations exceed those that we know interfere with $CO_2$ production, we increase the room ventilation until the ambient $CO_2$ decreases to acceptable levels; *3*) we measure $VO_2$ and $CO_2$ every month in one of our personnel; if the $CO_2$ production rate is >20 ml/min different from average, we remeasure her with another instrument to test for biological versus instrument issues; *4*) we use the same instrument for an entire protocol unless maintenance issues arise that require us to substitute another instrument; *5*) we track which instrument is used for each study and use these data as a way to track instrument performance. Body composition was measured using a combination of DXA (Lunar DPX-IQ) and a single slice abdominal CT scan at the $L_{2\text{-}3}$ interspace (15). Insulin and growth hormone concentrations were measured using chemiluminescent sandwich assays (Sanofi Diagnostics, Chaska, MN), and plasma epinephrine was measured using HPLC with electrochemical detection (16).

### Statistical analysis

Values are given as mean ± SD, where the data were normally distributed or as median (range), where the data failed to show normal distribution. To compare two sets of data, unpaired Student's *t*-test or nonparametric rank sum test were used where appropriate. For comparison of multiple groups of data with nonnormal distribution, Kruskal-Wallis one-way ANOVA on ranks was used. For analysis of correlation, Pearson Product Moment or Spearman Rank Order procedures was chosen based on the distribution of the data. The standard least squares method was applied. We also examined whether body fat, body fat distribution, and plasma concentrations of insulin, epinephrine, or growth hormone were related to plasma palmitate and RER using both univariate and multivariate regression analysis. Statistical software SigmaStat 2.03 (SPSS, Chicago, IL) was used. The measures of RER variability we examined included seven frequently used measures as indices of daily RER variability, range, variance, interquartile range, relative difference, coefficient of variation, SD, and the square of SD.

## RESULTS

### Protocol 1

*Subject characteristics.* The data from a subset of these participants have previously been published (7). The entire group's average age, BMI, and percentage of body fat are provided in **Table 1**.

*Predictors of postabsorptive RER.* The range of overnight postabsorptive RER values we observed on the first and second mornings in the GCRC were 0.65–0.92 (mean ± SD = 0.79 ± 0.06) and 0.66–1.01 (mean ± SD = 0.83 ± 0.06). The overnight postabsorptive RER measure on the second morning was best correlated with the average RER from the previous day ($r = 0.74$, $P < 0.001$; **Fig. 1**). Indices of daily RER variability (range of RER, CV, of RER) either were not correlated with the postabsorptive RER or were only weakly correlated. The variance of RER (greatest value to lowest value, divided by average daytime RER) was negatively correlated ($r = -0.37$, $P < 0.01$) with the second overnight postabsorptive RER. Separate analysis for data from men and women showed the same patterns.

This indicated to us that interindividual variations in overnight postabsorptive RER in the inpatient GCRC setting largely reflect the prior day's energy balance rather than differences in the ability to rapidly shift between carbohydrate and fat oxidation following meal ingestion.

### Protocol 2

*Subject characteristics.* **Table 2** provides the characteristics of Protocol 2 participants. Men and women had the same age and BMI but differed with regards to body composition as expected.

The overnight, postabsorptive RER values ranged from 0.72–0.95 despite efforts to obtain energy balance and despite providing a diet with identical macronutrient composition to all volunteers. Men had lower plasma palmitate concentrations ($P < 0.001$) and higher RER ($P = 0.003$) than women.

TABLE 1. General subject characteristics

| | Men | | | Women | | |
|---|---|---|---|---|---|---|
| | Protocol 1 | Protocol 2 | Protocol 3 | Protocol 1 | Protocol 2 | Protocol 3 |
| Age | 29 ± 7 | 24 ± 3 | 30 ± 7 | 28 ± 8 | 22 ± 3 | 31 ± 8 |
| BMI | 23.2 ± 2.1 | 24.9 ± 2.8 | 26.9 ± 4.3 | 20.6 ± 1.7 | 24.1 ± 2.8 | 26.5 ± 5.7 |
| Body fat (%) | 17.3 ± 4.5 | 22.3 ± 7.0 | 24.0 ± 7.4 | 28.6 ± 5.8 | 34.4 ± 6.5 | 38.5 ± 8.2 |

Values are mean ± SD.

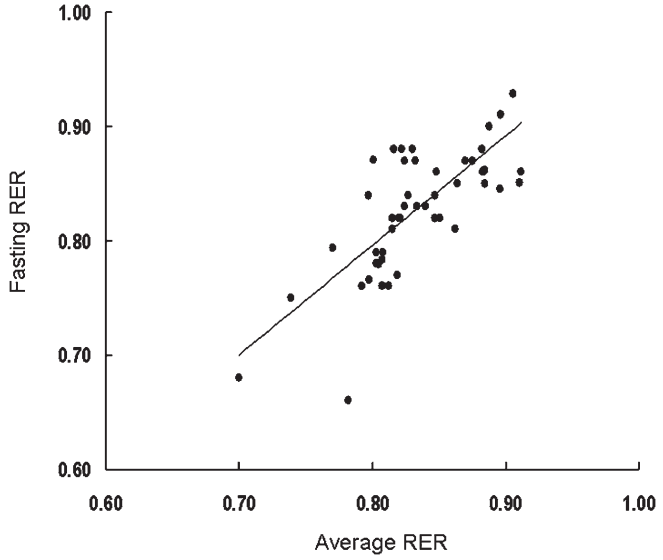


**Fig. 1.** The average of the daytime RER values from the previous day is plotted versus the fasting RER for volunteers in Protocol 1. There was a significant correlation ($r = 0.74$, $P < 0.001$) between the overnight postabsorptive RER and the average of 8–10 hly RER measurement from the previous day.

*Interindividual relationship between plasma FFA concentrations and RER.* RER was negatively correlated with plasma palmitate concentrations ($r = -0.39$, $P = 0.007$; **Fig. 2**), and this relationship was not affected or related to body fat, plasma hormone, or catecholamine concentrations in this group of nonobese men and women. The relationship was also significant for women ($r = -0.39$, $P = 0.037$) and men ($r = -0.37$, $P = 0.038$) separately. This is similar to what we observed in a lean and obese women and men (9) but does not address whether the interindividual variability in RER and the inverse relationship between RER and plasma FFA concentrations are constitutional.

### Protocol 3

*Volunteer characteristics.* The characteristics of participants in Protocol 3 have previously been published (6). There were no significant differences in plasma palmitate concentrations or RER between women and men.

*Interindividual relationship between plasma FFA concentrations and RER.* Plasma palmitate concentrations were inversely correlated with RER for all four measurement days (**Fig. 3**). The correlation coefficients ranged from −0.37 to −0.53 on the different days. The regression lines for the four separate days, together with the regression coefficients and $P$ values, are provided in **Fig. 4**. These relationships were not affected or related to body fat, body fat distribution, plasma hormone, or catecholamine concentrations, although plasma palmitate concentrations were independently correlated with plasma epinephrine concentrations as previously reported (6).

*Intraindividual relationship between FFA concentrations and RER.* To determine if day-to-day variations in RER and palmitate concentrations followed generally similar relationships within an individual as between individuals, we performed linear regression analysis using the 4 days of data from each of the 50 participants. The regression lines developed using each participant's data are depicted in **Fig. 5**. Using the four data points for each individual, the median coefficient of determination $R^2$ was 0.32. Five of the slopes were significantly different from zero (all were negative). The relationship between RER and plasma palmitate yielded a negative slope significantly more often than a positive slope ($\chi^2$ test; $P < 0.001$).

The average correlation coefficients were not significantly different between men and women; however, the slopes of the relationship between RER and palmitate concentrations were significantly more negative in men than women ($P = 0.028$; **Fig. 6**).

### Composite data

We compiled the available data from all of the volunteers included in these three protocols to create tertiles of overnight postabsorptive RER to test whether age or body composition differed between groups. **Table 3** presents the anthropometric, body composition, and fasting plasma triglyceride concentration data we collected arranged by tertile of RER. There is no significance between group differences in any of the variables.

## DISCUSSION

Much of the within and between individual variability in overnight postabsorptive FFA concentrations cannot be accounted for by traditional factors, such as diabetes and obesity (6, 17). We recently found a relatively strong interaction between RER and plasma FFA concentrations (9) but wished to reconfirm this finding and understand whether this relationship is largely constitutional versus environmental. We undertook this effort to understand the factors that relate to inter- and intraindividual variability in overnight postabsorptive RER and FFA concentrations.

TABLE 2. Subject characteristics: Protocol 2 participants

| Protocol 2 | All | Women | Men | $P$ |
|---|---|---|---|---|
| n | 60 | 29 | 31 | |
| Age (years) | 22 (18; 31) | 22 (18; 31) | 24 (19; 31) | 0.153 |
| BMI ($kg \cdot m^{-2}$) | 24.4 (19.6; 30.9) | 23.4 (19.6; 30.9) | 25.0 (19.7; 29.3) | 0.260 |
| Palmitate ($\mu mol \cdot l^{-1}$) | 82 ± 27 | 97 ± 28 | 68 ± 17 | <0.001 |
| REE ($kcal \cdot 24\ h^{-1}$) | 1,637 ± 272 | 1,471 ± 175 | 1,793 ± 255 | <0.001 |
| RER | 0.82 (0.72; 0.95) | 0.81 (0.72; 0.95) | 0.83 (0.74; 0.87) | 0.003 |

The median and range of age, BMI, and RER are provided together with mean ± SD of palmitate and REE. The $P$ value is the value for the nonpaired $t$-test for differences between the values of men versus women.

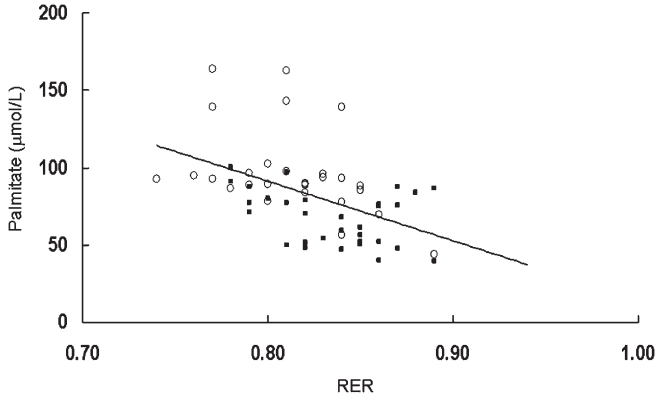


**Fig. 2.** Palmitate concentrations are plotted versus RER for participants in Protocol 2. Values for men are closed squares, and values for women are open circles. For all participants there was a significant, negative correlation ($r = -0.39$, $P = 0.007$) between RER and plasma palmitate concentrations.

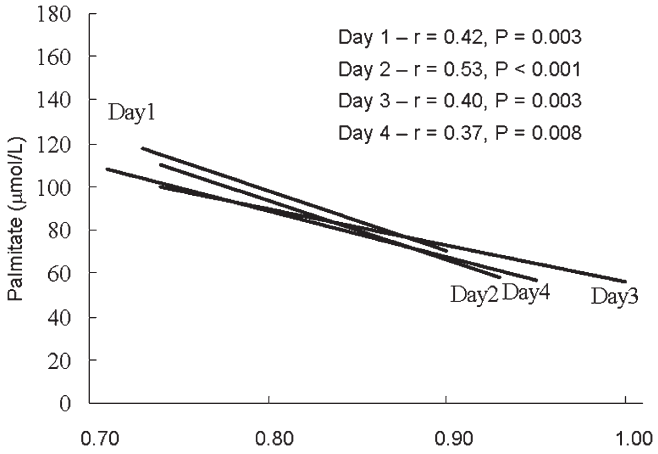


**Fig. 4.** The regression lines for palmitate concentration versus RER regression lines for each of the four separate study days for Protocol 3 are depicted.

The available data allowed us to address whether this association is related to constitutional and individual factors and/or the conduct of research studies.

We encountered substantial interindividual variability in postabsorptive RER even though our research volunteers consumed a fixed FQ, weight maintenance diet for 7 days prior to the studies. Although hyper- or hypoventilation, as well as technical problems with the indirect calorimetry unit, can create artificial differences in RER, we believe that the associations of fasting RER with FFA concentrations and the previous day's RER in our volunteers excludes these possibilities as the sole explanation for our findings. If perfect energy and body composition balance had been achieved, the average daily RER should reflect the FQ, and any interindividual difference in fasting RER would result only from difference in the ability to shift between carbohydrate and fatty acid oxidation. Instead, interindividual differences in fasting RER was related to the previous day's average RER rather than RER variability (Protocol 1). We take this as evidence that, like others (4, 5), our attempts to achieve energy balance are imperfect.

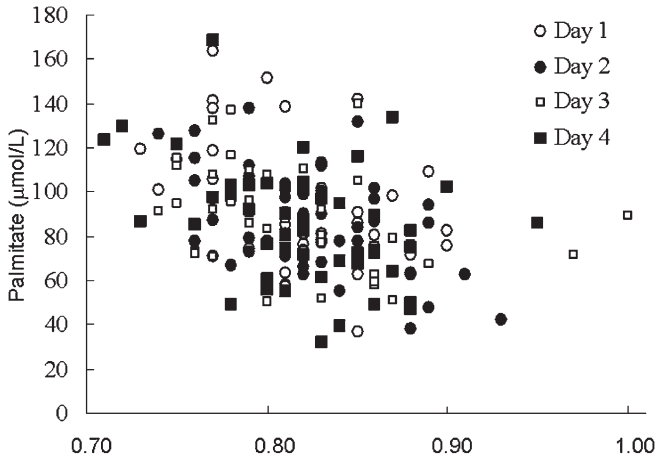


**Fig. 3.** Palmitate concentrations are plotted versus RER for all participants in Protocol 3 for each of the 4 days of measurement.

Although consistent with the accepted physiology that deficits or surpluses in energy balance drive greater fat or carbohydrate oxidation (18), respectively, these findings emphasize the impact relatively minor imbalances can have on standard metabolic measures. Given 10 days to feed a controlled diet, our metabolic kitchen is able to achieve energy balance within ~200 kcal of average daily energy expenditure as measured by double-labeled water (8).

The inability to achieve ideal energy balance could be caused by inaccurate prediction of one or more portions of total daily energy expenditure: REE, thermic effect of food, or activity energy expenditure. Although measured REE in our volunteers generally agreed well with that predicted using the Harris-Benedict formula, we found this formula overestimated the REE by an average of 4.5% ($P < 0.05$) for the volunteers in Protocol 2. We have previously noted that the thermic effect of food, although averaging

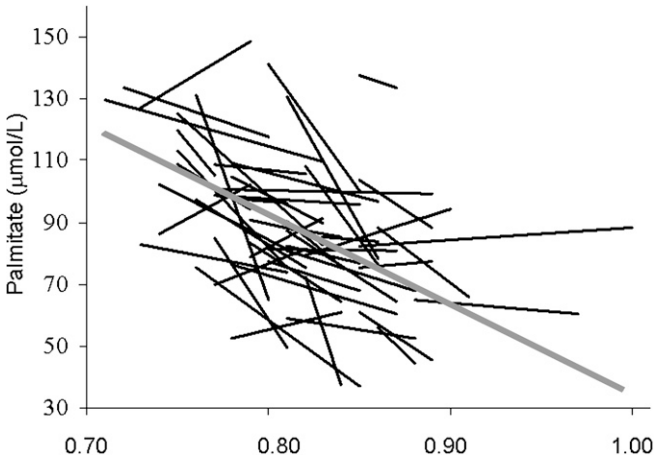


**Fig. 5.** The individual regression lines for each of the 50 participants in Protocol 3 are depicted separately, even if the relationship is not statistically significant. The length of the line represents the range of values observed for each individual. The gray line represents the mean slope and intercept of individual regression formulas.

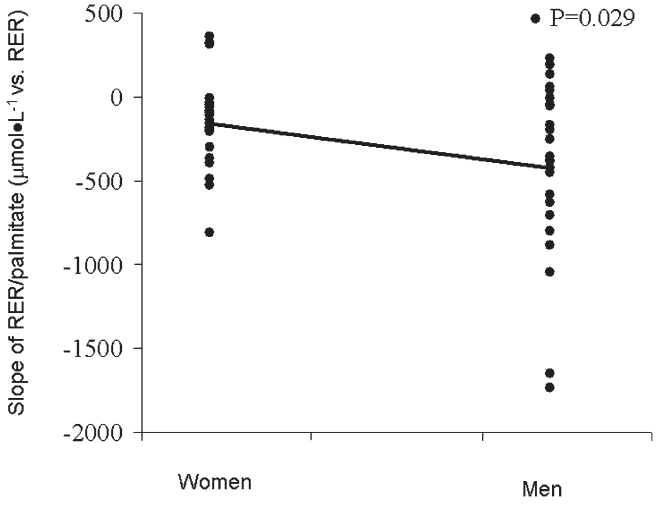


**Fig. 6.** The slope of individual palmitate-RER regression lines for men and women participating in Protocol 3 is depicted. Linear regression analysis indicated a significant sex effect.

10%, can range from ~5–15% (8). By assuming a 10% value for each volunteer, we might create an energy deficit or surplus of up to 5% in some individuals. Neither of these factors are likely to explain the data from Protocol 3, however, where intraindividual RER variability was observed over the course of four consecutive days. The substantial day-to-day variability in free-living physical activity (19) in the absence of compensatory changes in food intake seem likely to account for our observations. Literature reports on the relationship of postabsorptive RER with the day-to-day energy balance variation are scarce, but Goris and Westerterp (20) have reported a relationship between postabsorptive RER and weight change within the prior few days.

We reconfirmed the negative relationship between overnight postabsorptive RER and FFA concentrations (9) in a new data set (Protocol 2). The average fasting RER was significantly less in women than in men participating in this protocol, whereas plasma FFA concentrations were greater in women than men. Had we not measured RER, we might have falsely concluded that sex was the only reason for the differences in FFA between women and men. The reason for this discrepancy between men and women in this protocol likely relates to the fact that 3 days was not enough time to assure weight stability. Our GCRC meals often provide less sodium than the typical diet of our volunteers, which commonly results in some initial weight loss due to negative sodium balance. With the longer periods of controlled feeding, our dietitian staff was able to use daily weights to confirm energy intake was reasonably matched to energy expenditure, but with only 3 days they were more reliant on volunteer feedback regarding whether the quantity of food was excessive. The women participating in Protocol 2 were more likely than their male counterparts to indicate the GCRC meals were excessive. Without weight stability data to reinforce that the energy intake was appropriate, there appeared to have been more of a tendency to underfeed women in Protocol 2. The data from Protocol 3, where RER and FFA concentrations were not different between women and men (6), emphasize the importance of knowing RER when attempting to interpret group differences in FFA concentrations. The negative relationship between RER and FFA even within individuals fed standardized diets over 14 days (6) suggests that relatively small variations in daily energy balance contribute a substantial amount to variability in overnight postabsorptive fatty acid oxidation (RER) and plasma FFA concentrations.

Although the post hoc analysis of data, such as we have included in this report (6, 7), is often a cause for concern (21), we included additional data from new studies conducted in our laboratory to minimize the likelihood of a Type 1 statistical error. The validity of the statement about intraindividual relationship between palmitate concentration and RER (Protocol 3) is limited by the fact that for only 5 out of 50 volunteers was the slope significantly different from zero. Nevertheless, when only four data points per subject are available, $R^2$ over 0.90 is required to prove nonzero slope. The median power of intraindividual regression analyses 0.09 (0; 0.75) confirms the low informative value of negative result. In the context of the whole group, other characteristics of these individual analyses become more important. The consistently high degree of mutual determination between palmitate concentrations and the postabsorptive RER intraindividually as well as similar direction of individual regression lines should not be discounted. These two characteristics would be unlikely to occur in the majority of Protocol 3 subjects merely by chance.

The practical implications of our findings are that despite strict standardization of nutrition and daily regimens for research volunteers, obtaining unambiguous results in the studies of FFA metabolism and substrate oxidation in humans can be challenging. Although one might question whether a controlled diet is a useful precaution for studies of FFA metabolism, the ~15% CV in fasting plasma palmitate concentrations we observed following dietary control (6) is less than half that which we observed when no attempts were made at dietary control (17).

TABLE 3. Subject characteristics by RER tertiles

| | RER | | |
|---|---|---|---|
| | Low n = 54 | Mid n = 54 | High n = 53 |
| RER | 0.76 ± 0.03 | 0.81 ± 0.01 | 0.85 ± 0.02 |
| Age (years) | 29 ± 8 | 25 ± 5 | 26 ± 7 |
| Weight (kg) | 72.3 ± 14.4 | 72.6 ± 14.4 | 73.6 ± 16.6 |
| BMI (kg/m$^2$) | 24.0 ± 3.7 | 25.2 ± 4.2 | 23.8 ± 3.9 |
| Fat (%) | 27 ± 10 | 28 ± 10 | 27 ± 10 |
| Fat fee mass (kg) | 51.9 ± 11.8 | 51.3 ± 13.0 | 52.3 ± 13.1 |
| Body fat (kg) | 19.3 ± 9.5 | 19.5 ± 8.7 | 19.4 ± 9.1 |
| Abdominal subcutaneous fat (cm$^2$) | 123 ± 72 | 132 ± 91 | 137 ± 95 |
| Visceral fat (cm$^2$) | 59 ± 54 | 56 ± 43 | 63 ± 45 |
| Leg fat (kg) | 6.4 ± 1.9 | 7.3 ± 3.1 | 6.6 ± 2.8 |
| Plasma triglycerides (mg/dl) | 107 ± 72 | 109 ± 48 | 121 ± 80 |

Low, mid, and high refer to tertiles of RER. There were no statistically significant differences in any of the parameters between those in the three different RER tertiles.

In summary, we found that despite providing to our volunteers a standardized diet prepared in a metabolic kitchen, there was a wide variation in RER that was inversely related to plasma FFA concentrations both between and within subjects during repeated measurements. Mild fluctuations in energy balance were the most likely driving mechanism of this variability. On the basis of these results, we suggest that including high quality indirect calorimetry as part of experiments specifically designed to study FFA metabolism will be of benefit, even if significant efforts are made to minimize difference in energy balance.

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