

RESTING METABOLIC RATE DATA HANDLING RULES

Resting metabolic rate (RMR) is the primary outcome variable in CALERIE 2. RMR is measured using the Vista-MX/REE (Vacumed, Ventura CA) metabolic cart at six time points throughout the two-year intervention (Baseline 2A, Baseline 2B, Month 6, Month 12, Month 18 and Month 24) for the CR group and four time points (Baseline 2A, Baseline 2B, Month 12, and Month 24) in the control group. Stringent quality control testing was performed on each cart during the study to evaluate analyzer drift during a RMR test and long-term drift across the study. A small working group of study investigators versed in human energy metabolism expertise have reviewed the QC data and determined the data handling rules for RMR in CALERIE. The rules and supporting documentation are provided here. Six metabolic carts are in use across the multicenter study and data handling rules have been established by.

1) Raw data clean up

Resting metabolic rate data is collected from the metabolic cart each minute. During the test, trained research associates document any technical problems which may have occurred. The RMR test is 30 minutes in duration and the last 20 minutes of the test are used to calculate resting metabolic rate (kcal/day).

Initial clean up of RMR data occurs at the site by the research associates. Each minute of data is identified by a numerical identifier (flag). The following identifiers are used in CALERIE 2:

- 1= Discard data
- 2= CO₂ dilution data
- 3= Cal gas 1 data
- 4= Cal gas 2 data
- 5= Usable data

The first 10 minutes of participant data (minutes 5-14) are discarded as per the study protocol. Therefore each 60-second epoch in the first 10 minutes of the test are flagged with a "1". Minutes of data that correspond to the CO₂ dilution test or instrument calibrations are flagged as 2 through 4 accordingly. Only those data identified as usable '5' are used in analysis. The RMR test should be performed without any sneezing, coughing, talking, yawning and most importantly without sleeping or moving. Minutes of data that pertain to instrument error, operator error or subject will be identified as '1' and not included in the analysis. The accuracy of the identifiers used for each RMR test has been audited at DCRI by Lucy Piner, RMR Research Core Laboratory Coordinator under the direction of William E. Kraus, MD at Duke University.

2) Criteria for RMR test inclusion in statistical analyses

a. Usable minutes

For a resting metabolic rate test to be valid for use in statistical analysis, it must contain a minimum of 10 usable minutes of data. These minutes do not need to be continuous. RMR tests with fewer than 10 usable minutes will be omitted from statistical analyses.

b. RQ values

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During a valid resting metabolic rate test, the mean respiratory quotient (RQ) calculated from all usable minutes of data should fall within the range 0.65-1.05, inclusive. The test respiratory quotient = $\text{sum VCO}_2 / \text{sum VO}_2$ across all usable minutes of data. In cases where the test RQ is <0.65 or ≥ 1.05 , the test will be omitted from statistical analyses.

c. EE Values

During a valid resting metabolic rate test, energy expenditure (EE, kcals/day) should fall within 800-4000 kcal/day, inclusive. Test EE is calculated as the mean EE for all usable minutes of data. In cases where the test EE is <800 or $>4,000$, the test will be omitted from statistical analyses.

3) Handling of analyzer drifts

a. Individual Tests

Drifts in the $\%O_2$ and $\%CO_2$ measured by the analyzers may occur after pre-test calibrations are run. To evaluate analyzer drifts, each calibration gas is passed through the analyzer post-test for 4 minutes each without recalibration of the analyzers. The results collected during the last 3 minutes are used to evaluate analyzer drift. Analyzer drifts have been examined in all metabolic carts used in CALERIE 2 (see appendix). First, to determine if calibration drifts are affecting the individual results (RQ and RMR) we investigated the relationship between the analyzer drifts and measured physiological energy metabolism values (RQ from the individual tests and the $\%RMR$ deviates from the Harris-Benedict predicted value). RQ tells us about the fasting CHO/fat oxidation. $\% \text{ Harris Benedict}$ “tells us” whether a RMR is on the high or low range for a given sex, age and weight.

Second, to account for potential effects of analyzer drifts on the measured VO_2 and VCO_2 throughout a single resting metabolic rate test, we adjusted the data according to differences in the analyzer measurement of the calibration gas (span gas) measured before and after each test. Adjustments were made for each analyzer:

i. Adjustment of VO_2 data

Multiply mean VO_2 during a given test by $(\text{pre-test } O_2 \text{ delta span} / \text{post-test } O_2 \text{ delta span})$

ii. Adjustment of VCO_2 data

Multiply mean VCO_2 by $[\text{pre-tests } CO_2 \text{ span} / (\text{average pre-test, post-test } CO_2 \text{ spans})]$

Third, we compared the unadjusted or original RMR and RQ data collected in all subjects at baseline to values that were adjusted for O_2 and CO_2 analyzer drifts according to the methods above.

Recommendation: VO_2 and VCO_2 data measured directly by the Vista-MX/REE metabolic cart will be used in all analyses. No data will be recalculated or adjusted to account for possible drifts in the O_2 and CO_2 analyzers across the 30 minute RMR test.

Rationale:

- Adjustment of the VO_2 and VCO_2 data for analyzer drifts introduced more inherent variability in both RMR and RQ data.

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- Appropriate quality control testing (post-test gas calibration checks) was not performed in all tests and therefore analyzer drift adjustment cannot be uniformly performed on all tests in the study.

b. Long-term analyzer drift

Examination of the change in span gas (post-test minus pre-test) from the individual tests performed on the primary cart at each study site over time provides some indication of analyzer stability. The O₂ analyzers appear stable as do the CO₂ analyzers on Carts 4 and 6. We noted however a possible deterioration of the CO₂ analyzer on Cart 16 at PBRC. Ethanol combustion tests were performed every other week on the primary metabolic cart in an effort to review long-term analyzer performance. The set-up of the Vista-MX/REE metabolic cart does not allow for ethanol combustion to be accurately measured. We have insufficient QC data to evaluate analyzer drift over time.

Recommendation: No adjustment of RMR data should be made on the basis of possible analyzer drift over time.

Rationale:

- Combustion of ethanol is the gold standard methodology for evaluating performance of O₂ and CO₂ analyzers. The set-up of the Vista-MX/REE metabolic cart does not allow for ethanol combustion to be accurately measured.
- Insufficient QC data are available to evaluate analyzer drift over time.