19. QUALITY CONTROL ACTIVITIES

19.1 Introduction

The Manual of Procedures describes methods that are applied to implement the CALERIE Phase 2 protocol. It is detailed and specific, and outlines activities that must be applied for each of the evaluation procedures. Deviation in how procedures are conducted can lead to bias if systematic differences are observed. For example, if there is a downward trend in an outcome over time, and one site is perpetually late in performing this procedure, there will be a systematic deviation in the time effect at that site and therefore overall. Even if there is no bias, straying from study procedures can add variability in the outcome measures, thereby robbing the study of power to observe significant between-group differences.

In this section, we describe activities to be conducted in CALERIE to safeguard the fidelity and integrity of the CALERIE Phase 2 study. We describe committee membership and the scope of its activities, its *modus operandi*, and outline reports, analyses and summaries covering all evaluation components that are presented to the committee.

19.2 Scope of the Committee

This committee evaluates the fidelity with which the CALERIE Manual of Procedures (MoP) is being implemented. It scrutinizes adherence to these procedures, and evaluates consistency across staff, CALERIE sites and over time. Specifically, the following components come under scrutiny – the MoP section in which the procedure is described is provided in parentheses:

- Schedule of baseline and follow-up procedures (Ch. 6 & 10)
- Vital signs including pulse, temperature, respirations, systolic and diastolic pressure (Ch. 11.2)
- In-clinic anthropomorphic measures including height, in-clinic weight, natural and umbilical waist circumferences (Ch. 11.3)
- Metabolic procedures including core temperature and RMR (Ch. 11.4)
- 6-day food records (Ch. 11.5 & 21)
- DXA procedures (Ch. 11.6 & 19)
- Physical activity measures including VO_2 max, muscle strength and endurance, and the 7-day PAR (Ch. 11.7)
- DTH testing (Ch. 11.9)
- QoL and cognitive function including the BDI, MAEDS, Derogatis questionnaire and cognitive bias and CANTAB batteries (Ch. 11.10 & 11.11)
- Doubly labeled water procedures (Ch. 12 & 16)
- Blood draw and analysis at central biochemistry lab, plus the blood and urine repository samples (Ch. 13 & 17)
- Biopsy sample collection (Ch. 15)

Additionally, all protocol deviations reported on the Protocol Deviation case report form, such as eligibility violations and randomization errors, are reviewed by the Committee.

Routine reports provided to the Steering Committee (e.g., recruitment and retention summaries) as well as reports bearing on the safety of study participants are not dis-

cussed in this MoP chapter. Moreover, the fidelity in delivering the intervention is not discussed here, but is rather incorporated in the Intervention Manual.

19.3 Committee Membership

To provide effective oversight of all these activities, the QC committee is composed of investigators whose collective expertise covers the entire range of evaluation procedures outlined above. Investigators with broad expertise in a number of procedures are especially important. Moreover, there must be good representation from the following CALERIE constituents:

- The 3 CALERIE sites
- External labs and reading centers
- CALERIE Coordinating Center
- The NIA Project Office.

The exact committee membership is reviewed and approved by the CALERIE Steering Committee. The Chair is appointed by the Steering Committee at the same time.

19.4 Modus Operandi

The QC committee meets by teleconference at least once a month and optionally at the semi-annual CALERIE meetings. The list of procedures and activities reviewed by the committee on any call is rotated so that approximately one-third of the procedures is reviewed every month. This implies that a study procedure is reviewed once every quarter. Study procedures are rotated as follows:

Month "A" Procedures:

- Doubly labeled water procedures
- Metabolic procedures (core temperature, RMR)
- Vital signs (pulse, temperature, respirations, BP)
- In-clinic anthropomorphic measures (weight, waist circumference)

Month "B" Procedures:

- Schedule of baseline and follow-up procedures
- Summary of protocol deviations
- Blood draw and analysis at central biochemistry lab (including OGTT)
- Biopsy sample collection
- VO_2 max
- Physical activity measures (handgrip strength, knee extension, PAR)

Month "C" Procedures:

- DXA procedures
- 6-day food records
- DTH testing
- QoL and cognitive function

19.5 Roles and Responsibilities

Coordinating Center:

- Prepare summary reports as appropriate for review by the QC Committee including any reports arising from site monitoring activities.
- Provide the corresponding reports to the procedure's "champion" at least two weeks prior to the conference call. Provide final reports to the QC Committee at least one week prior to committee conference call.
- Participate in the conference calls and describe the reports and provide expert evaluation of their significance.

External Reading Center or Central Laboratory:

- Prepare summary reports as appropriate for review by the QC Committee.
- At least one week prior to committee conference call, provide the requisite reports to the QC Committee for review.
- Participate in the conference calls and describe the reports and provide expert evaluation of their significance.

CALERIE Clinical Sites:

- Prepare summary reports as appropriate for review by the QC Committee.
- At least one week prior to committee conference call, provide the requisite reports to the QC Committee for review.
- Participate in the conference calls and summarize their significance.

QC Committee:

- Review the reports and determine whether there are concerns about the fidelity and integrity of the evaluation procedures, and whether there is consistency across staff, CALERIE sites, between treatment arms and over time.
- Determine whether corrective action is necessary.
- Advise the CALERIE Steering Committee whether the study procedures are being conducted according to the CALERIE Phase 2 MoP, and provide recommendations on corrective action, if necessary.

19.6 Monthly Activity Report from Central Labs and Reading Centers

In what follows, "sample" is used generically to mean a set of urine vials for a DLW study; an electronic file with the DXA scan; for the Vermont biochemistry lab, a blood sample, urine sample and the different biopsy materials treated separately; or a dietary record for the Nutrition Reading Center.

A progress report is provided by personnel at the central lab or reading center to the CC at the beginning of every calendar month. It describes activity in the lab during the previous calendar month. This information is compiled by the CC and summarized for the QC committee. They are presented at "Month A", "Month B" or "Month C" according to the month in which the corresponding lab is discussed.

- Count of the number of subjects with new samples / records that have been forwarded from the sites this month, excluding replacement samples or revisions of old records.

- Count of the number of subjects whose samples have completed processing in the lab this month, including samples received this month as well as any samples which had been backlogged from previous months.

  Note that for DLW, DXA and food records, **pairs** of evaluations are performed during the baseline period (a pair of DXAs is performed at Month 6 in the CR group). To be comparable across all labs, a subject will only be considered "complete" when **both** samples have been processed. If one sample is processed, but the second is pending, then the participant is considered "backlogged" until **both** samples are completed.

For these two summaries, cumulative numbers, aggregated over calendar time, is compiled by the CC and plotted over time.

- Count of the number of subjects whose samples have not completed processing and are backlogged to the next month, including samples received this month as well as any samples backlogged from previous months.
- Of this count, the number of subjects whose processing has not been completed due to errors, problems and queries. This excludes subjects whose samples / records have not started processing or whose processing is proceeding normally.

These two summaries are not aggregated over calendar time. Rather the numbers reported in that calendar month are plotted over time. This provides the size the backlog existing in the lab / reading center at that point in calendar time, plus the number due to errors and problems.

19.7 Summary of Electronic Records Transferred from the Central Reading Centers to the CC

External reading centers include: (1) DLW lab; (2) DXA Reading Center; (3) Vermont laboratory (including repository materials); (4) Esoterix safety lab; and, (5) Nutrition reading center. "Record" is used generically to the results from an analysis conducted at the center. Reports are presented at "Month A", "Month B" or "Month C" according to the month in which the corresponding lab is discussed.

- Summary tables or plots, by site, treatment arm and protocol time point of the number of samples collected from study participants (derived from the corresponding CRF page).
- Summary tables or plots, by site, treatment arm and protocol time point, of the number of electronic records received at the CC from the laboratory (derived from the electronic data files transferred to the CC).

The following analysis is only performed if the lab does not "batch" the analysis.

- Summary tables or plots, by site, treatment arm and protocol time point, of the composite lag time from the sample collected at the site until the electronic record received at the CC.

19.8 Protocol Deviations

The following analyses are performed by the CC and reported on the "Month B" calls:

- Listing of all protocol deviations captured on the Protocol Deviation CRF page, including site, treatment arm and protocol time point, showing type and any text explanation. If there are too many, plots or summary tables are generated.

19.9 Schedule of Baseline and Follow-up Procedures

19.9.1 Variations in Clinic Visit Timings and Related Procedures

The following analyses are performed by the CC and reported on the "Month B" calls:

- Summary tables or plots, by site, of the lag time from the date of screening visit #1 to the date the baseline informed consent was signed (i.e., the elapsed time from starting screening to starting baseline visits).
- Summary tables or plots, by site for CR group (only), of the lag time from the date of randomization to "Day 1" of the intervention. This is a measure of the delay in getting started with the CR intervention.
- Summary tables or plots, by site, treatment arm and protocol time point, of the elapsed time from "Day 0" to the date of the first study procedure was performed at that protocol time point. This is a measure of whether follow-up visits are performed promptly at 3, 6, 9, … months from "Day 0".
- Summary tables or plots, by site, treatment arm and protocol time point, of the elapsed time from the first study procedure to the last study procedure at that protocol time point including baseline. This is a measure of how long it takes to complete the full set of procedures at that protocol time point.

In what follows, "most important study procedures" include the following:

- DXA (beginning of first DLW dose)
- DLW (first DLW dosing)
- Isometric / Isokinetic Knee Extension
- Clinic Weight (at Visit 1 only)
- VO_2 max
- 6-day food record
- Core temperature
- DTH date of injection
- Outcomes blood draw
- RMR
- BDI, MAEDS, Derogatis Interview
- Cognitive Bias, CANTAB Software
- Biopsy sample collection

The corresponding reports are provided by the CC on the QC call on which that procedure is scheduled for review, i.e., "Month A", "Month B", or "Month C",

- For the most important study procedures, summary tables or plots, by site, treatment arm and protocol time point, of the percentage of times the procedure was not performed (+ reasons why it was not performed).
- For the most important study procedures, summary tables or plots, by site, treatment arm and protocol time point, of the elapsed time in performing that procedure from "Day 0" to that protocol time point with a reference line at the appropriate length. This is a check that procedures being performed promptly relative to the timings in the protocol.

19.9.2 Deviations in the Timings of Study Procedures

The following analyses are performed by the CC and reported on the "Month B" calls:

- Summary tables or plots, by staff initials within site, and protocol time point, of the elapsed time from the last meal to the time of blood draw in the safety lab blood draw. Participant should be fasting at least 8 hours.
- Summary tables or plots, by staff initials within site, and protocol time point, of the elapsed time from the last meal to the time of blood draw in the outcomes lab blood draw. Participant should be fasting at least 8 hours.

- Summary tables or plots, by staff initials within site, and protocol time point, of the elapsed time from the date of the DLW dosing to the date of the Day 7 urine sample; similarly, for the elapsed time from the date of the DLW dosing to the date of the Day 14 urine sample. They should be exactly 7 and 14 days respectively. If there are a small number of deviations, then the percent of DLW studies in which the elapsed times were not equal to 7 and 14 days respectively can be reported.
- Summary tables or plots, by site, and protocol time point, of the difference between the date of the DLW dosing and the date of the first DXA scan at BL1, M6, M12, M18 and M24; and the date of Day 14 urine sample collection and the date of the second DXA scan at BL2 and M6 (for CR only). The first DXA scan should be performed on the dosing day, the second DXA (when performed) should be performed on Day 14. If there are a small number of deviations, then the percent of DXA studies not performed on the corresponding DLW date can be reported.
- Summary tables or plots, by site and protocol time point, of the difference between the Day 14 date of the DLW urine collection and the date the RMR was performed. The RMR should be performed on Day 14 of the DLW study. During baseline, this is Day 14 of the second DLW study. If there are a small number of deviations, then the percent of RMR studies not performed on Day 14 of the DLW urine collection can be reported.
- Summary tables or plots, by site and protocol time point, of the difference between the Day 7 date of the DLW urine collection and the date the first Stanford PAR was performed. If there are a small number of deviations, then the percent of Stanford recalls not performed on Day 7 of the DLW urine collection can be reported. Similarly for the difference between the Day 14 date of the DLW urine collection and the date the second Stanford PAR was performed.
- Summary tables or plots, by site and protocol time point, of number of 6-day food records for which the record quality corresponding to DLW Days 1-6 was coded as "missing". This would signify that food was not recorded on at least one of DLW Days 1-6 as specified in the MoP.
- Summary tables or plots, by site and protocol time point, of the percentage of participants for whom one or more home weights is missing. According to the MoP, home weights are required on Days 1-28 (not Day 0) during the two back-to-back baseline DLW studies. Post-randomization, CRs record weight from Day 0-14 of any DLW period; controls record their weight from Day 1-14. (They pick up the home scale on Day 0). If any of these home weights is missing, it signifies a protocol deviation.
- For women, summary tables or plots, by site and protocol time point, of the day in the menstrual cycle when the first blood draw for sex hormones was performed. According to the protocol, it should be performed on Days 19-21 (inclusive). Similarly for the day in the menstrual cycle when the last blood draw for sex hormones was performed.

19.10 Vital Signs and In-Clinic Anthropomorphic Measures

19.10.1 Summary of Reports Prepared by the Coordinating Center

Reports are prepared for the following data:

- Clinic weight (all replicates)
- Natural waist measurement (all replicates)
- Umbilical point waist measurement (all replicates)

- Pulse, temperature, respiration
- Systolic and diastolic pressures (all replicates)

The following analyses are performed by the CC and reported on the “Month A” calls:

- Summary tables or plots, by site, staff initials and calendar time (quarters), checking for digit preferences. This the last digit recorded in the data field. All the values recorded (including the replicates) in that quarter are pooled together. This could be presented as a bar chart with each bar partitioned into the 10 digits according to the percentages (the height of each bar is 100%). We can then observe whether the digits “0” and “5” or even numbers, for example, are over-represented.
- For clinic weight, natural waist measurement, umbilical point waist measurement and systolic and diastolic pressures, summary tables or plots, by site, staff initials and calendar time (quarters), of the maximum difference across the first two replicates, with a reference line at “0”. If there are few discrepancies, this could be presented as the percentage in which there is any discrepancy.

19.10.2 Summary of Reports Prepared by Site Monitoring

- The monitor reviews the ongoing calibration logs for the stadiometer, metabolic scale, tape measure, mercury sphygmomanometer, and those the sites are maintaining for calibration of the home scales at BL, 6 ,12, 18 and 24 months.

19.11 Metabolic Procedures

19.11.1 Summary of Reports Prepared by the Coordinating Center

The following analyses are performed by the CC and reported on the “Month A” calls:

- Core Temperature:
- The MoP indicates that only those temperatures from 8 pm on the day of administration to 8 pm on the following day are used in the analysis. All minute-by-minute temperatures during the 60 minutes after ingesting the pill should be discarded as unusable.
- Summary tables or plots, by staff initials within site, and protocol time point, of the number of non-missing, usable, minute-by-minute temperatures in this time interval with a reference line at 1,440 minutes (= 24 hours).
- The MoP indicates that core temperature recordings should be started no later than 7 pm and ended no earlier than 8 pm the following evening. Listing of all occasions when the recordings were not started or stopped by the appropriate times.
- RMR:
- This is the other primary outcome in CALERIE. The MoP indicates that RMR is conducted over a period of 30 minutes. Summary tables or plots, by staff initials within site, and protocol time point, of the number of non-missing, minute-by-minute records with a reference line at 30 minutes.
- VO_2 max:

The following analyses are performed by the CC and reported on the “Month B” calls:

- Tabulate the reasons for termination of testing (Q5) and the percentage of procedures not meeting at least two of the three VO_2 max criteria (Q8).

19.11.2 Summary of Reports Prepared by the CALERIE Sites

Additionally, there are three procedures that are conducted periodically during the study. They include:

- Daily calibration checks run after each participant test
- Alcohol consumption test run every two weeks
- O_2 baseline test run every two weeks.
- These procedures are described in detail in a separate document.

19.11.3 Summary of Reports Prepared by Site Monitoring

- RMR:
- The site RMR Calibration Log is reviewed at periodic monitoring visits to verify that gas analysis calibration has been completed according to the manufacturer's instructions before and after each test. Information obtained from review of the log is reported in the periodic monitoring visit report.
- VO_2 max:
- The site VO_2 max Log maintained by each site is reviewed at periodic monitoring visits to verify that gas analysis calibration has been completed before and after each test as described in MoP section 11.7.1. Information obtained from review of the log is reported in the periodic monitoring visit report.

19.12 6-day Food Records

19.12.1 Summary of Reports Prepared by the Nutrition Reading Center

Summary by clinical site of the following information

- No. of food records sent by the sites
- No. of queries sent to the site
- No. of NCC questions sent
- No. records completed
- Average time to completion

19.13 DXA Procedures

19.13.1 Summary of Reports Prepared by the Coordinating Center

There are two DXA scans performed at baseline (i.e., at the beginning of the first DLW period and at the end of the second DLW period), as well as two DXA scans at Month 6 (i.e., at the beginning and end of the DLW period) in the CR group (only).

- Summary tables or plots, by site, and protocol time point (baseline and Month 6), of the absolute change from the first DXA scan to the second DXA scan, restricted to whole body FM, FFM, and total mass.

19.13.2 Summary of Reports Prepared by the DXA Reading Center

The DXA QA Center provides regular reports to the CALERIE study regarding the status of the DXA participant scans and performance of the scanners. Reports include the following.

1. Training and Certification of DXA Operators:
    a. On site training (after completion of 3 sites)

b. Certification status (6 months; updated in end of visit reports – see #5)

2. Monitoring of Participant Scans (Every 2 months during BL visit; updated in end of visit reports after BL):
    a. # completed scans
    b. # unacceptable scans
    c. Feedback provided to each operator on scan acquisition
3. Cross Calibration (BL)
4. Precision Assessment of DXA Operators (BL)
5. End of Visit Reports (BL, 12-mo, 24-mo):
    a. Participant scans - # completed, # unacceptable
    b. DXA operator certification and performance
    c. Longitudinal scanner performance (spine and WB phantoms)
    d. Scanner maintenance and repair records
    e. 6-mo visit will be included in the 12-mo report; 18-mo visit will be included in the 24-mo report.

19.13.3 Summary of Reports Prepared by Site Monitoring

- DXA records maintained by the site is reviewed periodically for completeness. Information obtained from review is reported in the periodic monitoring visit report.

19.14 Physical Activity Measures

19.14.1 Summary of Reports Prepared by the CALERIE Sites

- Handgrip dynamometer calibration: This is to be performed monthly by the same person.
- Cybex/Biodex calibration: This is to be performed monthly.
- TM calibration: To be performed yearly.
- Report on the assignments of any new personnel to the study and training in these items (7-day PAR, Strength and Endurance testing, Peak VO_2 testing).

19.14.2 Summary of Reports Prepared by the Coordinating Center

The following analyses are performed by the CC and reported on the "Month B" calls:

Handgrip Strength:

- The MoP indicates that, "prior to each test, the dynamometer is inspected to be sure that the force gauge indicates zero when the dynamometer is not being squeezed. The 'zero' force value is recorded on the data form for each test." Reports any tests in which the "zero-meter check" is not checked.
- The MoP also indicates to, "… compare the two highest force values for each hand. If the two force values for a given hand are discrepant by more than15%, this suggests that (a) a force values was misread/misreported, (b) the subject did not give uniform effort with each trail, or (c) the instrument gave a spurious reading secondary to being bumped or jerked during the test. Report any tests in which in which the discrepancy between the two highest force values for each hand is >15% (i.e., is not within 15% of the lower value).

Knee Extension:

The MoP indicates the following:

- In the 60°/sec assessments, the average peak torque from the first two repetitions should always be greater than the average peak torque from the last two repetitions.
- The fatigue index values from the 180°/sec tests should always be positive.
- For each of the isometric knee extension and flexion assessments, the two greatest peak torque values should be discrepant by ≤ 10 n-m.

Report any tests in which these criteria were not satisfied.

Stanford 7-day PAR:

- The questionnaire includes questions on whether there were problems with the interview or it wasn't a valid interview (Q.5 and Q.6).

Report any tests in which these questions were checked "yes".

19.14.3 Summary of Reports Prepared by Site Monitoring

Handgrip Strength:

The site Dynamometer log is reviewed at periodic monitoring visits to verify that the Jamar Hydraulic hand dynamometer is calibrated every 3 months as described in MoP section 11.7.2.2. Information obtained from review of the log is reported in the periodic monitoring visit report.

Knee Extension:

The site Dynamometer Log is reviewed at periodic monitoring visits to verify that the dynamometer is calibrated monthly as described in MoP section 11.7.2.2. Information obtained from review of the log is reported in the periodic monitoring visit report.

19.15 Quality of Life Instruments

19.15.1 Summary of Reports Prepared by the Coordinating Center

- Listing by questionnaire, site, treatment arm, ID number and protocol time point of all occasions in which there was at least one missing item in that questionnaire.

19.16 CANTAB Software

19.16.1 Summary of Reports Prepared by the Coordinating Center

- Listing by site, treatment arm, ID number and protocol time point of all occasions in which there was at least one missing item in the battery.
- Box and whisker plots, by site and treatment arm, of the following variables at baseline:
  - MOT Mean Error
  - RTI Five choice accuracy score
  - VRM Recognition total correct – delayed
  - DMS Percent correct (all delays)
  - SWM Total errors
  - RVP Total hits
  - IED Completed stage trials

19.16.2 Summary of Reports Prepared by the CALERIE Sites

- Each site will perform a test run of the CANTAB for each time point (Baseline, and Months 6, 12, and 24) prior to running any subjects at that time point. The resulting test report will be sent (either by email or fax) to Tammy Scott for review. This is to ensure that the testing batteries and data report templates have been set up correctly.

19.17 Cognitive Functioning

19.17.1 Summary of Reports Prepared by the CALERIE Sites

As described in the MoP the following check is performed

- The Site Measurement Leader will submit a QC report to the DCRI every **3** months documenting that the QC procedures for the cognitive testing component outlined in the MoP are being followed.

19.18 Doubly Labeled Water Procedures

19.18.1 Summary of Reports Prepared by the Coordinating Center

The following analyses are performed by the CC and reported on the "Month A" calls:

- Verify the correct DLW dose. Take the participant's weight on the visit corresponding to the DLW dosing, and multiply by 1.5 gm/kg. Take the DLW dose weight given to the participant (Q.3) and subtract this amount. Summary tables or plots, by staff initials within site, and protocol time point, of this difference with reference lines at 0 and +5 gm (the maximum allowed in the MoP).

  Note that this amount was changed to 1.75 gm/kg on July 16, 2007. It does not apply to the second baseline DLW for any subject whose first baseline DLW was before that date. Only to pairs of DLWs at baseline and all post-randomization DLWs after that date.

- Using both DLW studies at baseline, plot the TEE vs. the clinic weight, distinguishing between the three clinical sites. Include a trend line for each site together with its R^2 value.

- Using the first DLW study at baseline, perform the following regression analysis: TEE is the *y*-variable; predictors include FM and FFM from the DXA performed prior to the DLW study, and the participant's gender and clinical site. Generate the ANOVA table together with the R^2 value.

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19.18.2 Summary of Reports Prepared by the DLW Lab

Two types of reports will be prepared by the Central DLW Lab: Progress Report and Quality Control Report.

The Progress Report will be submitted to the CC at monthly interval. The Progress Report will provide the number of DLW studies received at the lab; the number of DLW studies for which the analyses were processed in the lab; the number of processed DLW studies with no errors, problems or queries; and the number of processed DLW studies with at least one error, problem, query or other follow-up required with the sites.

The Quality Control Report will be submitted to the Quality Control Committee at quarterly interval. This quality control procedure is based on dose dilution measurements over the course of the phase 2 study and is specifically designed for the CALERIE phase 2 clinical trial in order to monitor the variability of the isotope ratio measurements

over time. Sufficient amount of the dose dilutions and the water used in the preparation of the dose dilutions have been saved so that the same dose dilutions and water could be used during the duration of the CALERIE phase 2 clinical trial. The quality control procedure is described as follows:

- The dose dilutions (~1:400 and ~1:1,500) and the water used in preparing the dose dilutions will be analyzed daily in duplicate over a period of 10 days.
- The logarithmic transformed isotopic enrichments of these measurements will be used to generate the hypothetical constants for the conversion of these enrichment values to produce theoretical fractional turnover rates of ^{2}H (k_H, 0.1) and ^{18}O (k_O, 0.13) or a difference in fractional turnover rates ($k_O - k_H$) of 0.03.
- The monthly average measurements of these same dose dilutions and water will be converted to k_H and k_O using the hypothetical constants.
- The differences in the fractional turnover rates ($k_O - k_H$) generated from the subsequent dose dilution measurements will be compared to the theoretical difference of 0.03.
- The comparison will be presented graphically with percentage difference from 0.03 on the y-axis and date of analysis on the x-axis.

19.19 Central Biochemistry Lab

19.19.1 Summary of Reports Prepared by the Coordinating Center

OGTT:

The MoP indicates the following:

- The OGTT should not be performed less than 96 hours (4 days) after the maximal treadmill exercise test. Take the date and time of the blood collection for outcomes minus the date and time of the VO_2 max test and convert to hours.

Summary tables or plots, by staff initials (of outcome blood collection) within site, and protocol time point, of this difference with a reference line at 96 hours (the minimum allowed in the MoP).

19.19.2 Summary of Reports Prepared by the Vermont Biochemistry Lab

The Vermont Biochemistry labs provides regular reports to the CALERIE study regarding the status of the blood samples and 24-hour urine samples collected from study participants. Reports include the following.

1. Baseline, 12-month and 24-month blood draws
   - No. of shipments received
   - No. of shipments received thawed
   - No. of sample sets received
   - Phlebotomy and Processing – counts of the no. of protocol deviations for the hot box, fasting blood sample, OGTT (30, 60, 90, and 120 minutes), and 24-hour urine sample:
     i) Missing or incomplete forms
     ii) Collection tubes filled

iii) >5 mins elapsed time between tubes (for Catecholamines)
iv) Blood collection timing exceeds acceptable limits
v) Number of tubes > 30 min plasma processing time
vi) Number of tubes > 40 min serum processing time
vii) Missing aliquots or tubes upon receipt
viii) Aliquots not frozen upright
ix) < 24 Hr urine collection period
x) > 24 Hr urine collection period

- Shipping and packaging:
  i) Notification and delivery on time
  ii) Shipping container damaged or improperly labeled
  iii) Insufficient dry ice
  iv) Samples thawed
  v) Not packaged according to IATA regulations
  vi) Aliquots incorrectly organized

2. Female Sex Hormones
   - No. of shipments received
   - No. of shipments received thawed
   - No. of female sex hormone kits received
   - Phlebotomy and Processing – counts of the no. of protocol deviations for off-cycle visit Day 1, off-cycle visit Day 2 and Day 2 collection:
     i) Missing or incomplete forms
     ii) Collection tubes filled
     iii) Blood collection time exceeds acceptable limits
     iv) Blood collection timing exceeds acceptable limits
     v) Number of tubes > 40 min serum processing time
     vi) Missing aliquots or tubes upon receipt
     vii) Aliquots not frozen upright
3. 3-month, 6-month and 18-month blood collection
   - No. of shipments received
   - No. of shipments received thawed
   - No. of sample sets received
   - Phlebotomy and Processing – counts of the no. of protocol deviations:
     i) Missing or incomplete forms
     ii) Number of tubes > 30 min plasma processing time
     iii) Number of tubes > 40 min serum processing time
     iv) Missing aliquots or tubes upon receipt
     v) Aliquots not frozen upright
4. 17-month, 23-month and unscheduled blood collection
   - No. of shipments received
   - No. of shipments received thawed

- No. of sample sets received
- Phlebotomy and Processing – counts of the no. of protocol deviations:
  i) Missing or incomplete forms
  ii) Number of tubes > 40 min serum processing time
  iii) Missing aliquots or tubes upon receipt
  iv) Aliquots not frozen upright

19.20 Computer Checks on CALERIE Data

In what follows, "most important study outcome variables" include the following:

• RMR (both values at baseline) • Core temperature • VO_2max	• Avg. diameter for ea. DTH antigen • TEE via DLW (both values at baseline & Mo. 6) • DXA: FM, FFM, %BF, BMD • 6-day food record: Avg. daily calories
• Serum triiodothyronine (T3) • Total & LDL cholesterol • CRP, TNF-α, IL6 • Sex hormones: LH, FSH, total testosterone (men), estradiol (women) • Serum insulin and glucose • Bone turnover: serum CTX	• Cognitive impairment: verbal recognition memory score • CANTAB: Variables in §19.16.1 • SF-36 – overall score + 8 subscales • Pittsburgh Sleep Index – overall only • Derogatis – overall only • Food Craving Inventory – overall only • Eating Inventory – three subscales • Weight Self-Efficacy – overall only
• EE from 7-day PAR • Mean handgrip strength – left & right • Knee extension / flexion: Muscle fatigue index	
• Clinic weight (avg'd. over replicates) • BMI • SBP & DBP (avg'd. over replicates)	• MAEDS – all domains • Body Shape Q're – overall only

19.20.1 Computer Checks for Outliers and Peculiar Values

In addition to the usual range and consistency checks performed by the Data Management group at the DCRI, a set of statistical analyses are performed to look for improbable and peculiar values. For each of the most important study outcome variables, the following check is performed.

- Box and whisker plots, by site, treatment arm and protocol time point, of the distribution of the variable. Outliers and peculiar values will be sent a query to verify the value.
- Because these checks are applied to outcomes post-randomization, they will not be reviewed by the QC committee. Rather, they will be reviewed as part of the internal QC process at the Coordinating Center. Any improbable or peculiar values will be queried using the usual procedures.
- To minimize the number of people unmasked by the results, the following safeguards will be applied by the CC statistician.
- The mean of the outcome variable in each treatment group at each site will be derived at the baseline visit.

- The distribution of the outcome variable at each protocol time point will be standardized to the baseline mean in that treatment group at that site. That is, the mean change from baseline in that treatment group at that site to the follow-up point is subtracted from the mean at that protocol time point. So, all mean values over time should be the same as that observed at baseline in that treatment group at that site. In this way, trends in mean values over time and between groups will not be apparent.
- Note that the treatment group will not be masked because treatment group will be obvious from the 2:1 allocation to the CR group.

19.20.2 Computer Checks for Longitudinal Data

Apply a mixed effects model to detect outliers in longitudinal data. But, there must be enough longitudinal data to make this worthwhile. This will be left to the second half of the study.

For daily home weights, as well as the most important outcomes, the following analysis is performed.

- The dependent variable is the vector of observations (including the baseline value) across all the subjects at that site.
- Include terms for the intervention, the time effect (treated as a categorical variable) and the intervention × time interaction. To account for variability among the subjects, add a random effect for each subject.
- Using this model, generate the best linear unbiased predictor for each observation and the residual for each subject at each time point. Derive the standardized residuals.
- Designate for review any observation for which the standardized residual is greater than 2.24 in absolute value. This corresponds to the central 97.5% of the standard normal distribution. We would expect 2.5% of all such "outliers" to be legitimate data values.

19.20.3 Computer checks for DTH Results

At each protocol time point, there are two dimensions measured for saline plus the three specific antigens (Tetanus toxoid, Candida, and Trichophyton), at two time points (i.e., at 24 and 48 hours post injection). The following reports are prepared:

- For saline and the three antigens, a bivariate scatterplot, by staff initials within site, and protocol time point, of the "B" diameter vs. the "A" diameter. Perform separate plots at 24 and 48 hours post injection. Because the "A" measurement is supposed to be the larger dimension, the values should all lie below a line at 45° angle.
- Compute the average of the two diameters for each antigen at 24 and 48 hours post injection. Perform a bivariate scatterplot for each antigen, by site and protocol time point, of the 48-hour average vs. the 24-hour average. Include a reference line at 45° angle.