

Appendix D. Oral Glucose Tolerance Test (OGTT)

Definition: An OGTT is commonly used in the U.S. The OGTT assesses how an individual uses glucose over time by measuring the participant's plasma glucose level before and at defined intervals for 2 hours after drinking a solution containing 75 grams (or 1.75 g/kg) of glucose. Samples for the measurement of C-peptide and insulin levels are also drawn, according to the sampling protocol defined below.

Exclusion Criteria for Performing an OGTT

Do **not** perform the OGTT if the participant:

- Has fasting blood glucose ≥ 250 mg/dl (if reading is by meter then a stat glucose should be performed in local lab if able). **Do not proceed with OGTT if the participant manifests unequivocal elevation of fasting plasma glucose (≥ 250 mg/dL) in the local laboratory.** In this case, draw all fasting samples to send to the core laboratories;
- Has an acute sickness or stress (e.g. surgery, injury or infectious disease in the past 2 weeks). The test should be postponed for at least two weeks after resolution of any intercurrent infectious illness or other stress. In addition, a participant who has fractured a bone within the past two weeks should not have the OGTT completed; or
- Is pregnant or suspects potential pregnancy.

**** Currently, the protocol for the TrialNet Immune Effects of Oral Insulin Study requires a 2 hour OGTT. Based on the experience in the DPT-1 Study, there will be subjects who have not been diagnosed with diabetes but who are discovered to have fasting plasma glucoses above 125 mg/dL (7.0 mM) on the day the OGTT is scheduled. It is recommended that the OGTT be performed in these cases provided the fasting plasma glucose does not exceed 250 mg/dL (13.9 mM), and the subject does not have ketonuria, ketonemia, or major clinical signs of uncontrolled hyperglycemia. This issue was considered by the Natural History/Pathway to Prevention and Oral Insulin Study Committees and subsequently approved by the TrialNet Steering Committee based on the following rationale:

1. There was consensus that the additional glucose rise would not be dangerous, where the dominant concern is precipitating ketoacidosis. In all cases, persons who are confirmed to have diabetes by the fasting and/or 2 hour plasma glucose levels need to be advised of the diagnosis and standard treatment including insulin injections, diet, and home glucose monitoring, will need to be quickly started; and
2. Because the OGTT also requires collection of samples for insulin and c-peptide levels, there was consensus that these additional variables could potentially provide further useful data on the natural history of beta-cell function in persons with pre-type 1 diabetes.

Although the OGTT is required by the protocol, we recognize that the local site Physician may choose not to proceed with an OGTT in persons with an unequivocally elevated fasting plasma glucose level on the day of the test. Thus, the final decision not to do the OGTT will be left up to the local study team. In those cases, we still recommend collection of samples for fasting glucose, insulin and c-peptide levels and explanation of the reasons why the full OGTT was not done be recorded in the comments section of the online OGTT Specimen Collection eCRF.

Subject Preparation for an OGTT

Because a large number of factors may affect the OGTT, care must be taken to prepare participants properly for the test.

Dietary Guidelines

The subject should follow a diet containing at least 150 grams (g) of carbohydrates for at least 3 days prior to the test. If the subject has not consumed sufficient dietary carbohydrates before the test, the insulin secretory response to the glucose may not be as great as it should be and the test results may be unreliable. Dietary sheets providing guidelines for the high carbohydrate diet should be given to each participant in time to prepare for the test.

The recommendation for children is to eat a usual diet, not restricted in carbohydrates prior to testing. It should be noted that 150 grams of carbohydrates is about 10 servings of starch, fruit, or milk exchanges and is not an unrealistic amount of carbohydrates to ingest except for very young children, perhaps less than 4 years old.

Other Preparatory Guidelines

Participants preparing for an OGTT should further prepare as follows:

- 10 hour fasting, except for water;
- 10 hour abstinence from cigarettes, alcohol, caffeine-containing drinks and vigorous exercise**;
- Hydrate with water consumption, especially for young children; and
- Adequate sleep the night beforehand.

Since the OGTT must be started between 6 AM and 10 AM, appointments should not be scheduled for mornings on which a subject has had to work during the preceding night.

**It is highly recommended that no prolonged or intense exercise should be performed within 24 hours of the OGTT. There is a scientific basis for this as the post-exercise recovery phase is characterized by accelerated glycogenic activity that continues for 12 to 24 hours after this type of exercise, characterized by alterations in insulin secretion, catecholamines, glucagon, growth hormone, and cortisol, all of which could affect the integrity of the test. Exercise should be avoided for one day prior to the OGTT to ensure an accurate profile of insulin secretory capacity.

OGTT Administration Eligibility Checklist

| | |
|--------------------------|---|
| <input type="checkbox"/> | Subject fasted (did not eat) after 10:00 PM the night before the test up until the start of the test. <i>Subject avoided all food and drink, with the exception of water.</i> |
| <input type="checkbox"/> | Subject abstained from consuming <i>coffee, tea, sodas, caffeine containing drinks, cigarettes, alcohol, or chewing gum</i> during the fasting period (10 hours before the test). |
| <input type="checkbox"/> | Subject refrained from vigorous exercise during the fasting period (10 hours before the test). |
| <input type="checkbox"/> | Subject refrained from working during the night preceding the test. |
| <input type="checkbox"/> | Subject ate a high carbohydrate diet (at least 150 grams) (<i>See Sample Menu in Appendix B for details</i>) for 3 days prior to testing. |
| <input type="checkbox"/> | PI reviewed medication list, including over the counter meds, to determine whether ok to proceed with test. Note, PI may contact TN20 CRA or Study Chair if he/she is uncertain about whether OGTT should be performed. |
| <input type="checkbox"/> | If subject has had an illness, surgery, or infection, the PI has evaluated the subject and determined whether the test should be done. Note, the PI may contact the TN20 CRA or Study Chair if he/she is uncertain about whether the OGTT should be performed |
| <input type="checkbox"/> | Subject is not pregnant, does not have any chronic illness such as cancer, nephritic syndrome, active hepatitis, or some other life threatening illness. |
| <input type="checkbox"/> | At start of OGTT, the subject's blood glucose was less than 250 mg/dL. |
| <input type="checkbox"/> | OGTT started after 6 a.m. and before 10 a.m. (i.e. -10 min samples were drawn after 6 a.m. and before 10 a.m.). |
| <input type="checkbox"/> | The entire amount of Glucola is consumed within 5 minutes. |

Clinic Preparation for an OGTT

Collect blood in 1.2 mL gray top K Oxalate/ Na Fluoride collection tube, 1.2 mL lavender top K2 EDTA collection tube, and 1.2 mL green top Li Heparin collection tube for each time point. To prevent sample degradation, extract plasma from each collection tube, transfer into the appropriately-colored cryovial and freeze at -20°C within one hour of collection.

Supplies:

Please refer to the TN20 Laboratory Manual of Operations for OGTT supplies.

Time Measurements: Record all times using the 24-hour clock format, using the key below:

| 12-Hour Clock | 24-Hour Clock |
|-----------------|---------------|
| 1:00 am | 01:00 |
| 2:00 am | 02:00 |
| 3:00 am | 03:00 |
| 4:00 am | 04:00 |
| 5:00 am | 05:00 |
| 6:00 am | 06:00 |
| 7:00 am | 07:00 |
| 8:00 am | 08:00 |
| 9:00 am | 09:00 |
| 10:00 am | 10:00 |
| 11:00 am | 11:00 |
| 12:00 pm (noon) | 12:00 |

| 12-Hour Clock | 24-Hour Clock |
|---------------------|-------------------|
| 1:00 pm | 13:00 |
| 2:00 pm | 14:00 |
| 3:00 pm | 15:00 |
| 4:00 pm | 16:00 |
| 5:00 pm | 17:00 |
| 6:00 pm | 18:00 |
| 7:00 pm | 19:00 |
| 8:00 pm | 20:00 |
| 9:00 pm | 21:00 |
| 10:00 pm | 22:00 |
| 11:00 pm | 23:00 |
| 12:00 am (midnight) | 00:00* (next day) |

OGTT Testing Procedure

Procedure:

Step 1. Ensure the subject is currently fasting

Step 2. Prepping Participant for OGTT

- a. The OGTT must begin between 6:00 and 10:00 a.m. for proper interpretation.
- b. Obtain the weight of the subject and calculate the glucose load to be administered. Individuals ≥ 43 kg will receive 75 g of glucose. Individuals < 43 kg will receive 1.75 g glucose per kg body weight to a maximum of 75 g glucose (See box below).

Glucose Dose Calculation for Subjects Under 50 kg:

Subject's weight in pounds _____ multiply by 0.454 = _____ kg
 Subject's weight in kg _____ multiply by 1.75 = _____ grams of glucose
 (do not exceed 75 grams)
 Grams of glucose _____ multiply by 3 (if using 100 gram glucola) = _____ ml *
 (total volume should not exceed 225 ml)
 multiply by 4 (if using 75 gram glucola) = _____ ml *
 (total volume should not exceed 300 ml)

* Glucose (Glucola) is generally supplied in both 75g/10oz and 100 g/10oz concentrations.
 Refer to concentration of glucose solution to determine volume to be administered in mls.

NOTE: When using 100 g/10 oz. bottle, up to a maximum of 7.5 oz. or 225 mLs may be given. When using 75 g /10 oz. bottle, up to a maximum of 10 oz. or 300 mLs may be given.

- c. Record the glucose dose in source documents.
- d. The subject should remain sitting or resting in bed quietly throughout the OGTT. The subject can engage in quiet, non-strenuous activities such as reading, playing cards, watching TV and may walk to the bathroom between blood draws if necessary, but should otherwise remain resting until the test is completed. It is recommended that participants not be asked to answer questions for the purpose of completing case report forms during the OGTT.
- e. Place an I.V. line into a peripheral vein, using an intracatheter/butterfly needle (usually 20 or 22 gauge depending upon the size of the subject). Maintain IV patency per your institutional guidelines.

Step 3. Glucose consumption and baseline samples

- a. The first sample should be taken at least 10 minutes after establishing the IV line and when the subject is calm and relaxed. This is the -10 minute sample. Throughout the procedure, record the actual time (24 hour clock) of each time point in source documentation. You may collect your autoantibody, hemoglobin A1C, and mechanistic (if applicable) samples at this time or at any point during the test. You can use blood from the adapter for your fasting glucometer specimen.
- b. Start the timer when the 0 minute sample is drawn, immediately before the subject starts to drink the glucola. Record this time in the online OGTT Specimen Collection eCRF as time 0. The dose of glucose must be completely consumed within five (5) minutes.
- c. If the subject is unable to finish drinking the solution within the five minutes, but the test is still believed to be valid, note in source documentation exactly how long it took the subject to ingest the solution and/or how much of the drink was consumed. If the test is not believed to

be valid, contact the TNCC and make a comment on the source document indicating the OGTT was completed but may not be valid due to inability to consume the glucola within the time allotted. The study coordinator can still send any samples collected to TrialNet labs for analysis.

- d. Obtain post-glucose blood samples: Draw appropriate samples at 30, 60, 90, and 120 min after time 0 and record the actual time of each draw on source documents. Do not start or stop clock throughout the procedure.
- e. Clogged lines, missed samples and other procedural issues should be noted in source documentation. If samples are missed, please select “not collected” in the online specimen collection form.

Sampling Protocol:

| Time (min) | Glucose Sample Taken 1.2 mL gray top tube | C-peptide Sample Taken 1.2 mL lavender top EDTA tube | Insulin Sample Taken 1.2 mL green top Li Heparin Tube |
|---------------|--|---|--|
| -10 | X | X | X |
| 0 | X | X | X |
| Drink Glucola | | | |
| 30 | X | X | |
| 60 | X | X | |
| 90 | X | X | |
| 120 | X | X | X |

Step 4. Termination of OGTT

- a. After drawing the final blood sample at 120 min, the IV line may be terminated. Apply pressure to the site, and then apply a sterile strip bandage. The subject should then be given a snack, such as peanut butter or cheese crackers, milk or ginger ale.

Step 5. Ensure the OGTT source document has been completed.

- a. **Please note** the source document references obtaining a blood glucose measurement by meter at -10 and 120 minutes. The measurement at -10 should be used to determine if it is OK to proceed with the OGTT as per OGTT exclusion guidelines (see section 6.4.2.1). The measurement obtained at 120 minutes is for safety purposes only and should not be shared with the participant.

OGTT Sample Processing, Storage, and Shipping

Please refer to the TN20 Laboratory Manual of Operations for OGTT sample processing, storage, and shipping instructions