This protocol has been designed to test for carriers for OCT deficiency in two ways:

- Firstly by collecting a fasting sample of urine (FASTING SAMPLE) for analysis of orotic acid/creatinine and comparison with our control population.
- Secondly by collecting three timed urine samples over the 4 hours of the test. SAMPLE 1 is collected immediately prior to taking the soy protein, SAMPLE 2 collected during 0 to 2 hours after that and SAMPLE 3 collected during 2 to 4 hours later. Analyses of orotic acid/creatinine on these samples are used to calculate the ratio of orotic acid excretion from 2 - 4 hrs over 0 - 2 hrs after the meal of protein. The absolute values of orotic acid/creatinine and the ratio are compared with results found in our control population. (see Journal of Inherited Metabolic Disease Vol 24, 1 pp 5-14).

It is ABSOLUTELY essential that the instructions for collection of urine are followed exactly.

Preparation of the Soy Protein Load

A. Height and weight of patient need to be supplied to the clinician or dietitian well before the test commences.

B. An amount of soy protein is prepared to give a calculated load of 1.0 g protein/kg body weight, if >40 Kg and <75 Kg. Give 35 g protein/M² surface area if body weight is <40 Kg or > 75 Kg. This calculation needs to be done by a dietitian. 100g Nature’s Way Instant Natural Protein contains 88g protein. Mixing instructions are 30g plus 1 cup water. Sugar based flavouring such as toppings can be added but not those containing milk protein.

Preparation of/by the Patient

A. Have nothing to eat or drink, except for water, from 10.00 pm. on the night before the test. Medications may interfere with the test. Any medications that are absolutely necessary must be discussed with the laboratory [Dr. Wilcken (02) 9845.3650; Dr. Carpenter (02) 9845.3123, Ms Green (02) 9845.3133].

The collections of urine start on getting up in the morning and continue until the end of the 4 hour test. All of each specimen passed must be collected, put into a clean container and labelled with name, time and date.

B. Collect the first urine passed after getting up, put all of it into a clean container and label with name, time and date (FASTING SAMPLE). The specimen should be kept cool. If at all possible, please refrain from passing urine again until the start of the test. If that is not possible, collect again into a separate clean container and label as before with the time, etc. A glass of water (250 mL) may be drunk.

C. On arrival at the appointed place, you will be asked to empty your bladder again (SAMPLE 1) and then immediately start having the soy and finish it within 25 minutes. It may be helpful to wash down the soy with a glass of water (250 mL). Ideally urine should be passed at exactly 2 hr (SAMPLE 2) and (SAMPLE 3) 4 hr after the soy protein. A glass of water (250 mL) may be drunk during this period.
DATA SHEET FOR THE SOY PROTEIN LOAD TEST

DATE OF TEST: .......................................................................................................................
NAME: .......................................................................................................................................
DATE OF BIRTH: .......................................................................................................................
HEIGHT (cm): .............................................   WEIGHT (Kg): ...................................................
SURFACE AREA (m²): ................................................................................... (if necessary)
CALCULATED SOY PROTEIN LOAD: ..........................................(the amount of solid needs to be calculated by
   a dietitian using the protein content of the soy powder and the patient's body surface area if required -
   1.0g protein/kg BODY WEIGHT if >40 Kg and <75 Kg;
   35g protein/m² SURFACE AREA if <40 Kg and >75 Kg)
ANY REACTION TO TEST: .........................................................................................(nausea, headache, etc.)

IMPORTANT
• It is essential to collect all of each specimen of urine passed.
• It is essential to record the time and total volume of each collection.
• It is essential to empty the bladder immediately before starting to eat.

PROCEDURE

<table>
<thead>
<tr>
<th>TIME</th>
<th>TOTAL VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

URINE COLLECTION BEFORE THE LOAD

FASTING SAMPLE  First urine after rising this morning
Other urine (if any) before appointment
SAMPLE 1  At the start of the soy protein load

TAKING THE LOAD (TO BE EATEN WITHIN 25 MINUTES)

Start of drinking
Finish of drinking
NOTE: If all of the load is not taken then measure the volume
   remaining.............................mL and CONTACT THE
   METABOLIC DIETITIAN

URINE COLLECTION AFTER START OF THE SOY LOAD

SAMPLE 2  Between 0 - 2 hours (if any)
SAMPLE 2  Bladder emptied at 2 hours after start
SAMPLE 3  Between 2 - 4 hours (if any)
SAMPLE 3  Bladder emptied at 4 hours after start

NOTE FOR LABORATORY SUPERVISING THE TEST: SAMPLES for Biochemical Genetics
After the total volume of each sample has been thoroughly mixed and carefully measured, approx. 20-30 mL may be transferred to
a small screw-cap container, labelled clearly with name, date, time, total volume and frozen.  Please keep the samples frozen
during transport to Biochemical Genetics.

Authorising Officer: Dr K Carpenter
Date Of Authorisation: 7th December 2006