**Study Protocol**

### Subjects

120 healthy participants (60 men and 60 women), aged 18 to 65yrs, BMI < 27 kg/m2 will be recruited from the general population in Dunedin. This age range is used so that the study findings will be applicable to Dunedin Study participants (past, present and future), and will also be generalizable to a wider New Zealand population for use in other adult cohort studies. Individuals with current major chronic disease will be excluded.

Participants will be required to attend a clinic at the Department of Human Nutrition for up to 60 minutes at weeks 0, and 10. At the first week informed consent will be obtained. At each clinic visit participants will be asked to provide two 5ml blood samples (for serum and red blood cell analysis), a hair sample (~50 hair strands, clipped close to the scalp, just inferior to the post-occipital protuberance).

At the first visit participants will also receive detailed instruction on collecting weighed diet records of everything consumed on one day per week for 7 weeks. Participants will be provided with a set of electronic scales and a set of paper-based food models to use during the 7 week data collection period. The diet records will be paper based. Participants will be randomly assigned a specific day of the week each time to complete the diet record so that each day of the week is captured by the time the 7DDR is completed.

Text and email reminders will be sent to participants 24hrs prior to each scheduled clinic visit and each diet recording day. A link to a short video explaining how to complete the diet record will be included in each email message. Participants will be asked to return the diet record each week by post, in person or by scanning the diet record and return it by email. Research assistants will review each diet record within 24hrs of its return and will contact the participant for further details if necessary to ensure accuracy and completeness.

In weeks 3 and 7 participants will be sent an email link to the electronic sugar-specific FFQ. This has been adapted from previously validated sugar-specific FFQs designed for Pacific and Māori populations in New Zealand (Teufl, 2013; Boniface, 2013; Furter, 2014; Walter, 2014) and uses the RedCap platform. The FFQ has been designed to reduce errors and requires that every question is completed. A paper version will be available for those who do not have ready access to a computer.

Figure 1 provides an overview of the study illustrating the data collection schedule.



Figure 1. Overview Study

#### Isotope Measurements

Bulk (i.e. total) carbon (C) isotopic compositions will be determined on 100 µl of freeze-dried red blood cells, and serum or 1mg hair, weighed into tin capsules. Carbon isotope ratios will be determined by combustion in a NA 1500 Elemental Analyser (CE Instruments, Milan), and measurement of the resulting N2 and CO2 gases made by a Thermo Finnigan Delta Advantage Isotopic Ratio Mass Spectrometer (EA-IRMS) at the Isotrace lab facility (Dunedin, New Zealand). The conventional method of expressing *δ*13C at natural abundance is in per mil (‰) abundance of 13C relative to an international standard (Vienna Pee Dee Belemnite, VPDB), as follows:

*δ*13C =(13C/12Csample-13C/12Cstandard)/13C/12Cstandard)

The instrument precision is 0.2 ‰ for C, based on multiple measurements of laboratory control material (EDTA). Data will be calibrated to the international scales using triplicate measurements of two reference materials (USGS41 and 41) run with each batch of samples.

For individual Amino Acid (AA) stable isotope analysis 50 µl samples of red blood cells and serum and 1 mg of hair, will be hydrolysed in 1 ml of 6 M HCl at 150 oC for 70 min. After hydrolysis, samples will be centrifuged (3000 rpm, 7 min) and supernatant extracted. Solvents will be removed at 60 oC under a stream of N2 and the AAs derivatised into *N*-acetyl isopropyl ester. After derivatization, samples will be prepared for isotope measurements. *δ*13CAA will be measured by gas chromatography/combustion/isotope ratio mass spectrometer (CG-IRMS), using a Thermo Trace gas chromatograph, the GC-IsoLink combustion interface, and a Delta-XP isotope ratio mass spectrometer (Thermo Fisher Scientific). Samples will be analysed in duplicate along with amino acid standards of known isotopic composition (measured on EA-IRMS). Samples will be calibrated against known standards and corrected. Raw *δ*13CAA values will be corrected for instrumentation drift and each measured *δ*13CAA will be corrected relative to the amino acid *δ*13CAA of standards to account for the exogenous C and kinetic fractionation introduced during derivatisation.

**Statistical Analysis**

Power calculations indicate that 100 participants should be sufficient to detect a correlation of 0.3 between free sugars intakes, estimated from weighed diet records, and *δ*13Calanine. We will allow for a drop out rate of 20% giving a sample size of 120 subjects.

Seven recording days are required to capture habitual dietary intake of participants. Diet records will be analysed for major nutrients including free and total sugars using Kai-calculator software and the New Zealand Food Composition Database, and the average daily intake for energy, macronutrients, free and added sugars, and dietary fibre will be calculated.

Relative validity of the *δ*13Calanine versus mean intakes of free sugars estimated weighed diet records (7 days total per person) will be assessed using Spearman correlation coefficients (SCC). Bland-Altman analyses will be performed to assess the strength of agreement between the *δ*13Calanine and 7d weighed diet record in measuring sugars intakes. Repeated measures two-way ANOVA will be used to assess the between-subject effect and to compare bulk δ13C and δ13Calanine in various tissues at each monthly time point with adjustment for potential confounders including age, sex, body size, hair thickness, physical activity level and total energy intake.

Correlation coefficients (*r*) measured between nutritional biomarkers and reported dietary intake vary widely in research trials, with typical *r* values ranging from 0.03-0.70, with a mean of 0.39[22](#_ENREF_22),[27](#_ENREF_27). These correlations may be interpreted as weak to modest, as they may underestimate the true validity of biomarkers due to the inherent inaccuracy of self-reported dietary intake measures[26](#_ENREF_26). *R* values of 0.5-0.7 are typically considered acceptable as a precision in dietary validation studies[27](#_ENREF_27).

**Subject Reimbursement**

Participants will be compensated $100 (in the form of grocery vouchers) for participating in the study once they have completed the study and returned all diet records. Compensation is intended to cover transport costs and to thank participants for volunteering their time.