

Appendix A. Methodology of the Diet and Nutrition Survey of Infants and Young Children (DNSIYC)

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Table of contents

A.1.	Ethical approval.....	1
A.2.	Sample design.....	3
A.3.	Overview of methodology.....	7
A.4.	Comparison of dietary to blood data.....	27
A.5.	Age at Stage 2.....	27
A.6.	Mobile unit.....	27
A.7.	Feedback to participants and General Practitioners (GPs).....	28
A.8.	Fieldwork quality control.....	29
A.9.	Tokens of appreciation.....	31
A.10.	Statistical methods.....	32

List of tables

Table A.1.	Clinic sites in DNSIYC.....	2
Table A.2.	Interviewer visits.....	10
Table A.3.	Clinic visit components.....	16

List of figures

Figure A.1.	Summary of stages included in DNSIYC.....	8
Figure A.2.	Measurement of length.....	15
Figure A.3.	Measurement of head circumference.....	15
Figures A.4a and A.4b.	Measurement of subscapular and triceps skinfold thickness.....	19
Figures A.5a and A.5b.	Stable isotope equipment and dose being consumed	20
Figure A.6.	Blood sampling.....	22



A.1. Ethical approval

Following reviews by the Medical Research Council Human Nutrition Research's (MRC HNR) Research Governance Committee, the ethical application for the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) was submitted on 4 November 2009, for review by the Cambridgeshire 4 Research Ethics Committee (REC) at their meeting on 26 November 2009. A favourable provisional opinion was given on 4 December 2009, subject to further information requested from the consortium and some administrative issues. Full ethical approval for the survey was received from the Cambridgeshire 4 REC on 18 January 2010.

Approval was subject to gaining site-specific approval for each host organisation for the clinic visit component and Research and Development (R&D) approval where the site was a National Health Service (NHS) site. Amendments were made following the Dress Rehearsal to comply with recommendations made in the Dress Rehearsal report.

Application was made on 20 November 2009 for DNSIYC to be considered a portfolio project since many of the clinics used were NHS Trust sites. Adoption as a portfolio project allows access to NHS support for England (including clinicians) for research purposes via the National Institute for Health Research Clinical Research Networks (NIHR CRN¹). The NIHR CRN supports clinical research and helps to facilitate the conduct of trials and other studies within the NHS. The NIHR CRN in England approved DNSIYC as a portfolio adopted study on 21 April 2010.

Stage 2 of DNSIYC involved 22 clinical sites across the UK including 2 mobile units. A site-specific application form was submitted to each local NHS R&D office for site-specific assessment. For non-NHS sites, such as Cambridge and the mobile units, an application for site approval was submitted to Cambridgeshire 4 REC. Site specific applications were approved for all sites. Trust R&D approval for various sites took variable, and at times lengthy, time to achieve. Approval for the non-NHS site, MRC HNR was obtained for the Cambridge site on 3 February 2010; the two mobile units used in the survey were given exemption from site specific approval; confirmation of this was received on 25 January 2010.

For the survey, the following sites were recruited:

Table A.1. Clinic sites in DNSIYC

Name of Site	Area	NHS Site
England		
Birmingham Children's Hospital, WTCRF*	Birmingham	Yes
Bradford Royal Infirmary	Bradford	Yes
St Michael's Hospital	Bristol	Yes
MRC Human Nutrition Research	Cambridge	No
Cheltenham General Hospital	Cheltenham	Yes
Leicester Royal Infirmary	Leicester	Yes
Alder Hey Children's Hospital	Liverpool	Yes
Great Ormond Street Hospital	London	Yes
Manchester Royal Infirmary, WTCRF	Manchester	Yes
Royal Victoria Infirmary, CRF**	Newcastle	Yes
Norfolk and Norwich University Hospital and University of East Anglia	Norwich	Yes
Nottingham City Hospital	Nottingham	Yes
Royal Berkshire Hospital	Reading	Yes
Sheffield Children's Hospital, CRF	Sheffield	Yes
Southampton General Hospital, WTCRF	Southampton	Yes
Mobile Units x 2	n/a	No
Scotland		
Royal Hospital for Sick Children, Children's CRF	Edinburgh	Yes
Royal Hospital for Sick Children (Yorkhill), CRF	Glasgow	Yes
Wales		
Nevill Hall Hospital	Abergavenny	Yes
Singleton Hospital	Swansea	Yes
Northern Ireland		
Carlisle Health and Wellbeing Centre	Belfast	Yes

* WTCRF - Wellcome Trust Clinical Research Facility

** CRF - Clinical Research Facility

References and endnotes

¹ http://www.crnc.nihr.ac.uk/about_us/processes/portfolio

A.2. Sample design

A named sample of infants and young children representative of the UK population aged 4 months up to 18 months was drawn using a multi-stage random probability design. The sample was selected from Child Benefit (CB) records (estimated uptake of 98%) provided by Her Majesty's Revenue and Customs (HMRC)¹; the Healthy Start² (HS) sample also used the HS recipient database (estimated to cover 22% of infants aged 4 to 18 months in the UK) provided by the Department of Health (DH). The CB and HS samples were stratified by Government Office Region, Index of Multiple Deprivation (IMD) scores and population density to ensure representativeness (see section A.2.3 and Appendix B).

The sample comprised three parts:

- 1) A core sample of children selected at random from CB records and covering all four countries of the UK.
- 2) A boost sample of children on the HS scheme selected at random from DH's HS database. This boost sample was combined with children on the HS scheme in the core sample to form the Healthy Start sample.
- 3) A boost sample of children in Scotland, also drawn from CB records completing Stage 1 only. This boost sample was combined with Scottish participants in the core sample to form the Scotland sample and results are reported in the Diet and Nutrition Survey of Infants and Young Children in Scotland³.

Weighting factors were applied to ensure that the results were representative of the population. More details about the sample selection process, including details on how the weighting factors were created, are provided in Appendix B.

A.2.1. Age

The sample drawn from the CB register was selected in two waves. This was done to try to ensure that the sample contained sufficient numbers of children at each end of the eligible age range, i.e. from 4 to 18 months. Interviews with the parents of participants aged 17 months were prioritised to reduce the number of children who would become ineligible through being beyond the specified age range at the time of interview. Similarly, interviews with the parents of participants aged four months were prioritised to maximise participation of infants of this age.

The age of the child was recorded during the home interview and again at the clinic, if a visit took place. Every effort was made to ensure that the clinic visit took place as soon after the home interview as possible to minimise the period

between the home and clinic visits and to maximise the number of children still being breastfed by the time of the clinic visit. However, some children who were being breastfed at the interview stage were no longer breastfeeding at the clinic stage. More information on the age of children at interview and at clinic visit is presented in section A.5 and Chapter 3 of the main report.

A.2.2. *Inclusion/Exclusion criteria*

As stated in the introduction of the main report, exclusive breastfeeding⁴ is recommended for around the first six months of an infant's life^{5,6}. However, complementary feeding⁷ often commences earlier than six months. Therefore, the named sample included infants and young children from four months to capture those who were receiving complementary foods earlier than six months. The full age range of the survey included children aged 4 to 18 months. Those who had turned 18 months by the time of the first interview were screened out.

Children with a birth weight under 2kg and/or those fed through a gastric tube at or after one week of age were excluded at the main interview stage. The latter criterion was chosen to exclude those individuals with congenital abnormalities likely to affect feeding practices⁸.

Participants who no longer lived at the selected address when the sample was drawn were still considered eligible to participate if they had moved locally and if a new address could be obtained. The parent of the participant who had the most involvement in the feeding of the child was selected as the interviewee. Only parents or legal guardians could be selected to complete the interview and to provide consent.

All children for whom at least three days of the food diary had been completed (i.e. fully productive participants) were regarded as eligible for a clinic visit.

A.2.3. *Selecting postcode sectors*

NatCen selected postcode sectors using CB claimant records provided by HMRC and information about HS recipients from DH. NatCen received postcode sector-level counts of children in receipt of CB and HS. These counts were used to create Primary Sampling Units (PSUs). PSUs were postcode sectors or groups of postcode sectors. Postcode sectors containing fewer than 50 eligible children were grouped with neighbouring sectors so that each PSU contained a minimum of 50 children in receipt of CB⁹.

The PSU sampling frame was then sorted, first by Government Office Region (with Wales, Scotland and Northern Ireland (NI) treated as separate regions) then by the IMD and population density. IMD is produced separately for each devolved country, there is no single UK index. All four indices were added to the

PSU sampling frame and the appropriate index was used to stratify within each Government Office Region (with Wales, Scotland and NI treated as separate regions). Within each Region the file was sorted and split into four groups based on aggregated IMD scores, and within each of these four groups the file was then sorted again by population density (the population in private households from the 2001 Census divided by the area of the PSU in hectares).

The PSUs for the core sample and HS boost were drawn together. The 178 PSUs were selected from the sorted PSU sampling frame and each PSU was to contain both core and HS children. Spreading the HS boost across 178 PSUs, rather than concentrating it within a small number of additional PSUs, reduced the effects of clustering. Thirty four additional PSUs were selected for the Scottish Boost sample. There was no overlap between the Scottish Boost and the HS boost.

A weighted selection approach was used to boost the number of HS children in the 178 PSUs. A weighting factor of 1.216 was applied to children on HS and all other children were given a weighting factor of 1 in order to obtain the required numbers of HS recipients. The weighted number of children was then generated for each PSU. Hence, a PSU containing 145 children in total, 120 of which were not on HS and 25 of which were on HS, got a weighted count of $120 + (25 \times 1.216) = 150.4$. The 178 core PSUs were selected with probability proportional to the weighted number of eligible children within them. This gave PSUs with a large number of HS children a higher chance of being selected.

A.2.4. *Selecting participants*

The core and HS boosts were selected from the same 178 PSUs but taken from two separate sources; the HS boost was selected by DH from the HS database and the core sample was selected by HMRC from CB records. The Scotland boost sample was also selected by HMRC from CB records.

Within PSUs children were sorted by postcode before the selection was done. This ensured the selected children were evenly spread throughout the PSU.

Twenty one children were randomly selected in total from each PSU but the ratio of core sample to HS boost varied across PSUs, depending on the overall proportion of families in that particular PSU claiming HS vouchers¹⁰. To avoid duplication between the sample selected from the CB records and the sample selected from the HS database, the two samples were merged at each wave and any duplicates were removed.

A.2.5. Benefits and Limitations of Child Benefit data

At the time of the survey CB was a universal benefit with a high rate of take up (around 98%); this made the CB records a highly comprehensive sampling

frame for parents and children. CB records contain information about the child for whom the claim is being made, allowing eligible households to be identified at the stage of sampling and making fieldwork more cost-effective.

A small number of CB recipients were excluded from the sampling frame before selection took place. The exclusions were made according to HMRC procedures. Reasons included: death of a child; cases where the child had been taken into care or put up for adoption; cases where the child did not live at the same address as the claimant; and cases where there had been correspondence by the recipient with the CB Centre (because the reason for correspondence could not be ascertained and might have been sensitive). These made up a small proportion of the overall population and should not have biased the sample.

Use of CB data may be limited in coverage of the youngest children since some parents may delay registration for the benefit and not be represented at the time the sample was selected. It is not known how long people typically take to register for CB. The fact that it is possible to backdate a claim for up to three months from the date the claim is sent, however, may encourage people to apply within the first three months.

There was a delay of 12 weeks between the date the sample was drawn and the delivery to NatCen due to the time taken for HMRC to prepare the data for external use.

Additionally, due to limited access to CB records it was not possible for fieldwork to span the entire year.

All HS claimants would also have been eligible for CB. As such, there was a small degree of overlap between the sample selected from the CB records and the sample selected from the HS database. At each wave the two samples were merged and any duplicates dropped to account for this overlap.

A.2.6. *Seasonality*

Ideally, it would have been desirable to assess seasonal variation in diet across the sample, however, it was not possible for fieldwork to span an entire year due to limited access to CB records. It was therefore not possible to detect or report seasonal variation in diet in DNSIYC. Intake and status of some nutrients will vary considerably by season, particularly vitamin D.

A.2.7. *Weighting the survey data*

DNSIYC required a set of weighting factors to adjust the sample for differences in sample selection and response. Details about weighting the sample can be found in Appendix B.

A.3. Overview of methodology

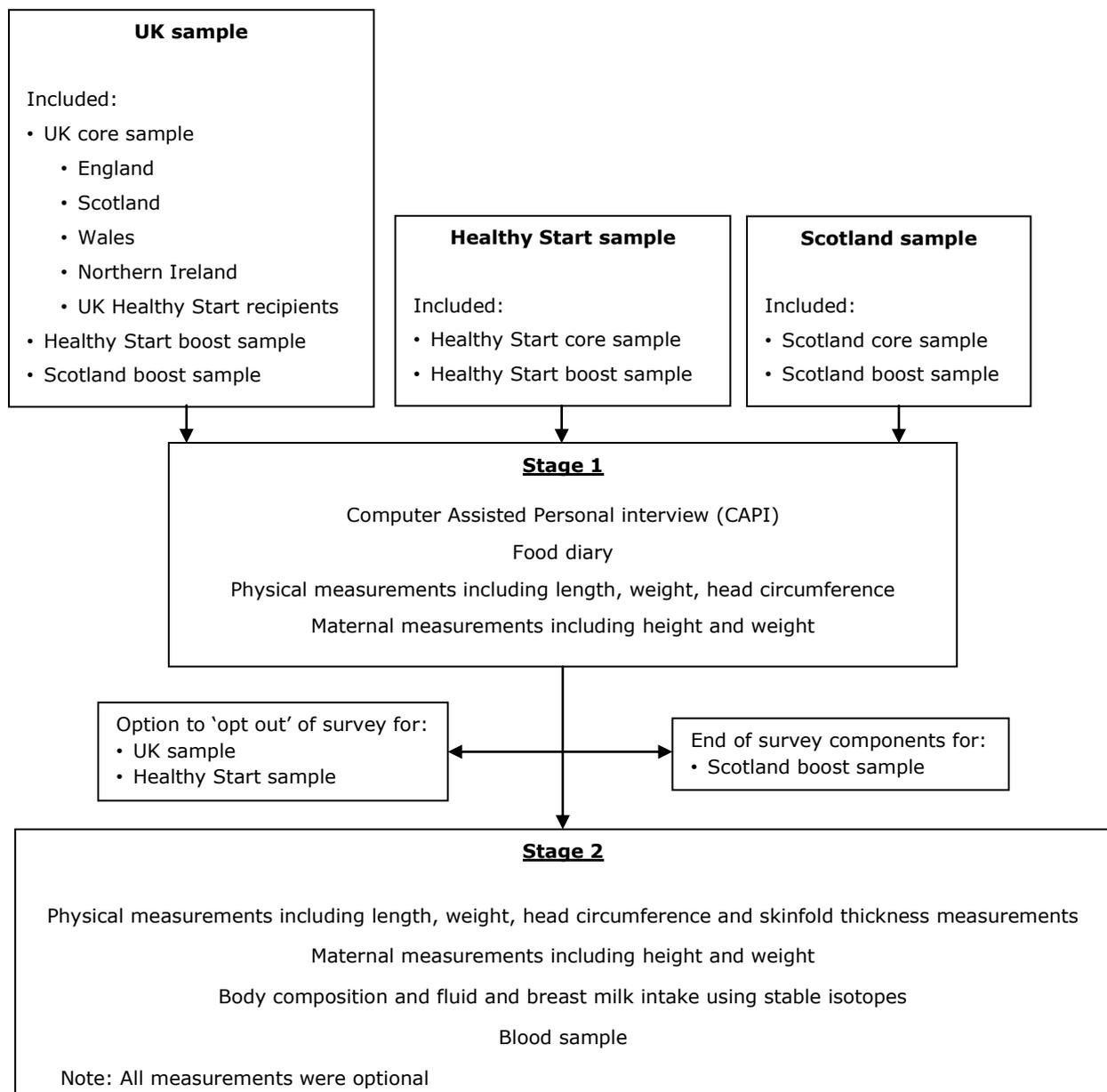
This section provides an overview of the methodology employed in the mainstage fieldwork of DNSIYC. The survey aimed to collect data from a representative sample of 2,246 infants and young children aged 4 to 18 months living in private households across the UK, including boosts. This sample size was chosen to enable robust analyses on different age groups and boosted samples. The key components were:

- Face-to-face interviews, conducted using Computer Assisted Personal Interviewing (CAPI) with the parent most involved in feeding the sampled child.
- Dietary data collection, using a four-day estimated food diary
- Anthropometric measurements (maternal height and weight; child length, weight and head circumference; and skinfold thickness).
- Collection of a blood sample for estimation of iron and vitamin D status.

Copies of all survey documents are included in Appendices C to I. The following diagram sets out the movement of participants through the survey.

Parents or legal guardians (hereafter referred to as 'the parent') of participants who took part in the CAPI interview and completed a food diary for at least three days were classified as 'fully productive' and were invited to attend a local clinic where, with consent, additional child and maternal anthropometric measurements and child blood samples were taken. In addition, parents were invited to take part in a stable isotope dose protocol in order to measure the child's body composition, fluid intake and breast milk intake (if breastfed) and participate in a urine collection protocol over 5 or 14 days, depending whether or not the child was being breastfed (refer to section A.3.3.4 and Appendix J).

Figure A.1. Summary of stages included in DNSIYC



A.3.1. *Survey components and fieldwork procedures*

Fieldwork assignments were issued to interviewers in two waves each consisting of two stages. The first stage was an interviewer visit to the participant's home. Having successfully completed Stage 1, the participant was transferred from the interviewer to HNR to arrange a hospital/clinic appointment. Stage 2 involved the participant visiting a clinic, followed up by a brief home visit by the interviewer.

Wave 1

Stage 1 - Interviewers: 6 January – 1 March 2011

Stage 2 - Clinics: 17 January – 25 May 2011

Stage 2 - Interviewers: 22 January – 1 June 2011

Wave 2

Stage 1 - Interviewers: 28 March – 23 May 2011

Stage 2 - Clinics: 11 April – 18 August 2011

Stage 2 - Interviewers: 15 April – 30 August 2011

The two main stages of data collection comprised the following:

Stage 1: Interviewer visit

- Detailed face-to-face interview using CAPI to collect background information on family dietary habits, socio-demographic status and health information and information about the child such as feeding practices, eating patterns, developmental stages, sunlight exposure and gastrointestinal symptoms (see Appendix C).
- Dietary data collection (estimated food diary, completed for four consecutive days) to provide a quantitative estimate of food consumption and nutrient intakes (see Appendices D and E).
- Physical measurements (height and weight of mother; length, weight and head circumference of child) in order to assess growth (see section A.3.3.3.).

Stage 2: Clinic visit

- Stable isotope assessment in order to estimate fluid intake, breast milk intake and body composition (see section A.3.3.4.).
- Skinfold thickness in order to measure body composition (see section A.3.3.3.).
- Blood sample collection for the analyses of iron and vitamin D status (see section A.3.3.6.).
- Follow up interviewer visit to the home to collect urine samples required for the stable isotope assessment (see section A.3.3.4.).

A.3.2. *Stage 1: Interviewer visit*

An advance letter describing the purpose of the survey was sent by post to all parents living at the selected addresses two weeks prior to the commencement of fieldwork. This letter provided the parents with the opportunity to opt-out of taking part in the survey prior to fieldwork commencing.

This was followed by a face-to-face visit by an interviewer to each address that had not opted out. The face-to-face visit enabled the interviewer to establish whether the sampled child lived at the address from the name, sex and date of

birth (DOB) of the child, plus the name of the 'benefit recipient' (parent) provided by HMRC and DH. The parent was then invited to take part in the survey.

Once co-operation had been secured, interviewers made up to three visits to the participating household. The interviewer introduced Stage 2 of the survey (the clinic stage) and asked for permission for the survey team at HNR to contact the parent of the participant to discuss this stage further and, if interested to arrange an appointment. The interviewer explained the stable isotope component and if the parent agreed, provided her/him with pre-clinic urine collection equipment. Further details about information collected during the interviewer stage and the fieldwork documents used can be found in Appendices D, E, F and G.

Throughout the interview process, interviewers were allowed some degree of flexibility about the timing of various procedures in order to fit in with participant availability and maximise cooperation. However, the preferred structure and order of tasks carried out at each visit was as follows:

Table A.2. Interviewer visits

1st visit	CAPI questionnaire (part 1). Four-day food diary placed with participant using to the Interviewer Diary Assessment Schedule (IDAS) prompt sheet as a guide to ensure appropriate points were covered.
2nd visit	Four-day food diary check-up (could be done by telephone ONLY if interviewer was sure this was appropriate) using the IDAS prompt sheet.
3rd visit	Four-day food diary collected and checked using the IDAS prompt sheet. CAPI questionnaire (part 2). Token of appreciation given (£30 in high street vouchers for food diary completion). Mother and child anthropometric measurements taken. Clinic visit introduced, agreement for HNR to contact obtained and for personal details to be transferred to HNR. Stable isotope introduced and pre-dose equipment provided if willing to take part. Interviewer completion of an evaluation form.

An additional interviewer visit was made to those participants who took part in the stable isotope element of the clinic visit protocol. At this visit, interviewers collected urine samples and accompanying paperwork and re-weighed the child (and the mother, if taking part in the breast milk protocol). Full details about the stable isotope element of the survey are in section A.3.3.4.

A.3.2.1. Computer Assisted Personal Interview (CAPI) questionnaire

The main interview was carried out using Computer-Assisted Personal Interviewing (CAPI). CAPI involves the interviewer reading questions from a laptop screen and entering parents' responses into designated fields. Details of the questions included in the CAPI are presented in Appendix C.

The CAPI questionnaire had two main elements:

- 'Household Structure'/composition interview, and
- Individual interview

The questionnaire was organised into a number of modules that could be accessed at different times at the interviewer's discretion. The 'Household Structure' interview allowed the structure of the household to be established, with questions about:

- Those living in the household's accommodation
- The relationship of each person in the household to everyone else
- The 'Household Reference Person' (HRP)
- The selected child, and
- The nature of tenure of the accommodation

The Household interview also established each person's sex, date of birth or age, work status and ethnicity and their relationship to other household members. The individual questionnaire had two parts and was asked of the parent best placed to answer questions about the child's feeding habits:

- Part 1, which was asked before the dietary data collection period, and
- Part 2, which was asked after the dietary data collection period

The individual questionnaire was divided into a number of sections including a Computer Assisted Self-completion Interviewing (CASI) module. The CASI module involved the interviewer handing the laptop to the respondent for completion by the interviewee independently. Questions asked in the CASI covered the parents' current smoking and drinking habits as well as details of the mother's smoking and drinking in pregnancy.

The various sections of the CAPI comprised the following:

PART 1 Sections

- Breastfeeding/complementary feeding practices
- Eating patterns
- Developmental stages

- Dietary supplements and medications currently taken by child (and by mother if breastfeeding)
- Assessment of exposure to sunlight of child over previous 12 months
- Details of childcare arrangements
- Health information
- Sleeping and minor gastrointestinal symptoms
- Smoking and drinking habits of mother (and partner, if applicable) both currently and during pregnancy (these questions were administered using a CASI)
- Socio-economic details of parents

PART 2 Sections

- Maternal dietary habits
- Maternal and child physical measurements
- Introduction to the clinic visit
- Stable isotope protocol: If a participant was eligible for, and parent agreed to take part in, the stable isotope part of the survey, there was a separate CAPI questionnaire component, which included administrative questions covering the collection of urine samples. It also included re-weighing the child (and the mother, if taking part in the breast milk volume protocol). This additional CAPI component took place after the participant had visited the clinic.

In order not to over-burden parents of participants, a particular concern with this target group, interviewers had the option to administer some modules from Part 1 during Part 2 instead. As long as the food diary had been placed, the interview could be terminated at any point and completed later.

The advantages of using a CAPI questionnaire are that survey questions, topics and routing can be more complex without affecting data quality, whilst also maintaining a high response rate. Interviewer effects on questions that are deemed to be more sensitive are offset by introducing the CASI module for current smoking and drinking and smoking and drinking during pregnancy as well as using show cards within the CAPI. The limitations of using a CAPI methodology are that full answers to open ended questions may not be consistently collected; hence, contextual information may be compromised. Initial set up costs of CAPI can also be higher.

A.3.2.2. Collection of dietary data: the four-day food diary

A randomised start day for the four-day food diary recording period was generated by CAPI. The parent who had most involvement with the child's feeding was chosen for the interview. If feeding was shared between two

parents, one would be selected for interview (based on availability), but ideally, both were present for the completion of the questionnaire.

Parents of participants were given a food diary with instructions to record all food and drink, which included breast milk and formula milk consumed by the child both in and out of the home for four consecutive days. Interviewers thoroughly explained the process of keeping an estimated food and drink diary using the IDAS prompt sheet as a reference tool to ensure that all aspects of the diary had been explained. The interviewer worked through an example diary day with the parent to assess their understanding after the diary had been explained.

Parents were asked to provide information on the eating context i.e. where the child was, who else was eating, whether the television was on and whether the child was at a table. Food and drink portions were estimated using household measures; parents were asked to collect food labels to improve accuracy when coding individual food and drink items.

On occasions when children consumed food and drink where the parent keeping the diary was not present, e.g. at a childminders, nursery, relative or a friend's house, other carer(s) were asked to help, by completing the diary or filling in a carer food and drink recording sheet. The carer food and drink recording sheet consisted of a simple A4 form for recording key details about what the child consumed whilst in their care, with instructions on the reverse side to assist other carer(s) in its completion. Carers were instructed to record the same details as would have been required in the diary and were also provided with a letter of explanation and provided with a plastic zip bag to gather food packaging. Four carer food and drink recording sheets were provided and the parent was asked to pass the recording sheets and bag to the other carer when dropping off their child and to collect them when picking up their child.

See Appendix D for information on the dietary data collection and editing, Appendix E for the food diary documentation, and Appendix M for information on the food groups.

A.3.2.3. Limitations to the quality of data collected using food diaries

There are a number of potential limitations to the quality of data collected using food diaries. It has been suggested that as a result of recording food and drink consumed, normal eating habits may be altered¹¹. Parents may change what the child eats in order for the child's diet to appear more socially acceptable. Alternatively, parents may change the foods consumed in favour of those which were easier to record, e.g. a jar of baby food or a packet of crisps. Participant burden may also affect the estimated food record. Parents were asked to record

food and drink at the time of eating, which may have resulted in a decrease of information over the recording period as motivation decreased.

The estimated food record provides greater flexibility and is especially useful for children who may have more than one carer¹² and where food may be more likely to be eaten outside the home. A study in toddlers indicated no difference in energy intakes when an estimated diary was compared to a weighed diary¹³. In order to address these potential problems, fieldwork interviewers were trained to provide parents of participants with a detailed explanation of how the food diary should be completed in and out of the home. Interviewers were also instructed to offer increased support to parents who were having difficulties completing the diary over the four-day recording period.

It is acknowledged that diary quality may be decreased if there is a lack of understanding¹². In order not to encourage change of normal diet, parents were told that the research team would not comment on the quality of their child's intake and that the priority was an accurate record, whatever the food intake might be. Parents were also assured that individual results would not be used in publications. Provision of dietary feedback was introduced partly to encourage an accurate record, since the information provided in the feedback related to the food consumption actually recorded (for details of dietary feedback, see section A.7.1 and Appendix K)

A.3.2.4. Collection of physical measurements: height, weight, head circumference and length

Various physical measurements were taken by interviewers.

Maternal measurements

Maternal height was measured using the portable 'Leicester' stadiometer¹⁴. Maternal weight was measured using calibrated Soehnle, Seca or Tanita digital scales. Height and weight measurements were taken in metric units following a standardised protocol. Interviewers recorded the measurements on a measurement record card, to be provided to the mother, if she wished to receive them.

Child measurements

Before any measurement was carried out, written consent was obtained from the parent of the child taking part in the survey.

Weight of the child was measured using calibrated Soehnle, Seca or Tanita digital scales. The child was weighed while being held by an adult – the interviewer first weighed the adult, and then weighed that adult holding the child. Both weights were entered into CAPI and the computer then calculated the weight of the child. As the scales used were only calibrated up to 130kg, where

the child and mother weight measured over 130kg the results had to be disregarded as unreliable.

Length of the child was measured using a Rollameter baby measure mat¹⁵. A maximum of three measurements were taken, the third being required if the difference between the first two measurements was greater than 0.5cm. The mean of the two closest measurements was recorded. Bulky clothing was removed and the Rollameter was used on any suitable flat, firm surface. The head was moved into a suitable Frankfort plane position and the legs were held straight by applying gentle downward pressure.

Figure A.2. Measurement of length



Occipito-frontal head circumference was measured using a Child Growth Foundation disposable head circumference tape¹⁶. The tape was placed around the child's head so that the tape lay across the frontal bones of the skull just over the occipital prominence at the back of the head (the widest part of the child's head).

Figure A.3. Measurement of head circumference



All measurements were taken in metric units following a standardised protocol. The interviewer recorded the child's measurements on a measurement record card for the parent, if the parent wished to receive them. For length and head circumference, a maximum of three measurements were taken, the third being required if the difference between the first two measurements was greater than

0.5cm. The readings were recorded to the nearest millimeter. A degree of variation was expected with measurements due to the difficulty of carrying out the procedure on young children (i.e. child wriggling or becoming upset). Spot checks in the CAPI were aimed at reducing variability in measurements and increasing reliability of the data.

Some error was expected on head circumference and length measurements because of challenges of taking these measurements on infants and young children and the newness of interviewers to the task. This was monitored through a Quality Control (QC) assessment held part-way through fieldwork. The QC assessment demonstrated that interviewers were confident and capable of performing these measurements accurately and showed acceptable levels of precision.

A.3.3. *Stage 2: the clinic visit*

Stage 2 of the survey was carried out in clinics and mobile units around the UK and took place at least two weeks and as soon as possible after the final Stage 1 interviewer visit. All participants completing three or four food diary days were eligible to take part in the clinic visit. Components of the clinic visit are detailed in the table below.

Table A.3. Clinic visit components

Physical measurements	Maternal height Maternal weight Child weight Infant length Infant head circumference Skinfold thickness measurements
Stable isotope	Protocol 1 – children fed any breast milk Protocol 2 – non-breastfed children or if unwilling/unable to participate in Protocol 1
Blood sample	Child venous blood sample

At the end of Stage 1, the parent of the participant was provided with information leaflets giving details of the clinic visit. If the parent was prepared to receive a phone call from HNR to discuss Stage 2 in more detail, the interviewer gained verbal consent to be able to pass on contact details to HNR. The interviewer then proceeded to discuss the stable isotope component of the clinic visit and if they were interested provided the parent with the relevant urine collection kit and explained how to collect a urine sample from the child.

Contact details of participants eligible for Stage 2 were securely transferred from NatCen to HNR via a secure file transfer protocol (FTP) and imported into the HNR DNSIYC survey database.

A.3.3.1. Clinic visit bookings

Daily meetings were held to allocate clinic booking calls to MRC HNR team members and to discuss any queries from the previous day.

A detailed telephone script was developed and provided to each team member to use for all clinic booking calls. This covered all the areas to be explained and discussed with the parent of the participant prior to booking an appointment, including the consent process, physical measurements and skinfold thickness measurements, the stable isotope component, the blood sample, travel to and from the clinic, other expenses, the urine collection visit from NatCen and the specific clinic locations and options available if the parent decided to take part. If an interest was expressed in the stable isotope component, collection of the pre-dose urine sample(s) was explained again. The parent was asked to take a clean, dry, ready to use feeding bottle to the appointment to help with administration of the stable isotope dose to the child.

Whenever an appointment was booked, the participant's home address was checked with the parent, a mobile phone number was obtained if available and General Practitioner (GP) details were obtained. Parents of the participant were advised of the free phone number for any queries regarding the clinic visit and if the appointment needed to be cancelled or postponed. A letter confirming the appointment was sent out in the post; this contained the address of the clinic chosen for the appointment. Reminder text messages were also sent, where possible, to the parent at one week and two days before the appointment to keep non-attendance to a minimum. The parent was encouraged to contact HNR to reschedule the appointment if they were unable to attend, for example due to the child being unwell on the day of the clinic visit.

The parent(s) of the participant had the choice of either making their own way to the clinic or having a pre-paid taxi provided. Parents choosing to take their cars or public transport to the clinic were informed how to claim back their expenses, including any food they may have purchased during the journey. Those who opted to travel by taxi were informed that their chosen clinic would contact them prior to their appointment to confirm their attendance and the taxi arrival time. In cases where the closest clinic was more than one hour travel for the parent and child, or where a parent expressed an interest in participating in the clinic visit but was unwilling or unable to travel to a clinic, an appointment was offered on one of the mobile units to allow the appointment to be carried out at the participant's home address.

Following the clinic booking call, the clinic components that the parent proposed to be involved in were recorded in the database. This information was used by the stable isotope team, who prepared the correct stable isotope dose(s) and issued them to the relevant clinic in time for the participant's clinic appointment. The clinic also had access to relevant information for participants booked to attend their clinic, and they were encouraged to call the parent prior to the appointment to confirm attendance and, where appropriate to remind the parent to collect the appropriate urine sample(s) before the clinic visit and to take these with them to the clinic appointment. The parent was also reminded to take the child's usual feeding bottle in a 'ready to use' state for the administration of the stable isotope dose.

A.3.3.2. Clinic visit consent

At the beginning of the clinic visit, the nurse took written consent for all relevant components. To ensure that consent was fully informed, the process was staged into sections to allow the parent to ask questions about each component and to ensure it was clear which sections were being opted into or out of. The sections were as follows:

General statements to confirm:

- that the participant information sheet was read and understood
- that the parent understood that participation was voluntary and that they could withdraw their child at any time
- consent to inform the child's GP with any clinical results, and
- that anonymised blood and urine samples taken as part of the study may be analysed outside MRC Human Nutrition Research

Consent for:

- physical measurements and skinfold thickness measurements
- measurement of fluid and breast milk intake and body composition
- blood sample

Provision of:

- contact details where the parent did not want results sent to them personally or to the GP, so that clinically relevant results could be discussed

The parent of the participant was not required to complete all survey components and could choose which measurements to take part in. They could withdraw or change their mind at any time.

A.3.3.3. Physical and anthropometric measurements

Height of the mother was measured using a fixed stadiometer¹⁵ with a sliding head plate. Weight was measured using electronic scales with a digital display.

Infant length was measured using a Rollameter baby measure mat¹⁶ and weight was measured using electronic scales suitable for children, if available. If not, having weighed the adult, the adult plus the child was measured using electronic scales with a digital display. In order to obtain the weight of the child, the weight of the adult was subtracted from the weight of the adult and child.

Occipito-frontal circumference was measured using the Child Growth Foundation disposable head circumference tape¹⁷. Skinfold thickness measurements were taken using the Holtain Tanner skinfold caliper¹⁷. See Appendix H for physical measurement protocols.

Figures A.4a and A.4b. Measurement of subscapular and triceps skinfold thickness

Figure A.4a.



Figure A.4b.



A.3.3.4. Stable isotope methods

Two protocols were followed for the stable isotope component of the clinic visit. The two protocols were protocol 1 (breast fed protocol) for those children fed any breast milk and protocol 2 (non-breast fed protocol) for those children without any reported breast milk intake, or if unwilling or unable to participate in protocol 1.

Refer to Appendix J for full details of the "dose-to-mother"¹⁸ and technical stable isotope methods.

Figures A.5a and A.5b. Stable isotope equipment and dose being consumed

A.5a



A.5b



Protocol 1 – Breastfeeding mothers

Each mother who reported some degree of breastfeeding was asked whether she would be willing to participate in the breast milk intake aspect of the survey. If willing, she was asked to collect a pre-dose (baseline) spot urine sample from both herself and her child one to two days before attending the clinic and to take these samples with her to the appointment. If the mother arrived at the clinic visit without the pre-dose urine sample for herself or her child, then she was asked to collect pre-dose samples at the start of the clinic visit. At the clinic the mother was then given an oral dose of 50g of approximately 20% deuterium-enriched water, and the child was given a 4gkg^{-1} oral dose of approximately 5% ^{18}O -enriched water. The child's dose was calculated from the child's body weight obtained at Stage 1. The mother then collected further spot urine samples from herself and her child each day for 14 consecutive days, starting the day after the clinic visit. A breastfeeding diary was also introduced to the mother at the clinic appointment. The nurse explained how to fill it in and that only breast milk feeds, either directly from the breast or expressed, should be recorded. At the end of this period the urine samples and breast milk diary were collected by a NatCen interviewer and dispatched to HNR for analysis. At this interviewer visit, the weights of the mother and child were re-measured and recorded.

Protocol 2 – Non-breastfeeding mothers and breastfeeding mothers who declined to participate in Protocol 1

Each parent who was not eligible for, or each mother who declined to participate in the breast milk volume assessment, was asked if they would be willing to participate in the fluid intake and body composition component of the survey. If willing, the parent was asked to collect a pre-dose spot urine sample from the child and take it to the arranged clinic visit. If the parent arrived at the clinic visit without the pre-dose urine sample for the child, then the parent was asked to collect a pre-dose sample at the start of the clinic visit. At the clinic, the child

was then given a 4gkg^{-1} oral dose of approximately 2.5% deuterium-enriched water, based on body weight taken at Stage 1. The parent then collected spot urine samples from the child each day for five consecutive days, starting the day after the clinic visit. At the end of this period the urine samples were collected by a NatCen interviewer and dispatched to HNR for analysis. At this interviewer visit the weight of the child was re-measured and recorded.

A.3.3.5. Stable isotope analytical methods

The urine samples were analysed for deuterium and ^{18}O content as described below.

Deuterium

A $200\mu\text{L}$ aliquot of the urine was placed in a nominal 3.7ml vial along with a platinum catalyst mounted on a hydrophobic substrate. The vial was then flush filled with hydrogen gas at approximately 200mBar pressure and left to equilibrate at $22\pm 0.1^\circ\text{C}$ for at least six hours.

After equilibration the gas was admitted to the sample bellows of an isotope ratio mass spectrometer fitted with a dual inlet (either a Sira 10, VG Instruments, Middlewich, Cheshire, or an Isoprime, GV Instruments Ltd, Wythenshawe, Manchester. Two point calibration of the instrument (Standard Mean Ocean Water (SMOW)/Standard Light Antarctic Precipitation (SLAP) correction) was achieved for each batch of samples by incorporation of standard waters prepared identically. These standards were directly traceable to the primary reference standards obtained from the International Atomic Energy Agency.

Oxygen

A $250\mu\text{l}$ aliquot of urine was placed in a nominal 12ml tube which was flush filled with 5% CO_2 in N_2 to ambient atmospheric pressure. Equilibration of the oxygen in the water and that in the CO_2 was achieved overnight at room temperature with constant agitation of the tube.

After equilibration the isotopic composition of the CO_2 was obtained by measurement on an AP2003 (Analytical Precision Ltd, Northwich Cheshire) continuous flow isotope ratio mass spectrometer. The raw data was Craig corrected to account for the presence of ^{13}C , and then standardised via a two-point calibration to the international standard V-SMOW (Vienna Standard Mean Ocean Water) in a similar way to the deuterium analysis using reference materials interspersed in the sample batches.

See Appendix J for full details of the technical stable isotope methods.

A.3.3.6. Blood sample

A 3.9ml non-fasting blood sample was taken by venepuncture, by a nurse or phlebotomist with paediatric phlebotomy experience. Blood samples were collected using the Sarstedt Monovette blood collection system with a butterfly needle and syringe. A maximum of two attempts were made where parental consent had been given. The parent of the participant was offered the use of anaesthetic cream to reduce discomfort felt by the child. Blood was collected in a syringe and placed into two tubes, a 1.2ml EDTA monovette and a 2.7ml Serum monovette, in that priority order (see Appendix H for further details).

Each participant who took part in the blood sample component was awarded a certificate to acknowledge their contribution and bravery for having a blood sample taken.

Figure A.6. Blood sampling



The EDTA monovette was sent to Addenbrookes Hospital in Cambridge by first class Royal Mail post on the day of sampling. Where at least 1ml of blood was achieved for this tube, a full blood count (FBC) analysis was undertaken.

Once analysed, the FBC results were sent both by post and electronically to HNR in Cambridge, where the results were entered into the DNSIYC database and checked for abnormal results. Abnormal results were reviewed by the DNSIYC survey doctor and followed up appropriately with the parent. They were later merged into feedback letters and sent to the parent of the participant and the GP, if consent was obtained for these. The serum monovette was processed at the clinic location (i.e. in the clinic laboratory or in the mobile unit). The sample obtained was left to clot for one hour at room temperature before being centrifuged to separate the serum from the red blood cells. The serum was then aliquoted into two microtubes and placed into a -80°C freezer or a -40°C if a -80°C freezer was not available. Where a partial sample was achieved, this was noted at the time of blood taking on the Blood Tracking Form (BTF).

Aliquots in the north mobile unit were transferred to a -80°C freezer at the MRC Human Genetics Unit (HGU) in Edinburgh at the end of each week, to ensure the

integrity of the samples over the weekend when the mobile unit was not staffed or powered. The aliquots remained stored at MRC HGU until transferred to MRC HNR. Aliquots in the south mobile unit were transferred directly to MRC HNR at the end of each week. Aliquots were couriered on dry ice from each clinic site to MRC HNR at intervals. When the aliquots arrived at MRC HNR, they were checked, along with the BTF, against a sample list, and any irregularities were reported and corrected before the aliquots were entered into Item Tracker¹⁹ and placed in a -80°C freezer for storage prior to analysis. The sample list contained specific details relating to each sample, and was used to log the sample into Item Tracker. Item Tracker is used to determine the location of any given sample in a freezer, to provide full traceability and so that it can be found quickly and efficiently.

A.3.3.7. Blood sample analytical methods

Details of the laboratory methods used for blood analysis can be found below.

Serum 25-hydroxyvitamin D (25-OHD)

Vitamin D is produced in the skin by the action of UV light and is ingested in the diet. In its active form as a hormone, it plays a role in calcium metabolism and is important for bone health. Measurement of circulating total 25OH vitamin D (25-OHD) is accepted as the best indicator of vitamin D status.

The DiaSorin Liaison method for quantitative determination of 25-OHD is a direct, competitive chemiluminescence immunoassay (CLIA). A specific antibody to vitamin D is used for coating magnetic particles (solid phase), and vitamin D is linked to an isoluminol derivative. During the incubation, 25-OHD is dissociated from its binding protein, and competes with labelled vitamin D for binding sites on the antibody. After the incubation, the unbound material was removed with a wash cycle. Subsequently, the starter reagents were added and a flash chemiluminescent reaction was initiated. The light signal was measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25-OHD present in calibrators, controls, or samples.

Serum ferritin

Ferritin is a high molecular weight protein that functions as the primary iron storage compound in the body. Circulating ferritin concentrations reflect iron stores. A low serum ferritin concentration is an early indicator of iron deficiency anaemia.

Ferritin concentrations are increased as part of the acute phase response and so individuals with infections or inflammatory processes may have a raised serum ferritin concentration which can mask a decrease caused by iron deficiency anaemia. Therefore serum ferritin concentration should be interpreted in the light of other markers of the acute phase response.

Ferritin was measured on the Siemens Dimension Xp and analyser by a "sandwich" immunoassay method; the serum was incubated with a solid-phase antibody and a β -galactosidase labelled antibody, each directed against different sites on the ferritin molecule. The "sandwich" formed in the presence of ferritin was separated from unbound reagents and quantitated colorimetrically.

Serum transferrin receptors

The transferrin receptor aids the uptake of transferrin-bound iron into cells, especially during the production of erythrocytes in the bone marrow. The concentration of soluble transferrin receptor circulating in the blood is raised when there is increased production of red blood cells and is a clinical indicator of iron deficiency in the tissues. Unlike ferritin, this concentration is not affected by the acute phase reaction.

The serum transferrin receptor assay is a "sandwich" immunoassay method in which the diluted serum is incubated with a solid-phase antibody (on a 96-well plate) and a second, horseradish peroxidase-labelled antibody in solution. A "sandwich" is formed in which the antibodies are joined together by the transferrin receptor molecule. The resulting immobilised enzyme is separated from excess unbound reagents by washing, and quantitated colorimetrically in a plate-reader.

The sample dilution and assay can be performed manually but in order to improve assay robustness, MRC HNR has automated the full process using the BEST 2000 (Launch Diagnostics) automated sample processor.

Serum C-reactive protein

C-reactive protein (CRP) is an acute-phase protein; its concentration increases in the early stages of inflammation. It was measured in this survey in order to highlight ferritin results which may have been elevated by an acute-phase reaction and therefore may not be a straightforward indicator of iron status.

CRP was assayed using a Siemens Dimension Xp and Clinical Chemistry Analyser. The CRP method was based on a particle enhanced turbidimetric immunoassay (PETIA) technique, giving high sensitivity by extending the detection range down to 1.1 mg/L. Latex particles coated with antibody to CRP aggregate in the presence of CRP in the sample. The increase in turbidity that accompanies aggregation is proportional to the CRP concentration.

Detailed haematology results can be found in Appendix N and details of laboratory quality control can be found in Appendix O.

References and endnotes

¹ HMRC supplied a sample of names, addresses of child benefit claimants under Paragraph 9, Schedule 5, Tax Credits Act 2002 which gives authority to supply information to other Departments for the purposes of provision of information for health purposes. CB records were used as a sampling frame and selected sample supplied to the Department of Health (DH) for the purpose of DNSIYC. The sample transfer between HMRC and DH was in line with Government security standards and with the agreement of the HMRC Data Guardian from the business area from which data is sourced. Data transfer was in adherence to the strict data transfer rules, and with the correct legal gateways in place.

² Healthy Start is a Government scheme set up to offer a nutritional safety net for pregnant women, new mothers and children under 4 years of age in very low income families, and encourage them to eat a healthier diet. The scheme provides vouchers to put towards the cost of milk, fruit and vegetables or infant formula, and coupons are also given to exchange for free Healthy Start vitamin supplements (see Annexe A).

³ Details of the Diet and Nutrition Survey of Infants and Young Children in Scotland, 2011 can be found in a separate report on the Scottish Government website. Available online: <http://www.scotland.gov.uk/Publications/Recent>

⁴ The children only receive breast milk without any additional food or drink, not even water.

⁵ World Health Organization. The optimal duration of exclusive breastfeeding: Report on an expert consultation. Geneva (2001)

⁶ Department of Health, Infant Feeding Recommendation, 2003 Available online at: http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4096999.pdf

⁷ Complementary foods/feeding: the period where infants make the gradual transition from liquid foods to eating solid and family foods.

⁸ A congenital abnormality that affects feeding is defined as a physical defect that was present at birth which is likely to impair feeding or growth.

⁹ Minimum sample per PSU was based on CB counts since all HS children are assumed to also be claiming CB.

¹⁰ For further details on how ratios were calculated see Appendix B.

¹¹ Biro G, Hulshof K, Ovesen L and Amorim Cruz JA (2002). Selection of Methodology to Assess Food Intake. *European Journal of Clinical Nutrition*. 56, 2 pp. 25-32

¹²

http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsStatistics/DH_128166 Accessed: 31/1/12

¹³ Lanigan JA, Wells JCK, Lawson MS & Lucas A (2001). Validation of food diary method for assessment of dietary energy and macronutrient intake in infants and children aged 6–25 months. *European Journal of Clinical Nutrition*. 55, pp. 124–129

¹⁴ http://www.medscope.co.uk/Seca_Leicester_Stadiometer_for_mobile_use~pp~428.htm Accessed: 11 August 2010

¹⁵ <http://www.healthforallchildren.com/index.php/shop/product/Length+Measure/3QG1RHR5CIDVYeQe/0> Accessed: 11 August 2010

¹⁶ <http://www.childgrowthfoundation.org/ghd.htm> Accessed: 11 August 2010

¹⁷ <http://www.holtain.com/tw.php> Accessed: 11 August 2010

¹⁸ Coward WA, Sawyer MB, Whitehead RG, Prentice AM, Evans J. New method for measuring milk intakes in breast-fed babies. *Lancet*. 1979 7:13-4

¹⁹ Item Tracker: the MRC HNR sample management and tracking software.

A.4. Comparison of dietary to blood data

The results in Chapter 6 are based on assessment of food consumption over four days (recorded in the food diary at Stage 1) and indicate dietary intake over a short period. Analysis of blood samples (collected at Stage 2) provides an indication of the nutritional status of the population usually over a long period. Nutritional status means the level of nutrients available to the body (after absorption) for use in metabolic processes and in this age group includes stores acquired in utero. In DNSIYC, dietary intake therefore cannot be compared directly to nutritional status in individuals or groups as status does not just reflect the intake of nutrients from the diet. In DNSIYC the focus was on two key nutrients (iron and vitamin D).

A.5. Age at Stage 2

The age of the participant was recorded during the home interview and again when the clinic visit took place. Every effort was made to ensure that the clinic visit took place as soon after the home interview as possible to minimise the age difference between these two components of the survey. To ensure the accuracy of results reported for Stage 2, the age of the participant at the time of the clinic visit has been used for presentation of results which relate to the clinic visit measures.

A.6. Mobile unit

Two mobile units were purchased for DNSIYC in order to reach the most rural participants, parents who requested a mobile unit visit to negate the need to travel with young children, and for those who had very limited time. One mobile unit (North mobile unit) was based at the MRC Human Genetics Unit in Edinburgh, Scotland and the other (South mobile unit) was based at MRC Human Nutrition Research in Cambridge, England.

The mobile units held all equipment necessary for a clinic visit and also allowed the processing and storage of blood samples. In one mobile unit the fridge and freezer (-40°C) ran from a 12V battery and alternator system and in the other mobile unit the electrics (including fridge and -40°C freezer) ran from a generator. In addition the mobile units were equipped with lighting, heating and air conditioning to ensure comfortable and appropriate conditions for participants and staff in all weather conditions.

The mobile units were used in the field primarily from Monday to Thursday and were driven back to their base locations on a Thursday evening. For blood samples taken, the EDTA monovette was posted that day to arrive the next day at Addenbrookes for immediate analysis. Clinic visits which included a blood sample were not possible on a Friday as weekend analysis was not available at

Addenbrookes. The serum monovette was transferred into a -80°C freezer at the relevant MRC location and stored until analysed.

In the field each mobile unit was staffed by one phlebotomist-research assistant and one driver-research assistant.

A.7. Feedback to participants and General Practitioners (GPs)

Individualised dietary and breast milk intake feedback was provided to parents of the participant with their agreement. Provision of feedback was seen to be an incentive to participate in the survey since it would provide parents with information about various aspects of their child's diet. For each participant where a blood sample was obtained, feedback of blood results was also sent to the parents and GP (if consent was given) with guidance for parents on how to interpret the results. (For examples of participant feedback see Appendix K).

A.7.1. Dietary feedback

Parents who agreed were provided with individualised dietary and breast milk intake feedback for their child. This feedback provided information on the child's intake of energy and four key nutrients (protein, vitamin C, iron and calcium) and how the intakes of these compared to recommendations for that age group. This information was provided in the format of a graph and was accompanied by information explaining the role of each of these nutrients in healthy development. A healthy eating advice letter was provided alongside the feedback letter, containing a list of healthy eating resources so that parents could find out more if they so wished.

Feedback letters differed depending on whether or not the child received any breast milk over the recording period. There were two versions of the feedback, one for those whom breast milk was part of the child's intake and one for those children who had no breast milk. Both were for children who were eating foods or drinks as well as milk. There were versions for children aged less than 12 months and for those aged 12 months and over. Each version of the feedback had appropriate wording and photographs and reference intakes for the appropriate age range. Those who were exclusively milk fed (breast and/or formula) received a letter explaining that all of the child's nutritional needs would have been met on this diet for the first six months of life.

Covering letters were provided for children who had been ill or who were on a special diet over the recording period, with a note for parents to consider this when reading the feedback. For instance, a child who was ill for three out of four days of the recording period may have consumed less food than usual, and this would be reflected in lower nutrient intakes. Alerting the parents to this put the feedback into perspective and prevented any unnecessary concerns.

A.7.2. *Breast milk volume feedback*

The feedback of breast milk intake was sent exclusively to the parents of participants who reported some degree of breastfeeding and took part in stable isotope Protocol 1. The aim of the breast milk feedback was to provide each breastfeeding mother with an indication of the volume of breast milk consumed by the child. Two pieces of information were provided within the feedback:

- 1) Volume of breast milk consumed per day (ml), and
- 2) Percentage of the total fluid per day which was consumed as breast milk

The feedback also included general information on sources of healthy eating advice for young children.

A.7.3. *Blood analyte feedback*

If consent was given at the clinic visit, feedback of clinically relevant blood results was issued to both the parent of the participant and the participant's GP by letter. Feedback of blood results was reported in two letters due to the timing of analysis. The first letter reported results from the full blood count analyses (measured by Addenbrookes) and was posted to participants in batches within two months of the clinic visit. The second letter contained results of the analyses for iron and vitamin D status; this was posted to participants within four months of the clinic visit.

A.8. Fieldwork quality control

Interviewer fieldwork in England, Scotland and Wales was carried out by NatCen. In Northern Ireland, fieldwork was carried out by interviewers working for the Northern Ireland Statistics and Research Agency (NISRA). All interviewers working on DNSIYC were briefed and trained before undertaking an assignment and were monitored during their assignment by a supervisor. Fieldworkers were also issued with comprehensive written instructions covering survey procedures and measurement protocols.

The CAPI program included various checks to ensure that the physical measurements were being taken accurately and correctly and that the protocol was being followed. The program also allowed space for interviewers to record any additional contextual information.

Food diaries were collected by interviewers and sent to HNR for coding via NatCen's Operations Department. Throughout fieldwork the standard of each interviewer's work was monitored closely by a series of "early work" checks

carried out by the Dietary Assessment Team. Timely feedback on quality issues was provided to the interviewers via NatCen.

Quality control days for infant length and head circumference measurements taken by interviewers were held part way through Stage 1 fieldwork to ensure that interviewers were demonstrating acceptable levels of precision when taking the anthropometric measurements (see Appendix L).

A.8.1. *Training for interviewers*

All DNSIYC interviewers attended a two-day training course where they were fully briefed on the protocols and administration of the survey.

The briefing covered background and content, questionnaire administration (including practice sessions) and placement and collection of the four-day food diaries, equipment and introducing the tracer water (stable isotope) component as well as the clinic visit. Interviewers at the briefings were also trained in taking maternal height and weight, and infant length, weight and occipito-frontal (head) circumference measurements. The briefings incorporated a formal accreditation process which all interviewers measuring infant length and head circumference had to complete and pass. These sessions were led by paediatric nurses from the Department of Paediatrics, University of Cambridge. NatCen interviewers were observed by the nurses performing each of the measurements on a life-sized doll. The purpose of the accreditation process was:

- to assess interviewer competence in performing measurements accurately and confidently
- to ensure that interviewers were comfortable performing each of the measurements and were confident carrying them out in the field
- to ensure that the measurements were carried out accurately

NatCen interviewers were not permitted to work on the survey until they had passed the accreditation process.

All interviewers completed a homework exercise which they were required to complete and return prior to the briefing. The exercise was aimed to familiarize interviewers with the four-day food diary.

A.8.2. *Training for nurses and other clinical staff*

At each site a clinician was recruited to have oversight of the procedures being conducted in the clinic visit at their site. The measurements were carried out by hospital/CRF staff. The role of the clinician was to ensure compliance with protocols, competency, to provide supervision and guidance and to become involved if performance or other issues arose with the clinic visit. For the

Cambridge site and the mobile units the role of oversight was given to two nurse supervisors under the supervision of the survey doctor.

Quality control for skinfold thickness measurements was carried out half way through the study to ensure that clinical staff at all sites were demonstrating acceptable levels of precision when taking these measurements (see Appendix L).

Clinics were provided with a full clinic protocol, document packs that contained all the necessary documents to carry out a single clinic visit and blood sample packs which contained consumables for collecting a blood sample. All equipment was provided to clinic sites prior to the training day and the start of the survey, so that everything could be viewed, discussed and explained. A disc containing instructions to install the survey database was provided to each site and passwords were issued separately. Clinics only had access to data relevant to their site and the booked appointments.

A one-day training was undertaken at each clinic location and covered the following:

- Background and aims of the survey
- Overview of Stage 1
- Informed consent
- Survey documentation/equipment
- Survey protocols
- Training on specific protocols, i.e. skinfold thickness and stable isotope protocols
- Survey identification (ID) labels
- Blood processing
- Data transfer

A further day of training was necessary for the mobile unit staff to brief them on the practical issues relating to the mobile units including use of the heating, battery charging systems, lighting, generator and use of electrical equipment.

An MRC HNR survey team member was present for the first day of clinic appointments at each clinic site to offer support and reassurance when the first visits with participants were undertaken.

A.9. Tokens of appreciation

At the final visit, the interviewer gave each participant's parent completing at least three food diary recording days a token of appreciation (£30 in high street vouchers).

Tokens of appreciation were also given for each component of the clinic visit. Each participant was given £10 in high street vouchers for taking part in Stage 2. A further £30 in vouchers was given for taking part in the stable isotope measurement or £50 for the more intensive breast milk intake protocol. An additional £30 was given to each participant's parent who took part in the blood sample component, where a sample was attempted, regardless of whether it was obtained. A toy was also used to distract the child to increase compliance for the components, and was given to the child to take home.

A.10. Statistical methods

A.10.1. *Statistical methods used in DNSIYC*

This section outlines the methods used to analyse the DNSIYC data. Different methods were used to compare estimates from independent sub-groups within the main sample and estimates from overlapping sub-groups. All statistical tests were carried out using statistical methods that allow for complex sample designs to be taken into account, these allow more accurate standard errors¹ to be generated.

A.10.1.1. Complex samples

DNSIYC has a complex survey design. The sample has been stratified by region, local deprivation indices and population density and is geographically clustered within postcode sectors. Stratification generally improves statistical precision. Stratification will reduce standard errors if the stratification variables are correlated with the survey outcomes. This should be the case in DNSIYC as there is a known relationship between diet and deprivation².

Clustering a sample will generally reduce statistical precision, particularly if the individuals within a cluster are very similar to each other but very different to those in different clusters. Despite this DNSIYC, like most large-scale surveys that use face-to-face interviewing, is clustered. This is because clustering a sample has huge practical advantages for fieldwork costs and interviewer management.

All statistical tests in the DNSIYC report were conducted using complex samples methodology. Using complex samples methodology means information on sample stratification and clustering is incorporated into the calculation of standard errors, making the standard errors more accurate. A standard error based on an assumed simple random sample (which is the default for statistical analysis carried out in packages without complex samples modules) would not incorporate this information and would be more conservative as a result.

The method of generating standard errors is important because standard errors are the basis for all statistical tests. If a standard error is conservative (i.e. smaller than it should really be), the resulting test will be more conservative and may give a negative result when a more accurate test would show a significant relationship. Significant relationships may go unreported as a consequence.

The availability of complex samples was, until relatively recently, not standard in statistical packages and complex standard errors were difficult to run. This meant it was customary in the past to ignore the sample design during analysis and treat the sample as if it was a simple random sample. Tables of design factors³ were generated and used as an accompaniment to the main report, this was done in the 2003 NDNS report, for example. Statistical packages are now able to generate complex standard errors as routine, removing the need for such technical tables. For DNSIYC we have used complex standard errors in all statistical tests.

All analysis of DNSIYC was carried out using SPSS (PASW version 18)⁴. The analysis was based on all fully productive interviews and with the appropriate weights applied to adjust for individual selection and non-response (see Appendix B for more details about the weights).

A.10.1.2. Comparisons of estimates from independent sub-groups

This section outlines the methods used to compare estimates from two independent sub-groups within the sample, for instance parents in managerial and professional, versus parents in intermediate occupations, parents in routine and manual occupations and parents in non-classifiable occupations.

Tables were run using a number of break variables, including NSSEC, ethnicity and age. Comparisons were made between the survey estimates from different categories of this break variable.

The first step was to test the variable of interest (the dietary or nutritional measure collected by the survey) for normality. This is done to ensure the data are normally distributed as this impacts on what statistical method can be used. If the data are normal a linear regression analysis has been used to test whether the variable of interest varied significantly between different sub-groups. If the variable of interest was not normally distributed then it was recoded into a binary variable, where 1 indicated the value was greater than the mean and 0 indicated otherwise. The recoded variable was analysed using a logistic regression model.

As the DNSIYC is stratified and weighted, the regression analyses were fitted using statistical methods that take into account the sample design. The regression coefficients in the linear regression analyses were estimated using

probability weighted least squares and those from the logistic regression used a pseudolikelihood approach. The standard errors of these estimates were estimated using a Taylor linearization method. These methods of estimation are available in SPSS as part of the Complex Samples option. The complex samples option in SPSS allows analysts to specify the cluster, strata and weight variables by setting up a plan file which is used by SPSS in the regression analyses to adjust the standard errors around the estimates and therefore obtain more reliable results.

Corrections for multiple testing, such as the Bonferroni correction, have not been employed here. These adjustments are used if testing sets of highly correlated outcomes, for example, comparing mean intakes for a large number of different food types.

While a number of the outcomes are correlated, this is not the case for all outcomes. In addition, the approach outlined above uses a regression model to test for differences across parameter estimates. This reduces the chances of finding significant results due to multiple testing across different subgroup categories, since it identifies where there are significant overall differences in the outcome variables across the subgroup, rather than comparing outcomes for each separate subgroup category. For example, the tests will show whether the calcium intakes for children vary significantly across NSSEC groups, they do not test for differences in the calcium intake of children with parents in managerial and professional occupations versus children with parents in intermediate occupations, and repeat the test for managerial and professional occupations versus routine and manual occupations, and so on (until all combinations had been tested).

Taking this approach means fewer tests are carried out. The latter analysis would require more tests to be carried out, as such it would be more likely to produce a significant result and would require an adjustment, such as Bonferroni, to be made.

A.10.1.3. Comparisons of estimates from over-lapping sub-groups

A different methodology was used to test differences between estimates from Scotland (or from the Healthy Start boost sample) and estimates for the whole UK. This is because the estimates being compared are taken from overlapping samples, meaning individuals in Scotland are counted twice.

The first step was to generate a point estimate for the difference (d) between the UK and Scottish estimates. This was simply the Scottish estimate minus the UK estimate and was calculated as follows:

$$d = \bar{x}_{Scotland} - \bar{x}_{UK}$$

The next step was to calculate a standard error for this difference. This will be used to generate confidence intervals that can be used as a significance test. The usual approach would be to combine the standard errors from both the Scottish and UK estimates. However, in this instance this cannot be done because the UK estimate represents a pooled estimate across all four countries – including Scotland. Instead we need to calculate a standard error for a 'UK' estimate based on data from England, Wales and Northern Ireland only. The standard error for the difference can then be calculated by the formula:

$$se(d) = \frac{r}{t} \sqrt{se(\bar{x}_r)^2 + se(\bar{x}_{Scotland})^2}$$

where r refers to the weighted sample size of UK minus Scotland and t refers to the weighted total sample size for the UK.

In summary, although the UK estimate is used in order to estimate the difference between Scotland and the UK, the confidence interval for that difference uses a standard error for the UK estimate that is computed without Scotland.

A 95% confidence interval for the difference between the estimates can then be calculated in the usual fashion:

$$d \pm 1.96 * se(d)$$

If the confidence interval did not include zero then the difference between Scotland and the UK was said to be significantly large. Our null hypothesis is that there is no difference between Scotland and the UK, hence we would expect the confidence intervals to lie around zero.

References and endnotes

¹ All samples contain error. There are two main types of error – random error and systematic error. A general discussion of the impact of these errors on survey results can be found in Appendix A of Ruston D et al, The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 4: Nutritional status (anthropometry and blood analytes), blood pressure and physical activity. TSO (London 2004)

² Using a combination of local deprivation indicators and population density ensures we have a good spread of deprived inner city, deprived rural and suburban areas in the sample.

³ The design factor shows the impact of the sample design on the standard errors and can be used to adjust the non-complex standard errors produced in the main body of a report.

⁴ NatCen uses SPSS version PASW (1200) 18.0 with the following add-on options: Tables Original (1201) 18.0; PASW Regression (1202) 18.00; 1210 (1210) 18.0 and PASW Complex Samples (1211) 18.0.