# NINUB (NIN and Univ. of Bristol) Collaborative Study: Effects of supplemental nutrition in pregnancy on cardiovascular disease risk in childhood

**Protocol v5**

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**&**

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## 1. Study background

### *1.1. Study background*

This research involves follow-up of children born to women participating in the ICMR-USAID collaborative study on Maternal Nutrition and Low Birth Weight, a study originally set up to examine the effects of supplemental nutrition during pregnancy on birth weight of the offspring. All women who became pregnant in a geographically defined population of 29 villages around Hyderabad city between 1987 and 1990 were invited to participate (following a pilot study in the last six months of 1986). A cluster of villages (15 and 14) with a total population of 30,000 was chosen from each of the two adjacent blocks, only one of which had in operation at the time, a programme of supplemental nutrition in pregnancy and childhood (ICDS programme). Detailed questionnaires and anthropometrical examination were carried out at several time points on the participating women during pregnancy and the newborns during the first year of life. Participation in the study is believed to be complete but impossible to determine accurately due to the cultural practice of delivering, most frequently in the case of firstborns, at the parental home of expectant mothers.

The aim of this follow-up is to study the role of early life nutrition in 'programming' an individual's cardiovascular disease risk and this aim will be achieved by relating the growth and nutrition data from the earlier survey to the cardiovascular disease risk factor data that we plan to collect now (age range 14-17 years).

### *1.2 Hypotheses and outcome measures*

The aim of this study is to investigate the role of early life nutrition towards programming of cardiovascular disease risk.

<u>Hypotheses to be tested</u>

1. The main hypothesis under consideration is that supplemental nutrition during pregnancy reduces the lifetime risk of cardiovascular disease amongst chronically malnourished populations.
2. The secondary hypotheses include the protective effects of dietary intake in pregnancy and childhood, supplemental nutrition in childhood, and birth

weight and growth during pregnancy (as indirect markers of nutrition in early life) towards risk of cardiovascular disease.

Outcome measures

*Main outcome measures*

- Arterial stiffness (pulse wave velocity and augmentation index)
- Blood pressure

*Other outcome measures*

- Central and generalised adiposity
- Atherogenic blood profile (fasting sugar, triglycerides, and cholesterol)
- Insulin resistance (HOMA)

Exposures

*Main exposure*

Nutritional supplementation during pregnancy

Possible confounders

- Timing of puberty
- Anaemia
- Dietary intake in pregnancy and childhood
- Nutritional supplementation in childhood
- Behavioural risk factors (sedentary lifestyle, smoking and substance abuse)
- Socio-economic status, especially maternal education
- Parental history of cardiovascular disease
- Maternal body size and current body size (for birth weight)
- Presence of ICDS programme (nutrition and health education, reduced infections through immunisation)
- Urbanisation

Possible mediators (factors altering as a consequence of nutritional supplementation)

- Dietary intake during childhood
- Current body size

### *1.3 Planned study investigations*

The study investigations on approximately 1,000 subjects will include:

Child (i.e. subject)

Interviewer completed questionnaire

Clinical examination

Blood investigations

Mother

Short interviewer completed questionnaire to corroborate early life data on the child

Height and possibly weight

Random 5-10% sample of study subjects (to compare the two main groups)

Validation studies – diet by detailed FFQ (or 24-hr diet recall), physical activity by vertical accelerometers, pubertal assessment in boys by direct examination (Tanner)

Village head

Interviewer completed questionnaire on village urbanisation

Census data

Change in urbanisation between 1991 and 2001.

**Table 1. Main outcome measures: NINUB study**

| Outcome | Instrument/Method | Comments |
|---|---|---|
| **Anthropometry** | | |
| Height | GPM anthropometer/Microtoise | |
| Sitting height | Above with stool | |
| Lower leg length | Chasmors knee height caliper | |
| Weight | Digital scale | |
| Waist circumference | BMI body tape/Metallic tape | |
| Hip circumference | BMI body tape/Metallic tape | |
| Mid arm circumference | BMI body tape/Metallic tape | |
| Thigh circumference | BMI body tape/Metallic tape | |
| Head circumference | Lasso tape/Metallic tape | |
| Skin folds (biceps, triceps, sub-scapular, and supra-iliac) | Holtain skinfold caliper | |
| | | |
| **Vascular physiology** | | |
| Blood pressure | OMRON HEM 705CP | Medium and small cuff |
| Augmentation index (general arterial stiffness) | Sphygmocor (carotid) | |
| Pulse wave velocity (aortic) | Sphygmocor (carotid-femoral) | |
| | | |
| **Biochemistry** | | External QC - WHO |
| Haemoglobin | | |
| Plasma viscosity | | |
| Fasting sugar | | |
| Fasting cholesterol (total and HDL) | | |
| Fasting triglycerides | | |
| Fasting serum insulin | Radio-immuno assay | |
| | | |
| **Diet and physical activity** | | |
| Questionnaire (short FFQ and activity questions) | Random 5-10% sample to compare the two main groups | Detailed FFQ (or 24 hr diet recall) for diet; Vertical accelerometers (CSM) for |

| | | |
|---|---|---|
| | | PA |
| | | |
| **Pubertal status** | | |
| Questions on timing of menstruation (girls) | | |
| Assessment of voice (text reading) & self assessed testicular volume (boys) | Using Prader orchiometer | Subgroup validation (5% sample) with full assessment in boys |
| | | |
| **Maternal anthropometry** | | |
| Height | GPM anthropometer/Microtoise | |
| ?? Weight | Digital scale | |
| | | |
| **Urbanisation** | | |
| Village head questionnaire | | |
| Census data comparison (1991 and 2001) | | |
| | | |
| **Other measures** | | |
| Room temperature | Digital thermometer | Inside-outside |

## 2. Follow up methods

### *2.1 Study cohort: sample size and tracing of the subjects*

In the existing main dataset, there are just over 3,500 unique women, and of these, names (women and husband) exist for 2,012. There are additional electronic and paper records, and these numbers are likely to change as the datasets get cleaned and records reconciled.

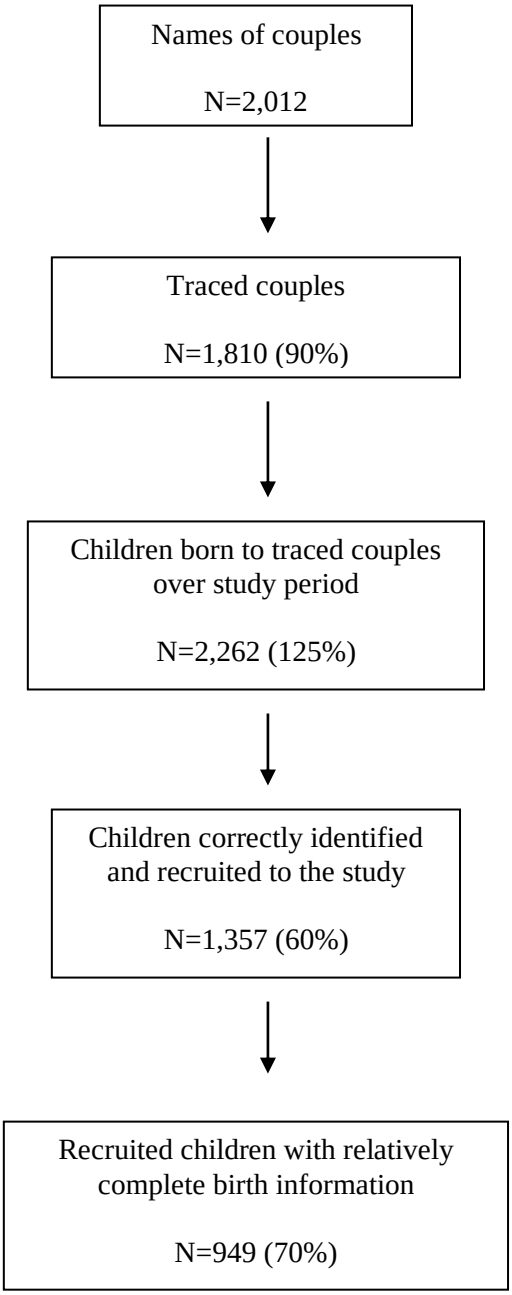
The couples will now be traced by village and linked to the women and child data in the dataset. As the villages are small, the uniqueness of the parents is relatively easy to establish and we expect to trace and identify 90% (n=1,810) of these. However, as exist no unique identification numbers/names for children, the children will be

matched to the existing data through other information such as date of birth and sex, birth order, and maternal recall of child being part of the earlier study.

To avoid bias being introduced due to the fieldworkers knowledge of the details of study child, this process will be carried out in a blinded fashion. The fieldworkers involved in subject tracing will be aware of the names of the couples but will not know which of their children is part of the study cohort. On finding a study couple, the fieldworkers will collect information for each of their children using a questionnaire (see Appendix), which will then be entered on to a database and matched on pre-established criteria by the study co-ordinators. A suggested algorithm for matching is presented; however, other existing information may also be used in this process.

The final dataset with relatively complete information for analyses is expected to be around 1,000 subjects (see flow chart).

**Fig. 1. Flow chart of study sample**




*Forecasted figures (likely to change after data reconciliation and depending on response rates)

### *2.2 Subject (child) identification algorithm*

The following algorithm will be used to trace subjects on the basis of existing information

(A) If ALL of the following four match perfectly:
Village name
Family name
Mother's first name
Father's first name

Then accept identity of the participant if either:

1. Age of the participant accurate to 6 months, **OR**
2. Age of the participant accurate to 1 year AND at least two of the following three match accurately
    - Birth order
    - Sex
    - Maternal recall of the participant being born in the study

(B) If THREE of the following four match perfectly:
Village name (compulsory)
Family name
Mother's first name
Father's first name

Then accept identity of the participant if:

1. Age of the participant accurate to 6 months, **AND**
2. At least two of the following three match accurately
    - Birth order
    - Sex
    - Maternal recall of the participant being born in the study

### *2.3 Framework of surveys*

The study team comprises of:

- **Two social workers** (a male and a female)
- **One village worker** (appointed locally in each village)

- **Doctor**
- **Nutritionist**
- **Biochemist**

The social workers will work as a pair and will be responsible for tracing and recruiting the subjects. Additionally, a local village worker will be recruited on a short-term basis in each village to provide bring local knowledge and support to the team. The doctor and the nutritionist will carry out the assessments on the participants.

The social workers roles will include tracing of the participants, securing their consent, organising appropriate venues to hold the clinics and arranging appointments for the participants, and ensuring arrival of the participants, in a suitably fasted state, to the clinic by either bringing them personally or arranging transport as required. In their spare time, they will also help with the clinic assessments, mainly questionnaire completion, and possibly data entry.

The doctor and the nutritionist will run the clinics, which could be based at some prominent place in the village itself or a district hospital or school. The doctor will manage the team, carry out arterial stiffness and blood pressure measurements, and take blood samples. The nutritionist will be responsible for questionnaire completion and anthropometrical examination. The clinics will start early, say 8 AM, and run only in the mornings for ease of ensuring fasting status and return of blood samples for processing within 4-5 hours. The doctor and nutritionist will devote the remainder of the afternoons to paper work and completing data entry.

<u>Social worker team</u>

1. They will arrive at each village with a master list of participants believed to be living in the village.
2. On arrival, they will meet the village head, explain the study and seek his/her approval and consent.
3. Once consent from the village head is secured, they will show the village list to him/her and seek information on the current residence status of each of the participants.
4. It is expected that the village head will know most people by name in a small size village but fewer people in the bigger villages. Where the village head is

unsure about a person's existence or whereabouts, the team will try to find their house and confirm their presence or otherwise.

5. During this first visit, the team will request the village head to arrange a meeting with all those likely to be invited to participate in the study and the village council.
6. At this joint meeting, the study will be explained followed by a question and answer session. If there is general consensus for the study to go ahead, discussions for possible venues and suitable timings will also be carried out
7. Following this meeting, on the same day or on subsequent visits, each of the participants will be met personally to get provisional verbal consent and agree some provisional dates for clinic visit. At this point, possible sites for clinics should also checked and most appropriate one selected on the basis of facilities such as availability of electricity, space and approachability for participants and study team.
8. Based on the numbers agreeing and dates, the team will arrange for a suitable venue for clinics and agree firm timings for clinic visits, explain fasting requirements, and agree transport arrangements. This is likely to be around 2-3 weeks before the clinic date.
9. A visit on the day before the clinic visit may have to be carried out (or alternative arrangements made through some village person) to remind the participants, especially about the fasting. This visit may also be used to double-check the venue for clinics.
10. The team may have to bring the participants either personally to the clinic or arrange for some form of transport to pick them. This is to ensure punctuality and prevent wastage of clinic time. For their return, the participants may have to be offered appropriate transport fares for return or arrangements made.
11. The team will be supported in their activities by the locally appointed village worker who will bring local knowledge and support to the study. The village worker will also be able to carry out follow up and tracing visits outside the 9-5 hours by virtue of living in the village.
12. The team may also have to help out in setting up the clinics and equipment.
13. The team may also help out with the reception duties at the clinic, organising the flow of participants in the clinic, and arranging for their departure after refreshments.
14. The team may also help out with the completion of questionnaires in the clinic, and may also help out with data entry depending on aptitude and time availability.

The clinical team

1. The clinical team will be made of the doctor and the nutritionist.
2. The clinics will start early and run in the mornings only, say 8-12 AM. For this, the team will have to leave early, say 7 AM to arrive at the clinic and set up equipment before arrival of the first participants at 8:00 AM.
3. Participants will be invited to come with their mothers preferably.
4. On arrival, the nutritionist will set up questionnaires and anthropometrical equipment and the doctor will do the same for arterial stiffness machine and blood sampling equipment.
5. Once the participant arrives, the nutritionist will explain the procedures and take voice recorded/written consent from the mother and the child for the study.
6. Following this, the doctor will take the blood sample followed by anthropometrical examination by the nutritionist.
7. After the anthropometrical examination, the doctor will carry out the arterial stiffness and blood pressure measurements.
8. Questionnaires may be completed at any stage by either member of the clinical team or even members of the social worker team depending on availability of time.
9. There may have to be certain flexibility in roles of the members of the clinical team as follows:
    a) Questionnaires – any member of clinical or social worker team
    b) Anthropometry and blood pressure – doctor or nutritionist
    c) Arterial stiffness – doctor only
    d) Blood sampling – doctor or biochemist
10. After completion of the assessments, the participants will be offered some refreshments, a small gift (educational material), and return fare as appropriate.
11. A small sample of participants pre-selected randomly will be invited to return for validation and repeatability studies.
12. On completion of a clinic, the nutritionist/biochemist will make arrangements for the transport of blood samples and the doctor will check the completed questionnaires and arterial stiffness data on the laptop.
13. On arrival back at the NIN, the biochemist will ensure that the samples are quickly centrifuged and processed or stored in aliquots at the laboratory with appropriate labels.

14. The clinical team will spend the remainder of the afternoon in filing the questionnaires and datasheets, downloading and saving the arterial stiffness and consent recording data, checking the data quality and helping with data entry as necessary and make preparations for the next day.
15. The day will end early on account of the early start say 3 PM.
16. Last day of the week, the team will carry out a small number of assessments relating to validation and repeatability studies, check for completeness and errors in data entry, and transmit a data file and completed summary sheet for the week to NIN and Bristol.

The time plan will be as follows:

- **Week 1:** Social workers and nutritionist start tracing of study participants
- **Week 4:** The doctor and biochemist join the study and spend the next two weeks in training and testing of the protocols.
- **Week 6:** Clinics begin. The doctor and nutritionist run the clinics and the social worker teams carry on participant recruitment.
- **Month 8-10:** Fieldwork completed

### *2.4 Consent*

- A village meeting with prospective study subjects and village elders will be arranged 3-4 weeks (at least 2 weeks) before the planned time of examinations to explain the study and allow a question-answer session for clarification of doubts. Written information sheet will be provided to the subjects. Those not able to make it to the meeting will be visited in person.
- Over subsequent weeks subjects will be visited personally to invite and arrange appointment for the study examinations. They will again be offered opportunity to clarify any doubts.
- At the time of the examinations, the study subjects (children and one of the parents) will be asked to provide written signed and/or verbal consent. Where they are not willing for either but agreeable to participate in the study, the researcher will sign the form on the behalf of the subject. Where parents do not come with the study subject at the time of the examinations, consent may be sought at the time of home visits where possible. Children will be asked to consent for themselves while one of the parents will be asked to consent on behalf of the family.
- The village head (or representative) will be asked to sign a consent form on the behalf of the village.
- We will also aim to invite an external observer at some stage of the study to observe and comment on the procedures being followed.
- A sample information sheet and consent form is included (see Appendix). This form will be translated into local language and back translated to ensure accuracy of language.

### *2.5 Blood sampling*

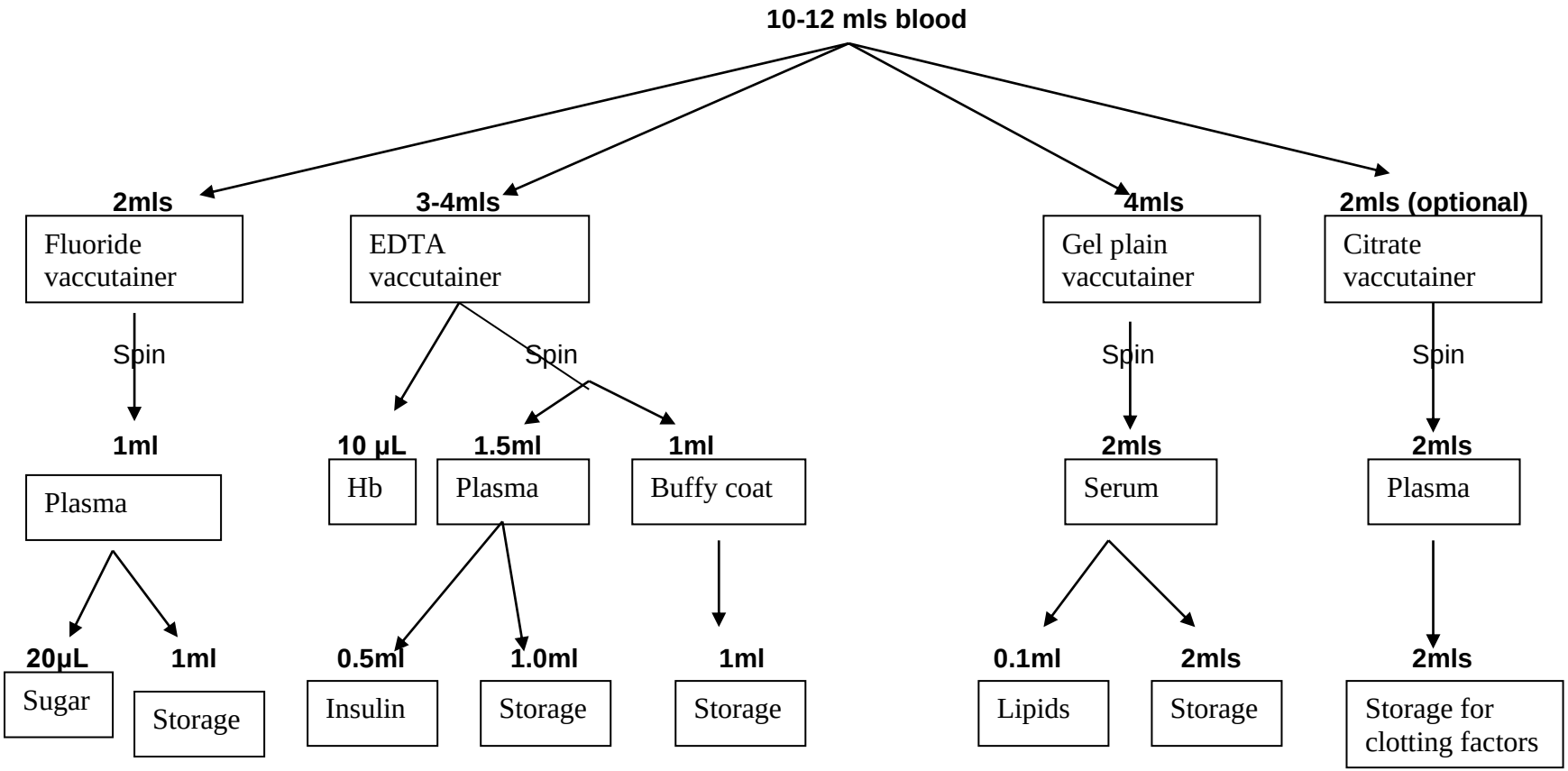
- Each participant will be asked to provide 10-12 mls of blood
- Tourniquet will be used for sample collection and use of anaesthetic cream will be offered to the subjects
- One ml of blood will be used immediately for assays and 5-7 mls will be available for storage. Blood will be stored after labelling with appropriate quality labels at –80 degree Celsius

- A maximum of two attempts, only one on any arm, will be made with the young person's permission. If the subject shows disinclination or appears distressed at any stage, the procedure will be abandoned.
- Blood samples may be centrifuged on site or back at base depending on the arrangements and time lag between collection and processing of blood samples
- The details of how blood will be spilt up and processed is included in the flow chart (see Figure 2)
- External quality control arrangements will be made with the WHO-QC provision.

### *2.6 Validity and reliability studies*

- Validity of measurements will be ensured by detailed protocols, regular training sessions (at the start and then every 3 months), and daily calibration of equipment that need calibrating as detailed under the section on measurement protocols.
- Asking one measurer to carry out the same measurement for all the subjects will ensure reliability. Monthly reliability studies will also be carried out at the end of each month between the lead measurer and the person designated as the back up measurer for a given assessment. Study will recommence only after the two measurers have reached a previously defined level of agreement between their measurements.
- Repeat measurements in sub-sample (??).

**Fig. 2. Blood collection plan: NINUB study**




Total blood collected: 10-12 mls; Required for study assays: 1 ml; Storage (-80 degree Celsius): 5-7 mls

## 3. Procedures for clinical examination and blood sampling

### *3.1 Clinical examination*

The procedures for measurements are described below:

Weight

Weight is measured using the electronic digital weighing scale (TANITA 305). Place the base plate of the scale on the most level and stable piece of ground possible. It should remain firmly on the ground when checked. Place the monitor at a higher level so the reading can be taken easily. Switch the scale on and ensure that the monitor reads 'zero'. Ask the child to stand on the scale with minimal clothing (only underwear should be worn). Subject should stand reasonably straight if possible - leaning to one side (or forwards) can affect the weight recorded. Weigh to nearest 100g. If the subject exceeds the maximum weight registering on the scales (200kg, 31stone) code them as “weight not attempted”. Do not attempt to weigh them.

Height and sitting height

Height will be measured with the Leicester Height Measure. Subjects will be asked to remove his/her shoes and socks and to stand on the stadiometer. The researcher should check for the following points:

FEET: flat on the centre of the base plate, ankles should be together and heels resting on the bar at the back,
BACK: should be as straight as possible, preferably against the rod but not leaning on it.
ARMS: should be resting by sides, not behind or in front,
HEAD: subject should be looking straight ahead (i.e. lower edge of orbit is in line with external auditory meatus i.e. ear hole). This position (the Frankfort plane) is important if an accurate reading is to be obtained.

Instruct the subject to keep their eyes focused on a point straight ahead, to breath deeply and to stretch to their fullest height. Assist this stretching by applying gentle upwards pressure beneath the mastoid. Care is needed to ensure that the subject does not stand on tiptoe. Lower the headrest.

When taking the reading try to get level with the scale to avoid errors due to parallax. Height must be recorded in millimetres. Record the measurement to the last completed millimetre and beware of digit preference. Record any problems that the patient has which may lead to underestimation of height in the 'adequate?' box. Reasons may include: problem with balance/standing, problem with posture, spinal curvature.

For sitting height, select a table that is high enough for the legs of the subject to be dangling over the edge when seated. Set up the table on a firm flat surface and ensure that it is stable and horizontal. Bring down the headrest of the height measure to the top of the table and record the table height. The subject should then be asked to sit on the table, and the measurement of height repeated using the same procedure as described above: ensure that the head is in the Frankfort plane, the back is straight, and the thighs horizontal or comfortably positioned so that the tendons of biceps femoris are approximately 1 cm clear of the table. The individual sits on the table with the legs hanging unsupported over the edge, hands resting on the thighs with the buttocks and shoulders relaxed.

Ask the subject to sit up tall and slide the headpiece down until it touches the subject's head. As with the standing height measurement, apply gentle upward pressure to the mastoid processes. Read the measurement while standing in front of the subject looking horizontally at the counter while applying the pressure and record to the last completed millimetre. Record any problems, which the patient has which may lead to underestimation of sitting height in the 'adequate' box. Reasons may include problems with posture, spinal curvature.

<u>Lower leg length</u>

Lower leg length (tibial length) will be measured on the left side using Chasmors calipers. While still sitting on the table and facing the observer, the subject is asked to rest his left ankle or calf on his right knee so that the medial aspect of the tibia faces upwards. Mark the proximal-medial border of the tibia and the distal border of the medial malleolus. The Chasmors caliper blades are then applied and measurement taken to the last completed millimetre.

<u>Head circumference</u>

Ask the child to stand erect and look straight ahead. Place the tape around the head, just above the eyebrows (or eyebrow ridges) anteriorly, and around the most

prominent bulge posteriorly. Check that the tape is not slant. Don't pull too tight – the tape should rest on the skin but not indent it. Hairstyles can be a problem and you may have to ask the subject to release their hair if it is tied up at the back. Read the tape to the last completed millimetre and repeat.

Waist circumference

This should be measured with weight evenly balanced on both feet, the subject standing with feet together. The arms should hang loosely at the sides. The measurement should be carried out on the bare skin and if the subject is wearing a corset or similar garment this should be removed for these measurements.

The waist should be identified as the mid point between the iliac crest below and the lower edge of the ribs (costal margin) above, i.e. measured at the sides. To locate the levels of the costal margin and the iliac crest use the fingers of the right hand held straight and pointing in front of the participant to slide upward over the iliac crest. Locate the right costal margin and the right iliac crest, run the tape measure from upper to lower point mark, mark the mid point (i.e measure the distance between the two points and divide in half). Repeat for the left side.

Pass the tape around the waist (for large subjects, ask them to help passing the tape around) and ensure the tape is horizontal. Ask subject to breathe out gently and to look straight ahead (this prevents the subject from contracting their muscles or holding their breath). Make sure the tape is not pulled too tight; it should be resting on the skin but not indenting it. Record measurement at the end of a normal expiration to the last completed millimetre. Repeat the measurement.

Hip circumference

The measurement should be carried out with the subject measuring underwear only, or over a single layer of light clothing e.g. sari petticoat, salwar or trousers. Apply the tape at the widest part, usually between the greater trochanter (top of thigh bone) and the lower buttock level, with the legs together. The tape should be horizontal and the gluteal muscles not contracted. Make several measurements of hip circumference and record the point of maximum circumference. Record to the last completed millimetre. Repeat the measurement.

THE FOLLOWING ARM AND SKIN FOLD MEASUREMENTS WILL BE MADE ON THE NON-DOMINANT SIDE OF THE SUBJECT. The side of the writing hand for the

literate subjects and the eating hand for the illiterate will be considered as the dominant.

Upper arm length and Mid arm circumference

The child stands with his/her back to the measurer arm being flexed at 90$^{o}$. The tip of the acromion (the point of the shoulder) is palpated and marked. Then with the subject's arm flexed at 90 degree, the olecranon (tip of the elbow) is palpated. Put the tape measure on the mark on the shoulder and drop it down to the tip of the elbow, by the side of the arm. Read the exact distance as if you had drawn an imaginary horizontal line from the bottom most point of the elbow to your tape measure. Measure and record the upper arm length and then mark a point halfway between the acromion and the olecranon. This marks the vertical level at which the circumference will be measured. It is important to ensure that the measurement is made with the arm flexed to avoid the tape taking an oblique course across the upper arm. The subject is then asked to relax, with the arm hanging by the side. The tape is placed around the upper arm such that its upper border is at the level of the marking. The tape should be horizontal all round, should be firmly resting on the skin, but should not be pulled too tight. Read the tape to the nearest completed millimetre and repeat. With the tape in position a horizontal line is drawn on the skin anteriorly and posteriorly at the level of the first mark for doing skin fold measurements.

Skin fold thickness

The child should be as relaxed as possible while doing these measurements. Holtain calipers (CMS Instruments, London) will be used for these measurements. Although, the dial of the Holtain caliper is calibrated to 0.2 mm, the measurements will be recorded to the last completed millimetre.

*Triceps skinfold thickness*

The tape is placed round the upper arm at the level of the mark done while measuring MUAC. With the tape in position, a horizontal line is drawn on the skin posteriorly at the level of the mark. Mark a vertical line on this at the most dorsal part of the upper arm. This level can be determined by 'eyeballing' the mid-point (the part that sticks out furthest posteriorly) or by a pen held vertically with one end on the olecranon process and the other end pointing towards the acromion. Make a vertical mark to form a cross.

The skin fold is picked up in a vertical tube at least 1 cm above the cross over the posterior surface of triceps muscle on a vertical line passing upward from the olecranon to the acromion. The calipers are applied below the fingers such that the marked cross is at the apex of the fold. The time should be noted and the readings taken at exactly 5 seconds after the application of the calipers jaws. Three readings to be taken, except in exceptional cases. (One reading in such cases)

*Biceps skin fold thickness*

The subject faces the measurer with their arms hanging and the (non-dominant) palm facing forward. An anterior horizontal line already marks the level at which the skin fold will be measured. As with the triceps you need to eye ball the point along the line where the arm bulges forward the most – the mid-point of the belly of the biceps muscle. Mark a vertical line here to form a cross. There is sometimes a prominent blood vessel here, but you can ignore it; the calipers will not damage it. The skin fold is picked vertically and the calipers applied at the level of the cross, with the cross on the apex of the fold.

*Subscapular skinfold thickness*

The subject stand with the shoulders and arms relaxed. The lowermost tip of the scapula is identified. If it is difficult to appreciate this, ask the subject to place the back of his/her hand on the lumbar region. Follow the medial border of the scapula downwards until the inferior angle is felt. Once it is identified, again ask the subject to relax with arms hanging by their side before you mark the skin immediately below the lower most tip (angle) of the scapula. The skinfold is picked up obliquely above the mark with the fold slightly inclined downward and laterally, in the natural cleavage of the skin. The caliper jaws are applied below the fingers, such that the marked cross is at the apex of the fold. Readings are taken at exactly 5 seconds after that. Take three readings, one reading if difficult.

*Upper suprailiac skin fold thickness*

Stand behind the subject. They should stand straight and relaxed with their arms folded in front of them. Locate the iliac crest, the large curving bone, just below the waist. In obese subjects you need to palpate firmly and in all subjects it helps to feel both sides together. Draw a horizontal line just above the crest at the side. Next find the mid axillary line: ask the subject to lift up their arm. The apex of the axilla is at the lowest point of the axillary hollow, just behind the thick fold made by the pectoral muscle. Drop an imaginary vertical line down from the apex of the axilla. This is the

mid-axillary line. Draw a line where this imaginary vertical line meets the horizontal line. Pick up the fold in the natural creases of the skin and apply the calipers at the level of the cross, with the cross on the apex of the fold. It may help to ask the subject to tilt towards you to ease the tension on the skin while picking up the skin fold.

Thigh length and Mid-thigh circumference

This measure will be used to compute the mid-thigh to waist circumference ratio, a predictor of metabolic disease risk.

This examination is on the RIGHT leg.

The subject should be supine with the legs straight and relaxed and the feet resting flat on the couch. The hands should be placed on the upper chest. Mark the outer edge of the right inguinal crease where it crosses the hip-flexing tendon. The outer edge of the right inguinal crease is usually near the anterior superior iliac spine. Mark the mid point of the right patella (knee cap). Measure and record the distance from the outer edge of the right inguinal crease to the mid point of the right patella, and divide by 2 to obtain the anterior mid-thigh location. Mark this with the pen. Then place the tape measure around the right thigh over the mid-thigh mark with the zero end of the tape just inferior to the mid-thigh mark. Check the tape is perpendicular to the long-axis of the thigh. Record the measurement to the nearest completed millimetre and repeat the measurement.

Blood pressure

Blood pressures will be measured in the brachial artery using an OMRON 705CP. The subject should be advised to avoid the following activities for at least 30 minutes before the BP measurement: strenuous exercise, eating, drinking of anything other than water, smoking, or taking drugs that affect the BP. Lie the subject on the couch. If the subject has breathing problems try elevating the head on a folded pillow, keeping the body as level as possible. The subject stays lying on the couch. Explain that you will leave him/her alone in the room for five minutes to relax; after that you will return to the room and take the blood pressure measurements. You will not speak to the participant while carrying out the recording. They should not smoke, eat, drink or read during this time.

Immediately after you have settled the subject down to rest for five minutes and prior to taking their blood pressures, set up the digital thermometer to take a reading. Just prior to recording the blood pressures, note the temperature. Always switch it off after taking a reading to avoid battery problems.

The subject should be strictly supine for 5 minutes before testing. Measure the mid-arm circumference and select the cuff size in accordance with arm circumference: small adult cuff if less than 25 cm, otherwise attach the adult cuff to the tubes. The widest cuff practicable should be used with the lowest edge of the cuff about 2 cm above the cubital fossa (elbow crease). The cuff should be placed around the right upper arm with the bladder centre over the brachial artery of the inner side of the right upper arm. Ensure that the arrow (green shaded area) on the cuff lies over the brachial artery and that the inflation tubing extends towards the middle finger. Use the left arm only if it is impossible to use the right. If the left arm is used, record this on the datasheet.

Do not put the cuff on too tightly as bruising may occur on inflation. Ideally, it should be possible to insert two fingers between cuff and arm. The cuff should not be applied too loosely, as this will result in an inaccurate measurement. It may be necessary to adjust for height if the arm is lower than that of the heart. To adjust for height place a pillow or towel under the elbow. Explain that you are starting the measurement. Check that subject is familiar with having his blood pressure taken. Explain that you plan to take 2 measurements one minute apart, the cuff will inflate and slowly deflate automatically, encourage the subject to keep the arm still and not to talk during measurement as this may affect their reading.

Blood pressure measurement with the OMRON705CP automated BP Recording Machine:
Set the inflation pressure on the OMRON BP monitor at 170mmHg.
Press the ON button and select START
Record the values of SBP, DBP, Mean BP & Pulse.
After the first measurement wait for one minute to allow redistribution of blood in the forearm.
Then repeat the measurement in exactly the same way as the first one.
Record the values of SBP, DBP, Mean BP & Pulse.
Whenever experiencing difficulties, the cuff must be completely deflated and at least one minute elapse before taking the second measurement.

*WHEN ERROR MARK APPEARS*

*-if you get an **ee** recording it is probably because 170 is too low. Increase the cuff pressure to 200 and repeat.*

*-when **e** appears this means that there has been movement during the recording. Repeat measure.*

***e.e** cuff has over-inflated. Relax and repeat.*

*IMPORTANT*

If you get an unusual recording - REPEAT reading

*And REMEMBER to replace all batteries at the one time (size C).*

Arterial stiffness

Arterial stiffness will be measured by the technique of applanation tonometry using Sphygmocor. Pulse wave velocity will be calculated by assessing the pulse waves in the carotid and femoral arteries and pulse wave analysis (augmentation index) will be carried out at the radial artery.

Make sure that the subject has not had a meal in the last 2 hours and that too only a small one. Subjects have to refrain from smoking and drinking beverages containing caffeine for at least 2 hours before assessments. Cancel measurements when subject's basal conditions are substantially altered like when outside temperature is high or immediately after strenuous exercise. Subjects should be resting in quite room for at least 10 minutes before assessments. Subject may neither speak nor sleep during assessments. All assessments are to be carried out in the supine position. Be aware of possible white coat arterial stiffness or variations due to time of day or position. Beware of possible disturbance of data due to cardiac arrythmia.

After the measurement of blood pressure in the supine position, enter the subject's details and the mean of the two blood pressure readings into the laptop. Place the ECG pads on the subject and attach the cables to the pads. Feel the carotid and femoral pulses and mark them with a pen. Measure the sternum to carotid pulse point and enter into the laptop as the proximal distance. Then measure and enter the sternum to femoral distance as the distal distance.

Before staring the assessments ask the subject to get quite comfortable because they will have to keep their head and body very still during the study. Tell them that you will keep talking to them but it's important that they don't speak back or we'll lose the picture. First carry out the pulse wave velocity measurement. Enter the sites into the laptop and get pulse waveforms at the femoral followed by the carotid arteries. Record the pulse wave velocity and its standard deviation on the datasheet after checking the quality. Next set the laptop to the pulse wave analysis and get the waveform at the radial artery. Record the augmentation and the augmentation index after checking the quality. To cover a complete respiratory cycle, the average of at least 10 successive measurements will be used for analysis.

Before accepting a reading check that the quality control values are within the set limits i.e. they are displayed in green. Also look at the overlayed data; there should be as little variability in pulses as possible. The quality index is an indicator of overall quality of the captured signal and should be 90% or above to be considered good. However, consider all quality control data when making an assessment of the quality. *Do not discard any measurements on the basis of quality index alone*. Visually inspect the waveform data to make a final decision.

If the pulse height variation or diastolic variation is outside the limits (i.e. displayed in red) examine the radial artery waveform trace to ensure no transients occurred during the averaging period that would make the averaging process invalid. Measurements will be accepted as valid only when standard deviation of beat-to-beat data is not exceeding 10% of mean. Values of parameters determined from ejection duration when the ejection duration values are outside the range 200-400msec should be disregarded.

Contra-indications:
The Sphygmocor process should not be used in persons with significant aortic valve stenosis (gradient >60mmHg)

***Blood sampling***

CHECK THAT THE CHILD HAS FASTED FOR AT LEAST 9 HOURS

If they had anything other than plain water to eat or drink, postpone the test to another day. It is important to check that they have eaten normally for the 3 days prior to the test.

**Applying the EMLA cream:** Area over the cubital fossa, from where the blood is to be drawn should be marked with a pen. Whole contents of EMLA cream from one tube should be applied over the marked area and left in place for 60 minutes, after covering the area. After the contact period, the area should be wiped clean with surgical cotton.

1. The blood sample will be taken at least half and hour before the examinations begins to allow subjects some refreshments after their overnight fast. The blood sample should be taken with the subject lying down.
2. Check with the subject when they last ate or drank and record time on the datasheet.
3. Check whether the subject has had previous problems with blood sampling.
4. Check samples are labelled with correct details.
5. Put on your rubber gloves at this point.
6. Alcohol swabs will be provided for skin cleaning - allow to dry after use.
7. Do not ask the subject to clench his/her fist.
8. A tourniquet may be used throughout. Wear the rubber gloves provided for taking the sample. A green 21 gauge vacutainer needle or butterfly should generally be used; a small supply of black 22 gauge needles will also be supplied for exceptional use.
9. If blood is not obtained at the first attempt, a single further attempt may be made in the opposite arm if the subject consents. If a second attempt is unsuccessful, NO FURTHER attempt to obtain blood should be made.
10. Each subject's blood collection tubes will be prepared in advance in a polythene bag with an identification label on the front and individual tube labels throughout. Please check the label against the data sheet. The tube labels will have the subject's full ID number, date of birth and initials for identification.

BEFORE DRAWING THE BLOOD, WRITE SUBJECT'S NAME AND THE SAMPLE TIME (WHERE APPLICABLE) ON THE VACUTAINERS.

After drawing the blood, mix the sample by GENTLY inverting the vacutainer several times. Green tube should be kept in ice and other samples are placed in a rack. The red vacutainers are allowed to stand for about 30 minutes before centrifuging. The samples are now ready to be processed.

- Always prepare the blood taking field carefully before taking blood to minimise the risk of accidents.
- Never re-sheath the needle after use.
- Dispose of needle immediately after use in sharps bin
- Do not allow the disposal box to become overfull as this can present a potential hazard.

***First-aid and abnormal results***

The team will carry a first aid box. The doctor will provide first aid as needed or refer the subject to the local or NIN hospital as needed.

The most common scenarios are likely to occur at the time of blood sampling and these include:

Fainting subjects

If a subject looks or feels faint during the procedure, it should be discontinued. The subject should be asked to place their head between their knees. They should subsequently be asked to lie down. If they are happy for the test to be continued after a suitable length of time, it should be done so with the subject supine and the circumstances should be recorded. They may wish to discontinue the procedure at this point, but willing to give the blood sample at a later time.

Subjects who are HIV or Hepatitis B positive

If a subject volunteers that they are HIV or Hepatitis B positive, do not take a blood sample. Record this as the reason on the datasheet. You should, never, of course seek this information.

Needle stick injuries

The wound should be encouraged to bleed. The wound should be washed with soap and warm water, if available. Other hand cleaner may be used if water is not available.

**Abnormal results**

The main abnormal result expected is severe anaemia. Haemoglobin levels of below 7 gm% will be referred to the local hospital for further management. Lesser levels of anaemia may be referred to the local PHC or iron tablets advised the team doctor. For other abnormal results, the subject will be seen by the team doctor and referred as appropriate to the local PHC or NIN hospital.

The other possibilities for abnormal blood results are high blood pressure or sugar. If the levels are abnormally high, again report to the PHC doctor.

## 4. Appendix of questionnaires

**Administrative questionnaire – NINUB study**

| | |
|---|---|
| 1. Subject ID: | |
| 2. Study village number: | |
| 3. Study household number: | |
| 4. Study serial number of woman in household: | |
| 5. Study serial number of woman in the village: | |
| 6. Village name: | |
| 7. Woman's family name (surname): | |
| 8. Woman's first (given) name: | |
| 9. Husband's first (given) name: | |

10. Date of questionnaire completion | | | | | 2 | 0 | 0 | |

11. Interviewer code (a) ☐ (b) Other, specify……..

[1=Nutritionist; 2=Male social worker; 3=Female social worker; 4=Village worker; 5=NIN staff; 6=Other]

---

**Verify the name of the woman and her husband.** Questions 12-15 need to be completed ONLY where the name printed above is incorrect/missing (otherwise leave blank)**. If you believe that the name is spelt incorrectly, please enter the correct version below. Enter all names in** BLOCK CAPITALS**.**

12. Woman's family name (surname)…….

13. Woman's first (given) name……...…….

14. Husband's first (given) name..……

15. Husband's surname if different from the woman's surname…….

16. Age of the woman (in **completed years**):……..(leave blank if don't know)

17. Informant (a) ☐ (b) Others, specify……

[1=Village head; 2=Anganwadi worker; 3=Family themselves; 4=Villagers; 5=Others]

18. Status of the family (a) ☐ (b) Other, specify…….

[1=Still living in the village; 2=Used to live in the village but have moved; 3=Never lived in the village; 4=Any other]

19. Current postal address of the family (if any):…….

……..

……...

20. Telephone number: (a) Landline with area code ……..….(b) Mobile…..……..

21. In the table overleaf enter details of **ALL** children in order starting from the **ELDEST**. Please verify that the list includes (a) **girls**, including those who are **married** (b) those **not living in the house** now, and (c) children who may have **died** after birth. NOTE: Enter **name** in **BLOCK CAPITALS** and **age** in **completed years**. Codes for questions (c), (h), (i), (j) and (k) are at the bottom of the table. Note additional information related to these under comments (l). Complete questions **(m) to (o) only** for children born during or earlier than 1990 (i.e. those aged **13 years and over)**. In **work/school** enter address of work/study and in educational address, include year (**grade/class)** of study. Enter address of **residence only if different** from family (otherwise leave blank). In **contact** enter additional information relevant to contacting the person in future i.e. **distance** to place of work/school/residence, **phone no**. & **timings** of availability. Where more than 8 children, write P.T.O and continue on the back of the page.

(a) Birth order ☐ (b) **First** name…….…….. (c) Sex ☐ (d) Age …… Date of birth: (e) ☐☐ (f) ☐☐ (g) ☐☐☐☐

(h) Status ☐ (i) Reason for move ☐ (j) Occupation ☐ (k) Study recall ☐ (l) Comments……

(m) Work/school……..…(n) Residence……..……

(o) Contact……..

(a) Birth order ☐ (b) **First** name…….…….. (c) Sex ☐ (d) Age …… Date of birth: (e) ☐☐ (f) ☐☐ (g) ☐☐☐☐

(h) Status ☐ (i) Reason for move ☐ (j) Occupation ☐ (k) Study recall ☐ (l) Comments……

(m) Work/school……..…(n) Residence……..……

(o) Contact……..

CODES: (c) Sex: 1=Male; 2=Female

(h) Status: 1=Alive & resident in the same village; 2= Alive & moved to Hyderabad; 3=Alive & moved relatively short distance (within 50 km of Hyderabad) but not to Hyderabad; 4=Alive & moved relatively long distance (i.e. greater than 50 km from Hyderabad); 5=Died; 6=Any other, specify in comments

(i) Reason for move (enter only if not staying with the family, otherwise leave blank) (where multiple reasons, enter primary reason): 1=Work; 2=Education; 3=Marriage; 4= Any other, specify in comments

(j) Occupation (main): 1=Full time education; 2=Full time employment; 3=Both education & employment; 4=Neither; 5=Any other, specify in comments

(k) Study recall (parental recall of child being born in the study by virtue of memory of): 1=Fieldworker visits (questionnaires completed); 2=Birth weight taken; 3=Both; 4=Neither; 5=Any other, specify in comments

22. Ask the respondent if she/he is aware of anybody else who took part in the same study or has children in the age range **13-17 years inclusive**. If yes, enter contact details.

Enter names in **BLOCK CAPITALS**.

| (a) Woman's family name (surname) | (b) Woman's first name | (c) Husband's first name | (d) Address/contact detail |
|---|---|---|---|
| | | | |
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**CONSENT FORM - NINUB STUDY**

This health research is being conducted by doctors from the National Institute of Nutrition (NIN) in Hyderabad and University of Bristol (UoB) in England. Some doctors believe that certain medical conditions of adult life such as heart disease and diabetes may be the result of poor nutrition in early life, especially from the time of being in the mother's womb. However, this is not known for sure and we want to carry out this research to find out whether it's true or not. Knowing this is important as it may help us to prevent these conditions by improving the mother's nutrition when she is pregnant.

You, the mother, and your child have been selected for this research because you took part in an earlier research study that was conducted by us, the NIN, approximately 15 years ago. At that time some of you (mothers) received food from the Anganwadi when you were pregnant and we had measured the weight of your child after birth to see whether the additional food had resulted in bigger babies. Now we want to see whether the children born bigger or those whose mothers were given extra food when they were in the womb are at a lower risk of heart disease and diabetes.

To carry out our research, we would like to use the information collected by the NIN on you and your child at the time and to collect new information now. To collect this additional information we will need to ask you and your child some health related questions, measure your height and weight, and perform medical examination and blood tests on your child. These tests will provide us with information about the 'heart health' of your child. An experienced team of researchers that includes a doctor will carry out these examinations and take a small blood sample (2 tea spoonful). Taking of blood sample should cause little or no discomfort to your child as we will use a fine needle and apply a special cream on the site that numbs the feeling. A total of 10 ml (2 tea spoonful) blood will be withdrawn. If you decide not to participate in this study, it is your right, and we will respect your decision. Even after you agree to participate, you are completely free to withdraw from the study at any time without giving reason. This research is being carried out to improve medical knowledge and may not be of any direct benefit to you or your child personally. Refusal to participate or withdrawal from the study at any stage will not affect your chances of receiving future medical or other care. An expert group of people at NIN whose role it is to ensure the welfare of the public have approved this study.

Q With all this information in mind, do you consent to you and your child participating in this study?

| | (a) Mother | (b) Child |
|---|---|---|
| 1.1 | Questionnaire … $Yes_1$ ☐ $No_2$ ☐ | Questionnaire…… . $Yes_1$ ☐ $No_2$ ☐ |
| 1.2 | Height & weight …$Yes_1$ ☐ $No_2$ ☐ | Medical examination …. $Yes_1$ ☐ $No_2$ ☐ |
| 1.3 | | Blood sample……$Yes_1$ ☐ $No_2$ ☐ |

All the information relating to you and your child will be kept confident and you will not be identified personally by name, or other details, in any report arising from this research. The information from this study and the earlier study will be stored in computers in Hyderabad and Bristol and used for medical research.

1.4 Do you consent to information being stored in this way and used for medical research? $Yes_1$ ☐ $No_2$ ☐

The blood samples that we collect will be stored in refrigerators in NIN, Hyderabad, and may be used later on do other tests for medical research including tests to find out whether diseases run in families.

1.5 Do you consent to us doing this without informing you again? ……$Yes_1$ ☐ $No_2$ ☐

Finally, we may try to contact you in future for further medical research.

1.6 Do you consent to being contacted in this way in the future? ……...$Yes_1$ ☐ $No_2$ ☐

Thank you for co-operation. You may contact us at any time if you have further questions.

Signature/ thumb impression (mother) …….…… Date…….

Signature/ thumb impression (child) …….…… …… Date…….

Signature (researcher) …….…….…… Date…….

Signatures or thumb impression (witness, if any) …… Date…….

Subject questionnaire (child) – NINUB study

| No. | Question | Answer | Coding | Skip |
|---|---|---|---|---|
| | **Interview details** | | | |
| | Date & time of questionnaire completion | __ __/ __ __/ __ __ __ __: __ __ __ __ | [dd/mm/yyyy:hhmm in 24-hr cycle] | |
| | Interviewer code | ___ | [1=Nutritionist; 2=Male social worker; 3=Female social worker; 4=Village worker; 5=NIN staff; 6=Other, 7=Doctor] | |
| | Interviewer initials | _____ | | |

| | | | | |
|---|---|---|---|---|
| | **Contact details** | | | |
| | *Verify the names above and complete the questions below only if incorrect* | | | |
| | Family name (surname) | _____ | | |
| | First name (given name) | _____ | | |
| | Middle name (if any) | _____ | | |
| | Mother's first name (given name) | _____ | | |
| | Father's first name (given name) | _____ | | |
| | Current postal address (if any) | _____ | | |
| | Phone number i(landline) | _____ | | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | Phone number (mobile) | _____ | | |

| | | | | |
|---|---|---|---|---|
| | **Demographic details** | | | |
| | Age last birthday | __ __ | [In completed years] | |
| | Date of birth (if known) | __ __ | [dd] | |
| | Month of birth | __ __ | [mm] | |
| | Year of birth | __ __ __ __ | [yyyy] | |
| | Sex | __ | [1=Male; 2=Female] | |
| | Birth order | __ __ | | |
| | Number of older siblings | __ __ | | |
| | Number of younger siblings | __ __ | | |
| | Current marital status | __ | [1=Never married; 2=Married but gauna not performed; 3=Married; 4=Widow/widower; 5=Separated/divorced] | |
| | Age at marriage (if married) | __ __ | | |

| | | | | |
|---|---|---|---|---|
| | **Education and employment** | | | |
| | Educational status | __ | [1=Illiterate; 2=Literate (can read & write) but no formal education; 3=Formally educated but now stopped; 4=Still in formal education] | |
| | Highest grade of school completed | __ __ | [00 for less than one year completed] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | School performance in last completed year (percentage score) | __ | [1=Less than 50%; 2=50-65%; 3=65-80%; 4=Above 80%; 5=NA] | |
| | School performance in last completed year (comparative performance) | __ | [1=Below average; 2=Average; 3=Above average; 4=Excellent; 5=NA] | |
| | Currently in full time employment outside the home | __ | [1=Yes; 2=No] | |
| | Primary occupation | __ | [1=Housewife; 2=Unemployed; 3=Unskilled manual labour, landless labour; 4=Semi-skilled manual labour, marginal landowner, rickshaw driver, army jawan, carpenter, fitter; 5=Skilled manual labour, small business owner, small farmer; 6=Trained, clerical, medium business owner, middle level farmer, teacher, maintenance (in charge), personnel manager; 7=Professional, big business, landlord, university teacher, class 1 ICS/services officer, lawyer] | |
| | **About the household: Standard of Living Index (SLI)** | | | |
| | Material used in construction of the house (roof/walls/floor) | __ | [1= Kutcha (made from mud, thatch, or other low quality material); 2= Semi-pucca (partly low quality and partly high quality material); 3= Pucca (high quality materials throughout, including roof, walls, and floor)] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | Total number of rooms in the house | __ __ | | |
| | House has a separate room for cooking | __ | [1=Yes; 2=No] | |
| | Main source of lighting in the house | | [1= Kerosene/gas/oil; 2= Electricity; 3=Other, specify] | |
| | Other, specify | _____ __ | Main source of lighting in the house | |
| | Main source of drinking water used in the house | __ | [1= Pipe, hand pump, or well (public); 2= Pipe, hand pump, or well in residence/yard/plot; 3=Other, specify] | |
| | Other (specify) | _____ ___ | Main source of drinking water used in the house | |
| | Main fuel used for cooking in the house | __ | [1= Coal, charcoal, or kerosene; 2= Electricity, liquid petroleum gas, or biogas; 3=Other, specify] | |
| | Other (specify) | _____ ____ | Main fuel used for cooking in the house | |
| | Nature of toilet facility in the house | __ | [1= Own flush toilet; 2= Shared/public flush toilet; 3= Own pit toilet/latrine; 4= Shared/public pit toilet/latrine; 5= No facility/bush/field; 6=Other, specify] | |
| | Other (specify) | _____ ____ | Nature of toilet facility in the house | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | Religion of the head of the household | __ | [1=Hindu; 2= Muslim; 3=Christian; 4=No religion, 5=Other, specify] | |
| | Other (specify) | - _____ ____ | Religion of the head of the household | |
| | Caste or tribe of the head of the household | __ | [1= Scheduled caste; 2= Scheduled tribe; 3= Other backward class; 4= None of them] | |
| | Other (specify) | _____ ____ | Caste or tribe of the head of the household | |
| | Household owns this house or any other house | __ | [1=Yes; 2=No] | |
| | Household owns any agricultural land | __ | [1=Yes; 2=No] | |
| | How much agricultural land does the household own | __ __ __ | [Land in acres; enter 000 for none] | |
| | Out of this land, how much is irrigated | __ __ __ | [Land in acres; enter 000 for none] | |
| | Household own any livestock | __ | [1=Yes; 2=No] | |
| | *Household own any of the following:* | | | |
| | A mattress | __ | [1=Yes; 2=No] | |
| | A pressure cooker | __ | [1=Yes; 2=No] | |
| | A chair | __ | [1=Yes; 2=No] | |
| | A cot or bed | __ | [1=Yes; 2=No] | |
| | A table | __ | [1=Yes; 2=No] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | A clock or watch | __ | [1=Yes; 2=No] | |
| | An electric fan | __ | [1=Yes; 2=No] | |
| | A bicycle | __ | [1=Yes; 2=No] | |
| | A radio or transistor | __ | [1=Yes; 2=No] | |
| | A sewing machine | __ | [1=Yes; 2=No] | |
| | A telephone | __ | [1=Yes; 2=No] | |
| | A refrigerator | __ | [1=Yes; 2=No] | |
| | A black and while television | __ | [1=Yes; 2=No] | |
| | A colour television | __ | [1=Yes; 2=No] | |
| | A moped, scooter, or motorcycle | __ | [1=Yes; 2=No] | |
| | A car | __ | [1=Yes; 2=No] | |
| | A water pump | __ | [1=Yes; 2=No] | |
| | A bullock cart | __ | [1=Yes; 2=No] | |
| | A thresher | __ | [1=Yes; 2=No] | |
| | A tractor | __ | [1=Yes; 2=No] | |
| | | | | |
| | **About the household: additional information** | | | |
| | Household type | __ | [1=Nuclear (parents & their children only), 2=Joint (with at least one grandparent); 3=Joint (with any other relative)] | |
| | Number of usual members of the household | __ __ | | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | Educational level of the father | __ | [1=Illiterate/no schooling; 2=Primary school/literate; 3=Middle school completion; 4=High school certificate; 5=High school + (HSC, ITI, Intermediate Ded, Post-high school diploma; 6=Other graduate (BA, BSc, BCom, DME, DHMS, BPNA); 7=Professional degree (MA, MSc, MCom, Ttech, MBBS, BE, MSW, PhD] | |
| | Educational level of the mother | __ | [Same as above] | |
| | Educational level of the husband (married girls) | __ | [1=Illiterate/no schooling; 2=Primary school/literate; 3=Middle school completion; 4=High school certificate; 5=High school + (HSC, ITI, Intermediate Ded, Post-high school diploma; 6=Other graduate (BA, BSc, BCom, DME, DHMS, BPNA); 7=Professional degree (MA, MSc, MCom, Ttech, MBBS, BE, MSW, PhD] | |
| | Primary occupation of the father/husband (married girls) NOW | __ | [1=Housewife; 2=Unemployed; 3=Unskilled manual labour, landless labour; 4=Semi-skilled manual labour, marginal landowner, rickshaw driver, army jawan, carpenter, fitter; 5=Skilled manual labour, small business owner, small farmer; 6=Trained, clerical, medium business owner, middle level | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | | | farmer, teacher, maintenance (in charge), personnel manager; 7=Professional, big business, landlord, university teacher, class 1 ICS/services officer, lawyer] | |
| | Primary occupation of the father at the time of subject's birth i.e. approx. 15 years ago | __ | [Same as above] | |
| | Primary occupation of the mother at the time of the subject's birth i.e. approx. 15 years ago | __ | [Same as above] | |
| | Average monthly income of subject from all sources | Rs _____ | [Estimated for the past 1 year] | |
| | Average monthly income of father/husband (married girls) from all sources | Rs _____ | [Estimated for the past 1 year] | |
| | **Benefits received by the household** | | | |
| | Subsidised food through ration card | __ | [1=Yes; 2=No] | |
| | Housing | __ | [1=Yes; 2=No] | |
| | Any other (specify)…… | __ | [1=Yes; 2=No] | |

| | | | | |
|---|---|---|---|---|
| | **Tobacco use** | | | |
| | Used tobacco in any form (smoked, chewed, snuff) on a regular basis i.e. at least once a day | __ | [1=No, never; 2=Yes, but don't any more (stopped over 6 months ago); 3=Yes, and still use it (up to last 6 months)] | |
| | Age at which started | __ | [Age in years] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | | | |
|---|---|---|---|---|---|---|
| | smoking/consuming any tobacco product regularly | | | | | |
| | | | | | | |
| | *Quantity and duration of tobacco use (present or at the time for ex-users)* | | *Duration of use [years]* | *Number of days per week* | *Number of use or number smoked per day* | |
| | Cigarette | __ [1=Yes; 2=No] | _____ | _____ | ____ | |
| | Beedi | __ [1=Yes; 2=No] | _____ | _____ | ___ | |
| | Tobacco chewing | __ [1=Yes; 2=No] | _____ | _____ | ___ | |
| | Pan with zarda | __ [1=Yes; 2=No] | _____ | _____ | ____ | |
| | Pan masala with zarda | __ [1=Yes; 2=No] | _____ | _____ | ____ | |
| | Gutka | __ [1=Yes; 2=No] | _____ | _____ | ____ | |
| | Snuff | __ [1=Yes; 2=No] | _____ | _____ | ____ | |
| | Other, specify…… | __ [1=Yes; 2=No] | _____ | _____ | ____ | |
| | Exposed to tobacco smoke from others at home or workplace regularly | __ [1=Yes; 2=No] | _____ | _____ | ____ | |
| | **Alcohol habit** | | | | | |
| | Consume alcoholic beverages regularly | __ | [1=No, never; 2=Yes, but don't any more (stopped over 6 months ago); 3=Yes, and still use it (up to last 6 months)] | | | |
| | Age at which started | __ | [Age in years] | | | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | | | |
|---|---|---|---|---|---|---|
| | consuming any alcoholic drink regularly | | | | | |
| | *Quantity and duration of alcohol use (present or at the time for ex-users)* | | *Duration of use [years]* | *Frequency per week* | *Quantity in ml per occasion [1 small peg=30 ml; 1 large peg=60 ml; 1 beer glass=325 ml)* | |
| | Local spirits (desi, arrack, toddy, etc) | __ [1=Yes; 2=No] | | | | |
| | Branded spirits (whisky, rum, brandy, gin, vodka) | __ [1=Yes; 2=No] | | | | |
| | Beer | __ [1=Yes; 2=No] | | | | |
| | Other, specify…….. | __ [1=Yes; 2=No] | | | | |

| | | | | |
|---|---|---|---|---|
| | **General health** | | | |
| | Self-reported health at present, compared to other boys/girls of the same age | __ | [1=Excellent; 2=Good; 3=Fair; 4=Poor] | |
| | In the preceding one year, number of days missed from school/work due to ill health | __ __ | | |
| | Suffer from asthma | __ | [1=Yes; 2=No] | |
| | On any medication | __ | [1=Yes; 2=No] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | *Name/reason for medication* | *Name* | *Reason for taking it* | |
|---|---|---|---|---|
| | | _____ ___ | _____ | |
| | | _____ ___ | _____ | |
| | | _____ ___ | _____ | |

| | | | | |
|---|---|---|---|---|
| | **Parental health** | | | |
| | *Mother* | | | |
| | Age of MOTHER last birthday | __ __ | [In completed years] | |
| | Mother ever suffered from any of the following | | | |
| | Heart disease | _____ _____ | [1=Yes; 2=No] | |
| | High blood pressure | _____ _____ | [1=Yes; 2=No] | |
| | Diabetes mellitus (high blood pressure) | _____ _____ | [1=Yes; 2=No] | |
| | Stroke (paralytic attack) | _____ _____ | [1=Yes; 2=No] | |
| | *Father* | | | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | Age of FATHER last birthday | __ __ | [In completed years] | |
| | Father ever suffered from any of the following | | | |
| | Heart disease | _____ | [1=Yes; 2=No] | |
| | High blood pressure | _____ | [1=Yes; 2=No] | |
| | Diabetes mellitus (high blood pressure) | _____ | [1=Yes; 2=No] | |
| | Stroke (paralytic attack) | _____ | [1=Yes; 2=No] | |

| | | | | |
|---|---|---|---|---|
| | **Pubertal status** | | | |
| | **Male participants only** | | | |
| | Ask to read text | | | |
| | Male adult voice (not just cracking) | __ | [1=Yes; 2=No] | |
| | Age at which adult voice developed fully | __ __ | [Age in years] | |
| | **Female participants only** | | | |
| | About the periods (menstruation) | __ | [1=Never had periods; 2=Used to get periods but not anymore; 3=Still get periods] | |
| | Age at which periods started | __ __ | [Age in years] | |
| | Reason why periods stopped (if relevant) | __ | [1=Pregnancy; 2=Lactation; 3=Oral contraceptive pill; | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | | | 4=Any other, specify] | |
| | Any other (specify) | _____ | Reason why periods stopped | |

| | | | | |
|---|---|---|---|---|
| | **Diet** | | | |
| | Is vegetarian | __ | [1=Yes; 2=No] | |
| | Main staple diet | __ | [1=Rice; 2=Wheat; 3=Ragi, 4=Other, specify] | |
| | Other (specify)…… | | [Main staple diet] | |
| | *Frequency of consuming the following items over the past 3 months* | | | |
| | Meat (red: beef, pork, mutton) | __ | [1=Twice per day or more; 2=Once a day; 3=Once a week; 4=Thrice a week or more; 5=Once per month; 6=Twice per month; 7=Sometimes; 8=Never] | |
| | Meat (white: chicken) | __ | [Same as above] | |
| | Fish | __ | [Same as above] | |
| | Eggs | __ | [Same as above] | |
| | Milk (plain – including dahi, lassi) | __ | [Same as above] | |
| | Milk in tea/ coffee/ other (bournvita) | __ | [Same as above] | |
| | Tea/ coffee/ bournvita | __ | [Same as above] | |
| | Fresh fruit | __ | [Same as above] | |
| | Raw (salad) vegetables | __ | [Same as above] | |
| | Shop-bought sweets | __ | [Same as above] | |

| | | | | |
|---|---|---|---|---|
| | Shop-bought savoury snacks | __ | [Same as above] | |
| | Pickles | __ | | |
| | *Frequency of adding the following items to cooked food (over the past 3 months)* | | | |
| | Ghee | __ | [Same as above] | |
| | Butter | __ | [Same as above] | |
| | Salt | __ | [Same as above] | |
| | Proportion of weekly meals consist of food cooked outside the home | __ | [1=Occasional/never; 2=Less than 25%; 3=25-50%; 4=More than 50%] | |

| | | | | | |
|---|---|---|---|---|---|
| | **Physical activity** | | | | |
| | **Activities related to work outside home** | | | | |
| | Nature of work | __ | [1=Almost/entirely sedentary; 2=Mainly sedentary, some walking/standing; 3=Mainly standing/squatting (static); 4=Mainly standing (active); 5=Mainly moving around/walking; 6=Heavy physical work, load carrying, pushing] | | |
| | Average number of hours spent at work per day | __ | [Hours per day] | | |
| | Activities related to domestic work at home | | *Number of times per week* | *Time spent per episode [minutes]* | |
| | Sweeping/swabbing | __ [1=Yes; 2=No] | | | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | | |
|---|---|---|---|---|---|
| | Hand-washing clothes | __ [1=Yes; 2=No] | | | |
| | Manual pounding/grounding of cereals | __ [1=Yes; 2=No] | | | |
| | Manual washing of utensils | __ [1=Yes; 2=No] | | | |
| | Cooking and serving food | __ [1=Yes; 2=No] | | | |
| | Gardening at home | __ [1=Yes; 2=No] | | | |
| | Carrying groceries from market | __ [1=Yes; 2=No] | | | |
| | Drawing water from well | __ [1=Yes; 2=No] | | | |
| | Fetching water from well/river/tap | __ [1=Yes; 2=No] | | | |
| | Cutting fire wood from tree | __ [1=Yes; 2=No] | | | |
| | Fetching firewood | __ [1=Yes; 2=No] | | | |
| | Caring for animals (feeding, milking, washing) | __ [1=Yes; 2=No] | | | |
| | Breast feeding | __ [1=Yes; 2=No] | | | |
| | *Caring of children below 10 years* | | *Number of children below 10* | | |
| | | __ [1=Yes; 2=No] | __ | | |
| | **Travel related activity** | | | | |
| | *Activity* | | *Total time spent on travelling to work/school and return* | | |
| | Walking | __ [1=Yes; 2=No] | __ | [1=Less than 15 minutes; 2=15-29 minutes; 3=30-59 minutes; 4=Over 60 minutes] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | | |
|---|---|---|---|---|---|
| | Cycling | __ [1=Yes; 2=No] | __ | [Same as above] | |
| | **Leisure related activity at work/school/home** | | | | |
| | *Activity* | | *Number of times per week* | *Time spent per episode [minutes]* | |
| | Slow walking | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | Brisk walking | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | Jogging/running | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | Cycling | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | Aerobic/gym | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | Yoga | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | Physically active games, specify below: | | | | |
| | (a) _____ | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | (b) _____ | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | (c) _____ | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | Other, specify…… | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | **Leisure related screen viewing** | | | | |
| | Watching TV/Video/DVD | __ [1=Yes; 2=No] | __ __ | __ __ | |
| | Sitting on computer (internet/computer games) | __ [1=Yes; 2=No] | __ __ | __ __ | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

Subject questionnaire (mother) – NINUB study

| No. | Question | Answer | Coding | Skip |
|---|---|---|---|---|
| | **Interview details** | | | |
| | Date & time of questionnaire completion | __ __/ __ __/ __ __ __ __: __ __ __ __ | [dd/mm/yyyy:hhmm in 24-hr cycle] | |
| | Interviewer code | ___ | [1=Nutritionist; 2=Male social worker; 3=Female social worker; 4=Village worker; 5=NIN staff; 6=Other, 7=Doctor] | |
| | Interviewer initials | _____ | | |

| | | | | |
|---|---|---|---|---|
| | **Contact details** | | | |
| | *Verify the names above and complete the questions below only if incorrect* | | | |
| | Family name (surname) | _____ | | |
| | Child's first name (given name) | _____ | | |
| | Child's middle name (if any) | _____ | | |
| | Mother's own first name (given name) | _____ | | |
| | Husband's first name (given name) | _____ | | |
| | Current postal address (if any) | _____ | | |
| | Phone number (landline) | _____ | | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | Phone number (mobile) | _____ | | |

| | | | | |
|---|---|---|---|---|
| | **Food supplementation from Anganwadi** | | | |
| | In pregnancy | __ | [1=Never; 2=Occasionally (less than three times a week; 3=Regularly] | |
| | Reason for taking infrequently or not at all (pregnancy supplementation) | __ | [1=Not available; 2=Difficulty in collecting; 3=Did not like the taste; 4=Did not feel the need for it; Any other, specify] | |
| | Shared anganwadi food with other members of the family (pregnancy supplementation) | __ | [1=Yes; 2=No; 3=Don't know] | |
| | Child below the age of five years | __ | [1=Never; 2=Occasionally (less than three times a week; 3=Regularly] | |
| | Reason for taking infrequently or not at all (under five supplementation) | __ | [1=Not available; 2=Difficulty in collecting; 3=Did not like the taste; 4=Did not feel the need for it; Any other, specify] | |
| | Shared anganwadi food with other members of the family (under five | __ | [1=Yes; 2=No; 3=Don't know] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | supplementation) | | | |

| | | | | |
|---|---|---|---|---|
| | **Breast feeding** | | | |
| | Child ever breast fed | __ | [1=Yes; 2=No] | |
| | Age till exclusively breast fed | __ | [Age in years] | |
| | Age breast feeding stopped completely | __ | [Age in years] | |

| | | | | |
|---|---|---|---|---|
| | **Immunisation** | | | |
| | *Child received any of the following vaccinations* | | *Number of doses* | |
| | BCG against TB (injection in the left shoulder that caused a scar) | __ [1=Yes; 2=No; 3=DK] | NA | |
| | DPT against diphtheria, whooping cough, and tetanus (given as an injection) | __ [1=Yes; 2=No; 3=DK] | __ | |
| | Polio vaccine (drops in the mouth) | __ [1=Yes; 2=No; 3=DK] | __ | |
| | Injection against measles | __ [1=Yes; 2=No; 3=DK] | __ | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | | |
|---|---|---|---|---|
| | **General health** | | | |
| | Child's general health and fitness in the first 10 years of life (compared to your other children) | __ | [1=Less healthy, 2=Same as the other children; 3=More healthy; 4=No other children] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

# Subject status record sheet – NINUB study

| | | | |
|---|---|---|---|
| | Visits to establish contact with the subject (maximum 5) | | **1.7** Visit notes |
| | Date and time of visit (ddmmyyyy:mmhh) in 24-hr clock | | |
| **1.1** | __ __/__ __/__ __ __ __: __ __ __ __ | | |
| **1.2** | __ __/__ __/__ __ __ __: __ __ __ __ | | |
| **1.3** | __ __/__ __/__ __ __ __: __ __ __ __ | | |
| **1.4** | __ __/__ __/__ __ __ __: __ __ __ __ | | |
| **1.5** | __ __/__ __/__ __ __ __: __ __ __ __ | | |
| **1.6** | Contact established…… ☐ [1=Yes; 2=No] | | |
| **2.1** | Contact status…… ☐ [1=Contactable; 2=Avoid contact (refused); 3=Dead] | | **2.4** Contact notes |
| **2.2** | Date contact status __ __/__ __/__ __ __ __ | | |
| **2.3** | Date review status __ __/__ __/__ __ __ __ | | |
| | **(a)** Study component completed | **(b)** Date completed | **3.5** Study component notes |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | | | |
|---|---|---|---|
| **3.1** | Consent…..☐ [1=Yes; 2=No] | __ __/__ __/__ __ __ __ | |
| **3.2** | Questionnaire… ☐ [1=Yes; 2=No] | __ __/__ __/__ __ __ __ | |
| **3.3** | Physical exam…☐ [1=Yes; 2=No] | __ __/__ __/__ __ __ __ | |
| **3.4** | Blood sampling…… ☐ [1=Yes; 2=No] | __ __/__ __/__ __ __ __ | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | Consent | **(a)** Verbal<br>[1=Yes; 2=No] | **(b)** Recorded<br>[1=No; 2=Yes] | **(c)** Written<br>[1=No; 2=Yes] | **4.8** Consent notes |
|---|---|---|---|---|---|
| **4.1** | Questionnaire (mother) | ☐ | ☐ | ☐ | |
| **4.2** | Height/weight (mother) | ☐ | ☐ | ☐ | |
| **4.3** | Questionnaire (child) | ☐ | ☐ | ☐ | |
| **4.4** | Physical examination (child) | ☐ | ☐ | ☐ | |
| **4.5** | Blood sampling (child) | ☐ | ☐ | ☐ | |
| **4.6** | Data storage/use | ☐ | ☐ | ☐ | |
| **4.7** | Blood storage/use | ☐ | ☐ | ☐ | |
| **4.8** | Further follow up | ☐ | ☐ | ☐ | |
| **5.1** | Date and time of appointment (ddmmyyyy:mmhh) in 24-hr clock: .__ __/__ __/__ __ __ __ ____: __ __ __ __ | | | **5.2** Did the subject attend? ……..☐<br>[1=Yes; 2=No] | |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| | |
|---|---|
| **5.3** | Appointment notes |
| **6** | General notes |

1. Subject ID:_____; 2. Study village number: __
3. Mother's ID: _____; 4. Village name: _____
5. Subject family name (surname): _____; 6. Subject first (given) name: _____
7. Mother's first (given) name: _____; 8. Father's first (given) name: _____
9. Age last birthday (in completed years): __ __; 10. Sex __ [1=Male; 2=Female]; 11. Birth order: __

| 1. Recording ID as shown on the recorder | 2. Date/Time in 24-hr format dd/mm/yyyy:hhmm | 3. Subject ID | 4. Subject's initials | 5. Father/husband's initials | 6. File name (name of the file as saved on the PC under C:\consents\) |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Please note that items 1-5 must be filled jut prior to the beginning of the recording. Item 6 should be filled after downloading the recording to the PC and renaming each file using the following convention: subjectID_ddmmyyyy_subjectinitials_fatherhusinitials