PROBIT III

Promotion of Breastfeeding Intervention Trial III

MANUAL OF PROCEDURES

ISRCTN37687716

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Questions and Answers from Training Workshop, September 2007

Blood Collection

For blood collection, how do I disinfect the finger?
Please ask the child to wash their hands before blood collection, then put their hand in the warm water for 3 minutes, wipe to dry the hand thoroughly. Then perform the finger prick, and collect blood for glucometry and dried blood spots. Then apply disinfectant to the puncture site after all blood collection is completed.

Which finger do I lance for blood collection?
The medial side of the fourth or middle finger of the non-dominant hand.

How do I retrieve a blood glucose result if the glucometer switches off?
Switch the glucometer on and press the **left** arrow ONCE only. At the top left hand corner of the display screen, you will see the word "Mem 1". This will tell you that you have retrieved the last reading that was taken. Please use this function only if it is essential, as it is easy to confuse readings. It is always preferable to write the result in the Interview Questionnaire when it first appears on the screen of the glucometer (when the blood is first applied to the test strip, the glucometer beeps when the result is ready).

Do I fill in the child's details on the blood spot card before or after taking the blood spots?
First, confirm the child will have their blood taken. Then fill in the details on the blood spot cards. Please try to use one card per child only, as they are very expensive. If the child has an incomplete collection of blood spots from a first visit, at their second visit use a new card and complete section 20 of the Interview Questionnaire, as the date of blood collection will be different.

What is the minimum number of blood spots?
Ideally we would like all eight bloodspots to be taken. The minimum number to ensure that the essential assays can be measured is five. Therefore, if you obtain 5 blood spots then the child does not need to return for a second visit. We would like you to take the glucose measurement first and then take the blood spots onto the filter paper.

If I need to call back a child for another attempt at blood collection, what should I do?
The child should be encouraged to fast (as previously) before the second visit. When the child arrives, collect blood in the same manner as on the first attempt and complete section 20 of the Interview Questionnaire. If the child does not want to fast but is willing to return to give a finger-stick blood sample, please ask the child to return for unfasted bloods. At the time of the second finger-stick blood sample, record the date and time the child last had something to eat or drink apart from water and continue with the blood glucose measurement and blood spot samples.
If the child agrees to return for a second attempt at blood collection:
1. Tick **Yes** to question 2.20 on the Interview Questionnaire.
2. Fill in section 1 ('Child identifying information') and questions 20.01 and 20.02 of the **green** 'SECOND BLOOD COLLECTION' Form and keep this form for the second visit. Questions 20.01 and 20.02 record whether a glucose reading has already been taken and how many blood spots have been filled after the first visit. This will remind you what has already been done.

3. Send the Interview Questionnaire and any blood spot cards from the first visit to the Centre in the usual way.
4. At the second visit, take the blood spots and complete questions 20.03 to 20.21 of the 'SECOND BLOOD COLLECTION' form. Only measure blood glucose at the second visit if it has not already been measured at the first visit.
5. At the second visit you should take the blood spots on a second blood spot card. Label this second card with the letter 'R' to indicate it is the second attempt at blood collection.
6. Send both the green form and the blood spot card to the Centre in the usual way.

Other Questions

At what age range should I see the PROBIT child for PROBIT III?
Please try to see all the oldest children first, so that as many as possible are seen before they enter puberty (aim for an age range of 10.5 to 11.5 years; try not to let any child reach the age of 12 before seeing them for this study).

Can I perform the examination on children who were not recruited to PROBIT I?
No; we only want data on those children who were in PROBIT I.

What do I do with the consent form?
Please keep the consent form at your polyclinic. Please fill in the appropriate part of the Interview Questionnaire to record that you have a copy of the consents/assent.

Is the order of the questions/measurements in the Interview Questionnaire important?
The order of the Interview Questionnaire is deliberate - Please answer the questions and take the measurements in the order they are printed. The blood collection is first as we need to collect blood before the child eats. We also need to measure bioimpedance when the child is fasted. Height is required for the bioimpedance measures. After glucometry and blood collection, measure the child's height, then weight and bioimpedance. As soon as the blood and bioimpedance measures are taken the child can eat. The time it takes to review the medical history questions will allow the child to rest quietly for 5 minutes before the blood pressure measurement. If you complete the family medical history questions by phone, please ensure the child sits quietly to rest for 5 minutes before you measure his or her blood pressure. The child's arm length is used to measure the location for the mid-upper arm circumference. The mid-upper arm circumference is used to decide which size of blood pressure cuff to use for the child. Then the blood pressure readings, other circumferences and skinfolds, and finally Tanner staging are measured. The chart review can be done without the child being present.

Helpful Tips

- It is useful to say all values out loud until you record them. This tip includes numbers that you measure (e.g. height, skinfold thicknesses) as well as those you read from a machine (e.g. blood pressure, glucose, weight, and bioimpedance). Repeating the measurements out loud helps to ensure you write down the correct values.
- Label the three blood pressure cuffs small, medium and large. This will make writing the cuff size on the Interview Questionnaire easier.
- When you phone the parent to get consent for the child to participate in the study, inform those mothers who are planning to accompany the child of the plan to take her weight and blood pressure so that she is prepared to bare her upper arm and to take off socks/stockings.

A. DESCRIPTION OF THE STUDY

A1 Overview

Children from 31 maternity hospitals were randomly selected and followed for 12 months from time of birth (PROBIT I) and again when the children were 6½ years of age (PROBIT II) at a polyclinic affiliated with each study maternity hospital. PROBIT III is a follow-up of these children at 11 years of age.

Objective of the study: The principal objective of the proposed study is to examine whether the experimental breastfeeding promotion intervention introduced in Belarus in 1996-98 has effects on obesity, high blood pressure, atherogenic lipoproteins, glucose homeostasis and insulin sensitivity, and growth factors detectable at 11 years of age. The comparison of the experimental and control groups, when analyzed by intention to treat, will allow the most rigorous examination to date of the causal relationship between the duration and exclusivity of breastfeeding and these important health outcomes.

A2 Information to be collected

During the interview, the information collected will include:

1. Blood collection information.
2. Weight and bioelectrical impedance.
3. Breastfeeding history
4. Medical histories of the child, the child's mother and father, and the child's extended family.
5. Measurements of child's blood pressure; standing and sitting height; head, waist, hip, and mid-upper arm circumferences; triceps and subscapular skinfolds.
6. Pubertal development.

The polyclinic chart will also be reviewed to record data on past heights and weights, weaning, and hospitalizations from 12 months to 7 years of age, ***only*** for those children who ***did not*** participate in PROBIT II. Hospitalizations since 7 years of age will be recorded for ***all*** children.

A3 Logistics and roles

Polyclinic paediatrician(s):

In each clinic, the participating polyclinic paediatrician(s) will be responsible for:

1. Verifying that each child included in the study is followed up according to the protocol. The main role will consist of identifying mothers and children and asking them to come to the polyclinic. This task is very important for the study. The paediatrician's clinical responsibilities are listed in detail in section B.
2. Making sure that for each study child, the following information is collected and sent by driver every 4 weeks to the Data Centre in Minsk:
    (i) PROBIT III Interview Questionnaire
    (ii) PROBIT III Chart Review Questionnaire
    (iii) Dried filter paper blood spots (frozen)
3. Storing the filter paper blood spots in a specially designated –18°C freezer in the polyclinic.
4. Arranging all paper work and blood spot samples according to the child's Subject number order before collection by the driver.
5. Correcting errors and completing missing information identified by the Data Centre.

Data Centre:

The Data Centre is responsible for data entry and management. It will receive the forms on a 4-weekly basis on average from each of the participating polyclinics and proceed to data entry. The Data Centre will generate, on a weekly basis, a list of errors identified on the forms that have been entered. There are two types of errors: missing values and incorrect values (incompatible with what is expected).

The polyclinic paediatricians will be contacted to correct the errors. The corrections will then be sent back to the Data Centre to be entered into the database.

Polyclinic patient representative:

Each polyclinic will identify a polyclinic patient representative to address any ethical or other concerns raised by the study children or their parents.

B. ORGANIZATION OF THE STUDY

B1 Locating the Children

By the end of September 2007, each participating polyclinic paediatrician will attempt to locate each PROBIT child and document if the child is willing to participate in PROBIT III. It is essential that the oldest children (i.e. those around 11 years) are located and examined first, so that as many as possible are examined before they reach puberty.

The paediatrician will telephone the parents of the children in the study; they will obtain verbal consent for the child to take part in the PROBIT III, and this consent must be recorded on a specially designed form (See Appendix 1). The paediatrician will check whether or not the child is diabetic. If the child is **not** diabetic, the paediatrician will give detailed information regarding fasting as below:

'We are interested in measuring some important factors in the blood to do with diet. So, we would like to take a blood sample before your child consumes anything in the morning. To do this we would like your child **not** to eat or drink anything, apart from water, after 10 PM on the night before the appointment until **after** they have had their blood test. (Please do not give them juice or tea or chewing gum). Please bring a snack from home to give to your child once we have done this part of the examination.'

If the child **is diabetic** then please let him or her eat and drink as normal.

The appointment should be given as close to this phone call as possible. An instruction letter about fasting will be provided for you to send to the mother before the child attends the clinic. You may wish to send this to the mother via your polyclinic nurse (Appendix 2a).

B2 The Visit

The polyclinic paediatrician will receive the Interview Questionnaires after the training workshop in Minsk.

The polyclinic paediatrician will:

1. Ensure that children come to the polyclinic visit.
2. Ensure that children fast after 10 PM the evening before the visit.
3. Ensure that the phone consent has been administered and signed.
4. Ensure a pre-examination instruction letter has been sent to the parents.
5. Ensure that children with diabetes are excluded from fasting.
6. Ensure that the parent consent and child assent forms are signed.
7. Ensure that the PROBIT III data forms are completed.

8. Ensure that blood spots are properly collected, frozen, and stored.
9. Measure the blood glucose using a glucometer.
10. Every 4 weeks, send copies of the data forms and frozen filter paper blood spots to the Republican Centre for Maternal and Child Health in Minsk.
11. Correct or complete information when errors are detected.
12. Attend PROBIT III workshops.
13. Participate in audit and quality assurance monitoring (see Appendix 6).

C. DURING THE VISIT

1. Ask the accompanying parent to sign the consent form and the child to sign the assent form [Appendix 2b]; record the relationship of the adult to the child. If the parent has not accompanied the child, then the paediatrician must check that the telephone consent sheet obtaining verbal consent from the parent is fully completed. If not, please phone the parent to obtain consent and record this on the telephone consent sheet.
2. Measure the blood glucose of the child using the glucometer and complete Section 2 of the data form. If the glucose measurement is found to be abnormally high or low, inform the accompanying parent and perform any clinically indicated follow-up testing and treatment (see Section 2 of this manual).
3. Collect the blood spots from the child onto the filter paper and complete Section 2 of the blood collection data form.
4. Let the blood spots air dry on the filter paper, then place the filter paper in the plastic bags that have been provided and store in the special polyclinic freezer at –18ºC.
5. Ask the child to remove their clothing to their underpants and bra and give them a gown to wear when not carrying out measurements
6. Measure height, weight and bioelectrical impedance of the child and mother (if present), and complete Section 3 in the data form.
7. Ask the child to eat the snack brought from home and ensure that they are sitting quietly for at least five minutes before their blood pressure is measured.
8. Interview the accompanying parent, following the order of questions on the PROBIT III data form.
9. Examine the child and complete the remaining parts of the data form.
10. When the interview and examination have been completed, give the child the gift.
11. Complete the Chart Review form using the child's polyclinic chart.
12. Every 4 weeks: send the data forms and frozen blood spots (on dry ice) by driver to the Republican Centre for Maternal and Child Health in Minsk.

D. RULES FOR COMPLETING THE DATA FORMS

1. Write the responses in the designated areas or tick the appropriate boxes.
2. Dates should be written in Arabic figures starting with day, then month and year.
3. All figures should be entered by filling the provided spaces on the right, and zeroes entered in the empty cells on the left.
4. If it is not possible to obtain data on certain questions, write 'unknown' or cross out the question and write 'unknown.'
5. All corrections made to data in the questionnaires should be initialled by the examining paediatrician.
6. Use a 24-hour clock for recording times.
7. Please answer the questions in the order they are printed.

Interview Questionnaire

SECTION A: Children's eating attitude test

Give this part of the questionnaire to the child to fill in, while they are waiting to be examined.

SECTION 1: Child identifying information

1.01 Hospital number

Each polyclinic has a specific number from 01 to 34. The hospital number for each child is the number that was originally assigned in PROBIT I. This number is also part of the identifier that serves to uniquely identify each child during the study. It is therefore necessary to report this number on all data forms. It is also crucial to keep the original hospital number to ensure a link between the three databases (PROBIT I, PROBIT II, and PROBIT III). This number will already have been stamped onto the questionnaire by the research staff in Minsk. Please verify that the identification details are correct for each child and inform the data centre if they are not.

1.02 Subject number

Each child has a subject number from 0001 to 9999, which was assigned at the maternity hospital for all children whose mothers originally agreed to participate in PROBIT. This number is also part of the identifier that serves to uniquely identify each child during the study. It is therefore necessary to report this number on all data forms. It is crucial to keep the same subject number to ensure a link between the three databases (PROBIT I, PROBIT II, and PROBIT III). This number will already have been stamped onto the questionnaire by the research staff in Minsk. Please verify that the identification details are correct for each child and inform the data centre if they are not.

Example: **32_0010** would be the identifier for polyclinic 32 and child number 10

1.03 Child's last name

Write in this order: last name

1.04 First names

Then write first name.

1.05 Child's date of birth

Write the day, month and year of birth: Day Month Year.

1.06 Participated in PROBIT II

Please tick the appropriate box as to whether or not the child participated in PROBIT II.

1.07-1.11 Who is accompanying the child

Please tick the appropriate box or boxes.

1.12-1.14 Consent and assent

Please tick the appropriate boxes. In order to proceed with the visit, the telephone consent must have been given and recorded and the assent form must be signed by the child (Items 1.12 and

1.14 both ticked “yes”). Item 1.13 may be “no”, but ONLY if the parent or legal guardian did not accompany the child and 1.12 is ticked “yes.”

SECTION 2: Blood collection

Overview

Blood sample collection will be by finger-prick using lancets to obtain blood spots, which are collected onto filter card, dried and stored in a –18°C freezer, and transported every 4 weeks to Minsk by driver. Whole blood glucose will be measured at the time of the clinic visit using the first drop of the finger-prick blood sample placed on a glucose dehydrogenase test strip, inserted in a Roche Advantage Accu-Chek Glucometer.

Finger-prick collections MUST be made using the specific procedure described below to minimize the potential for haemolysis or the dilution of finger-prick specimens by interstitial fluid.

The procedure is summarized below and then described in more detail in Appendix 3a and 3b.

1. At the start of the day, turn on the glucometer and perform the Internal Quality Control check. Write the results of the Quality Control check in the Interview Questionnaires of each of the children to be seen that day.
2. For each child:
    a) Finger-prick the child using protocol below.
    b) Take one drop of blood for glucose measurement by glucometer
    c) Take eight drops of blood onto the blood spot card
    d) Record the glucometer reading on the Interview Questionnaire
    e) Label the blood spot card with the identifiers for the child and paediatrician
    f) Store the blood spot card in the –18ºC freezer (to ensure that the stored blood spot cards do not thaw do not remove the box from the freezer).
3. Periodically:
    a) Transport of blood spot cards and Interview Questionnaires to Minsk: fortnightly
    b) Undertake External Quality Control of the glucometer: 3 monthly
    c) Code the glucometer: with each new box of glucometer strips

Important notes

1. Only one pack of Roche test strips should be opened at any one time.
2. Make sure the test strip vial cap is tightly replaced every time a strip is removed
3. Keep the glucometer free from dust and protect it against extreme cold or hot weather. The operating range of the glucometer is between 14°C and 40°C.
4. When a battery symbol appears on the display, the battery needs replacing immediately. Spare batteries will be supplied.
5. Do not use the memory function on the glucometer.

Detailed Protocol for each child

<u>1. Blood glucose measurement</u>

Explain the procedure to the child and reassure them.

a) At the start of each day perform the Internal Quality Control check **[see Appendix 3a]**. Write the results of the Quality Control check in each of the Interview Questionnaires of

the children to be seen that day. If the reading is acceptable then see the first child of the day, otherwise see Appendix 3a.

b) When you are ready to take the blood sample from the child, put on gloves.

c) In order to disinfect the child's hand, ask the child to wash their hands before blood collection. Perform the finger prick, and collect blood for glucometery and dried blood spots as directed below. Then apply disinfectant to the puncture site after all blood collection is completed.

d) Sit the child in a high chair so that the hand is hanging freely below the level of the heart. Use the child's non-dominant hand. **Warm the child's hand by placing it in a bowl of warm water for 3 minutes (while waiting undertake steps e to j).**

e) Label the blood spot card with the child's hospital number and subject number, initials, date of birth, and today's date in ballpoint pen. Tick Box A if you are the sole paediatrician taking part in the study in your polyclinic, otherwise you will be given a letter (A or B) at the training workshop. Tick the appropriate box on the blood spot card. It is important to write on the blood spot card **before** the blood spots are collected, to reduce the chance of contamination.

f) Check the expiry date of the test strips. Switch the glucometer on.

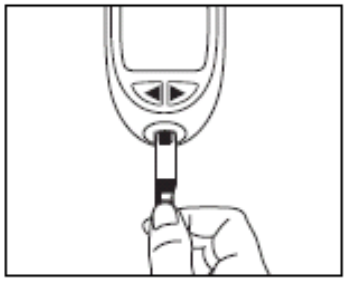
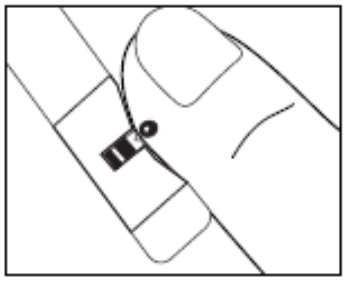
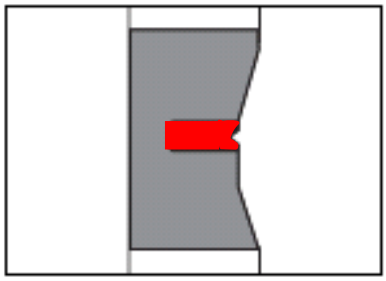
g) Press and hold down the ON/OFF button to check that the display is working.

h) Check that the code on the display screen of the meter matches the code on the test strip pot.

i) Open the test strip pot and remove a test strip. Replace the lid firmly back on test strip pot.

j) Within 30 seconds, insert the glucose test strip into the glucometer (yellow window on the test strip facing up).

k) Once the glucose test strip is inserted, a "drop of blood" display will appear on the screen. You have 90 seconds to apply the sample before the meter will switch off.

l) Twist the purple cap off the lancet device. Set the depth gauge on the lancet to **level 1 (the shallowest depth).** Dry the child's hand and fingers thoroughly with a sterile gauze pad. Place the lancet firmly against the side of the **middle** finger, going no lower than the nail bed and avoiding the fingertip. Hold the finger to prevent the child pulling away. Avoid using the child's index finger and thumb for the procedure.

m) Press the purple firing button firmly.

n) Allow 5 seconds to elapse after pricking the finger. Ensure that the hand is always hanging down freely so that blood can flow by gravity. Massage the palm of the hand towards the pricked finger so that a hanging drop of blood appears. DO NOT SQUEEZE AT THE SITE OF THE PUNCTURE.

o) When a hanging drop of blood appears, touch the droplet of blood to the curved side of the glucose test strip and keep it in place **until the yellow target area is completely covered**.

p) An "hourglass" symbol rotates on screen until the measurement is completed and the result is displayed. While you are waiting for the result to appear, collect a further eight blood spot samples onto the filter paper card as described below.

q) The glucometer displays the result for 90 seconds before it turns itself off. After this time you will need to switch the glucometer back on and press the **left** arrow once to recall the

result [If you press the left arrow once, you should see the word "**Mem 1**" at the top left hand corner of the display screen; this will tell you that you have correctly retrieved the last reading that was taken. Please use this function only if it is essential, as it is easy to confuse readings]. Please record the glucometer result in the PROBIT III Interview Questionnaire for this subject when it is displayed [the glucometer beeps when the result is ready], and then continue to collect ALL eight blood spots.

r) Dispose of the test strip and used lancet into a suitable clinical waste container.
s) Switch off the glucometer.
t) If it is not possible to obtain a glucometer reading and blood spots on this occasion, give the child another appointment to come back after fasting to take the glucometer reading and blood samples.

2. Collection of blood spot samples

a) Once a drop of blood has been collected on a glucometer test strip, continue to collect blood spots onto the filter paper card by allowing the blood to drop onto one side of the filter paper. Do not allow the finger to touch the filter paper. Allow the blood to soak through and completely fill the circle.
b) To enhance blood flow, gently apply intermittent pressure to the area surrounding the puncture site. Avoid excessive squeezing of the finger.
c) Collect eight separate spots of blood into the pre-printed circles indicated. Do not apply multiple drops of blood to fill each circle. One good hanging drop of blood is sufficient to fill each circle. See **Appendix 3b** for examples of valid and invalid blood spots.
d) Once the blood has been taken from the child, wipe the puncture site with a dry, clean material such as gauze.
e) Be careful not to contaminate the filter paper circles with spillages or by touching them before or after blood collection.
f) If blood flow is diminished repeat steps in Section 1 c, j, k, and l.
g) Allow the blood spots to completely air-dry on the drying rack for at least 30 minutes and preferably 3-4 hours at room temperature. Avoid contact between the table and the wet blood spots. Nothing should touch the wet filter paper.
h) Once the blood spot card is dry, wrap the cover over the filter paper circles to protect them. Then place the card in one of the protective zip lock bags with two desiccant packs that have been provided for the study. One blood spot card only per plastic bag.

3. Storage and Transportation of blood spot samples

a) After air-drying and by the end of the morning clinic, store the bagged samples collected each day in the –20°C study freezer. Do not allow the stored blood spot cards to thaw. Arrange all paper work and blood spot samples into Subject number order before collection by the driver.
b) Blood spots will be collected by the driver every 4 weeks and transported on dry ice to Minsk for central storage and analysis.

4. What to do if you fail to obtain enough blood spots for glucose measurement or to fill the filter paper circles.

Try and obtain as much blood as possible using the protocol above. If having repeated the finger-prick once, you are still unsuccessful, ask the child and the mother if they would be willing to return to a future clinic visit for one more attempt (see point 7 below). When the child visits again give them another small gift (provided) to say thank you. If they do not wish to return, then record how much blood was taken and send off the partially completed form and blood spot card.

5. What to do if glucometer results are abnormal.

If the child's glucose levels are too high (i.e. at or above 6.0 mmol/L) or too low (i.e. at or less than 3.5 mmol/L), you must immediately inform the child's parent on the day of the visit and arrange for a repeat blood test by venepuncture to confirm the abnormal result. If still abnormal, arrange for a full oral glucose tolerance test and/or further diagnostic work-up as appropriate.

Thresholds for immediate action are < 2.6 mmol/L or >10.0 mmol/L, which should be followed by immediate venepuncture to confirm the abnormal result. If the results are still abnormal, perform a full oral glucose tolerance test and/or further diagnostic work-up as appropriate.

6. Maintaining the quality of blood spot collection

The minimum number of blood spots required is five.
At the laboratory in Minsk the blood spots will be assessed for quality, by measuring:

- The number of blood spots
- If the circle widths are completely filled with blood
- If the full thickness of the filter paper circle is filled with blood
- If the blood spot is uniform
- If there is spotting
- If there is contamination

The quality of the dried blood spots is very important for the validity of the laboratory assays. If any problems with quality are found then the paediatrician will receive feedback, advice and possibly be offered further training.

7. Second blood collection attempt

If you need to call back a child for another attempt at blood collection, you should follow the following steps. The child should be encouraged to fast (as previously) before the second visit. When the child arrives, collect blood in the same manner as on the first attempt and complete section 20 of the Interview Questionnaire. If the child does not want to fast but is willing to return to give a finger-stick blood sample, please ask the child to return for unfasted bloods. At the time of the second finger-stick blood sample, record the date and time the child last had something to eat or drink apart from water and continue with the blood glucose measurement and blood spot samples.
If the child agrees to return for a second attempt at blood collection:

1. Tick **Yes** to question 2.20 on the Interview Questionnaire.
2. Fill in section 1 ('Child identifying information') and questions 20.01 and 20.02 of the **green** 'SECOND BLOOD COLLECTION' Form and keep this form for the second visit. Questions 20.01 and 20.02 record whether a glucose reading has already been taken and how many blood spots have been filled after the first visit. This will remind you what has already been done.
3. Send the Interview Questionnaire and any blood spot cards from the first visit to the Centre in the usual way.
4. At the second visit, take the blood spots and complete questions 20.03 to 20.21 of the 'SECOND BLOOD COLLECTION' form. Only measure blood glucose at the second visit if it has not already been measured at the first visit.
5. At the second visit you should take the blood spots on a second blood spot card. Label this second card with the letter 'R' to indicate it is the second attempt at blood collection.
6. Send both the green form and the blood spot card to the Centre in the usual way.

SECTION 3: Weight, Height and Bioelectrical Impedance

It is important to use standardized procedures consistently. Remove clothes, shoes and socks down to underwear before taking the measurements.

Standing height

Standing height is measured with a wall-mounted stadiometre with a movable headboard. Hair ornaments, jewellery, and braids should be removed from the top of the head in order to measure height properly. The child stands with the heels of both feet together so the medial malleoli are touching (unless the child has knock knees), and the backs of the heels are touching the base of the wall. Feet should be flat, so the undersides of the heels are in contact with the ground. The toes are pointing slightly outward at an approximately 60-degree angle.

The paediatrician should check the position of several points of body contact with the wall. The first contact point is the heels, then calves, followed by the buttocks, the scapula or shoulder blades, and finally the back of the head. Depending upon the overall body shape of the child, all points may not touch. The trunk of the body should be positioned vertically above the waist with the arms and shoulders relaxed and arms loose with palms facing medially. The head should be aligned in a Frankfort horizontal plane. The head is in a Frankfort plane when the horizontal line from the ear canal to the lower border of the orbit of the eye is parallel to the floor and perpendicular to the wall. Many children will assume this position naturally, but for some it might be necessary to make a minor adjustment. If required, the paediatrician may gently tilt the head up or down until a proper alignment is achieved with the eyes looking straight ahead. Once correctly positioned, the headboard is lowered, and the child is instructed to take a deep breath and stand as tall as possible. A deep breath allows the spine to straighten, yielding a more consistent and reproducible height measurement. Ask the child to 'relax the shoulders and stretch up but keep the heels on the ground'. Assist this stretching by applying gentle upwards pressure beneath the mastoid processes. Check that the heels are still touching the ground. The headboard is positioned firmly on top of the head with sufficient pressure to compress the hair. The measurement is read in centimetres and recorded to the nearest millimetre (0.1cm). The child then relaxes and steps away from the stadiometre.

3.01

Measure the child's height twice. If the measurements differ by more than 0.5 cm, check technique and take a third and fourth measurement. Record all readings.

3.02-3.03

Measure and record the mother's height, if she is present. Otherwise, record the mother's height (if known) reported verbally by the attending father / guardian.

Sitting height

Sitting height is a measure of the length of the trunk of the body from the buttock to the top of the head when a subject is sitting upright. To record this measure, a stadiometre and a box or stool of known height are used. It is essential you use the box or stool provided so that we know its height. After measuring standing height, the child sits on the box with the posterior aspect of the buttock, the shoulder blades and the back of the head touching the wall. Similar to the procedure followed for standing height, the head is positioned in the Frankfort plane and thighs are horizontal. Support the feet on the footrest so that the knee is at right angles, the hands are resting in the child's lap, and shoulders relaxed. The child is instructed to take a deep breath and sit up as tall as possible. The head piece of the stadiometre is lowered to the top of the head and the hair is compressed. Ask the child to 'relax the shoulders and stretch up'. Assist

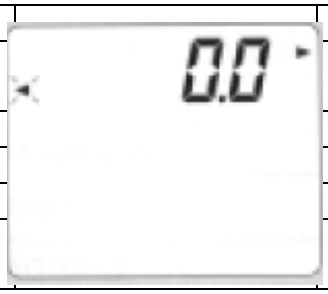
this stretching by applying gentle upwards pressure beneath the mastoid processes. Read the measurement while standing in front of the table looking horizontally at the counter while applying pressure. The reading is taken to the nearest millimetre (0.1 cm).

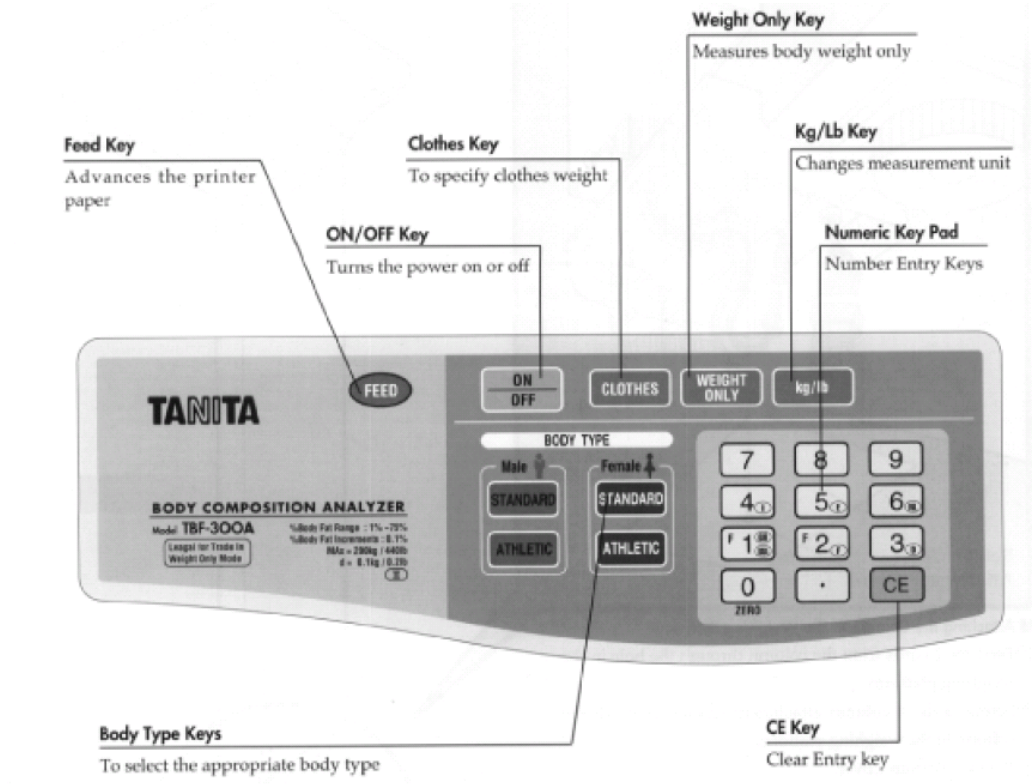
3.04
Measure the child's sitting height twice to the nearest 0.1 cm. If the measures differ by more than 0.5 cm, check technique and take a third and fourth measurement. Record all readings.

3.05-3.06
Record if a study stool was used. If a non-study stool was used record the height of the stool.

Weight and Bioelectrical Impedance

Background: The Tanita TBF 300GS body-fat analyser, which provides a measure of bioelectrical impedance from foot to foot, will be used to measure weight, total body water, % body fat, fat mass and fat free mass. This bioelectrical impedance system consists of two main units. The first is a platform scale on which two subdivided stainless steel foot pad electrodes are mounted. While the subject stands with bare feet making pressure contact with the foot pads, a small current (50 KHz/0.8 mA) is transmitted through the anterior part of the foot pad electrodes and the difference in voltage across the legs is measured by the posterior (heel) electrodes. The second unit is a tabletop indicator with a digital keyboard through which height and sex can be entered into a microcomputer. The unit measures body weight and impedance simultaneously and the measured impedance (W) is converted into the output of total body water, % body fat, fat mass and fat-free mass

WEIGHT	ВЕС		кг	kg
CLOTHES	ОДЕЖДА		фунты/стоуны, фунты	lb/st.lb
MALE	МУЖЧИНА		см	cm
FEMALE	ЖЕНЩИНА		футы, дюймы	ft. in
ATHLETIC	СПОРТСМЕН		ВОЗРАСТ	AGE
STEP ON	СТАНЬТЕ НА ПРИБОР		ЖИР, %	%FAT



Feed key Advances the printer paper	**Клавиша загрузки** Подает бумагу в принтер
ON/OFF key Turns the power on and off	**Клавиша ВКЛ/ВЫКЛ** Включает и выключает питание
Clothes key To specify clothes weight	**Клавиша «одежда»** Для указания веса одежды
Weight only key Measures body weight only	**Клавиша «только вес»** Измеряет только вес тела
Kg/Lb key Changes measurement unit	**Клавиша «кг/фунты»** Выбирает единицу измерения
Numeric key pad Number entry keys	**Клавиатура нумерации клавиш** Номера клавиш ввода
Body type keys To select the appropriate body type	**Клавиши выбора типа тела** Для выбора типа тела
Standard Athletic Male Female	Стандартный Атлетическо Мужчин Женщин
CE key Clear entry key	**Клавиша очистки** Клавиша очистки ввода

Procedure: Place the platform of the Tanita machine on a stable flat surface, clean with a disinfecting wipe, and switch on. The paediatrician asks the child to pass urine (if possible) and remove clothes, shoes and socks down to underwear before taking the measurements. The first prompt asks for the weight of the child's clothing, enter 0.0 for **ALL** subjects. Now select either STANDARD MALE or STANDARD FEMALE. Enter the child's age in years (remember to enter the leading zero if the child is less than 10: i.e. 09 for 9 years). Record the child's standing height in centimetres to the nearest centimetre (up to 0.4 is rounded down and 0.5 is rounded up). The child steps slowly on the metal sole plates of the machine without touching anything else. It is essential that the child stands on the metal plate with clean bare feet. Heels should be placed directly on the posterior electrodes and the front part of the foot should be in contact with the anterior electrodes, with feet parallel. The child should stand reasonably straight if possible - leaning to one side (or forwards) can affect the weight recorded. Once the printer prints out the results, the child steps off the machine.

The Tanita scale sometimes reads an error with very thin boys when using the 'male standard' setting. If this occurs please take a reading using the 'female standard' setting. Record this information on the interview questionnaire.

3.07-3.16 Bioelectrical Impedance results for child

Using the results from the printout, record the child's weight, impedance, % fat, fat mass, fat free mass and total body water onto the data form. In addition, staple the printout onto the data sheet; make sure the printout is readable, if not, correct any printer problems and repeat the measurement. One measurement is taken.

If the child exceeds the maximum weight registering on the scales (200kg) do not attempt to weigh them but code as "weight not attempted". If the child has a pacemaker or internal medical device, code as "weight not attempted". Do not attempt to weigh them using the Tanita machine. If the Tanita machine cannot be used, measure weight using the standard clinic scales.

ТАНИТА АНАЛИЗАТОР ТЕЛА TBF – 300GS	
ТИП ТЕЛА	**СТАНДАРТНЫЙ**
ПОЛ	**МУЖСКОЙ**
ВОЗРАСТ	**26 лет**
РОСТ	**182 см**
ВЕС	**82,0 кг**
ИМТ	**24,8**
ИОО	**10810 кДж 1932 ккал**
ИМПЕДАНС	**449**
ЖИР, %	**16,1%**
ВЕС ЖИРА	**13,2 кг**
ВБЖ	**68,8 кг**
ОВО	**50,4 кг**
ЖЕЛАТЕЛЬНЫЙ ДИАПАЗОН	
ЖИР, %	**14-20%**
ВЕС ЖИРА	**11,2-17,2 кг**

TANITA
BODY COMPOSITION
ANALYZER
TBF - 300GS

BODY TYPE	STANDARD
GENDER	MALE
AGE	26
HEIGHT	182cm
WEIGHT	82.0 kg
BMI	24.8
BMR	10810 kj
	1932 kcal
IMPEDANCE	449
FAT%	16.1 %
FAT MASS	13.2 kg
FFM	68.8 kg
TBW	50.4 kg
DESIRABLE RANGE	
FAT%	14 - 20 %
FAT MASS	11.2 - 17.2 kg

3.17-3.25 Bioelectrical Impedance results for mother

Repeat the procedure for the mother (if present), only shoes and socks need to be removed.

Now the child may eat the snack bought from home. Please do not let the child have tea or coffee, as this may interfere with blood pressure readings. Give a task or something to occupy the child, as they may get bored while the parents answer the following questions.

SECTION 4: Breastfeeding history

4.01-4.02

Please record answer to the nearest completed month.

SECTION 5: Child's medical history

5.01

Please tick the appropriate box. If 'No,' go to Section 6:

5.02

Please give the date.

SECTION 6: Maternal medical history

8.07 to 6.10

Please tick the appropriate box. If someone other than the child's mother accompanied the child, please ask him/her to answer these questions on behalf of the mother to the best of his/her knowledge.

SECTION 7: Paternal medical history

7.01 to 7.06

Please tick the appropriate box. If someone other than the child's father accompanied the child, please ask her/him to answer these questions on behalf of the father to the best of her/his knowledge.

SECTION 8: Extended family medical history

8.01 to 8.06

Please tick the appropriate box. Ask the person accompanying the child to answer these questions to the best of her/his knowledge.

8.07 to 8.11

Please tick the appropriate box.

SECTION 9: Medications

Please indicate if the child has taken any of the medications listed and the last time it was taken. Tick ONE BOX ONLY in each row.

SECTION 10: Mother's blood pressure

10.01 to 10.04

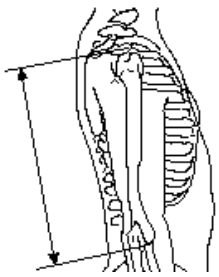
The mother's blood pressure should be taken if she is present.The systolic and diastolic blood pressure are measured in the right arm in the sitting position using the fully automated (digital read-out) OMRON 705IT device. **The appropriately sized blood pressure cuff should be used i.e. the bladder of the cuff should encircle at least 80% of the upper arm (but NOT more than 100%)**. The following protocol for adult blood pressure should be followed:

1. The subject should sit down; rest their right arm on the table, palm of hand facing upwards and upper arm at chest level. Ensure that the subject is sitting with their feet on the ground and that the legs are not crossed.
2. The cuff should be airless and the air tube inserted into the right side of the OMRON 705IT device.
3. The cuff should be placed around the **bare** right upper arm. Avoid rolling sleeves up as this can cause a constriction to the upper arm.
4. The cuff should be at the same level as the heart. The green marker on the cuff should lie over the brachial artery on the inside of the arm. The air tube should run down the side of the arm. The lower end of the cuff should be 2 cm above the antecubital fossa (elbow crease).
5. Pull the cuff so that the top and bottom edges are tightened evenly around the arm. When the cuff is positioned correctly, close the Velcro fastener firmly. Make certain the cuff fits snugly around the arm. The cuff should make good contact with the skin. The cuff should be positioned firmly around the arm but must not be too tight: i.e. you should be able to insert a finger between the cuff and arm.
6. Check that subject is familiar with having her/his blood pressure taken.
7. Explain that you will inflate the cuff, which will then slowly deflate automatically.
8. Be sure there are no kinks in the air tubing.
9. Press the 'on' button. Then press the 'start' button.
10. As the cuff begins to inflate, the monitor automatically determines the ideal inflation level. Because the monitor detects the pulse even during inflation, ask the subject not to move the arm or talk, but remain still until the entire measurement completes.
11. Inflation stops automatically and blood pressure measurement is started. As the cuff slowly deflates, decreasing numbers appear on the display and the heart symbol flashes at every heartbeat. In rare circumstances, a higher inflation may be necessary. In those cases the monitor automatically reinflates the cuff up to 30mmHg higher than initial inflation and restarts the measurement.
12. It is very important that the subject keeps the arm still and does not to talk during measurement. Arm movement is a very common cause of error readings on the blood pressure machine and talking will distort the blood pressure reading.
13. When the measurement is complete, the cuff completely deflates and the blood pressure (and pulse rate) is displayed.
14. Record the systolic and diastolic blood pressure readings exactly as on the machine (DO NOT ROUND THE FIGURES UP OR DOWN).
15. Clear the memory by pressing the 'M' button and the start button simultaneously. This MUST be done before the child's blood pressure is taken.

Before taking the child's blood pressure, allow the child to rest for 5 minutes, including the time taken to complete sections 4-11. Allow the child to empty their bladder if needed (a full bladder will affect blood pressure readings).

SECTION 11: Child's upper arm length and mid upper-arm circumference

11.01 Child's upper arm length



To locate the middle of the upper arm, the child should stand with the right arm flexed 90 degrees at the elbow. The palm faces up and the fingertips point straight ahead. The paediatrician stands behind the subject. The uppermost edge of the posterior border of the acromion process is located on the right scapula, and a horizontal line is drawn at this point. The zero end of the measuring tape is held on this mark. The tape is extended down the midline on the posterior surface of the arm to the tip of the olecranon process at the elbow. This is the child's upper arm length and is recorded to the nearest millimetre (0.1 cm).

Record child's upper arm length **once** to the nearest 0.1 cm. It is not necessary to repeat this measurement. Record the reading.

11.02 Child's mid-upper arm circumference.

The distance between the mark at the acromion and the tip of the olecranon is divided by two. A horizontal mark is made at the mid-point on the posterior aspect of the arm before the measuring tape is removed. This mark is then crossed with another line extending from the acromion to the olecranon. This point defines the site at which both mid-upper arm circumference and the triceps skinfold is measured. Arm circumference is measured with the child standing and the right arm hanging loosely and relaxed. It is important to be certain that the muscle of the arm is not flexed or tightened, which could yield a larger and inaccurate reading. The paediatrician stands to the right side of the child and places a measuring tape around the upper arm perpendicular to the long axis of the arm at the marked point. The measuring tape is held gently on the skin surface. The two ends of the measuring tape are pulled together, taking care not to compress the skin and underlying subcutaneous tissue. The arm circumference is recorded to the nearest millimetre (0.1 cm).

Record mid-upper arm circumference twice to the nearest 0.1 cm. If the measures differ by more than 0.5 cm, check technique and take a third and fourth measurement. Record all readings.

SECTION 12: Child's sitting blood pressure (1)

12.01

Record the time at which the blood pressure measurement is taken.

12.02

Record the temperature of the room with an electronic thermometer

12.03-12.04

The child's systolic and diastolic blood pressure is measured three times during the examination on the right arm in the sitting position using the fully automated (digital read-out) OMRON 705IT

device. It is important to select the correct blood pressure cuff size. You should choose the cuff that is appropriate to the circumference of the arm as measured above in **11.02**.

Small cuff is used for ≤ 22cm arm circumferences
Standard cuff for > 22 to ≤ 32cm arm circumferences
Large cuff for > 32 cm arm circumferences

Follow the detailed instructions in Section 10, with particular attention to the following:
The child should sit comfortably at a table, feet uncrossed and flat on the floor, with their right arm on the table so the upper arm is at chest level (this ensures the cuff will be at approximately heart level). The right arm should be relaxed with palm upwards. Explain what is going to happen as for the adult blood pressure measurement.

Make sure the arm is bare i.e. child is wearing a vest only. The cuff should be placed securely around the upper arm with the bladder centre over the brachial artery. There should be a two-finger gap between the antecubital fossa and the bottom edge of the cuff. Do not place the cuff too tightly as bruising may occur on inflation. Ideally, it should be possible to insert a finger between the cuff and arm, but the cuff should not be applied too loosely, as this will result in an inaccurate measurement. Keep the air tube parallel with the child's middle finger.

Encourage the child to keep the arm still and not to talk during measurement. Press 'start' and the cuff will inflate automatically. Do not put your hand on the cuff, as this will interfere with the readings. The cuff will deflate automatically. Read the display and record accurately what is displayed - DO NOT ROUND THE FIGURES UP OR DOWN. For further readings repeat this process.

12.05

Tick the appropriate box. Note that measurements should be taken in the **right** arm. If the right arm cannot be used or you are unable to obtain a reading in the right arm after two attempts using the OMRON 705IT, then move to left arm and make two further attempts using the OMRON 705IT. If this fails, use the mercury sphygmomanometer on the right arm.

12.06

Tick the appropriate box. Note that measurements should be taken using the OMRON 705IT. The main reasons why blood pressure readings using this device fail are:

- Positioning the cuff incorrectly
- Not keeping the arm still or child is talking
- Using an incorrectly sized cuff
- Placing a hand on the cuff while pumping
- Arrythmia (rare)
-

If there are difficulties obtaining readings with the OMRON 705IT, repeat having checked that you are not making one of the mistakes listed above. As a last resort, a mercury sphygmomanometer may be used, but only after multiple unsuccessful attempts with the OMRON 705IT. (See 13.6).

We recommend that standard nomograms are used to determine if the child's blood pressure (averaged over 3 readings) is in excess of current Belarusian recommendations and that standard protocols for their management are followed (see Appendix 4).

12.07

Tick the appropriate box to record the size of the cuff used to measure the child's blood pressure.

Repeat blood pressure readings after waiting 1 minute between measurements.

SECTION 13: Child's sitting blood pressure (2)

See Section 12

SECTION 14: Child's sitting blood pressure (3)

14.01-14.04

See Section 12

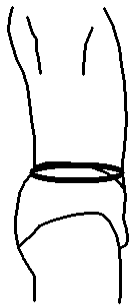
14.05-14.09

Tick the appropriate response for item 14.05. If you ticked "yes," then go on to item 14.06-14.09 to tick the answer that best fits the problem you encountered.

SECTION 15: Circumference and skinfold measurements

15.01 Child's waist circumference

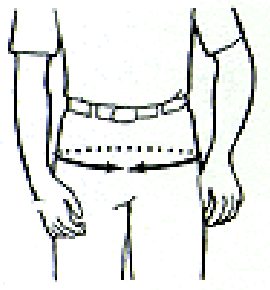
This should be measured with the subject standing with feet together and weight evenly balanced on both feet. The arms should hang loosely at the sides. The paediatrician, who is positioned to the right of the child, locates the right ilium. Just above the uppermost lateral border of the right ilium, a horizontal mark is drawn and then crossed with a vertical mark on the mid-axillary line. The paediatrician places the measuring tape around the trunk at the level of the mark on the right side. The paediatrician then inspects all sides to make sure the measuring tape is at a level horizontal plane. The tape is then tightened slightly, but without compressing the skin and underlying subcutaneous tissues. The measure is made at minimal respiration and is recorded to the nearest millimetre (0.1cm). Ask the child look straight ahead, be relaxed, and not to pull the tummy in.



Record waist circumference twice to the nearest 0.1 cm. If the measures differ by more than 0.5 cm, check technique and take a third and fourth measurement. Record all readings.

15.02 Child's hip circumference

The child is asked to stand upright with feet together and weight evenly distributed. The paediatrician is positioned on the right side with eye level at the hip region of the child. The cloth measuring tape is placed around the hip and anchored at the maximum protuberance of the buttock. The measuring tape is held snugly but not pulled tight, and the measure is recorded to the nearest millimetre (0.1 cm).



Record hip circumference twice to the nearest 0.1 cm. If the measures differ by more than 0.5 cm, check technique and take a third and fourth measurement. Record all readings.

15.03 Head circumference

A plasticised cloth tape is used to measure head circumference. Any hair ornaments or any hair arrangements such as braids that may interfere with the accuracy of the measure should be removed. The paediatrician stands to the right side of the child. The tape is placed around the head. On the face, the lower margin of the measuring tape is placed just above the eyebrows. On the side of the head, the tape extends above the ears to the back of the skull, where it is centred over the occipital prominence. While holding the tape in place over the eyebrows, the tape is moved up or down as necessary on the posterior aspect of the skull. The objective is to locate the maximal circumference of the head at the occipital prominence. The two ends of the measuring tape should be pulled firmly to compress the hair and the underlying soft tissues. The measurement is recorded to the nearest millimetre (0.1 cm).

Record the child's head circumference twice to the nearest 0.1 cm. If the measures differ by more than 0.5 cm, check technique and take a third and fourth measurement. Record all readings.

Skinfolds

Lange spring-loaded callipers are used loaded with a constant pressure of 10g/mm^2. Measurements are made vertically for the triceps skinfold and diagonally to follow the natural tissue contour for the subscapular skinfold. Take skinfold measurements directly on skin – not through clothing. Pick up and hold skinfold with one hand, while measuring the skinfold thickness with callipers held by other hand. Locate and measure each skinfold with care. Results may vary if measurements are not consistently taken at the exact location.

Prior to measuring the skinfolds, each site is carefully marked on the right side of the child's body. For each site, the skinfold is lifted up at a location 2 cm above the point at which the calliper tips will be placed. The thumb and index fingers separate the subcutaneous fat from the underlying muscle. Just the skin and adipose tissue are taken to form a distinct fold. The jaws of the calliper are placed perpendicular to the length of the fold while the paediatrician continues to hold the skinfold. The actual measurement is read from the calliper dial 2-3 seconds after the calliper tips are applied to the skin, and the tension is then released from the calliper handle.

The skinfold thickness is measured in millimetres to the nearest 0.5 millimetre. On some individuals it is not possible to separate the fat from the muscle tissue. When a distinct separation between a fold of skin and subcutaneous fat cannot be made with confidence, an appropriate note should be added on the data form (17.13 or 17.14), and the measurement value should not be recorded.

15.04 Triceps skinfold

The triceps skinfold is measured on the right upper arm at the point previously marked for the mid-upper arm circumference. The child stands upright with feet together, shoulders relaxed, and the arms hanging loosely at the side. The paediatrician stands behind the child and gently lifts the triceps skinfold with fingertips just above the mark. The triceps skinfold is held parallel to the long axis of the upper arm. The tips of the calliper jaws are placed perpendicular to the line of the fold, 2 cm from the fingertips and centred over the marked point.

Measure the right triceps skinfold twice to the nearest 0.5 mm, each time after a 2-3 second equilibration (relax) period. If the measures differ by more than 1 mm, take a third and fourth measurement. Record all readings.

15.05 Subscapular skinfold

The subscapular skinfold is measured with the child standing upright, shoulders relaxed, and arms hanging loosely at the side. The paediatrician stands behind the child and gently palpates the inferior angle (lower-most tip) of the right scapula. A mark is made on the inferior angle of the scapula. The paediatrician gently lifts a fold of skin and subcutaneous adipose tissue with the index finger directly above and medial to the mark at the inferior angle of the scapula, and with the thumb reaching toward the spine. The skinfold follows the normal subcutaneous tissue contour, forming a line extending diagonally toward the right elbow. The jaws of the calliper are placed perpendicular to the length of the fold, 2 cm from the fingertips, with the tip of the calliper jaw directly on the mark at the inferior angle of the scapula.

Measure the right subscapular skinfold twice to the nearest 0.5 mm, each time after a 2-3 second equilibration period. If the measures differ by more than 1 mm, take a third and fourth measurement. Record all readings.

15.06 to 15.19

Please indicate if there were any problems taking any of the anthropometric measurements. If 'yes,' specify the problem (for example, scoliosis, broken leg, etc.) and indicate which measurements were affected.

SECTION 16: Girls' pubertal development report and

SECTION 17: Boys' pubertal development report

Tanner staging is the standard used for sexual maturation in adolescents. The primary characteristics are best described in relationship to growth of the breasts in females, the genitals in males and the development of pubic hair in both. We suggest that during Tanner staging there is a chaperone (such as the child's parent) in the room, regardless of the sex of the physician or child. In order to avoid embarrassment and anger, before Tanner staging is performed the child must be told what the doctor will be observing. Tanner staging should be described as a procedure to ascertain the stage of growth and development; the procedure should never be described as looking at the size of the various parts. In order to limit the intrusiveness of the Tanner staging it must be performed in a professional manner as a routine part of the exam. (The child does not need to be completely undressed as the doctor should be able to assess maturation by discreet arrangement of the appropriate article of clothing). After completion of the Tanner staging, a simple statement assuring the child that everything looks normal would be appropriate. Tick the box that looks most like the child now. Tick one box only per section.

SECTION 18 Examiner details

18.01 Initials of Examiner

Please enter your initials.

18.02 Date physical examination completed

This is the date on which the physical examination is completed: Day Month Year.

END OF INTERVIEW

Give a small gift (provided) to say thank you. Remember to follow-up any unexpected findings. A list of equipment required in the clinic is listed in Appendix 5.

SECTION 19 Chart Review Questionnaire

PART 1

This part should be completed for all children

Hospitalizations since 7 years of age

Transcribe the dates of all hospitalizations for gastrointestinal infection, pneumonia and asthma since 7 years of age.

PART 2

This part should be completed for only those children who did not participate in PROBIT II

Heights and weights from 12 months to 7 years of age

S1.1 to S1.14 Heights

Record the child's height in centimetres to the nearest 0.1 cm for all heights measured from 12 months to 7 years of age

S2.1 to S2.14 Weights

Record the child's weight in kilograms to the nearest 0.1 kg for all weights measured from 12 months to 7 years of age

S3.1, S3.2 Weaning

Record the age at weaning, to the nearest completed month.

S4.1 to S6.4 Hospitalizations from 12 months to 7 years of age

Transcribe the dates of all hospitalizations for gastrointestinal infection, pneumonia, or asthma from 12 months to 7 years of age.

SECTION 20 Second attempt at blood collection

This section will only be completed if a child returns for a second attempt at blood collection. Ensure that the child's identifying information is completed.

20.01-20.02

Record whether a glucose reading has already been taken and how many blood spots have been filled after the first visit. This will remind you what has already been done.

20.03-20.04

Record the date and time that the child last ate or drank anything other than water.

20.05-20.06

Record the date and time that the second attempt at blood collection was completed.

20.07-20.21
Complete the blood collection according to the protocol in Section 2 above. Only measure blood glucose at the second visit if it has not already been measured at the first visit.

Check all forms have been filled in clearly. Order all paper work and blood spot samples into subject number order before collection by driver

Appendix 1

Telephone consent form (for office/reception use)

PROBIT III

Telephone consent form (for office/reception use)

This form is to be used when the parent is giving consent over the telephone, shortly before the test is done. It is completed by the paediatrician who has made the telephone call.

I (parent/legal guardian full name) ……

confirm that I am happy for my child (name) ……

to attend the visit and consent for the study.

My relationship to child is as parent /legal guardian (delete as appropriate).

Signed at parent's direction by (paediatrician) ……

Name (Please print)……

Date…… Time……

Appendix 2a

Pre exam instruction letter re fasting:

PROBIT III

Dear <<insert_parent_and_child_name>>

Thank you for agreeing to take part in the Probit III study. We hope that you will enjoy your visit, which will take about 30 minutes.

If your child is **not** diabetic we are interested in measuring some important factors in the blood to do with diet. So, we would like to take a blood sample before he/she eats anything in the morning. To do this we would like your child **not** to eat or drink anything, apart from water, after 10pm on the night before the appointment until he/she has seen the doctor. (Please do not give him/her juice or tea or chewing gum). Please could you bring a snack from home to give to your child once we have done this part of the examination.

If he/she forgets, and eats or drinks something by mistake, please phone us and we will rearrange the appointment for another day.

If your child **is diabetic** then please let them eat and drink as normal.

We look forward to seeing you both at the clinic on <<invite_date>> at <<time>>.

With very best wishes

<<sign here>>

Appendix 2b

Parent consent form:

PROBIT III

BREASTFEEDING DURATION AND EXCLUSIVITY:
Impact on atherosclerosis and diabetes risk factors in childhood

Project Sponsors: Canadian Institutes of Health Research, The European Union, The USA National Institute of Health

Consent Form (English version)

Purpose of Study
You are invited to participate in a research study that seeks to follow up children who participated in PROBIT, the Promotion of Breastfeeding Intervention Trial, which started in 1996-98 in 31 Belarusian maternity hospitals and their affiliated polyclinics. The first phase of PROBIT involved the follow-up of the children through the age of 12 months. During the second phase (PROBIT II), the children were followed up at 6½ years of age to further evaluate their growth, health, and development.

We are now inviting you to participate in a third follow-up (PROBIT III) of the same children at 11 years of age. This follow-up seeks to analyse the effects of infant feeding on such problems as obesity, high blood pressure, and cholesterol and other lipids (fats) in the blood, which are risk factors for atherosclerosis (hardening of the arteries that can lead to a heart attack or stroke) and diabetes.

The project is funded by the Canadian Institutes of Health Research and the European Union, and the principal investigators are Drs. Konstantin Vilchuk (Belarus), Michael Kramer (Canada), Matthew Gillman (USA), and Richard Martin and George Davey Smith (UK).

What Will Happen

- If you agree to participate, we will ask you to bring your child to his or her polyclinic after an overnight fast for a 30-minute visit, where the pediatrician will first do a blood test on your child. Blood will be collected from a single finger-prick using a minimally invasive procedure specially designed for children to minimize pain. The blood drops collected will be dried on filter paper and then transported to Minsk for analysis for insulin, cholesterol carrying proteins, and hormonal growth factors.

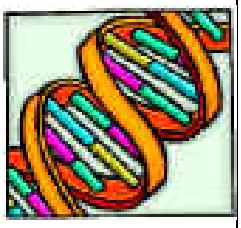
- A simple test of your child's blood sugar level will also be made using one of the blood drops at the time of the visit. Your pediatrician will review the result and may ask for follow-up tests if the result is either too high (above normal) or too low (below normal). These follow-up tests will not form part of the research but willbe a part of routine clinical care.

- Your child will be required not to consume any food or drink other than water after 10 PM the night before the blood test. After the blood test, your child should eat a snack brought from home. However, if your child has **diabetes** and is taking insulin, please let him/her eat and drink as normal.

- The pediatrician will also measure your child's standing and sitting height, weight, waist circumference, hip circumference, arm and upper back skinfold thickness, blood pressure, and percent body fat (using a device that looks like a weighing scale and passes a very small and imperceptible electric current) and assess his or her stage of pubertal development (genitals and breasts).

- The dried blood spot cards will be stored for the duration of the study. Long-term storage (15-20 years beyond the study duration) will be subject to prior approval by the Ministry of Health of the Republic of Belarus. No further tests will be performed on the blood spots, other than those outlined above, without your prior consent and approval by the Ministry of Health of the Republic of Belarus.

Test Results
If your child's blood sugar result is found to be too high or too low, you will be notified by the pediatrician on the day of your visit. None of the other tests planned have any known signification for clinical care, and thus will not be communicated to the pediatrician or to you.



Risks and benefits
With the exception of the discomfort of the finger-prick, none of the measurements or tests carry any risk to your child. Other than the information we obtain on your child, he/she is not expected to derive any benefit from participating in the follow-up study. Rather, the benefits of the study will accrue to future children in Belarus and elsewhere, because no study like PROBIT has been carried out anywhere else in the world. The information we obtain from the new proposed follow-up will help doctors and policy makers in planning their future practices concerning infant feeding. To thank you and your child for your participation, we will give your child a gift.

Confidentiality
All of the information collected in the course of this study will be kept confidential and anonymous. This means that the results of all the tests and answers to questionnaires will be kept separate from your child's name. No-one who works with samples or answers to questionnaires is allowed to know where they came from. The name of no parent or child will be used in any publication or report, either inside or outside the Republic of Belarus. No one will sell any of the samples or information you have given us.

Voluntary Participation
Although we hope you will consent to your child's participation in this follow-up, his/her participation in the first or second phase of PROBIT does not oblige you or your child to participate in this third phase, nor will your declining participation in the follow-up diminish the quality of

care that your child receives at the polyclinic now or in the future.

_____

Contact Information

If you have any questions regarding the study, you may contact Dr. Natalia Bogdanovich at (17)-233-32-85 or Nina Gusina at (17)-288-06-85. If you have any ethical concerns regarding the study that you would like to discuss, you may contact the polyclinic patient representative, _____ , at _____.

I have read the above consent form and have been given the opportunity to ask questions and request additional information.

I understand the purpose and procedures of the proposed study, and I hereby consent to my child's participation.

Signature | Date

Print name | ☐ Parent ☐ Legal guardian ☐ Other

Paediatrician | Date

Print name

Child's assent form:

PROBIT III

BREASTFEEDING DURATION AND EXCLUSIVITY:

Impact on atherosclerosis and diabetes risk factors in childhood

Child's Assent Form (English version)

Why is this study being done?

We are inviting you to take part in a research study. Research is a way to test new ideas and learn new things.

In our research study, we want to learn more about breastfeeding. We want to test if giving a baby breast milk only for the first few months of life helps to prevent certain problems like obesity (overweight) and heart problems later in life.

This research study is a continuation of a study that your parents included you in when you were born. We are inviting you to take part in our study one more time so we can learn more about how breastfeeding affects the growth and health of children your age. There is so much we want to find out, and you can help!

What will happen?

If you agree to take part in our study, we will ask you to see your doctor with your mother or father in the polyclinic for a visit of about 30 minutes. The doctor will first ask you to have a blood test. The night and morning before the test, you must not eat or drink any food or liquid after 10 PM. On the morning of the test, a doctor or nurse will prick your finger to take a few drops of blood. Immediately after the test, you can eat the snack you brought from home.

The doctor will then measure your height, weight, body fat, and physical development using standard scales, tape measures, and other instruments. In addition, you will stand on a machine that looks like a weighing scale that measures your body fat by passing a tiny amount of electrical energy through you. This test is completely harmless and you will not feel anything.

The doctor will ask you to undress to examine the development of your genitals and (for girls) breasts.

Will taking part in the study hurt?

The blood test will hurt a little, but only for a few moments. None of the other measurements will cause any pain. To thank you for your help, we will give you a small gift.

Will being in the study help you?

What we learn in this research will not help you now but will help future children in Belarus and around the world.

Who will know that you are in this study?

The information collected about you in this study will be kept safely locked up. No one will be able to see this information except the researchers, and no one will ever report your individual results or mention you by name.

Do you have to be in this study?

Taking part in this study is your choice. You can say "yes" or "no". Whatever you choose, your doctor will still take good care of you.

What if you have any questions?

Take the time you need to decide. You can ask questions at any time.

Child's Statement

My doctor has told me about the research. I had a chance to ask questions. I know I can ask questions at any time. I agree to take part in this study.

Child's Name

Child's Signature — Date

Paediatrician/Witness — Date

Appendix 3a

Glucometer Maintenance, Coding and Quality Control Procedures

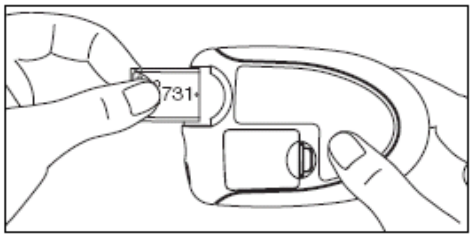
1. Introduction

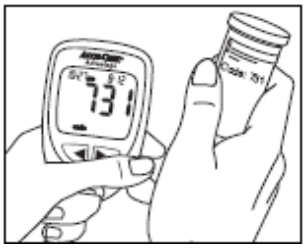
The Roche Advantage Accu-Chek is a blood glucose monitoring system

2. Start-up & coding of the meter

1. Before you use the meter for the first time and every time you open a new box of test strips, you need to code the meter to match the box of test strips.
2. A new code key comes with every pot of test strips. Each code key provides the meter with specific information it needs to accurately measure blood glucose.
3. **You will need to insert a new code key**:
   - whenever you open a new box of strips;
   - whenever an Error code (Err) message appears on the display panel.
4. **To code the meter**:
   - Ensure that the meter is turned off
   - Remove the old code key from the back of the device

- Insert the new code key with the code number facing towards you
- Make sure the code key snaps into place
- Turn on the meter and read the 3- digit code number displayed on the screen
- This number must match the code number on the pot of test strips. If it does not, repeat step 4.





NOTE: If the meter is used despite incorrect coding, inaccurate blood glucose readings could result. The coding chip must remain in the meter until all the test strips of the relevant pack have been used.

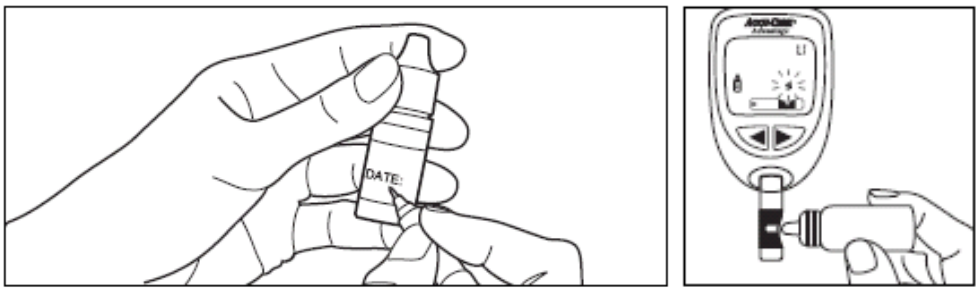
3. Quality Control

It is important to check that the glucometer is performing properly before assaying patient samples. This is achieved using internal and external quality control materials as described below. When the quality control solution bottles are first opened, please write the date of opening on the label of the bottle, as the solution can be used for 3 months only or to the expiry date, whichever comes first.

a. Internal Quality Control check

This must be performed daily using two check strips and two control solutions supplied by Roche as follows:

1. Check the expiry date of the strips. Switch meter on. Check that the code on the meter matches the code on the pot of test strips.
2. Within 30 seconds, insert the test strip (yellow window facing up) into the meter.
3. The screen will then display a blood drop symbol. Press and release the right arrow button once to select a L1 (Level 1) control, or twice to select a L2 (Level 2) control.
4. Take the bottle of Level 1 solution and gently invert a few times
5. Squeeze the bottle gently until one small drop of solution appears at the tip of the bottle
6. Touch the drop to the curved application point of the strip so that the yellow application area is completely covered. An hourglass figure flashes on the display screen until the measurement is complete.
7. Tightly replace the cap on the control solution.
8. If the result is within the acceptable range, 'OK' will appear on the screen
9. If the result is not within the acceptable range, the error code (Err) + a reading will appear on the screen. The quality control procedure should be repeated, after checking that the quality control solutions and test strips are in date and that you followed the testing steps exactly. If the error message persists, contact Dr Nina Gusina for further advice.
10. Remove the test strip after the result has been accepted. Insert new test strip wait for test strip and flashing drop of blood symbol. Press and release the right arrow button twice to select a L2 (Level 2) control. Repeat steps 5-9 for the Level 2 control solution.



Checking Quality Control Values

The Quality control ranges are on the side of the test strip pot, (make sure you read mmol/L column not the mg/L) this range will vary according to the batch number of the strips. If the readings are not within the appropriate control solution range, check the battery level and replace if necessary. Check the consumables eg are the QC solution and test strips in date, check technique, and repeat the test. If the glucometer is not functioning it will not allow the operator to proceed to the measurement stage. Contact Dr Nina Gusina at the Republican Centre for Maternal and Child Health in Minsk for another instrument. Use the back-up glucometer while waiting for a replacement. If there is no back-up, recall the child to a subsequent clinic visit for the blood test after fasting.

b. External Quality Assessment (EQA)

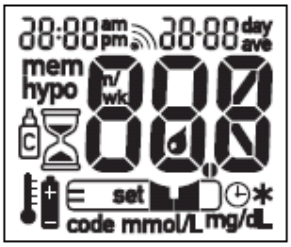
A solution of EQA will be sent on a 3 monthly basis by Dr Nina Gusina to each clinic. This EQA solution has a known glucose concentration, but you will not be told in advance what it is. Apply the EQA solution to the test strip as you would do for a blood glucose test. Do not press the buttons as for an Internal Quality Control check. Follow the same steps as for Internal Quality Control to obtain a value for the glucose concentration. Record the results, which should be returned to Dr Nina Gusina. Feedback on whether or not the correct result was obtained will be provided to each clinic.

4. Trouble-shooting

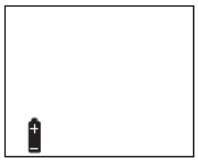
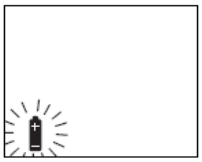
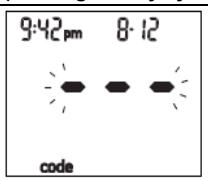
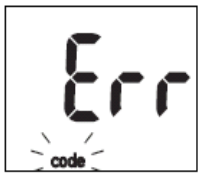
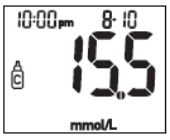
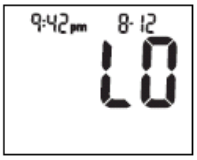
The following section lists the more common error messages seen. Any further problems should be reported to Dr Nina Gusina. Faulty meters must not be used. Always store a spare set of new batteries with the glucometer.

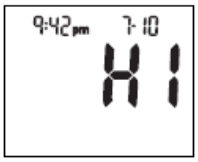
Display check

This verifies that all parts of the display are working. Make sure the meter is off. Press and hold down the ON/OFF button. The display should look exactly like the picture below. If you drop the meter, check the display.



Common error messages seen

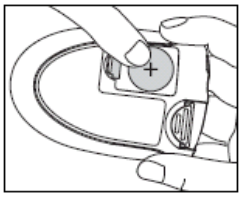
DISPLAY SYMBOL	EXPLANATION	REMEDY
 (battery symbol not flashing)	Low power	Replace batteries
 (flashing battery symbol)	No further tests can be performed until batteries are replaced	Replace batteries
	Meter needs to be coded or Code Key not inserted correctly	Check Code Key
	Code Key is faulty or was removed when device was on	Check Code Key
 **or** 	Failed QC check (for Level 1)	Check control solution, test strips and procedure and repeat check. If problem persists contact Dr Gusina
	Ambient temperature outside operating range of the meter.	Check environment
	Temperature is above or below the operating range of the meter.	Move to an area between 14° and 40°C, wait 5 minutes, and perform a test. Do not artificially heat or cool the meter.
	Blood sugar may be extremely low, an incorrect amount of blood was applied, or the test strip is damaged or inserted improperly.	Repeat test
	Blood glucose <0.56 mmol/L	



Blood glucose ≥33.3 mmol/L

5 Maintenance

- The meter should be cleaned regularly after use by wiping with a damp cloth, ensuring that the meter is first switched off (and preferably batteries removed). **Do not use alcohol swabs to clean**.
- Take care not to let liquid drip into the meter and take extra care to ensure that no moisture comes into contact with the code key slot and the test strip guide. The meter should be stored at room temperature, away from extremes of temperature and humidity and in a dust-free environment.
- Replace control solution from Dr Nina Gusina at Republican Centre for Maternal and Child Health in Minsk (See cover for contact details).
- Replace Batteries as required (e.g. as soon as a low power error message is displayed).



Appendix 3b

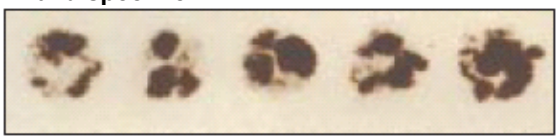
Simple Spot Check

Valid specimen:

Allow a sufficient quantity of blood to soak through to completely fill the preprinted circle on the filter paper. Fill all eight circles with blood. Do not layer successive drops of blood or apply blood more than once in the same collection circle. Avoid touching or smearing spots.

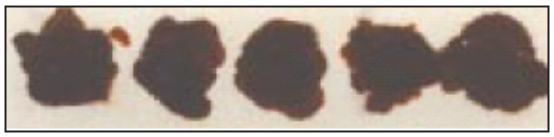
Invalid specimen:

Possible causes:



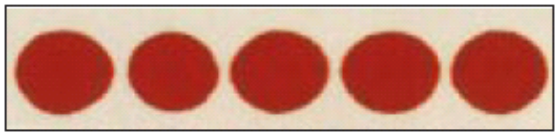
1. Specimen quantity insufficient for testing.

- Removing filter paper before blood has completely filled circle or before blood has soaked through to second side.
- Applying blood to filter paper with a capillary tube.
- Touching filter paper before or after blood specimen collection with gloved or ungloved hands, hand lotion, etc.
- Allowing filter paper to come in contact with gloved or ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection.



2. Specimen appears scratched or abraded.

- Applying blood with a capillary tube or other device.



3. Specimen not dry before mailing.

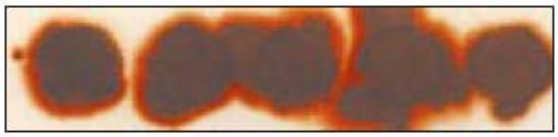
- Mailing specimen before drying for a minimum of four hours.

4. Specimen appears supersaturated.

- Applying excess blood to filter paper, usually with a device.
- Applying blood to both sides of filter paper.

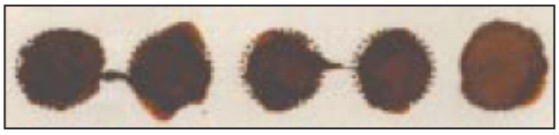
5. Specimen appears diluted, discolored or contaminated.

- Squeezing or “milking” of area surrounding the puncture site.
- Allowing filter paper to come in contact with gloved or ungloved hands or substances such as alcohol, formula, antiseptic solutions, water, hand lotion or powder, etc., either before or after blood specimen collection.
- Exposing blood spots to direct heat.



6. Specimen exhibits serum rings.

- Not wiping alcohol from puncture site before making skin puncture.
- Allowing filter paper to come in contact with alcohol, hand lotion, etc.
- Squeezing area surrounding puncture site excessively.
- Drying specimen improperly.
- Applying blood to filter paper with a capillary tube.



7. Specimen appears clotted or layered.

- Touching the same circle on filter paper to blood drop several times.
- Filling circle on both sides of filter paper.

Appendix 4

Blood pressure for boys age and height percentile

Age years Blood Pressure percentile	Systolic Blood Pressure, mmHg Percentile of height							Diastolic Blood Pressure, mmHg Percentile of height						
	5	10	25	50	75	90	95	5	10	25	50	75	90	95
9														
50th	95	96	98	100	102	103	104	57	58	59	60	61	61	62
90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89
10														
50th	97	98	100	102	103	105	106	58	59	60	61	61	62	63
90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90
11														
50th	99	100	102	104	105	107	107	59	59	60	61	62	63	63
90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90

Blood pressure for girls age and height percentile

Age years Blood Pressure percentile	Systolic Blood Pressure, mmHg Percentile of height							Diastolic Blood Pressure, mmHg Percentile of height						
	5	10	25	50	75	90	95	5	10	25	50	75	90	95
9														
50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10														
50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88
11														
50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89

Reference: National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004; 114(2 Suppl 4th Report):555-576.

Appendix 5

Inventory of equipment for each centre

Blood collection		
	1	Sharps bin
	1	Glucometer
		Test Strips and Code Key
		Internal Control solution level 1 and 2
		Gloves
		Disposable lancets
		Sterile gauze pads
		Pre-printed filter paper cards
	1	Racking
		Disinfectant
		Air-tight zip closure plastic bags
		Desiccant packages
		Storage box
		-18°C Freezer
Bio impedance:		
	1	Tanita TBF 300GS Body Fat analyser
	1	Cleaning wipes
		Printer rolls
Blood Pressure:		
	1	Omron 705IT blood pressure monitor
	3	Omron cuffs (small, medium and large)
	1	Thermometer for room temp
Anthropometry:		
	1	Wall-mounted stadiometre
	1	Standard stool for sitting height
	1	Harpenden cloth tape measure
	1	Lange callipers
	1	Marking pencil

Appendix 6

Quality assurance Protocols

1 Monitoring & test-retest protocol

1. Monitoring Visits will occur in first 3 months after the rollout of the PROBIT III. We will also consider repeating the monitoring visits half-way through the recruitment period to ensure compliance with the protocol is being maintained.
2. On a specified day, specifically trained monitors will observe the paediatricians to check adherence to the blood taking and examination protocols.
3. During the observed visits, monitors will repeat the pediatrician's circumference and skinfold measurements. These measurements will be recorded on the Monitoring Report Form.
4. Monitors will provide feedback to the paediatricians at the end of the monitoring visit.
5. Monitors will also provide a feedback report to the study investigators who will collate the reports to see if a generic problem requires a further specific retraining day in Minsk.
6. In total 190 children will be retested in the first 3 months and possibly half-way through the recruitment period (5 children per paediatrician = 38X5 = 190 children).

2 Audit

1. Audits will be carried out at the end of the study recruitment period as in PROBIT I and II.
2. Approximately 5 children per paediatrician (n = 190) will be randomly selected for re-examination, administration of the questionnaire and blood sampling by trained auditors.
3. Repeat examination should only be undertaken with the parent's consent and child's assent. This will require second consent and assent forms labelled AUDIT to be signed.
4. Recording sheets and blood spot cards for the monitoring visits must be clearly marked 'AUDIT' to avoid confusion with the main study data.
5. Provide the child with an additional gift for the extra test.