PART A
Research Design and Methods

A. Research Design

This is a one-point in time cross-sectional study (or a supplement nested within an existing prospective cohort study) of anthropometric status of newborns up to six-year-old children by ethnicity, gender and study location. The study locations include nine study sites and may include measurements in the home, community center or clinic.

Subjects:

Eligibility criteria:

Mothers: aged 49 years or younger; non-institutionalized; and of ethnic group membership as required or available by each study site.

Infants and children: Generally healthy; and living with and the same ethnicity as the mother.

Infant and child Health status at the time of the measurements: Afebrile; Not having suffered from an acute bout of diarrhea or other illness associated with weight loss nor having been acutely ill within the past week.

Methods:

Screening: Eligibility screening procedures may differ by site dependent on the population base for participant selection i.e. availability of a cohort, another population, or for the NCS in particular.

Data Collection Materials:

Demographic and other lifestyle information: (Please see the appendix: questionnaire)
Amongst the nine sites, several sites have ongoing cohort studies from which demographic and other relevant data can be abstracted while others will administer the questionnaire to mothers. The form of administration, i.e. in-person, has been specified.

Anthropometry: A team of two staff members (either nurses or other trained staff) makes measurements: one is the holder, and the other is the measurer. The staff team reverses their positions when making a second set of replicate measurements. All measurements will be collected on the right side of the participant. All measurements will be taken in metric units and recorded on the measurement form (See next page: measurement form). Make sure the checklist for each measurement is handy; the holder reads it aloud as measurements are performed (See appendix for checklists).

The goal for performing replicate measures is approximately 10% by location (home/clinic) and age-group. A second goal is to capture as many pairs of measurers as possible over the ~~full time~~ course of this formative research in the replicate substudy. When taking the replicates, please have the person acting as holder or recorder for the first set of measurements become the measurer when taking the replicates. This will allow for estimation of inter-observer measurement reliability. Make sure that the second observer starts anew by wiping off the existing bony landmarks using an alcohol wipe and then re-identifying the landmarks. Indicate on the measurement form that the second set of measurements recorded constitute replicate measurements.

I. Standard Measurements

Weight: SECA or other scale at site. Each scale should be calibrated daily using a standard weight (identified by brand and actual weight of the standard). Calibration occurs every morning and is entered into a log. If the scale differs from the standard weight by 100 grams a second calibration is done. If the second comparison weight differs by more than 100 grams from the standard, then do not use the scale in the field. Otherwise retain the measurements in the log and use in the field. Assessments of the calibration of scales should occur weekly to identify scales that require repair.

Please measure weight (in infants wearing dry diaper or undiapered or in children wearing underpants) in duplicate to the first decimal point in kilograms.

Height/length: Infantometers to measure recumbent length and stadiometers to measure standing height. (NB thru age 36 months will be measured on the infantometers while standing height (age 24 months and older) will be measured on the stadiometers in **duplicate** to the first decimal point in centimeters. Children aged 24.0 months-36.0 months will be measured recumbent and standing using both types of equipment. If the stadiometer allows calibration, the calibration by a rod of fixed length can be used. See page 5 for further details on measurements.

Recumbent length is measured with a subject in supine position and body extended.
1) Place crown of head with steady pressure against the vertical wall – the headboard- and position the head looking upwards using an object or having the mother to direct the attention of the child to look upward.
2) Gently extend legs fully (no bend in the knees) and dorsiflex ankles to approximately 90 degrees (toes pointing to the sky).
For the neonate and infant, gently press one leg (usually the left leg) to full extension and position the foot to 90 degrees dorsiflexion.
3) Bring the vertical foot board up to the heel and call out the measurement to the nearest 0.1 cm. or nearest millimeter.
4) Repeat for total of two measurements.

Standing height is measured using a stadiometer.
1) Shoes are removed.
2) Place the stadiometer head platform firmly on the top of the head (make sure any head pieces are removed from the participant's head before measurement) ;
3) Subject should stand tall with head in Frankfort plane (gaze angled slightly downward), shoulders relaxed, abdomen sucked in, ask them to take a breath and hold it, and neck stretched ("like a giraffe").
4) Head, shoulders, and buttocks should be against the wall or stadiometer vertical piece with arms hanging freely at the sides.
5) Heels are together and touching the vertical wall /stadiometer; feet are angled slightly away from each other. If there is a position for feet at the base of the stadiometer, check that feet are in place.

II. Experimental Measurements

A. Upper Extremity Measurements

Arm span is measured by 1 method: steel or plasticized (non-stretch) tape measure.
For standing participants aged 36 months and older: A participant stands against a wall with heels at the base of the wall and buttocks as close as possible against the wall; arms are stretched laterally at the level of the shoulders and parallel to the floor while the palms of the hands are facing out and the tip of

the middle finger of the right hand is touching an adjacent wall or block while a sticky note is taped to the wall where the tip of the left middle finger is touching and marked on the sticky note. The measurement is made to the nearest 0.1 centimeter.

For recumbent infant: 1) The tape measure is placed taught on a table or floor or other flat surface.2) The subject lies supine on a table or floor, with shoulder flat against the surface and the body extended.
3) Both arms are stretched laterally outward, perpendicular to the long axis of the body, palms facing upward; gently press your thumb against the elbow to maximize arm extension, and extend the fingers maximally.
4) Measure between the tips of the extended middle fingers with tape measure to the nearest 0.1 centimeter.

Ulna length (Right arm) is measured by 3 methods: calipers, tape measure, and marking on paper-grid or ruler.
1) The patient is sitting in the mother's lap or lying supine with right elbow flexed approximately 90 degrees and arm positioned comfortably alongside the body (elbow touching the table, arm pointing upward)
2) Flex the elbow approximately 90 degrees with wrist straight (unbent), hand in the same axis as the forearm, and fingers bent with the extended thumb pointing to the right shoulder (hitch-hiker position).
3) Palpate and mark in ink a line of about 1 cm at the distal end of the ulna (the end of the head of the ulna, a.k.a. styloid process)
4) Ulna-Caliper measurement. Place the zero end of the caliper against the olecranon and move the arm of the caliper against the distal end of the ulna, apply steady pressure, and take the measurement to the nearest 0.1 centimeter.
5) Ulna-Tape measurement. Place the zero end of the tape at the olecranon and measure to the end of the head of the ulna (styloid process), following the contour of the arm, and read to the nearest 0.1 centimeter.
6) Ulna-Grid Measurement. The patient is sitting in the lap of the mother (for younger children) or sitting on his/her own if they are able to remain still. With elbow bent to approximately 90 degrees, place the forearm on the grid secured on a thin rigid board (or on table if sitting), with the olecranon at the zero point of the grid and the hand palm down with the thumb pointing at the body. (See picture of arm on the grid to aid in clarity.) On the grid, mark the location of the distal end of the ulna.
6a) NOTE: Ulna-ruler measurement: For NB to 3 months of age, measure the distance between the length points and between the width points using a ruler to the nearest 0.1 cm.
7) Width of the forearm using the grid: Have the arm straightened and pointing outward from the body, palm down, lateral aspect of forearm aligned along the zero vertical axis of the grid. Read the maximal width of the forearm on the grid for the measurement.

8) Forearm (brachial) circumference (right arm) is measured by 1 method: SECA retractable insertion tape measure.
) 1) The patient lies supine, standing or sitting with right elbow extended and the forearm positioned so that it is freestanding (not resting on the table or the body).
2) Measure the maximal forearm circumference with the tape measure to the nearest 0.1 cm. The insertion tape should be snug with plug in the hole and tape taut and perpendicular to the long axis of the arm.

B. Lower Extremity (Lower Leg) Measurements.

Five measurements are to be taken at the lateral side of the right lower leg: length of the lower leg by calipers and tape measure; length of fibula by calipers and tape measure; and maximal circumference of the lower leg by tape measure. Start with the following steps.

1) Subject is supine or sitting.
2) Place the lower leg in a comfortable position with knee bent approximately 90 degrees – the heel is held by the mother or assistant and bent (flexed) at 90 degrees as well.
3) Mark in ink the two ends of the fibula: at the lower end (most distal extension) of the lateral malleolus, and at the top (most proximal extension) of the apex of the head (styloid process) of the fibula, located on the lateral/outside at the level of the inferior edge of the knee.

B1. Lower leg length (right leg) is measured by 2 methods: calipers and tape.

1) Dorsiflex the foot approximately 90 degrees at the ankle; this is important to position the heel in a reproducible fashion.
2) Press the arms of the caliper against the base of the heel and against the peak of the flexed knee (anterior surface of the distal femur) with steady pressure on both ends and take the measurement to the nearest 0.1 cm. The calipers should be parallel with the long-axis of the leg.
3) Tape measurement: Replace the caliper with the tape. Place the zero end of the tape at the base of the heel and read thelower leg length at the top of the bent knee to the nearest 0.1 cm. (This one requires practice to decide the optimal method.)

B2. Fibula length (lateral right leg) is measured by 2 methods: calipers and tape measure.

Caliper measurement. Place the arms of the caliper with steady pressure at the marks at both ends of the fibula and read the measurements to the nearest 0.1 cm. The calipers should be parallel to the long axis of the leg.

Tape measurement. Place the zero end of the tape at the marked distal end of the fibula (lateral malleolus) and measure to the marked proximal end of the fibula; this measurement may be slightly longer than the caliper measurement because of soft tissue curvature of the leg contour. (This one needs practice to decide the optimal positioning of the zero end of the tape.)

B3. Lower leg (calf) circumference (right leg) is measured by 1 method: tape measure.

Measure the maximal calf circumference with the tape measure to the nearest 0.1 cm. The tape should be perpendicular to the long axis of the leg and the automatic retention of the retractable tape should be used to make the tape snug against the leg.

Research Protocol

A. **Objectives:** The purpose of this study is to determine the effectiveness of different segmental length measurements as predictors of length or height in infants through children 5 years of age. Measurements will include length or height, and tibia and ulna length. Regression equations will be created to predict height based off of each of the two segmental length measurements. Bland-Altman plots will then be developed comparing the difference between the length/height measurements based on traditional anthropometry versus length/height measurement based on the individual regression equations. Accuracy of the segmental measurements will be verified utilizing dual energy x-ray absorptiometry (DXA).

B**. Participants:** Study participants will be recruited from 3 different locations (SDSU, Utah, Cincinnati Children's Hospital Medical Center). We will measure 300 healthy infants and children within this age range (100/center) with equal numbers of males/females. There will be no restriction on BMI. Overall, approximately one-half of the population will be Caucasian, ¼ African-American and ¼ Asian. Approximately one-third of the infants and children will be asked to join the DXA portion of the study. A separate informed consent form will be made for this part of the study, and all risks and benefits of participation will be explained. Parents or legal guardians of the participants will be required to sign an informed consent prior to their infant's or child's participation in the study. The following numbers will be recruited into the study:

Age (months)	Total/Center	Total (# males)	Total DXA/ Center	Total DXA (#males)
Preterm (<38 weeks)	10	30 (15)	4	12 (6)
0-11.9 (term)	18	54 (27)	6	18 (9)
12-23.9	18	54 (27)	6	18 (9)
24-35.9	18	54 (27)	6	18 (9)
36-47.9	18	54 (27)	6	18 (9)
48-59.9	18	54 (27)	6	18 (9)
Total Number	**100**	**300 (150)**	**34**	**102 (51 males)**

Medically stable preterm infants will be recruited while they are in the NICU and those with DXA measurements will be measured and scanned close to discharge.

C. **Time Required for Individual Participants:** Participants in the segmental measurements portion of the study will have one visit which will take approximately 30 minutes to complete and will be done in the home. Participants who enroll in the DXA portion of the study will have an additional 30 minutes of testing which would bring their total time commitment to 60 minutes, and will be done at SDSU EA Martin Program. If there are more than two participants in more remote locations, we will take our mobile research unit to a more central location in order to minimize travel time for the participants.

D. **Compensation to Participants:** Parents will be given $25 as monetary compensation for participation in the study, and an additional $10 will be provided for the DXA component of the study.

E. **Benefits to Participants:** None

F. **Methods:**

We will obtain the following information on all infants and children:

1) Demographic information (age, sex, race)
2) Anthropometric measurements (length or height, weight, ulna & tibia length; all in triplicate)

On a subset of these children we will use the IVA software on a Hologic Discovery system to determine the true ulna and tibia length. This software can be completed in 15 seconds and has a 0.5 mm resolution. This will allow us to determine how accurate the measurement of these bones are using segmentometer. It is possible that the difference between the "true" bone length and the segmentometer measured length will increase with increasing BMI (or weight) since identification of the positioning landmarks may be more difficult in infants and children with greater body fat.

Traditional anthropometric measurements will include:

Length or Height. For comparison, as well as the development of the prediction equation, infant length and children's height will also be measured using the 2 person technique in infants and a standard stadiometer in the toddlers.

Ulnar Length. Ulnar measurements will be taken with the child either sitting or lying supine depending on their age and developmental stage. All measurements will be taken on the right side of the body. The child's arm will be positioned with the elbow bent at 90 degrees and the palm lying flat on the table. The examiner will palpate the olecranon process and the styloid process of the ulna and make marks with a cosmetic pencil at each location to serve as reference points for the measurement. A segmentometer will be used for the measurement with one point being placed on the styloid process mark and one point being placed on the olecranon process mark.

Lower Leg Length. Lower leg length will be measured with the child lying in a supine position. A segmentometer will be used to measure the distance from the superior edge of the lateral condyle of the tibia down to the inferior point of the lateral malleolus of the fibula.

Data Management & Statistical Analysis. All data will be double-entered and compared using SAS Proc Compare. We will determine between and within coefficients of variation for each method, and use Bland-Altman plots and linear regression analyses to determine the relationships among measurements. Multiple regression analysis also will be used to test the reliability of these measurements across different ages and

across different BMI. We also will utilize multiple regression analysis to determine if these measurements are consistent between males and females as well as across the different races (tested using -sex and –race interaction).

We will develop a regression equation for predicting length and height from the more precise of the two measurements (ulna vs. tibia). We also will determine whether there are any sex or race differences in the consistency of a height prediction (tested as a sex or race-by-limb length interaction). Additional goals of this proposal are to determine if the reproducibility and accuracy of the limb measurements is greater than that of the infant length board measurement, and whether the agreement between the ulna and tibia measurements measured by segmentometer and ulna and tibia measurements obtained by regional DXA scans. A final goal of this proposal is to determine the cost effectiveness and trainability of utilizing a segmentometer for these measurements. This will be done by training 5 NCS field staff at the South Dakota and Utah Study Centers and measuring intra-observer variability in measuring length or height, and ulna and tibia lengths. All statistical analyses will be completed at SDSU Study Center.

G. **Risks to Participants:** All participants in the study have a small risk of a loss of confidentiality due to their participation in the study. Participants enrolled in the DXA portion of the study will have a small exposure to radiation. A Hologic Discovery DXA scanner will be used in IVA scan mode. This procedure has an estimated effective dose of 1 millirem (mRem). This is less than the effective dose received during a normal chest X-ray (5.0 mRem), or a transcontinental round trip flight (5.0 mRem). The effective dose is also within the negligible individual risk limit (NIRL) of 1 mRem which has been established by the National Council for Radiation Protection. The FDA also has regulations regarding the maximum acceptable effective dose which is allowable from medical research in children. These regulations are a single whole body dose of 300 mRem or an annual whole body dose of 500 mRem. Based on these data, participants in this study are at no greater than minimal risk by participating in this project.

H. **Risk Reduction:** Only trained personnel will operate the DXA equipment.

I. **Confidentiality**: All individuals who have access to the data have signed an affidavit of nondisclosure stating that under no circumstance will they share any participant information with anyone not directly involved with the study. In addition, all individuals working on the NCS have completed CITI Human Subjects and IT security training, and have also passed a class D background check. Access to the offices where data will be kept at Wecota Hall is regulated through card swipe access and all hard copy data will be kept in a locked filing cabinet and all electronic data will be kept on a secure database on password protected and FISMA compliant computers.

J. **Recruitment:** Premature infants will be recruited through the neonatal intensive care units at Sanford Health and Avera Women's. Term infants will be recruited through hospitals and clinics and through a contact database of people who have inquired about participation in the NCS but were not at an eligible address.

However, if a NCS mother is interested; her infant could be eligible to participate in this formative research project. Older children will be recruited through contact with childcare facilities, preschools, and through the above listed database of people who inquired about participation in the NCS but were not at an eligible address.