

RESEARCH PROTOCOL

STUDY TITLE

Low cost body composition measurement for nutrition assessment using Near Infrared (NIR) light reflection from birth up to 2 years.

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LAY DESCRIPTION

Globally there are millions of under-five who are stunted (162M), underweight (99M), wasted (51M) or overweight (44M). The timing of child malnutrition reveals a 1,000 day period for meaningful intervention – before/during pregnancy, immediately following birth and up to 2 years of age - if the immediate effects on health and child mortality and the life-long impact of early onset adult disease are to be prevented.

Assessment of body composition is vital to deliver improved nutritional management of under and over nourished infants & children. However, there is a lack of convenient, accurate and low cost devices for direct monitoring of nutritional status. This leads to difficulties for health workers to identify undernourished and overnourished infants and children and in monitoring changes in nutritional status following interventions. Our aim is to produce a suitable device for direct monitoring of nutritional status from birth to two years of age.

BACKGROUND AND RATIONALE

Malnutrition, particularly undernutrition, is a major public health problem that is associated with substantial increases in mortality and morbidity. Malnutrition affects all communities, however, neonates and young children are the most vulnerable because of their high nutritional requirements for growth and development (1). The first two years of life and predominantly the neonatal period offer the greatest window of opportunity to prevent childhood undernutrition which often leads to irreversible damage. Malnourished neonates and children have a lowered resistance to infection and are more likely to die from diarrhoeal diseases, respiratory infections (1) and severe neurological complications due to a lack of energy soon after birth (2). The majority of the 4 million neonatal deaths worldwide each year occur on the first day and within the first week after birth. Furthermore growth reduced babies that survive are at risk of long term health outcomes, including hypertension, stroke, type 2 diabetes, obesity and cardiovascular disease (3, 4).

According to the 2008 *Lancet* series on undernutrition, 'Maternal and child undernutrition is the underlying cause of 3.5 million deaths, 35% of the disease burden in children younger than 5 years and 11% of total global disability-adjusted life-years (DALYs)' (5). The most recent data from another *Lancet* series published in July 2013 reported that undernutrition, including fetal growth restriction, suboptimum breastfeeding, stunting, wasting, and deficiencies of vitamin A and zinc are attributed to 45% of child deaths (6). It was further reported that globally 165 million children are stunted due to undernutrition underlying 3.1 million deaths in children younger than five years (7). In particular, for children under the age of two, the consequences of undernutrition are particularly severe, often irreversible, and reach far into the future. Although many of underweight neonates are born prematurely, there are a large proportion of full-term neonates who are small because of poor growth in utero. This presents a major difficulty in low and high income countries in determining which neonates are truly undernourished and those that are constitutionally small. As yet, there are limited techniques available in neonates and children to determine their nutritional status.

Accurate assessment of body composition provides further information on individual components which contribute to total body weight including water, fat, protein and mineral providing valuable parameters for the assessment of nutritional status and indicating degree of under and over nutrition for clinicians and health workers (8, 9). A variety of methods are available to assess body composition in neonates and children. The most precise way of

assessing body composition is through direct chemical analysis of cadavers (10). However, there are a limited number of studies using neonates and children (10). By contrast, the most practical means of measuring body composition is by non-invasive and indirect methods, which may be based on different models, such as 2, 3 or 4 compartments. In the 2-compartment model, the body is divided up into fat mass and fat free mass (FFM) (9). The 3-compartment model adds a value for skeletal or bone mass, whereas the 4-component model includes the composition of fat, mineral, protein and water and is obtained by integrating data from various techniques (9). Some of the commonly evaluated techniques for use in infants and children include; anthropometric measurements such as skinfold thickness, TBW, bioelectrical impedance analysis (BIA), dual energy X-ray absorptiometry (DXA), imaging including ultrasound and air displacement plethysmography (ADP) (11) .

The usual screening method for detecting poor growth in neonates is to detect weight for gestational age (weeks) less than the tenth percentile. Newborns are then checked for adequate breast milk intake and, checked by heel prick to ensure glucose levels are safe. Screening requires accurate measurements of weight and gestational age. In children nutritional status assessment is typically calculated using weight-for-length/height z scores and mid-upper arm circumference (MUAC)-for-age along with clinical signs such as bilateral oedema (12). However, for both neonates and children at least one or all of these measurements are usually either not known or inaccurate in developing countries where homebirths are frequent, conception dates and date of birth often not known and ultrasound and other anthropometric equipment and expertise unavailable. Furthermore, in Australia and in developed countries generally, newborns can still be undernourished but with a weight above the tenth percentile. These “long lean” newborns are the least detected and most at risk of low glucose levels as glucose stores are exhausted and the newborn dependant on mobilizing energy from meagre fat stores (13, 14). Thus, there is an urgent need for a device to quickly indicate if a newborn baby or child has low fat levels and thus energy stores, without the need for accurate gestational age, weight and length measurements to avoid severe complications.

AIMS OF THE STUDY

1. To investigate a novel low cost body composition technique based on the effect of subcutaneous fat thickness using Near-Infrared (NIR) light reflection against a range of criteria with reference to a gold standard.
2. To determine whether this proposed NIR device can provide rapid, accurate, easy recognition of nutritional status for clinical point of care in both low and high income countries.

OBJECTIVES

1. To compare the 3-compartment model (Deuterium dilution and DXA) against NIR at 3, 6,12,18 & 24 months
2. To compare the 3-compartment model (ADP and DXA) against NIR at birth (within 48 hours)
3. To determine the best prediction equation for NIR body composition assessment without the need for age and length measurements.
4. To examine differences between gender, weight-for-age and ethnicities.
5. To determine the minimum required wavelengths for the development of a low cost 'community' device.
6. To investigate anthropometric variables in relation to body composition measurements
7. To investigate subcutaneous fat thickness as measured by ultrasound at the abdominal and thigh anatomical sites in comparison with NIR

STUDY DESIGN

A cross-sectional population-based validation study recruiting infants and children from birth up to 2 years of age.

SAMPLE SIZE

The sample size is based on a true mean difference between BF% measured by a criterion method (TBW and / or ADP and DXA) and BF% measured by NIR using a mean difference of 2.0 BF% with a standard deviation of 3.0 BF%, at a significance level of 0.05 with 80% power (15), a sample size of 28 infants and / or children at each age point are required to complete all aspects of the study. Thus with an expected non-compliance rate based on previous studies by Fields et al (15), we estimate the total number required across the age groups from birth to 24 months would be approximately ~ 720 in total to evaluate the accuracy, precision, bias, and reliability of the NIR device.

STUDY POPULATION

The infants and children enrolled in this study will be recruited from the Soweto pregnancy and follow up clinics or associated child care centres over a 12 month time period. Throughout the end of pregnancy and if an infant / child is aged at the eligible age groups (3, 6, 12, 18, 24 months) parents will be approached by a research team member and invited to participate in the study. The aims and procedures of the study will be explained to the parents and an information sheet will be distributed. Neonates and children will only be enrolled into the study after parental written consent is obtained and the eligibility criteria are met.

INCLUSION & EXCLUSION CRITERIA

INCLUSION CRITERIA

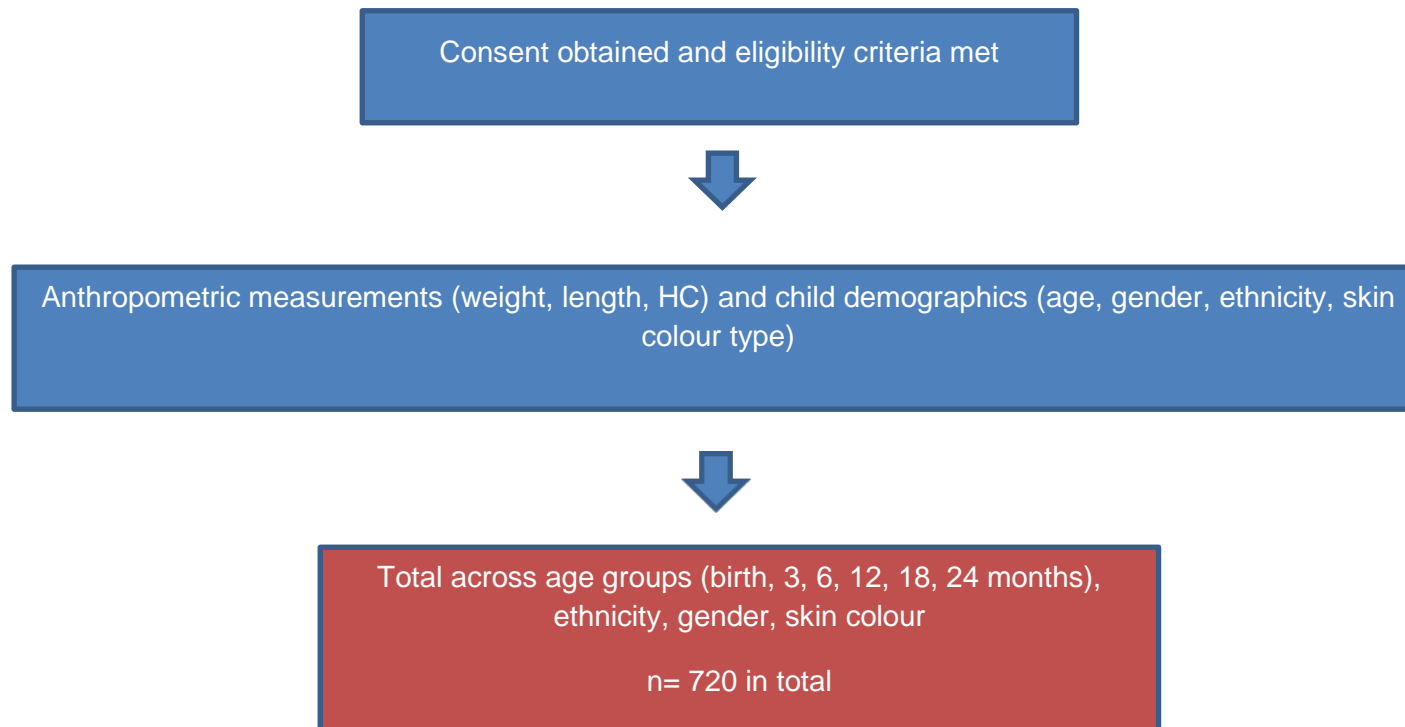
1. Written parental informed consent
2. Term gestational age (GA) ($\geq 37^{+0} - 41^{+6}$ weeks GA)
3. ≤ 2 years (24 months) of age
4. Mother > 18 years old age
5. Singleton pregnancy
6. Mother lives within the study area
7. Can read and understand English

EXCLUSION CRITERIA

1. Any maternal condition or impairment with inability to give parental informed consent
2. *Neonates*: Prematurity ($\leq 36^{+6}$ weeks GA) and/ or the presence of any major congenital anomalies or morbidity that may contraindicate the neonatal body composition measurements
3. *Infants and/or children*: Birth defect (congenital anomalies, preterm birth ($\leq 36^{+6}$ weeks GA))
4. Physical or intellectual disability in infants and /or children, influencing physical activity or food habits
5. Chronic health problem in infants and / or children, influencing weight and length
6. If currently enrolled in any other interventional drug trial
7. If any DXA scans completed in the past 12 months

ALLOCATION OF PATIENTS

Each age group (birth, 3, 6, 12, 18, 24 months) will then be stratified by gender, ethnicity and skin colour type as shown in the flow chart below. A screening form will be completed (see Attachment 1) and the allocation of patients as show below will allow distribution in the sample groups for ethnicity, gender and skin colour type.



CONDUCT OF THE STUDY

As the study is cross-sectional in design parental consent and relevant testing procedures (anthropometry and body composition measurements) will be required at *any* of the specified time points (between birth and 24 months); with only one clinic visit required.

	REQUIRED PROCEDURES AT ANY OF THE FOLLOWING AGES: BIRTH (UP TO 48 HOURS), 3, 6, 12, 18, 24 MONTHS OF AGE
INCLUSION / EXCLUSION CRITERIA	X
INFORMED PARENTAL CONSENT	X
SCREENING FORM ANTHROPOMETRY (WEIGHT, LENGTH HEAD, ARM, ABDOMEN AND THIGH CIRCUMFERENCE) ALLOCATION OF PATIENT ACCORDING TO ETHNICITY, GENDER AND WEIGHT-FOR-AGE Z SCORES	X
PARENTAL AND INFANT / CHILD QUESTIONNAIRES	X
NEAR-INFRARED LIGHT REFLECTION (NIR) AT UP TO 4 SITES	X
SKINFOLDS AT UP TO 4 SITES	X
ADP <u>AT BIRTH ONLY</u> <u>(WITHIN THE FIRST 48 HOURS AFTER BIRTH)</u>	X
DEUTERIUM DILUTION <u>FROM 3 MONTHS OF AGE</u>	X
DXA	X
ULTRASOUND	X

STUDY PROCEDURES

DEMOGRAPHIC DETAILS

Parents will complete a short interviewer-administered questionnaire to determine background, pregnancy specifics, feeding status, bath and products applied to the skin and delivery details of the infant / child (see Attachment 2 and 3).

ANTHROPOMETRY

During the one required clinic visit the infant and/or children will have their weight, recumbent length, head, abdomen, thigh and arm circumference measured using WHO Child Growth standards. The infant and/or children will also have a skinfold measurement at the thigh, subscapular, triceps and flank measured using WHO Child Growth standards.

BODY COMPOSITION

NEAR-INFRARED LIGHT REFLECTION (NIR)

The NIR reflection method is non-invasive and simple and has been used to study body composition in adults, however there is limited research surrounding neonates and young children (16). NIR technology works because the human skin is transparent to NIR light over a range of wavelengths. This is the basis of pulse oximetry where the haemoglobin and oxyhaemoglobin absorb NIR at different wavelengths. Fat has been shown to absorb NIR in several studies (16-19). It has been demonstrated that the thickness of fat can be determined by NIR at two wavelengths in phantoms and in a small pilot trial (20).

NIR will be measured at birth up to 24 months through the skin surface in up to 4 sites including the thigh, subscapular, triceps and flank. Fat, water and haemoglobin have clearly distinguished spectral shapes that can be detected using NIR laser based reflectance spectroscopy (NITS). NIR measurements can be conducted with the infant in their cot beside their mother, or held by their parents or for children lying flat (Figure 1, page 18). (Refer to Attachment 4 for the NIR device standard operating procedures (SOP)).

AIR DISPLACEMENT PLETHYSMOGRAPHY (ADP)

At birth infants' body composition will be measured using the PEA POD system (COSHED, Concord, CA), a method that is based on ADP which has been validated against the four-compartment model and biological and physical phantoms and is considered the criterion method for determining BF% in neonates (21). ADP noninvasively, accurately, and quickly measures body composition in infants.

The infant's mass is measured using the integrated scale to the nearest gram. The infant is then placed in the test chamber tray for the volume measurement. During the entire volume measurement period (about 2 minutes), the infant is clearly visible at all times through the acrylic window. The infant's body composition (fat and fat-free mass) are calculated from body density (Density = Mass /Volume) with Age and gender-specific densities computed (22).

DEUTERIUM DILUTION

After 3 months of age, body composition will be assessed using deuterium dilution to determine Total Body Water (TBW) which includes both intracellular fluid and extracellular fluid. If we have an estimate of TBW, we can estimate the amount of fat-free mass (FFM). Body fat mass (FM) is the difference between body weight and FFM. Stable isotope techniques have been used in studies of infants and young children (23). Deuterium is a stable (non-radioactive) isotope of hydrogen, with the symbol ^2H . It is given orally as deuterium oxide ($^2\text{H}_2\text{O}$) and after mixing with body water is eliminated from the body in urine, saliva, sweat and human milk. Deuterium oxide is handled in the body in the same way as water, and is dispersed through the body water within a matter of hours. Body water can be sampled in the form of saliva, urine, plasma or human milk and the enrichment of deuterium can be measured by isotope ratio mass spectrometry (RIMS) using dual-inlet or continuous-flow RIMS, or Fourier transform infrared spectrometry (FIR). Refer to Attachment 5 for the Deuterium Dilution SOP.

DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA)

From birth body composition will be assessed using DXA scanning. DRA is a full body scan which determines muscle, fat and bone mass. The infant / child will be wrapped in a light cotton blanket and placed supine on the scanning bed and then a machine will move over him/her (it does not touch the baby) and sends an image to the computer of the infant / child's bone, fat and muscle composition. The levels of radiation in DRA are extremely low (please see Risks and side effects). DRA scans will be performed only once as part of the study at birth, 3, 6, 12, 18 and 24 months of age.

ULTRASOUND

From birth measurements using high-resolution ultrasound will examine the abdominal subcutaneous and visceral fat thickness and the subcutaneous thickness of the anterior thigh. Ultrasound or sonography involves exposure of the body to high-frequency sound waves which reflect off the structures and tissues of the body and are detected by a transducer. A scanner uses the amplitude, velocity and frequency of these reflected sound waves to convert them into real-time images of the interior of the body (24). Ultrasound has the ability to distinguish between visceral and subcutaneous fat which is not possible while using anthropometric measurements (25). Ultrasound can measure subcutaneous and preperitoneal fat which provides good approximation of visceral fat using electronic callipers (24).

STANDARDISATION

Standardisation training will be conducted as part of the study to enable observers to measure accurately without bias (26). To achieve this, researchers will be trained to obtain measurements that are on average equal to the values produced by an expert who is considered the 'gold standard' taken on several subjects (26). The degree of accuracy will be assessed using intra and inter reliability scores. Standardisation will be used for the NIR device and anthropometric factors including circumferences and skinfolds.

ETHICAL IMPLICATIONS

REQUIREMENTS OF THE RESEARCHERS AND PARTICIPANTS

Families will be provided with a detailed parent information sheet which provides contact details of all the investigators involved. Researchers involved will communicate with the participants the details of the research study in their chosen language. All results will be explained to the participants. Any abnormal research findings that may affect the infant and/or child's care will be communicated to the families' clinician or medical officer. All infants and children in the study must have informed consent prior to any clinical measurements being undertaken.

RISKS AND SIDE EFFECTS

ADP Near-Infrared light reflection (NIR) and ultrasound involve no risks or side effects. In particular, NIR is based on measurements that are commonly used in the neonatal and paediatric ward; both NIR and ultrasound are low energy and low frequency and not associated with heating. All associated devices are designed to meet IEC60601 medical safety regulations and have been rigorously tested for medical safety. For correct anatomical positioning for the anthropometric, NIR and ultrasound measurements the infant / child's skin will be marked with a surgical non-toxic ink pen (TGA approved ARTG 162327). The infant/child's skin will be wiped with an alcohol wipe before application of the NIR device to remove any oil or moisturiser on the skin.

There are no risks of deuterium use to infants. All procedures have been shown to be safe. **Heavy water**, formally called **deuterium oxide** or $^2\text{H}_2\text{O}$ or D_2O , is a form of water that contains a larger than normal amount of the hydrogen isotope deuterium, (also known as heavy hydrogen, which can be symbolized as ^2H or D) rather than the common hydrogen-1 isotope (called protium, symbolized as ^1H) that makes up most of the hydrogen in normal water. Some or most of the hydrogen atoms in heavy water contain a neutron, making each hydrogen atom about twice as heavy as a normal hydrogen atom (though the weight of the water molecules is not as substantially affected, since about 89% of the molecular weight resides in the unaffected oxygen atom). The increased weight of the hydrogen in the water thus makes it slightly denser. **Heavy water is not radioactive at all.** In its pure form, it has a density about 11% greater than water, but otherwise, is physically

and chemically similar. The human body naturally contains deuterium equivalent to about five grams of heavy water, which is harmless, thus the technique to be used in this study is noted as “enrichment” (final deuterium levels –baseline deuterium levels of infants)

DXA measurements involve exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 to 3 millisieverts (mSv) each year. The DXA scanner uses beams of very low-energy radiation to determine body composition and bone density. The effective dose in any year from birth to 18 years is 0.5 mSv. The amount of radiation is small, using the infant whole body mode the radiation exposure is approximately 0.009 mSv for neonates and 0.008 mSv for 1 year olds (27). The test is quick, painless, and considered completely safe.

POTENTIAL BENEFITS

There is no direct benefit to participants. However, the increased knowledge gained from investigating low cost and novel methods of detecting body composition may be beneficial for others in the future.

PRIVACY AND CONFIDENTIALITY

All subjects will be assigned a study number and de-identified patient data will be entered into a secure database. Electronic data will be located on a secure password-protected department server. Copies of hardcopy data will be stored in locked filing cabinets until converted into electronic format. Only authorised personnel will have access to this data. Information will be stored for ten years after the results have been published. Any publications arising from this study will report group data only; there will be no identification of individuals.

REPORTING OF ADVERSE OR UNEXPECTED OUTCOMES

Any Adverse Events (AEs) for example excessive movement while using the device, Serious Adverse Events (SAEs) including any skin irritations during and following the use of the NIR device and Unanticipated Serious Adverse Device Events (USADEs) such as if an infant

child becomes unwell during and following the use of the NIR device will be monitored in the clinical site in Soweto, South Africa by the Principle Investigator (PI). Any AEs will be submitted through the University of Sydney and University of Witwatersrand HREC's annual reporting systems. Any SAEs will be reported by the PI at the clinical site in Soweto, South Africa to the lead HREC (University of Witwatersrand) and will notify the Chief investigator / project team at the University of Sydney local site for relevant HREC reporting within 72 hours. For USADEs the PI in the clinical site will report to the lead / responsible HREC (University of Witwatersrand) and the Chief investigator / project team at the University of Sydney local site for relevant HREC reporting within 72 hours if the incident has an impact on the ethical acceptability of the study and / or action is planned. Otherwise, the USADEs will be included in the relevant summary reporting for all USADEs relating to the device as per HREC requirements.

DATA STORAGE

There will be two datasets:

1. Measurement data of the new device, these will be raw voltage measurements and the time of measurement in a csv format and calculated optical densities.
2. De-identified subject information: age, sex, location, weight, length, % fat, fat mass, fat free mass, volume in a csv format.

These include meta data in the header file: subject ID, measurement time, location, calibration information. Both datasets will be archived on The University of Sydney's research data store within a Redcap database and meta-data will be published on the publicly accessible Research Data Australia <https://researchdata.ands.org.au/> as per the Data Management Policy of the University of Sydney.

DATA ANALYSIS

The data will be analysed by the following groups for each age group which are stratified by gender, ethnicity and skin colour type

For validation purposes, Pearson's correlation coefficients will be used to describe the associations between NIR against the 3 compartment model and /or ultrasound measurements. Coefficient of determination or R^2 will be used to assess the best prediction equation and minimum wavelength for NIR body composition assessment

Multiple and logistic regression will be used to analyse the relationship with anthropometric factors including circumferences and skinfolds.

For prediction of under and over nutrition positive and negative predictive values (PPV and NPV) and the sensitivity, specificity, and likelihood ratios with 95% confidence intervals. Receiver Operating Characteristic (ROC) curves will be used along with the ease of use, cost and portability of each method to determine the most appropriate tool for determining body composition.

OUTCOMES

Scientific publications in peer-reviewed journals.

Validation of a NIR low cost device for detecting body composition against criterion methods for measuring body composition across multiple age groups (birth up to 2 years).

RESEARCH SITE

In order to test populations with melanin variation, diversity including low range body fat percentages and in varied income settings; the testing site will be in Soweto, South Africa in collaboration with Professor Shane Norris.

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FIGURES



Figure 1. Measurement conducted on the anterior thigh using the Ocean Optics QEPro NIR device