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Nutrition Core Protocol and IRB Language

Anthropometric Assessment of Growth and Body Composition

The Nutrition Core Laboratory has the capability of performing a wide array of anthropometric measurements of infants, children, and adults to assess growth, body dimensions, and body composition. Anthropometric examination protocols are modified to meet the needs of the investigator and the study population. Dr. Zemel, Director of the Center for Human Phenomic Science (CHPS) Nutrition Core Laboratory, is available for consultation on the selection of measures, study design, computations of derived measures, and selection of reference data for interpretation. Pubertal status by self-assessment questionnaire may be also obtained in conjunction with the anthropometric examination with the aid of a full length mirror (for the self-assessment) and privacy in the anthropometry room.

Protocol Description

The anthropometric examination to assess growth, body size, and body composition may include the following: body weight (accurate to 0.1kg; infant accurate to 0.01kg), measured on a Scaletonix (Scaletonix, Skaneateles, NY) digital scale without shoes and in light clothing; standing height (0.1cm) measured on a stadiometer (Holtain, Crymych, UK) without shoes and with hair adornments removed; infant length and/or crown-rump length (accurate to 0.1cm) measured on a neonatometer or infantometer (Harpenden, Crymych, UK); sitting height (0.1cm), measured on a stadiometer and sitting platform.

Head, waist, hip and mid-upper arm circumferences are measured with a non-stretchable fiberglass tape (0.1 cm; Weigh and Measures, LLC, Olney, MD) without interfering clothing. Hip circumference is measured over light clothing. Standard skinfold thicknesses (0.1mm) are measured at the triceps, biceps, subscapular, and suprailiac sites for subcutaneous fat stores, fat distribution and/or body composition (i.e., fat mass, fat-free mass and percent body fat) with a skinfold caliper (accurate to 0.2mm; Holtain, Crymych, UK). Other skinfolds can be acquired upon request. Sagittal abdominal diameter, an anthropometric correlate of abdominal fat, is measured with the Holtain Kahn Abdominal Caliper (0.1 cm; Holtain, Crymych, UK).

Elbow and wrist breadth and biacromial diameter are measured with sliding calipers (0.1 cm; Holtain, Crymych, UK). Segmental length of the upper arm, lower arm, and lower leg are measured with a segmometer (0.1 cm; Weigh and Measures, LLC, Olney, MD) for an assessment of body proportions and alternative measures of linear growth. A high precision measurement of the length of the lower leg from the heel to the superior surface of the knee can be obtained using a specialized knee-height measuring device for the assessment of short-term growth of the lower leg (0.01 mm).¹

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All anthropometric measurements are taken and recorded in triplicate (unless otherwise specified by a research protocol) and the mean used in analyses. All measurements follow the methods described in The Anthropometric Standardization Manual of Lohman, et al.² The anthropometric examination takes approximately 15 to 30 minutes.

Pubertal status by a self-assessment questionnaire originally designed by Morris and Udry³ can be used that provides pictograms and descriptions for breast stage in girls, genital stage in boys, and pubic hair development in both boys and girls based on the sexual maturity stages defined by Tanner.⁴ The questionnaire is explained to the study participant (with or without a parent present) in advance, and then they are left to complete the questionnaire in private. The anthropometric exam room is equipped with a full length mirror to aid in the self-assessment process.

Consent Description

We will measure your body length (children 2 years and younger) and/or height, sitting height, and weight. We will also measure the size of your head, waist, hip, upper-arm, and calf using a tape measure. The size of your body (body breadth) at the elbow, shoulders, and abdomen will be measured with a sliding ruler to estimate body size. We will measure the length of your upper and lower arm and lower leg with a sliding ruler. We will also measure the amount of fat stored beneath the skin (we take a “fold” of fat and measure it with a device called a skinfold caliper) in four different areas. It will take approximately 30 minutes to complete these measurements. These measures are important for assessing body size and patterns of growth.

If you are seven years of age or older, you will be asked to complete a questionnaire that asks questions about your current pubertal status (the changes in your body as you become more mature) based on a description sheet and pictures that we will provide. A private room will be provided for completion of this self-assessment by yourself or with the help of your parents. Female subjects will also be asked several questions regarding menstrual periods.

Consent Risks

There are no known risks associated with the measurement of growth and body size (i.e., height, weight, limb lengths, body circumferences, and skinfold thickness). None of the measurements involve pain or physical discomfort. You may feel pressure where the growth measurements are being taken with calipers. You may feel embarrassed or uncomfortable while completing the puberty questionnaire. You will be given the questionnaire and instructions on completing it by trained professionals



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who will try to minimize your embarrassment or discomfort and you will be able to complete this in private.

Body Composition

The Nutrition Core Laboratory has several methods available for body composition assessment.

Whole Body Dual Energy X-ray Absorptiometry (DXA)

Protocol Description

Body composition will be assessed by whole body DXA scanning using a Hologic fan-beam bone densitometer (Marlborough, MA) in array mode operating in Apex software. The analyses will report regional and whole body estimates of bone mineral content and density, fat mass, fat free mass, lean body mass, percent body fat, and fat distribution. Visceral adipose tissue area is also estimated in older children and adults. DXA uses a very low-dose x-ray exposure ($\sim 10 \mu\text{Sv}$ / scan) and measurements are based on the attenuation of the dual energy x-ray beams as they pass through the body. The method is acceptable for use in infants, children, adolescents, and adults. Females of reproductive age are required to have a negative pregnancy test prior to the scan. A quality control protocol is completed weekly using a whole body phantom with fields of acrylic and aluminum of varying thickness and known absorptive properties that simulate body composition and tissue thickness.

Consent Description

We will measure your bone mineral content, bone density, and body composition by a method called dual energy x-ray absorptiometry (DXA). This method uses a very low-powered x-ray beam to scan your entire body. The results are analyzed by computer, which provides an estimate of the amount and density of your bones, and the amount of fat and muscle in your body (body composition). The test is done while you are lying on the soft tabletop of the DXA machine. The whole body scan test takes less than 5 minutes. The radiation used in this machine is very minimal. This is the standard method for measuring bone mineralization and body composition, and it is considered safe for children. If you are a female of reproductive age, you will have a urine pregnancy test before the DXA scan.

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Consent Risks

The whole body DXA scan involves exposure to radiation. The radiation dose you will receive is less than the amount of radiation dose you would receive from a round trip airplane flight from New York to California. The risks from this radiation dose are too small to be measured accurately.

All female participants ages 10 and older will be asked to provide a urine sample for pregnancy testing prior to the whole body DXA scan. Young and adult women who know that they are pregnant or who think they might be pregnant are ineligible to participate in this part of the study. Participants are required to disclose the possibility of pregnancy to the investigator to ensure the safety of the unborn child.

Pea Pod Air Displacement Plethysmography

Protocol Description

Infant body composition testing using air displacement plethysmography with the Pea Pod (COSMED, Concord, CA) is a safe, noninvasive, reliable, accurate and brief (approximately 7 minutes) method to estimate fat mass, fat-free mass, and percent body fat using menu driven software.⁵ Body mass, volume, density, and surface area are also determined. Sedation is not required, and it can accommodate infants weighing between 1 kg and 8 kg (17.6 lbs.).

Consent Description

We will measure the total amount of fat and muscle in your baby's body with a device called the Pea Pod. Your baby will be undressed, weighed on a scale and then placed into the warmed Pea Pod. From air measurements in the Pea Pod we can estimate the amount of fat and muscle in your baby's body. The entire test takes 5-7 minutes. This is safe, and there is no radiation exposure.

Consent Risks

There are no known risks to the Pea Pod measurements.

Multifrequency Bioelectrical Impedance Analysis

Protocol Description

Multifrequency bioelectrical impedance analysis (BIA) using HYDRA- ECF/ICF BIA (Xitron Technologies, Inc., San Diego, CA) will be used to obtain non-invasive, rapid measurements of total body water, fat-free mass, fat mass, percent body fat of the



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total body and body segments based on resistance and reactance measurements. The device is small and portable. Measurements are taken with the study participant in a supine position on a gurney or examination table after a period of rest. Alcohol swabs are applied to clean the skin surface prior to placing the four electrodes needed to obtain the measurement. Electrodes are usually placed on the hand, wrist, ankle, and foot, although this may vary by protocol. It takes approximately 5 minutes to complete the procedure.

Consent Description

Bioelectrical impedance analysis (BIA) measures the amount of muscle and fat in the body. BIA measures are taken by passing a faint electrical current through the body. You cannot feel this and it is not painful. While you are lying down, electrodes (stickers) will be placed on four locations on your body, typically your right ankle and wrist <<this may vary according to protocol>> in order to do this test. This is safe, and there is no radiation exposure.

Consent Risks

There may be minor skin irritation associated with the use of the electrodes during the BIA measurement.

Bone Health Assessment

The Nutrition Core Laboratory offers three techniques for bone health assessment.

Peripheral Quantitative Computerized Tomography (pQCT)

Protocol Description

Peripheral quantitative computerized tomography (pQCT) is a research tool that measures trabecular and cortical bone dimensions and strength in the peripheral skeleton (Stratec XCT 2000, Birkenfield, Germany). The Stratec XCT 2000 is a rotate-translate QCT device with 12 detectors using a slice thickness of 2.3 mm with a standard voxel size of 0.4mm and a scan speed of 25mm/second. Quality control is monitored by using the manufacturer-provided phantom composed of hydroxyapatite encased in acrylic. Measurements are typically taken of the distal tibia at the 3%, 38%, and 66% from the distal reference line at the tibia growth plate or can be customized to sites specified by the study investigator. Comparable measurements can be obtained in the distal radius at the 3%, 30%, and 65% from the distal reference line at the radial growth plate. The 3% site is primarily trabecular and gives a volumetric measure of trabecular bone mineral density (vBMD) (g/cm³). Total vBMD at the 3% site provides an integrated measure of cortical and trabecular density at a



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distal site. The 38% (radial 30%) site provides measures of cortical vBMD, as well as structural parameters such as periosteal and endosteal circumference (mm) and cross-sectional moment of inertia, an index of bone strength (mm⁴). The 66% site of the tibia (65% radius) is the location of maximal muscle circumference and may be used to evaluate the effect of local body composition (i.e., muscle cross-sectional area) and muscle density on bone. Electronic data sets of scan results with quality assurance ratings and derived measures of cortical and trabecular bone density, geometry, and strength are provided to investigators.

The effective radiation dose for the p-QCT measurements is very low (< 0.01μSv per slice). Phantoms are scanned daily to monitor quality control of the device. The CHPS Nutrition Core Director, Dr. Babette Zemel, reviews all scans for quality assurance. The coefficient of variation for QCT measurements in children range between 0.5 to 2.8%.⁸⁻¹⁰ The procedure takes approximately 15 minutes to complete depending on the number of sites scanned. Local reference data is available on approximately 500 to 700 healthy control children.

Consent Description

A method called peripheral quantitative computerized tomography (pQCT) will be used to measure your bone density. This instrument uses a low energy x-ray beam to measure the density of bone at the forearm and the lower leg. It is different from the DXA because it measures the hard outer layer of bone (cortical bone) separately from the inner layer of bone (trabecular or “spongy” bone). The forearm and/or lower leg will be measured three times. The radiation exposure for each scan is very low, and the scan is completed in approximately 30 minutes. These p-QCT tests are the most advanced and accurate methods of measuring bone health and are considered safe for children. Females of reproductive age are required to have a negative pregnancy test prior to the procedure.

Consent Risks

This research study involves exposure to radiation from the pQCT studies and therefore you will receive a radiation dose. The radiation dose you will receive is less than the amount of radiation dose you would receive from a round trip airplane flight from New York to California. The risks from this radiation dose are too small to be measured accurately.

All female participants ages 10 and older will be asked to provide a urine sample for pregnancy testing prior to the whole body DXA scan. Young and adult women who know that they are pregnant or who think they might be pregnant are ineligible to



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participate in this part of the study. Participants are required to disclose the possibility of pregnancy to the investigator to ensure the safety of the unborn child.

Dual Energy X-ray Absorptiometry (DXA)

Protocol Description

DXA is the technique most commonly used in the clinical diagnosis of osteoporosis in adults and low bone mass in children. Bone mineral content (BMC) and areal bone mineral density (aBMD) of the anterior-posterior (AP) spine, lateral spine, proximal femur, lateral distal femur, forearm, and whole body can be assessed by DXA using a Hologic Horizon bone densitometer (Hologic, Marlborough, MA) operating in Apex software. The whole body and lumbar spine are the standard measurement sites for bone density assessment in children,¹¹ but other sites may be measured, including the proximal femur, forearm and lateral distal femur. For adults, the lumbar spine and proximal femur are recommended sites.¹² Whole body DXA is also used for body composition assessment as described above. All scans are obtained following standardized procedures as recommended by the manufacturer. It takes approximately 20 minutes to complete all DXA measurements. Instant Vertebral Assessment (IVA) for determination of spine deformities is also available.

DXA uses very low-dose X-ray exposures (1 to 10 μSv per scan) and measurements are rapid, making this the preferred technique for measurement of bone mineralization in children. Quality control scans are performed daily using a simulated L1-L4 lumbar spine made of hydroxyapatite encased in epoxy resin and thrice weekly using a whole body phantom. The in vitro coefficient of variation is < 1% and the in vivo coefficient in children is < 2%. All scans are reviewed by or under the supervision of the CHPS Nutrition Core Director, Dr. Babette Zemel, for quality assurance.

The CHPS Nutrition Core is staffed by research technicians who are expertly trained in acquiring DXA scan images in children and adults. Electronic data sets with scan results for bone area, content and density, and body composition from whole body scans are provided to investigators.

Consent Description

We will measure your bone mineral content and bone density by a method called dual energy x-ray absorptiometry (DXA). This method uses a very low-powered x-ray beam to scan the whole body and then the regions lumbar spine, hip, forearm and/or lower thigh. The results are analyzed by computer, which gives estimates of the amount and density of your bones, and your fat mass and muscle mass (body composition). The test is done while lying on the soft tabletop of the DXA machine.



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Each scan test takes less than 5 minutes, and the total time for the bone health test is approximately 15 to 30 minutes <<depending on the number of scans>>. The radiation used in this machine is very minimal. This is the standard method for measuring bone mineralization and body composition, and it is considered safe for children. <<add pregnancy statement>>

Consent Risks

This research study involves exposure to radiation DXA scans and therefore you will receive a radiation dose. The radiation dose you will receive is less than the amount of radiation dose you would receive from a round trip airplane flight from New York to California. The risks from this radiation dose are too small to be measured accurately.

All female participants ages 10 and older will be asked to provide a urine sample for pregnancy testing prior to the whole body DXA scan. Young and adult women who know that they are pregnant or who think they might be pregnant are ineligible to participate in this part of the study. Participants are required to disclose the possibility of pregnancy to the investigator to ensure the safety of the unborn child.

High Resolution Quantitative Computerized Tomography (HR-QCT)

Protocol Description

HR-QCT provides sufficient resolution for 3D in vivo imaging of bone. The Xtreme CT II (SCANCO Medical AG, Bruettisellen, Switzerland) yields 3D measurements of bone geometric parameters and cortical and trabecular microarchitecture that correlate with bone strength.¹⁵⁻²⁰ It uses a low dose x-ray dose (< 5 μ SV per scan) to measure both bone density and microarchitecture. HR-pQCT achieves sufficient resolution for building microstructural finite element (μ FE) models to assess bone strength, a direct measurement of bone's resistance to fractures.^{15, 16, 20-22} Each scan of the distal radius or tibia takes less than 4 minutes, and a complete visit, including instructions to patients, scout view, and full scans of both the distal radius and tibia requires less than 30 minutes.

The HR-pQCT scan procedure requires immobilizing the non-dominant distal radius or distal tibia in a carbon fiber shell to scan the limb. A scout film is obtained first to allow manual placement of a reference line at the proximal edge of the distal end of the metaphysis/growth plate for measures in children and at the distal end of the epiphysis/endplate for measures in adults where the metaphysis is no longer visible. Scan sites can be adapted to study specifications. Typically, for the non-dominant radius, the ultradistal measurement is obtained at a site that is 3% of ulnar length, and a midshaft measurement of cortical bone is obtained at 30% of ulnar length <<or



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as specified by the study protocol>>. For the tibia, the ultradistal measurement is obtained at a distance that is 3% of tibia length, and the midshaft measurement is acquired at 30% of tibia length <<or as specified by the study protocol>>.

Consent Description

The HR-pQCT gives detailed information about the strength of bones. It gives us information about the hard, outer shell of bone (cortical bone) and the inner “spongy” looking (trabecular) bone. This additional information tells us more about how healthy and strong your bones are compared to the usual bone density tests.

We will measure your bone density and strength using HR-pQCT. This instrument uses a low energy X-ray beam to measure the amount of bone in your lower arm (radius) and the lower leg (tibia). The radiation exposure for each scan is low, and the scans will be completed in approximately 30 minutes.

Consent Risks

This research study involves exposure to radiation from the HR-pQCT studies and therefore you will receive a radiation dose. This research study involves exposure to radiation from the HR-pQCT studies and therefore you will receive a radiation dose. This radiation dose is not necessary for your medical care and will occur only as a result of your participation in the study. At doses much higher than you will receive, radiation is known to increase the risk of developing cancer after many years. At the doses you will receive, it is very unlikely that you will see any effects from the radiation dose.

Measures of Muscle Strength

The Biodex Dynamometer

Protocol Description

The Biodex Dynamometer (Biodex Medical Systems, Shirley, NY) is used to measure muscle strength across joints. The procedure requires a standardized warm-up period consisting of 5 minutes of treadmill walking. The subject then performs an isometric strength test. Typically, knee and/or ankle flexion and extension are measured although other sites are possible. Peak torques will be recorded during isometric and isokinetic contractions. First, a maximal pain free range of motion (ROM) is established from a neutral position. The subject is then assessed in multiple positions depending on the total available ROM <<or as specified by the study protocol>>.



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Rest is given between repetitions and a between sets. Trained technicians perform the test.

Consent Description

We measure the strength of your leg muscles using a Biodex machine. You will sit in a chair and perform a series of leg extensions (leg straightens) and flexions (leg bends) with the left knee and/or ankle. The equipment is like that used for weight-lifting exercises. The amount of force produced will be measured. This procedure will take approximately 10 minutes.

Consent Risks

The Biodex test may result in mild discomfort to the knees, ankles, or muscles in the leg.

Hand-Grip Dynamometry

Protocol Description

The Smedley III Digital Grip Strength (Takei Scientific Instruments Co., Ltd., Tokyo, Japan) is a handheld device capable of measuring instantaneous hand strength as a function of time for periods of up to 300 seconds. The subject stands upright with arms extended and is instructed to grip the dynamometer and exert full force. Three trials are performed using each hand. The dynamometer digitally displays the force production (kgf) by the subject.

Consent Description

Muscle strength will be measured using a hand-grip strength dynamometer. You will squeeze the handle on the device with each hand and the strength of the "squeeze" will be measured. This measure will take approximately 5 minutes to complete and does not involve any radiation.

Consent Risks

The hand-grip dynamometer test may result in mild, temporary hand or forearm discomfort.

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Force Plate Jump Mechanography

Protocol Description

The Kistler Multicomponent Force Plate (Kistler Instruments AG, Amherst, MA) measures jump power, force, and height, primarily to determine the functional strength and muscle loading of the lower limbs, but other applications are possible. Following a warm-up period consisting of 5 minutes of treadmill walking, the study participant performs the force plate test using a computer driven standardized jump protocol such as a squat jump or counter-movement jump. The subject first completes three practice jumps followed by three test jumps. An electronic database for each test is generated and provided to the investigator.

Consent Description

We will measure the muscle strength in your legs using a force plate. A force plate is like a large, square scale. You will jump on the force plate, and the force plate will electronically record a measure of your muscle strength used in jumping. First, you will be asked to walk on the treadmill (with shoes on) for 5 minutes to warm up before the test. Next, you will take your shoes off and do some practice jumps on the force plate. When you are ready, real jumps will be done for measurement of strength. The test will take about 10 minutes to complete, along with time needed for warm-up.

Consent Risks

The force plate test may result in mild, temporary discomfort to the muscles in the lower leg.

Indirect Calorimetry

Resting Energy Expenditure

Protocol Description

REE will be assessed by open circuit indirect calorimetry using a VMAX computerized metabolic cart (CareFusion, Yorba Linda, CA). Resting energy expenditure (REE) tests for children and adults are performed in the early morning in an awake, fasted state, with minimal physical activity prior to the test. A standardized evening meal will be provided based on subjects' food preferences and caloric needs, as assessed by the CHPS Bionutritionists. A 60-minute REE test is performed between 7:00 a.m. and 10:00 a.m. with the subject resting quietly under a clear, plastic hood watching a videotape. The results are edited to eliminate measurements during the initial period



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of acclimation to the test, and any other periods of significant physical movement or coughing. The remaining measurements are averaged to obtain the mean REE calculated from the modified Weir equation,²³ using oxygen consumption and carbon dioxide production.

REE is subsequently compared to predicted values derived from the World Health Organization that adjust for age, gender, and weight²⁴ and Schofield equations that adjust for age, gender, weight, and height.²⁵ Respiratory quotient is also obtained from the test. For infants, sleeping energy expenditure is measured while napping following a day-time feed. The test can also be used to assess energy expenditure under other circumstances such as the metabolic response to a meal. The metabolic cart is somewhat portable and tests can be performed at the bedside for inpatient subjects.

Consent Description

REE is calculated by measuring the amount of oxygen and carbon dioxide that is in the air that you breathe out. The test will be done shortly after you wake up in the morning. You must not eat or drink anything except water for 12 hours prior to the test. This test requires that you put on a large, comfortable, clear plastic hood, and simply breathe regularly. You will be breathing regular room air. The test lasts about 60 minutes. During that time you must lie quietly, but you may watch movies or listen to music while you are resting. This test will determine the amount of energy (calories) you use in a normal resting state.

Consent Risks

There are no known risks associated with the REE measurement.

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