4.1. Primary Outcome

4.1.1. Background and Rationale

The measure used as the primary outcome variable of all four COPTR trials is body mass index (BMI). BMI assesses body weight adjusted for height and is correlated with percent body fat as assessed by dual energy x-ray absorptiometry (Bray 2001, Dezenberg 1999, Pietrobelli 1998, Daniels 1997). When calculated using measured anthropometrics BMI is highly reliable. BMI has demonstrated clinical validity in its associations with type 2 diabetes mellitus (Pinhas-Hamiel 1996, Scott 1997), hyperinsulinemia (Freedman 1999), blood pressure and hypertension (Dwyer 1998, Freedman 1999, Daniels 1998), adverse lipoprotein profiles (Dwyer 1998, Freedman 1999, Teixeira 2001) and early atherosclerotic lesions (McGill 1995, Berenson 1998) among children and adolescents. Importantly, BMI can be assessed easily in clinical and public health settings and is generally accepted and well understood.

4.1.2. Objective

The objective of the BMI measures is to provide a precise and accurate measure of the impact of the intervention on relevant aspects of body size in the children studied in COPTR.

4.2.3. Methods

All consented index children in the COPTR study have weight and height measured at the beginning and end of the intervention (36 months) and at two common interim time points (12 and 24 months). All baseline anthropometric data will be collected prior to randomization. Weight and height are measured with the participant in light clothing without shoes. Weight is measured to the nearest 0.1 kg using research precision grade, calibrated, digital scales and height is measured to the nearest 0.1 cm using a free-standing or wall mounted stadiometer. BMI is calculated as weight in kilograms divided by the square of height in meters.

All height and weight measurements are collected by trained and certified staff. COPTR will use a “train the trainer” model. Each field center will designate one or more “Master Trainers” who participate in a central training organized by the RCU at the University of North Carolina at Chapel Hill on April 16-18, 2012. These Master Trainers are responsible for training and certifying the data collection staff at their center.

4.2. Anthropometric Secondary Outcomes

Anthropometric secondary outcomes differ by site as detailed in Table 1. Variables measured in the index child at all sites include waist circumference and triceps skinfold. All sites are measuring height and weight in at least one adult family member of the index child and some sites are measuring siblings. Secondary outcomes that will be calculated from anthropometry in at least one site include BMI z-score, waist-to-height ratio (WtHR), and percent body fat.

Table 1. Anthropometric Common Measures by Research Center

Anthropometric Measure	Case	Minnesota	Stanford	Vanderbilt
Index Child				
Weight	x	x	x	x
Height	x	x	x	x
Waist circumference	x	x	x	x
Triceps skinfolds	x	x	x	x
Other Children				
Weight	--	x*	x†	--
Height	--	x*	x†	--
Waist circumference	--	--	x†	--
Triceps skinfolds	--	--	x†	--
Other Adults				
Weight	x	x*	x	x
Height	x	x*	x	x
Waist circumference	--	--	x	x
Triceps skinfolds	--	--	--	x

* Minnesota: All children and adults in household.

† Stanford: Only study eligible children

4.2.1. Background and Rationale

BMI z-scores provide a method for evaluating the weight status of children adjusted for age and gender. The measure is commonly used in clinical practice to track body size trajectory. However, several authors have cautioned against the use of BMI z-scores for research using longitudinal designs citing concerns that their use could result in spurious differences between groups (Cole 2005, Berkey 2007). One reason for this problem is that children at the extreme ends of the BMI distribution require substantially greater changes in weight than their thinner counterparts for the same change in z-score. Also because the BMI z-score curves were constructed using only data between the 3rd and 97th percentiles, the CDC recommends extreme caution when using the growth curves outside this range (Kuczmarski, 2000). Finally, Berkey et al. noted that the difference between z-scores reflect larger differences in BMI in older compared to younger children. For these reasons the COPTR investigators have chosen to study BMI z-score as a secondary rather than a primary outcome.

Abdominal adiposity is associated with metabolic risk factors in children (Freedman 1987, Freedman 1989, Caprio 1995, Caprio 1996) although evidence to date suggests that anthropometric measures tend to only moderately predict visceral fat (Goran 1998a, Goran 1998b). Waist circumference is a feasible non-invasive measure of abdominal fatness for community-based assessments of children. It has also been shown to be sensitive to change in response to prevention interventions (Robinson 1999).

Waist-to-height ratio (WtHR) is a simple index that has recently received increased interest from investigators (Browning, 2010). After the age of four years, waist and height appear to simultaneously increase during childhood and adolescence (Kahn, 2005). Thus, WtHR could provide a practical estimate of adiposity that could be consistently applied to a wide range of age groups. Recently Browning et al. (2010) published a systematic review of waist to height ratio as a screening tool for cardiovascular and diabetes-related outcomes. In their examination of 13 cross-sectional studies in children they found that waist-to-height ratio compared favorably with waist circumference and BMI. In a cross-sectional study of 1,511 youth 8 to 17 years of age McMurray et al. (2010) found that waist circumference performed well as a predictor of insulin resistance in boys but not girls. Better performance was observed when waist circumference was divided by height, producing an index that was highly associated with insulin resistance in both genders and over a range of ages. Kahn et al. (2005) and Savva et al. (2000) have suggested a WtHR cut point of 0.49 to distinguish high and low levels of risk, however, McMurray et al. (2010) suggest that a WtHR of 0.54 may result in fewer misclassifications. WtHR can also be analyzed in the continuous form. COPTR can provide an opportunity to further evaluate this index using both cross-sectional and longitudinal designs.

Triceps skinfold thickness is a measure of subcutaneous fat and is a component of equations used to predict percent body fat. COPTR investigators are using data from the NHANES study to develop a prediction equation for percent body fat that uses triceps skinfold along with other anthropometric variables collected in COPTR (height, weight and waist circumference) together with demographic variables to predict percent body fat. Equations were developed in children in the age ranges being studied by Case Western and Stanford. Preliminary work indicates that this equation has an R^2 of over 0.8. Unfortunately estimates of percent body fat from DEXA are not available in children less than 8 years of age in NHANES. Therefore Vanderbilt and Minnesota will estimate percent body fat in younger children in their study using the prediction equation created by Dezenberg (1999) (R^2=0.95 as compared to DEXA, Model SEE=0.46) using data from White and African American 4 to 11 year old children. This method has been shown to have higher validity across subgroups than other equations (Slaughter 1988, Goran 1996) and has been validated in 3 to 8 year old White and Hispanic children.

Obesity has been shown to cluster in families such that having obese parents increases the risk of obesity in children (Silventoinen, 2009; Barness, 2007; MacFarlane, 2009). This clustering is due to both shared environment and genetic factors. The collection of anthropometric variables in the families of the index children in COPTR provides an opportunity to examine longitudinal changes within families in the family members and to assess any impact of the intervention on family members.

4.2.2. Objective

The anthropometric secondary outcomes are assessed to provide a richer understanding of the changes in body size characteristics associated with the COPTR interventions.

4.2.3. Methods

Waist circumference and triceps skinfolds will be measured at the beginning and end of the intervention (36 months) and at two common interim time points (12 and 24 months). Measurement details have been determined with guidance from the 2007 NHANES anthropometry procedures manual (Centers for Disease Control, 2007, (http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf). Waist is measured to the nearest 0.1 cm just above the uppermost lateral border of the right ilium using a Gulick II tape measure, model 67020.

The triceps skinfold is measured using a Lange skinfold caliper (or a Harpenden caliper if the measurement exceeds capacity of the Lange skinfold caliper) in the midline of the posterior aspect (back) of the arm, over the triceps muscle, at a point midway between the lateral projection of the acromion process of the scapula (shoulder blade) and the inferior margin (bottom) of the olecranon process of the ulna (elbow). Skinfolds are measured to the nearest 0.1 mm.

References

Barness, L.A., Opitz, J. M., Barness, E. G. (2007) Obesity: Genetic, molecular, and environmental aspects. *American Journal of Medical Genetics* Part A, 143A , 3016–3034.

Berenson, G. S., Srinivasan, S. R., Bao, W., Newman, W. P.,3rd, Tracy, R. E., & Wattigney, W. A. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. the bogalusa heart study. *The New England Journal of Medicine, 338*(23), 1650-1656. doi:10.1056/NEJM199806043382302

Berkey, C. S., & Colditz, G. A. (2007). Adiposity in adolescents: Change in actual BMI works better than change in BMI z score for longitudinal studies. *Annals of Epidemiology, 17*(1), 44-50. doi:10.1016/j.annepidem.2006.07.014

Bray, G. A., DeLany, J. P., Harsha, D. W., Volaufova, J., & Champagne, C. C. (2001). Evaluation of body fat in fatter and leaner 10-y-old african american and white children: The baton rouge children's study. *The American Journal of Clinical Nutrition, 73*(4), 687-702.

Caprio, S., Hyman, L. D., Limb, C., McCarthy, S., Lange, R., Sherwin, R. S., . . . Tamborlane, W. V. (1995). Central adiposity and its metabolic correlates in obese adolescent girls. *The American Journal of Physiology, 269*(1 Pt 1), E118-26.

Caprio, S., Hyman, L. D., McCarthy, S., Lange, R., Bronson, M., & Tamborlane, W. V. (1996). Fat distribution and cardiovascular risk factors in obese adolescent girls: Importance of the intraabdominal fat depot. *The American Journal of Clinical Nutrition, 64*(1), 12-17.

Cole, T. J., Faith, M. S., Pietrobelli, A., & Heo, M. (2005). What is the best measure of adiposity change in growing children: BMI, BMI %, BMI z-score or BMI centile? *European Journal of Clinical Nutrition, 59*(3), 419-425. doi:10.1038/sj.ejcn.1602090

Daniels, S. R., Khoury, P. R., & Morrison, J. A. (1997). The utility of body mass index as a measure of body fatness in children and adolescents: Differences by race and gender. *Pediatrics, 99*(6), 804-807.

Davison, K. K., & Birch, L. L. (2002). Obesigenic families: Parents' physical activity and dietary intake patterns predict girls' risk of overweight. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 26*(9), 1186-1193. doi:10.1038/sj.ijo.0802071

Dezenberg, C. V., Nagy, T. R., Gower, B. A., Johnson, R., & Goran, M. I. (1999). Predicting body composition from anthropometry in pre-adolescent children. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 23*(3), 253-259.

Dwyer, J. T., Stone, E. J., Yang, M., Feldman, H., Webber, L. S., Must, A., . . . Parcel, G. S. (1998). Predictors of overweight and overfatness in a multiethnic pediatric population. child and adolescent trial for cardiovascular health collaborative research group. *The American Journal of Clinical Nutrition, 67*(4), 602-610.

Freedman, D. S., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (1999). The relation of overweight to cardiovascular risk factors among children and adolescents: The bogalusa heart study. *Pediatrics, 103*(6 Pt 1), 1175-1182.

Freedman, D. S., Srinivasan, S. R., Burke, G. L., Shear, C. L., Smoak, C. G., Harsha, D. W., . . . Berenson, G. S. (1987). Relation of body fat distribution to hyperinsulinemia in children and adolescents: The bogalusa heart study. *The American Journal of Clinical Nutrition, 46*(3), 403-410.
Freedman, D. S., Srinivasan, S. R., Harsha, D. W., Webber, L. S., & Berenson, G. S. (1989). Relation of body fat patterning to lipid and lipoprotein concentrations in children and adolescents: The bogalusa heart study. *The American Journal of Clinical Nutrition, 50*(5), 930-939.
Goran, M. I. (1998b). Measurement issues related to studies of childhood obesity: Assessment of body composition, body fat distribution, physical activity, and food intake. *Pediatrics, 101*(3 Pt 2), 505-518.
Goran, M. I., Driscoll, P., Johnson, R., Nagy, T. R., & Hunter, G. (1996). Cross-calibration of body-composition techniques against dual-energy X-ray absorptiometry in young children. *The American Journal of Clinical Nutrition, 63*(3), 299-305.
Goran, M. I., Gower, B. A., Treuth, M., & Nagy, T. R. (1998a). Prediction of intra-abdominal and subcutaneous abdominal adipose tissue in healthy pre-pubertal children. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 22*(6), 549-558.
Kahn, H. S., Imperatore, G., & Cheng, Y. J. (2005). A population-based comparison of BMI percentiles and waist-to-height ratio for identifying cardiovascular risk in youth. *The Journal of Pediatrics, 146*(4), 482-488. doi:10.1016/j.jpeds.2004.12.028
Kuczmarski, R. J., Ogden, C. L., Grummer-Strawn, L. M., Flegal, K. M., Guo, S. S., Wei, R., Johnson, C. L. (2000). CDC growth charts: United states. *Advance Data, (314)*(314), 1-27.
MacFarlane, A., Cleland[1], V., Crawford, D., Campbell K., Timperio, A. (2009). Longitudinal examination of the family food environment and weight status among children. *International Journal of Childhood Obesity*, 4, 343-352.

McGill, H. C.,Jr, McMahan, C. A., Malcom, G. T., Oalmann, M. C., & Strong, J. P. (1995). Relation of glycohemoglobin and adiposity to atherosclerosis in youth. pathobiological determinants of atherosclerosis in youth (PDAY) research group. *Arteriosclerosis, Thrombosis, and Vascular Biology, 15*(4), 431-440.
McMurray, R. G., Bangdiwala, S. I., Catellier, D. J., & Harrell, J. S. (2010). The relationship between insulin sensitivity and surrogates for adiposity in youth. *Int J Child Adolesc Health, 3*(1), 93-98.
Pietrobelli, A., Faith, M. S., Allison, D. B., Gallagher, D., Chiumello, G., & Heymsfield, S. B. (1998). Body mass index as a measure of adiposity among children and adolescents: A validation study. *The Journal of Pediatrics, 132*(2), 204-210.
Pinhas-Hamiel, O., Dolan, L. M., Daniels, S. R., Standiford, D., Khoury, P. R., & Zeitler, P. (1996). Increased incidence of non-insulin-dependent diabetes mellitus among adolescents. *The Journal of Pediatrics, 128*(5 Pt 1), 608-615.
Robinson, T. N. (1999). Reducing children's television viewing to prevent obesity: A randomized controlled trial. *JAMA : The Journal of the American Medical Association, 282*(16), 1561-1567.
Savva, S. C., Tornaritis, M., Savva, M. E., Kourides, Y., Panagi, A., Silikiotou, N., . . . Kafatos, A. (2000). Waist circumference and waist-to-height ratio are better predictors of

cardiovascular disease risk factors in children than body mass index. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 24*(11), 1453-1458.
Scott, C. R., Smith, J. M., Cradock, M. M., & Pihoker, C. (1997). Characteristics of youth-onset noninsulin-dependent diabetes mellitus and insulin-dependent diabetes mellitus at diagnosis. *Pediatrics, 100*(1), 84-91.
Silventoinen K., Rokholm, B., Kaprio, J., Sørensen, T.I.A. (2009) The genetic and environmental influences on childhood obesity: a systematic review of twin and adoption studies. *International Journal of Obesity*, 34, 29–40. doi:10.1038/ijo.2009.177
Slaughter, M. H., Lohman, T. G., Boileau, R. A., Horswill, C. A., Stillman, R. J., Van Loan, M. D., & Bemben, D. A. (1988). Skinfold equations for estimation of body fatness in children and youth. *Human Biology; an International Record of Research, 60*(5), 709-723.
Teixeira, P. J., Sardinha, L. B., Going, S. B., & Lohman, T. G. (2001). Total and regional fat and serum cardiovascular disease risk factors in lean and obese children and adolescents. *Obesity Research, 9*(8), 432-442. doi:10.1038/oby.2001.57

4.3. Accelerometry

4.3.1. Background and Rationale

Physical activity (PA) will be measured objectively using a commercially available ActiGraph GT3X+ (all youth). For parents and other adults GT3X+ accelerometers (Vanderbilt) or GT3X accelerometers (Minnesota) will be used. (ActiGraph, Pensacola, FL). The rationale for using ActiGraph is that among currently available devices it provides consistent and high quality data supported by feasibility, reliability, and validity testing in children and adults.

ActiGraph monitors have been used in numerous studies to assess PA in children (Cliff, 2009; De Vries, 2009; Freedson, 2005; Reilly, 2010). The validity of the ActiGraph has been examined in several studies involving children aged 2 to18 years. ActiGraph has been validated using direct observation (Hands, 2006; Kelly, 2004; Sirard, 2005), doubly labeled water (DLW) (Montgomery, 2004; Reilly, 2006), indirect calorimetry (Choi, 2010; Garcia, 2004; Pate, 2006; Schmitz, 2005; Trost, 2006) and other accelerometers (Garcia, 2004; Kelly, 2004) as reference methods. Correlations between ActiGraph counts and observed activity was moderate to high (r = 0.52-0.77) in older ActiGraph models (Hands, 2006; Kelly, 2004; Sirard, 2005) and higher in a newer ActiGraph (GT1M) model and when using more advanced algorithms (Choi, 2010). Although the validity of ActiGraph GT3X and GT3X+ models in populations including children has not be reported, it is expected to be at least as high or higher than the GT1M and older ActiGraph models.

The GT3X+ and GT3X contain electronic motion sensors consisting of piezo-electric sensors that generate an electric charge in response to a mechanical force, thus, acceleration. They do not respond to constant acceleration. Their major advantage is that no power supply is required, except for data storage, resulting in a considerable reduction in the size and weight of the device. Both monitors provide activity counts, vector magnitude, and inclinometry data. Other data calculated by the ActiGraph manufacturer-provided software includes activity intensity levels, energy expenditure (METs) and number of steps.

The GT3X+ collects data in the raw format at a pre-defined sample rate from 30 to 100 Hertz (Hz). When collecting data at 40 Hz, the battery life is stated to be 13 days and the data memory lasts for 16 days. The GT3X has the ability to collect 1-second epoch data for at least 7 days. The GT3X does not have adequate data storage capacity to collect raw data for multiple days.

Accelerometry technology is still improving and mathematical models to predict PA and PA-related energy expenditure are being developed. We expect these advances to continue. Thus, COPTR investigators will collect raw acceleration data in the index child that could be used to measure physical activity and sedentary behavior using both currently existing algorithms and new algorithms/approaches that emerge during the study (next 6 years). Table 1 summarizes the specifications of the GT3X devices.

Table 1. Specifications of the GT3X+ and GT3X devices

Specifications	**GT3X+**	**GT3X**
Transducer	Tri-axis, solid state accelerometer	Tri-axis, solid state accelerometer
Dynamic Range	+/- 3G	+/- 3G
Dimensions	4.6cm x 3.3cm x 1.5cm	3.8cm x 3.7cm x 1.8cm
Capacity	16 Days (Raw data at 40 Hz)	16MB or 400 Days (60 sec epoch)
Battery Life	13 Days (Fully Charged at 40 Hz)	20 Days (Fully Charged)
Weight	19 g	27 g
Resolution	12-bit A/D conversion; 1.46 mG (Raw Data)	12-bit A/D conversion; 1.46 mG (Raw Data)
Sample Rate	30Hz-100 Hz	30 Hz

Limitations of accelerometry

Accelerometers are the best currently available relatively simple and precise device for objectively assessing physical activity and sedentariness. However, they do not provide information on types of activities, nor can they be used to assess lifestyle activities such as raking and shoveling, static activities such as bicycling and weight lifting, and aquatic activities such as showering and swimming. These limitations may be addressed as new algorithms emerge during the course of the study. Other limitations are related to use and application of collected data in device-specific arbitrary counts (PA counts) or more comparable approach of using acceleration (m/sec^2) to summarize accelerometry data.

4.3.2. Objective

Accelerometry monitoring will provide an objective measurement of the amount and patterns of physical activity and sedentary behavior.

4.3.3. Methods

Accelerometry data on children and parent (Minnesota and Vanderbilt) will be collected at four common data collection time points – baseline, 12 months, 24 months and 36 months. All baseline accelerometer data will be collected prior to randomization. The GT3X+ will be set to 40-Hertz frequency and the GT3X will be set to 1-second epoch.

The index children in the study will wear the GT3X+ monitor on the right hip for seven complete days (including while sleeping and naptime) except during water activity (e.g., bathing, swimming, showering). The responding parent in Minnesota and Vanderbilt will also wear the GT3X and GT3X+ monitor, respectively for seven days on their right hip. A consensus has been reached that the monitoring period should include two weekend days and five weekdays. In some cases, participants may be able to provide only 6 days of data, which is acceptable. If the participant does not wear the activity monitor for four days, it may be necessary to have the participant wear the monitor again in order to get valid data. The valid wear time criteria (minimums) are 4 days (3 weekdays and 1 weekend day) of at least 6 hours of awake time with 33% non-zero epochs per hour. For some participants, accelerometer data for the 2 wears will be combined in

order to meet the minimum wear time criteria. Table 2 summarizes the recommended accelerometer parameters by age group.

Table 2. Recommended accelerometer parameters by age group

Parameter	Children (2-5 years)	Children (6-18 y)
Field Monitoring Period	≥7 days	≥ 7 days
Placement	1 monitor – waist/hip	1 monitor – waist/hip
Epoch (Data Reduction)	10 - 15 sec	10 – 15 sec
Non Wear	60 min consecutive 0 counts with allowance for 1–2 epochs of non-zero counts.	60 min consecutive 0 counts with allowance for 1–2 epochs between 0 and 100
Minimal Wear Time	50%-75% of waking hours > 5 h and < 18 h	> 600 min
Minimal # Valid Days	4 or more	4 or more
Outcome Variable	Time spent in SED, LPA, MPA, VPA, MVPA, LMVPA	Time spent in SED, LPA, MPA, VPA, MVPA
ActiGraph Cut-Point Recommendation	Pate (2006) (15 sec) SED: < 25 MOD: >= 420 VIG: >= 842	(Evenson, 2008) (15 sec) SED: < 25 MOD: >= 574 VIG: >= 1003

SED = sedentary activity; LPA = low physical activity; MPA = moderate physical activity; VPA = vigorous physical activity, MVPA= moderate to vigorous, LMVPA = light- moderate- and vigorous physical activity
Table Courtesy of Stewart Trost, December 2011 COPTR Webinar

Any major updates in the ActiLife software version used during the trial will be made as a collaborative decision by the Diet and Physical Activity Working Group. If a change does occur, it will be on the same calendar day for all Field Sites. Regular (minor) updates in the ActiLife software will be done by each Field Site as they are released by ActiGraph. The Accelerometer Manual of Procedures will be updated only after major updates in the ActiLife software (e.g. Version 6.0 to Version 7.0).

COPTR will use a “train the trainer” model. Each field center will have at least two activity monitor master trainers who will participate in a central in-person training organized by the RCU at the University of North Carolina at Chapel Hill on April 16-18, 2012. Following part 1 of the training session, the master trainers will wear the accelerometer for at least 8 hours. The certification process requires the master trainer to successfully initialize, download and transfer accelerometer data. The master trainers will train and certify additional research staff at their site. Data collectors/staff do not initialize or download accelerometer data until after they have been trained and certified.

References

Choi, L, Chen, KY, Acra, SA, Buchowski, MS. (2010). Distributed lag and spline modeling for predicting energy expenditure from accelerometry in youth. *J Appl Physiol, 108*(2): 314-327. doi: 10.1152/japplphysiol.00374.2009

Cliff, DP, Reilly, JJ, Okely, AD. (2009). Methodological considerations in using accelerometers to assess habitual physical activity in children aged 0-5 years. *J Sci Med Sport, 12*(5): 557-567. doi: 10.1016/j.jsams.2008.10.008

De Vries, SI, Van Hirtum, HW, Bakker, I, Hopman-Rock, M, Hirasing, RA, Van Mechelen, W. (2009). Validity and reproducibility of motion sensors in youth: a systematic update. *Med Sci Sports Exerc, 41*(4): 818-827. doi: 10.1249/MSS.0b013e31818e5819

Evenson, KR, Catellier, DJ, Gill, K, Ondrak, KS, McMurray, RG. (2008). Calibration of two objective measures of physical activity for children. *J Sports Sci, 26*(14): 1557-1565. doi: 10.1080/02640410802334196

Freedson, P, Pober, D, Janz, KF. (2005). Calibration of accelerometer output for children. *Med Sci Sports Exerc, 37*(11 Suppl): S523-530.

Garcia, AW, Langenthal, C.R., Angulo-Barroso, R.M., Gross, M.M. (2004). A comparison of accelerometers for predicting energy expenditure and vertical ground reaction force in school-age children. *Meas Phys Educ Exerc Sci., 8*(3): 119-144.

Hands, BP, Parker, H., & Larkin, D. (2006). Physical activity measurement methods for young children: a comparative study. *Measurement in Physical Education and Exercise Science, 10*(3): 203-214.

Kelly, LA, Reilly, JJ, Fairweather, SC, Barrie, S, Grant, S, Paton, JY. (2004). Comparison of two accelerometers for assessment of physical activity in preschool children. *Pediatr Exerc Sci, 16*(4): 324-333.

Montgomery, C, Reilly, JJ, Jackson, DM, Kelly, LA, Slater, C, Paton, JY, Grant, S. (2004). Relation between physical activity and energy expenditure in a representative sample of young children. *Am J Clin Nutr, 80*(3): 591-596.

Pate, RR, Almeida, MJ, McIver, KL, Pfeiffer, KA, Dowda, M. (2006). Validation and calibration of an accelerometer in preschool children. *Obesity (Silver Spring), 14*(11): 2000-2006. doi: 10.1038/oby.2006.234

Reilly, JJ. (2010). Low levels of objectively measured physical activity in preschoolers in child care. *Med Sci Sports Exerc, 42*(3): 502-507. doi: 10.1249/MSS.0b013e3181cea100

Reilly, JJ, Kelly, LA, Montgomery, C, Jackson, DM, Slater, C, Grant, S, Paton, JY. (2006). Validation of Actigraph accelerometer estimates of total energy expenditure in young children. *Int J Pediatr Obes, 1*(3): 161-167.

Schmitz, KH, Treuth, M, Hannan, P, McMurray, R, Ring, KB, Catellier, D, Pate, R. (2005). Predicting energy expenditure from accelerometry counts in adolescent girls. *Med Sci Sports Exerc, 37*(1): 155-161.

Sirard, JR, Trost, S.G., Pfeiffer, K.A., Dowda, M., Pate, R.R. (2005). Calibration and evaluation of an objective measure of physical activity in preschool children. *J Phys Act Health, 3*: 324-336.

Trost, SG, Way, R, Okely, AD. (2006). Predictive validity of three ActiGraph energy expenditure equations for children. *Med Sci Sports Exerc, 38*(2): 380-387. doi: 10.1249/01.mss.0000183848.25845.e0

4.4. Dietary Assessment

4.4.1. Background and Rationale

The 24-hour recall is the most widely used method to assess diet in studies of populations, and is used in national food consumption surveys such as the NHANES. This method allows assessment of all foods, beverages and dietary supplements consumed during the 24-hour period obtained – typically beginning with the first item consumed the previous day. The 24-hour method, which can be performed face-to-face or by telephone, has been validated in lean and obese individuals (Conway, 2004; Conway, 2003). In face-to-face interviews, the use of visual aids such as food models, food portion booklets and measuring utensils improves the accuracy of estimation of quantities consumed (Moshfegh, 1999). For telephone interviews, visual aids and instructions are often mailed to subjects (Posner, 1992). Whether in-person or by telephone, 24-hour recalls provide little subject burden and are not likely to alter food consumption behavior. In addition, with a trained interviewer, they are relatively quick and easy to administer. An important strength of the 24-hour recall method is that it allows comparison of groups of individuals by demographic variables such as age, gender, race/ethnicity or geographic region. Another strength is that the 24-hour recall (Nutrition Data Systems for Research or NDSR) has been used to generate Healthy Eating Index scores, and thus to assess dietary quality (Miller, 2011). The main limitations of capturing quantitative dietary intake information by use of 24-hour recalls are: 1) the variability in day-to-day dietary intakes; 2) reliance on subject memory; and 3) the potential of over or underreporting of intakes. To compensate for these possible limitations, interviewers typically capture data on more than one day of the week which includes both weekdays and weekend days, and use the *United States Department of Agriculture* (USDA) 5-step multi-pass method (Moshfegh, 1999).

4.4.2. Objective

The purpose of performing dietary intake assessment is to capture quantitative nutrient information on all the foods, beverages and dietary supplements that study subjects consume. The dietary intakes are analyzed for: volume of food, total energy, macronutrients, micronutrients, water, dietary fiber, added sugars and specific food groups. We will also examine glycemic load, dietary energy density, nutrient adequacy ratios, and dietary pattern and quality. Examples of diet quality indices used in children are shown in Table 1.

The Dietary Assessment protocol was approved by the COPTR Steering Committee on Feb.23, 2012.

Table 1. Examples of dietary quality indices used in children

Citation	Subjects			Diet	Group/Index	Methods
	N	Sex	Age	Assessment		
(Daniels, 2009)	1,810	m/f	2y	24 hr recall	Diet Diversity score(DDS-10g) - FAO (score 1-9)	Cross-sectional: 1 pt per 10g of a each food group or 1 pt for 1g oil.
(Feskanich, 2004)	16,452	m/f	9-14y	132 item FFQ	Youth HEI- 13 components (score 0-100)	Modified HEI and compared to YHEI (Note: YHEI not strongly related to energy intake).
(Freedman, 2010)	17,311	m/f	≥2y	24hr recall	HEI-2005: 12 dietary components	NHANES ('01-'04) data- 3 part model (they create) based on Tooze 2- part model(Tooze, 2006) in >1000 subjects.
(Guenther, 2008)	8,650	m/f	≥2y	24 hr recall	HEI-2005	NHANES ('01-'02) compared HEI-2005 assessed validity through 4 methods (concluded valid).
(Kennedy, 2007)	3,164	m/f	24-71 mo	24 hr recall	Diet Diversity Score (DDS) – 10 food group & DDS-10g	Filipino Nutrition Database. DDS summed unique food groups for score. DDS-10g required minimum amounts (see: Daniels, 2009).
(Manios, 2009)	2,287	m/f	2-5y	24 hr recall + weighed records + food diaries	HEI- 10 component	Weighed records were used in nurseries and recalls or diaries were used outside nurseries. Summed individual scores- used quartiles of the scores for analysis.
(Steyn, 2006)	2,200	m/f	1-8y	24 hr recall	DDS- following FAO guidelines Food Variety Score (FVS) (Score 0-45)	Secondary analysis of NFCS in South Africa. 1 24 hr recall by caregivers. Also used nutritional adequacy ratio and mean adequacy ratio.
(Serra-Majem, 2003)	3,166	m/f	6-24y	24 recall +16 item FFQ	KIDMED- Mediterranean diet measure (Score: -3 to 12)	Assessed diet from Spanish children has high, med, low KIDMED.
(Kranz, 2006)	5,437	m/f	2-5y	24 recall	Created new- RC-DQI	Continuing Survey of Food intakes by individuals (1994-1996, 1998) components chosen based on My Food Pyramid, ADA, and APA recommendations (Nutrient-based)
(Hurley, 2009)	317	m/f	11-19	131 item- youth/ adolescent FFQ	Compared HEI and YHEI	Compared the indices to body composition and found HEI better correlated with body composition and disease risk.
(LaRowe, 2010)	135	m/f	2-5	24 hr recall	My Food Pyramid	Great Lakes Inter-Tribal Council Head Start programs- baseline data from HCSF intervention.
(Cheng, 2010)	376	m/f	6-8y	3-day weighed record	Nutritional Quality Index (NQI)- Density measure RC-DQI- nutrient based	German Cohort

4.4.3. Methods

Dietary Intakes will be measured using 24-hour recalls that are conducted on two weekdays and one weekend day per study time-point using NDS-R version 2012. Any update in the NDS-R version during the trial will be made as a collaborative decision by the Diet and Physical Activity Working Group. If a change does occur, it will be on the same calendar day for all Field Sites with one caveat. Participants who have already completed 1 or 2 recalls in the old version of NDS-R will have their remaining recalls conducted using the same older version of NDS-R such that all 3 recalls are collected using the same version of NDS-R.

Dietary assessment data will be collected at baseline, and 12, 24 and 36 months during the study. All baseline dietary assessment data will be collected prior to randomization. Table 2 summarizes the specific data collection plans for each Field Site. To avoid collecting days with similar foods, recalls should not be conducted on consecutive days. In addition, in order to capture variability of food supplies in the home, all three recalls should not occur within a seven day period. The third recall needs to be collected more than one week after the first recall. All three recalls must be collected within 30 days. This is a hard deadline. While the goal is to collect three dietary recalls per participant, it is possible that a limited number of participants at each Field Site may only have two dietary recalls completed within the 30 day window. All efforts will be made to obtain a minimum of two recalls (1 weekday and 1 weekend) for each participant. All dietary intakes (i.e., food, and beverages including water~~)~~ will be collected. For Diet Recall of young children, those responsible for child feeding (e.g. parents, daycare providers) will be the reporter. Details of the procedures to be used in dietary assessment are in the COPTR Manual of Procedures for Dietary Assessment.

COPTR will use the “train- the- trainer” model. Each Field Site will have at least two diet master trainers who will participate in a central in-person training organized by the RCU at the University of North Carolina at Chapel Hill on April 16-18, 2012. Following the training session, the master trainers will complete two dietary recalls for certification by the RCU. The master trainers will train and certify additional research staff at their site. No diet recalls will be conducted until after the trainer has been trained and certified.

Table 2. Site Specific 24-hour dietary recall data collection plans

	Case	Minnesota	Stanford	Vanderbilt
Number of recalls	3	3	3	3
# weekdays	2	2	2	2
# weekends	1	1	1	1
Recaller	Child & parent	Parent & day care provider	Child & parent	Parent & day care provider
How collected (1st, 2nd, 3rd)	In-person Telephone Telephone	In-person In-person/Telephone In-person/Telephone	In-person Telephone Telephone	Telephone Telephone Telephone
Announced/ Unannounced	Announced	Announced	Unannounced	Announced
Language administered	English	English, Spanish	English, Spanish	English, Spanish
Portion Size Device	Food Booklet	Food Booklet	Food Booklet	Food Booklet

References

Cheng, G, Gerlach, S, Libuda, L, Kranz, S, Gunther, AL, Karaolis-Danckert, N, Kroke, A, Buyken, AE. (2010). Diet quality in childhood is prospectively associated with the timing of puberty but not with body composition at puberty onset. *J Nutr, 140*(1): 95-102. doi: 10.3945/jn.109.113365

Conway, JM, Ingwersen, LA, Moshfegh, AJ. (2004). Accuracy of dietary recall using the USDA five-step multiple-pass method in men: an observational validation study. *J Am Diet Assoc, 104*(4): 595-603. doi: 10.1016/j.jada.2004.01.007

Conway, JM, Ingwersen, LA, Vinyard, BT, Moshfegh, AJ. (2003). Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. *Am J Clin Nutr, 77*(5): 1171-1178.

Daniels, MC, Adair, LS, Popkin, BM, Truong, YK. (2009). Dietary diversity scores can be improved through the use of portion requirements: an analysis in young Filipino children. *Eur J Clin Nutr, 63*(2): 199-208. doi: 10.1038/sj.ejcn.1602927

Feskanich, D, Rockett, HR, Colditz, GA. (2004). Modifying the Healthy Eating Index to assess diet quality in children and adolescents. *J Am Diet Assoc, 104*(9): 1375-1383. doi: 10.1016/j.jada.2004.06.020

Freedman, LS, Guenther, PM, Krebs-Smith, SM, Dodd, KW, Midthune, D. (2010). A population's distribution of Healthy Eating Index-2005 component scores can be estimated when more than one 24-hour recall is available. *J Nutr, 140*(8): 1529-1534. doi: 10.3945/jn.110.124594

Guenther, PM, Reedy, J, Krebs-Smith, SM. (2008). Development of the Healthy Eating Index-2005. *J Am Diet Assoc, 108*(11): 1896-1901. doi: 10.1016/j.jada.2008.08.016

Hurley, KM, Oberlander, SE, Merry, BC, Wrobleski, MM, Klassen, AC, Black, MM. (2009). The healthy eating index and youth healthy eating index are unique, nonredundant measures of diet quality among low-income, African American adolescents. *J Nutr, 139*(2): 359-364. doi: 10.3945/jn.108.097113

Kennedy, GL, Pedro, MR, Seghieri, C, Nantel, G, Brouwer, I. (2007). Dietary diversity score is a useful indicator of micronutrient intake in non-breast-feeding Filipino children. *J Nutr, 137*(2): 472-477.

Kranz, S, Hartman, T, Siega-Riz, AM, Herring, AH. (2006). A diet quality index for American preschoolers based on current dietary intake recommendations and an indicator of energy balance. *J Am Diet Assoc, 106*(10): 1594-1604. doi: 10.1016/j.jada.2006.07.005

LaRowe, TL, Adams, AK, Jobe, JB, Cronin, KA, Vannatter, SM, Prince, RJ. (2010). Dietary intakes and physical activity among preschool-aged children living in rural American Indian communities before a family-based healthy lifestyle intervention. *J Am Diet Assoc, 110*(7): 1049-1057. doi: 10.1016/j.jada.2010.04.009

Manios, Y, Kourlaba, G, Kondaki, K, Grammatikaki, E, Birbilis, M, Oikonomou, E, Roma-Giannikou, E. (2009). Diet quality of preschoolers in Greece based on the Healthy Eating Index: the GENESIS study. *J Am Diet Assoc, 109*(4): 616-623. doi: 10.1016/j.jada.2008.12.011

Miller, PE, Mitchell, DC, Harala, PL, Pettit, JM, Smiciklas-Wright, H, Hartman, TJ. (2011). Development and evaluation of a method for calculating the Healthy

Eating Index-2005 using the Nutrition Data System for Research. *Public Health Nutr, 14*(2): 306-313. doi: 10.1017/S1368980010001655

Moshfegh, AJ, Borrud, L, Perloff, B, LaComb, R. (1999). Improved Method for the 24-hour Dietary Recall for Use in National Surveys. *FASEB Journal., 13*(4): A603.

Posner, BM, Smigelski, C, Duggal, A, Morgan, JL, Cobb, J, Cupples, LA. (1992). Validation of two-dimensional models for estimation of portion size in nutrition research. *J Am Diet Assoc, 92*(6): 738-741.

Serra-Majem, L, Ribas, L, Garcia, A, Perez-Rodrigo, C, Aranceta, J. (2003). Nutrient adequacy and Mediterranean Diet in Spanish school children and adolescents. *Eur J Clin Nutr, 57 Suppl 1*: S35-39. doi: 10.1038/sj.ejcn.1601812

Steyn, NP, Nel, JH, Nantel, G, Kennedy, G, Labadarios, D. (2006). Food variety and dietary diversity scores in children: are they good indicators of dietary adequacy? *Public Health Nutr, 9*(5): 644-650.

Tooze, JA, Midthune, D, Dodd, KW, Freedman, LS, Krebs-Smith, SM, Subar, AF, Guenther, PM, Carroll, RJ, Kipnis, V. (2006). A new statistical method for estimating the usual intake of episodically consumed foods with application to their distribution. *J Am Diet Assoc, 106*(10): 1575-1587. doi: 10.1016/j.jada.2006.07.003

4.5. Questionnaires – Demographics, Mediators and Moderators

4.5.1. Background and Rationale

Self-reported information will be collected from COPTR index children and other household members by obtaining responses to written or verbalized questions. Although we refer to “questionnaires”, as discussed in the methods section below, several methods are used to collect these data, and only a minority of the data is collected through the use of paper questionnaires. The information obtained is used to describe the study population or as a confounder, mediator, moderator or secondary outcome of intervention effects.

In general, the mediators chosen for measurement are targeted by the intervention, are expected to change as a result of the intervention and to result (directly or indirectly) in change in BMI. In COPTR, each Field Site's intervention is unique and many of the mediator variables are site-specific because they serve as explanatory constructs for the site-specific theoretical model. A moderating variable is defined as a variable that could influence the primary or secondary outcomes because the variable interacts with the intervention to change study outcomes. In other words, the intervention affects people differently, depending on their status on the moderator variable. These variables are evaluated at the beginning and the end of the intervention, and in some cases as interim measurements.

4.5.2. Objective

The purpose is to describe the characteristics of participants, to determine possible mediators and moderators of intervention effects and to study secondary outcomes that are impacted by the intervention.

4.5.3. Methods

The demographic, household, mediators and moderators survey is administered to parents/primary caregivers of the participating child and/or to the participating child. Table 1 summarizes the location where the questionnaire will be administered and administration format in each site. To accommodate the sample being studied some sites administer questionnaires in Spanish.

Table 2 lists the questions used to collected common questionnaire data and shows which sites are collection each item. All of the common survey questions are not administered at all Field Sites. The source of the 55 common questions and the responses are listed in Table 3. In addition to the common survey questions, each Field Site has site specific mediator and moderator questions (Tables 4-7). There will be four common measurement time points – baseline, 12 months, 24 months and 36 months. All common data collection will occur between May 2012 and March 2017. All baseline data collection will occur prior to randomization. Measurement data collectors are not intervention staff unless data are collected prior to randomization.

A “train the trainer” model is used to prepare staff to collect questionnaire data. Each Field Site designates two or more “Master Trainers” who participate in central trainings conducted by the RCU at the University of North Carolina at Chapel Hill on April 16-18,

2012. These Master Trainers are responsible for training and certifying the data collection staff at their Field Site. To be certified, Master Trainers attends the central training, reads the protocol and manual of procedures, complete the questionnaire and administer the questionnaire. The data collectors are certified by a Master Trainer who will describe the data collection process, insure that the protocol and manual of procedures are read and observe the questionnaire being administered to a volunteer.

Table 1. Characteristics of questionnaire administration by Field Sites

	Field Sites			
	Case Western	**Minnesota**	**Stanford**	**Vanderbilt**
Administration Location	Clinic	Home	Community center, Home, or Clinic	Community center
Administration Format	Interviewer administered	Interviewer administered	Interviewer administered (child) and mix of interviewer and self-administered (parent)	Interviewer administered
Data collection format	Computer	Computer	Paper Computer	Computer
Languages	English	English Spanish	English and Spanish (parents) and English (child)	English only in pilot; English and Spanish in main trial
Respondent	Parent or primary adult caregiver and participating child	Parent or primary adult caregiver	Parent(s) or primary adult caregivers and participating child	Parent or primary adult caregiver

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Table 2. Questionnaire Common Measures by Field Site

Construct	Item	Case	Minnesota	Stanford	Vanderbilt
Household Configuration	For all children and adults living in your household, please tell me:				
	Gender,	X	X	X	
	Birth date, or age	X	X	X	
	Relationship to the participating child.	X	X	X	
Child's date of birth	Child's date of birth	X	X	X	X
Child Sex	What is this child sex?	X	X	X	X
Child Ethnicity	Is this child Hispanic, Latino/a or of Spanish origin?	X	X	X	X
Child Race	Which of the following best describes your child?	X	X	X	X
Parent Ethnicity	Are you Hispanic, Latino/a or of Spanish origin?	X	X	X	X
Parent Race	Which of the following best describes you?	X	X	X	X
Parent Country of Birth	In what country were you born?		X	X	X
Child Country of Birth	In what country was this child born?		X		X
Years Parent Lived in USA	How many years total have you lived in the United States?		X	X	X
Employment Status	What is your employment status?	X	X	X	X
Marital Status	What is your current marital status?	X	X	X	X
Access to Car	Is there a car that you can use whenever you need to?	X	X		X
Frequency of Speaking English at Home with Family	How often do you speak English at home with your family? (Choose one.)		X	X	
	If you do not always speak in English at home with your family, what languages do you speak the rest of the time?	X	*X*		
WIC	Do you participate in WIC? WIC stands for Women, Infants, and Children, a Federal assistance program.	X	X		X
Food Stamps/ SNAP	Does anyone in your household receive food stamps or SNAP? SNAP stands for Supplemental Nutrition Assistance Program.	X	X	X	X
Unemployment/ Social Security/	Does anyone in your household receive Unemployment, Social	X	X	X	

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Construct	Item	Case	Minnesota	Stanford	Vanderbilt
Disability	Security, or Disability Benefits?				
Education Completed	What is the highest degree or level of school that you have completed?	X	X	X	X
	What is the highest degree or level of school that your child's other parent living in the household or adult caregiver living in the household has completed?	X	X	X	X
Child Care	In a usual week, how much time does this child spend being cared for by someone other than parent/guardian?				
	in your own home		X	X	X
	in someone else's home		X	X	X
	in childcare center/after school program		X	X	X
Household Income	What was your total household income from all sources before taxes last year? By "household", we mean that you should report the combined income of everyone in your home.	X	X	X	X
Child Health Insurance	Is your child covered by a health insurance plan?	X	X	X	
	Which type of plan are they covered by?	X	X	X	
Free or Reduced Price Breakfast or Lunch	Does any child in your household receive free or reduced price breakfast or lunch at school?		X	X	
Maturation Status	Has your daughter started having her menstrual period?	X		X	
	When did she have her first menstrual period?	X		X	
Breastfeeding/ Pregnancy Risk	Did <this child> breastfeed for more than a month?	X	X		X
	How old was <this child> in months when he/she first received a bottle of formula, cow's milk, water, juice, tea, or cereal at least once a day?	X	X		X
	How much did this child weigh at birth?	X	X		X
	Did a doctor say that <you/birth mother> had diabetes when pregnant with <this child>?	X	X		X
	Did a doctor say that <you/birth mother> had hypertension (high	X	X		X

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Construct	Item	Case	Minnesota	Stanford	Vanderbilt
	blood pressure) when pregnant with <this child>?				
Food Security	"The food that (I/we) bought just didn't last, and (I/we) didn't have money to get more." Was that often, sometimes, or never true for (you/your household) in the last 12 months?	X[3]	X	X	X
	"I/we couldn't afford to eat balanced meals." Was that often, sometimes, or never true for (you/your household) in the last 12 months?	X[3]	X	X	X
	In the last 12 months, since (date 12 months ago) did (you/you or other adults in your household) ever cut the size of your meals or skip meals because there wasn't enough money for food?	X[3]	X	X	X
	How often did this happen --almost every month, some months but not every month, or in only 1 or 2 months?	X[3]	X	X	X
	In the last 12 months, did you ever eat less than you felt you should because there wasn't enough money to buy food?	X[3]	X	X	X
	In the last 12 months, were you ever hungry but didn't eat because you couldn't afford enough food?.	X[3]	X	X	X
TV & Media	How many working TVs do you have in your home?	X[1]	X	X	
	Is there a working TV in the room where <this child> sleeps?	X[1]	X	X	X
	Is there a computer in your home?	X[1]	X	X	X
	Is there a computer in the room where <this child> sleeps?	X[1,2]	X	X[2]	X
	Is there a video game player in your home?	X[1]	X	X	
	Is there a video game player in the room where <this child> sleeps?	X[1]	X	X	X
	Do you have Internet access in your home?	X[1]	X		
	On an average WEEK day, how many hours does <this child> watch TV?		X		X
	On an average WEEKEND day,		X		X

Construct	Item	Case	Minnesota	Stanford	Vanderbilt
	how many hours does <this child> watch TV?				
	On an average day, how many hours does <this child> play video or computer games, or use a computer for something that is not school work? (Include activities such as Play Station, Xbox, hand held video games, computer games, and the Internet.)		X		X
Food Norms	During the past seven days, how often did your family eat breakfast together?		X		X
	During the past seven days, how often did your family eat lunch together?		X		X
	During the past seven days, how often did your family eat dinner together?		X		X
Weight Status	How would you classify your own weight?	X	X	X	X
	How would you classify <this child's> current weight?	X	X	X	X

1 – The TV/Media questions for Case are derived from a group of embedded scale questions
2 – Case and Stanford uses the term “desktop” computer in their question.
3 – Case questions are embedded into a survey and are not administered as an interview.

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Table 3. Source and Response Sets of Questionnaire Common Measures

Construct	Item	Response Options	Source
Household Configuration	For all children and adults living in your household, please tell me:		Developed
	Gender,	Male; Female	
	Birth date or age	MMDDYYYY; __ __ yrs	
	Relationship to the participating child.	Mother; Father; Stepmother; Stepfather; Other male CG, (list); Other female CG, (list)	
Child's date of birth	Child's date of birth	MMDDYYY	Developed
Child's sex	What is this child's sex?	Male; Female	HHS data standards (Dorsey, 2011)
Child Ethnicity	Is this child Hispanic, Latino/a, or of Spanish origin? (Choose all that apply.)	No, not of Hispanic, Latino/a or Spanish origin; Yes, Mexican American, Chicano/a; Yes, Puerto Rican; Yes, Cuban; Yes, Another Hispanic, Latino/a or Spanish origin	HHS data standards (Dorsey, 2011)
Child Race	Which of the following best describes your child? (Choose all that apply.)	American Indian or Alaskan Native Asian; Black or African American; Native Hawaiian or Pacific Islander; White; Other (please describe)	(US Census Bureau, 2010)
Parent Ethnicity	Are you Hispanic, Latino/a, or of Spanish origin? (Choose all that apply.)	No, not of Hispanic, Latino/a or Spanish origin; Yes, Mexican American, Chicano/a; Yes, Puerto Rican; Yes, Cuban; Yes, Another Hispanic, Latino/a or Spanish origin	HHS data standards (Dorsey, 2011)
Parent Race	Which of the following best describes you? (Choose all that apply.)	American Indian or Alaskan Native Asian; Black or African American; Native Hawaiian or Pacific Islander; White; Other (please describe)	(US Census Bureau, 2010)
Parent Country of Birth	In what country were you born?	USA; Mexico; Somalia; Laos/Thailand/Vietnam; Other (please describe)	Adapted from (Marin, 1996; Norris, 1996)
Child Country of Birth	In what country was this child born?	USA; Mexico; Somalia; Laos/Thailand/Vietnam; Other (please describe)	Adapted from (Marin, 1996; Norris, 1996)
Years Parent Lived in USA	How many years total have you lived in the United States?	_ _ yrs	Adapted from (Marin, 1996; Norris, 1996)
Employment Status	What is your employment status?	Working full time; Working part time; Not working for pay	Developed

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Construct	Item	Response Options	Source
Marital Status	What is your current marital status?	Married or living as married; Single	Developed
Access to Car	Is there a car that you can use whenever you need to?	Yes and I drive; Yes but I don't drive; No	Developed
Frequency of Speaking English at Home with Family	How often do you speak English at home with your family? (Choose one.)	Never; Sometimes; About ½ the time; Most of the time; Always	Adapted from (Marin, 1996; Norris, 1996)
	If you do not always speak in English at home with your family, what languages do you speak the rest of the time?	*Free text*	
WIC	Do you participate in WIC? WIC stands for Women, Infants, and Children, a Federal assistance program.	Yes; No; Don't know	Developed
Food Stamps/ SNAP	Does anyone in your household receive food stamps or SNAP? SNAP stands for Supplemental Nutrition Assistance Program.	Yes; No; Don't know	Developed
Unemployment/ Social Security/ Disability	Does anyone in your household receive Unemployment, Social Security, or Disability Benefits?	Yes; No; Don't know	Developed
Education Completed	What is the highest degree or level of school that you have completed? (Choose one answer.)	6th grade (elementary school) or less; 7th - 8th grade (attended some middle school/junior high); 9th - 12th grade (attended some high school); High school graduate (received diploma or the equivalent, GED for example); Completed some college credit, (or technical school) but no degree; Technical degree; Associate's degree; College degree; Master's, Professional, or Doctoral degree	Modified U.S. Census, 2010
	What is the highest degree or level of school that your child's other parent living in the household or adult caregiver living in the household has completed? (Choose one answer.)	6th grade (elementary school) or less; 7th - 8th grade (attended some middle school/junior high); 9th - 12th grade (attended some high school); High school graduate (received diploma or the equivalent, GED for example); Completed some college credit, (or technical school) but no degree; Technical degree; Associate's degree; College degree; Master's, Professional, or Doctoral degree	Modified U.S. Census, 2010

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Construct	Item	Response Options	Source
Child Care	In a usual week, how much time does this child spend being cared for by someone other than parent/guardian…		Developed
	in your own home?	0 Hours; 1-10 Hours; 11-20 Hours; 21-30 Hours 31-40 Hours; 41+ Hours	
	in someone else's home?	0 Hours; 1-10 Hours; 11-20 Hours; 21-30 Hours 31-40 Hours; 41+ Hours	
	in childcare center/after school program?	0 Hours; 1-10 Hours; 11-20 Hours; 21-30 Hours 31-40 Hours; 41+ Hours	
Household Income	What was your total household income from all sources before taxes last year? By "household", we mean that you should report the combined income of everyone in your home.	$14,999 or less; $15,000 - $24,999; $25,000 - $34,999; $35,000 - $49,999; $50,000 - $74,999; $75,000 - $149,999; $150,000 - $199,999; $200,000 or more; Don't know; I prefer not to answer	Developed
Child Health Insurance	Is your child covered by a health insurance plan?	Yes; No; Don't know	
	Which type of plan are they covered by?	Medicaid, Medicare, CHIP, state funded, or other federally funded; Private - through work or purchased individually; Military; Other, type unknown; Don't know	
Free or Reduced Price Breakfast or Lunch	Does any child in your household receive free or reduced price breakfast or lunch at school?	Yes; No; Don't know	Modified from TAAG2
Maturation Status	Has your daughter started having her menstrual period?	Yes; No; Don't know	Developed
	When did she have her first menstrual period?	MMYYYY	Developed
Breastfeeding/ Pregnancy Risk	Did <this child> breastfeed for more than a month?	Yes; No; Don't know	(Schwarz, 2010)
	How old was <this child> in months when he/she first received a bottle of formula, cow's milk, water, juice, tea, or cereal at least once a day?	_ _ mos.	(Schwarz, 2010)
	How much did this child weigh at birth?	_ _ lbs _ _oz	(Schwarz, 2010)
	Did a doctor say that <you/birth mother> had diabetes when pregnant with <this child>?	Yes; No; Don't know	(Schwarz, 2010)
	Did a doctor say that <you/birth mother> had hypertension (high blood pressure) when pregnant with <this child>?	Yes; No; Don't know	(Schwarz, 2010)

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Construct	Item	Response Options	Source
Food Security	"The food that (I/we) bought just didn't last, and (I/we) didn't have money to get more." Was that often, sometimes, or never true for (you/your household) in the last 12 months?	Often true; Sometimes true; Never true; Don't know; Refused	USDA (Bickel, 2000)
	"I/we couldn't afford to eat balanced meals." Was that often, sometimes, or never true for (you/your household) in the last 12 months?	Often true; Sometimes true; Never true; Don't know; Refused	USDA (Bickel, 2000)
	In the last 12 months, since (date 12 months ago) did (you/you or other adults in your household) ever cut the size of your meals or skip meals because there wasn't enough money for food?	Yes; No; Don't know; Refused	USDA (Bickel, 2000)
	How often did this happen -- almost every month, some months but not every month, or in only 1 or 2 months?	Almost every month; Some months but not every month; Only 1 or 2 months; Don't know; Refused; Not asked	USDA (Bickel, 2000)
	In the last 12 months, did you ever eat less than you felt you should because there wasn't enough money to buy food?	Yes; No; Don't know; Refused	USDA (Bickel, 2000)
	In the last 12 months, were you ever hungry but didn't eat because you couldn't afford enough food?.	Yes; No; Don't know; Refused	USDA (Bickel, 2000)
TV & Media	How many working TVs do you have in your home?	*text*	Derived from (Borzekowski, 1999; Robinson, 1999; Robinson, 2010)
	Is there a working TV in the room where <this child> sleeps?	Yes No	
	Is there a computer in your home?	Yes No	
	Is there a computer in the room where <this child> sleeps?	Yes No	
	Is there a video game player in your home?	Yes No	
	Is there a video game player in the room where <this child> sleeps?	Yes No	
	Do you have Internet access in your home?	Yes, No, Don't Know	
	On an average WEEK day, how many hours does <this child> watch TV?	None Less than 1 hour per day 1 hour per day 2 hours per day 3 hours per day 4 hours per day 5 or more hours per day	(Schmitz, 2004)
	On an average WEEKEND day, how many hours does <this	None Less than 1 hour per day	(Schmitz, 2004)

Construct	Item	Response Options	Source
	child> watch TV?	1 hour per day 2 hours per day 3 hours per day 4 hours per day 5 or more hours per day	
	On an average day, how many hours does <this child> play video or computer games, or use a computer for something that is not school work? (Include activities such as Play Station, Xbox, hand held video games, computer games, and the Internet.)	None Less than 1 hour per day 1 hour per day 2 hours per day 3 hours per day 4 hours per day 5 or more hours per day	Modified (Schmitz, 2004)
Food Norms	During the past seven days, how often did your family eat breakfast together?	0 times 1-2 times 3-4 times 5-6 times 7 times	Developed
	During the past seven days, how often did your family eat lunch together?	0 times 1-2 times 3-4 times 5-6 times 7 times	Developed
	During the past seven days, how often did your family eat dinner together?	0 times 1-2 times 3-4 times 5-6 times 7 times	Developed
Weight Status	How would you classify your own weight?	Very Underweight Underweight Normal Overweight Very Overweight	Modified (Birch, 2001)
	How would you classify <this child's> current weight?	Very Underweight Underweight Normal Overweight Very Overweight	Modified (Birch, 2001)

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Table 3. Case Western Reserve University Site-Specific Mediators and Moderators

Construct	Respondent	# Questions
Treatment Self-Regulation Questionnaire	Child	6
Brief scale for sedentary equipment in the home	Child	9
Brief scale for physical activity in the home	Child	14
Active Where? Survey – Rules for TV	Child	8
Parental Monitoring Scale	Child	6
Social Support and Exercise Survey	Child	10
Self-efficacy scale for physical activity barriers	Child	4
Modified Rosenberg Self-Esteem Inventory	Child	6
Center for Epidemiological Studies Depression Scale for Children	Child	20
Neighborhood environment walkability scale – Safety scales	Child	13
Youth Risk Behavior Survey physical activity	Child	3
Modified activity questionnaire	Child	10
Active Where? Survey – Active transportation to school	Child	2
Active Where? Survey – Food scale	Child	18
Active Where? Survey – Rules for eating	Child	12
Child Food Security Survey	Child	9
School wide food practices scale	Child	7
Social support and eating habits survey	Child	20
Children's self-efficacy for eating habits survey	Child	15
Modified -Youth Eating Disorder Examination Questionnaire	Child	3
Family Ritual Questionnaire – dinnertime subscale	Child	7
Sleep evaluation questionnaire (SEQ)	Child	10
Adolescent sleep wake scale (ASWS)	Child	28
Pediactric Daytime Sleepiness Scale	Child	8
Perceived Stress Scale	Child	10
Systems Thinking Scale	Child	16
Index of Self-Regulation	Child	7
Impact of weight on quality of life (IWQOL)	Child	27
Health Utilities Index	Child	40
Physical Exam	Child	7
PACER Test	Child	1
Brief scale for sedentary activity equipment in the home	Parent	9
Brief scale for physical activity equipment in the home	Parent	14
Active Where? Survey – Rules for TV	Parent	8
Child Behavior Checklist – social problems subscale	Parent	7
Center for Epidemiological Studies Depression Scale	Parent	20
Family Assessment Device	Parent	12
Neighborhood environment walkability scale – Safety scales	Parent	13
Active Where? Survey – Active transportation to school	Parent	2
Active Where? Survey – Rules for eating scale	Parent	12
The Child Feeding Questionnaire	Parent	31
Obstructive Sleep Apnea screen	Parent	8
Systems Thinking Scale	Parent	20
The Stress Index for Parents of Adolescents	Parent	34
Family Health History	Parent	20
Maternal History	Parent	5
Medical History	Parent	9

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Table 4. Minnesota Site-Specific Mediators and Moderators

Construct	**Respondent**	**# Questions**
Child Ethnicity	Parent	1
Parent Ethnicity	Parent	1
Living Situation	Parent	1
Smoking	Parent	2
Breastfeeding duration (age stopped)	Parent	1
Perceived Home Physical Activity Environment	Parent	6
Parental enjoyment of physical activity	Parent	1
Types of Child Physical Activity	Parent	3-13
Participation in Parenting classes	Parent	1
Perceived neighborhood environment	Parent	6
Parental support for child physical activity	Parent	4
Child eating behavior	Parent	20
Fast food	Parent	2
Parent feeding	Parent	7
Food and Physical Activity neighborhood resource use	Parent	4
Parenting styles	Parent	10
Social networks	Parent	TBD
Verbal test	Child	Series of questions

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Table 5. Stanford University Site-Specific Mediators and Moderators

Construct	Respondent	# Questions
Short diet questionnaire on product use	Parent	8
Transportation to school	Child	4
Eating with screens/homework	Child	7
Time spent in sedentary behavior	Child	48
Hunger after eating / 2nd / 3rd helpings	Child	3
McKnight over-concern with body size and shape	Child	5
CDI Depressive symptoms	Child	10
Implicit theory questions – body weight, general habits, sports ability and eating habits	Child	15
Do you think of yourself as an athlete	Child	1
Tanner	Child	2
Child's most recent grades	Parent	1
Child's after school physical activity/sports program	Parent	2
Child school lunch eaten in a typical week	Parent	1
Child meals (breakfast, dinner) eaten outside of home in a typical week	Parent	2
Child Home TV/media environment	Parent	22
Children's sleep habits questionnaire	Parent	17
Child's unsupervised time at home on typical week and weekend	Parent	3
Family members' weight status	Parent	1
Acculturation status of other parent	Parent	2
Employment status of other parent	Parent	1
Medrich Household TV	Parent	4
Washburn physical activity	Parent	3
Washburn physical activity of other parent	Parent	3
Implicit theory questions – body weight, general habits, sports ability and eating habits	Parent	15

The Questionnaires protocol was approved by the COPTR Steering Committee on March 9, 2012.

Table 6. Vanderbilt University Site-Specific Mediators and Moderators

Construct	**Respondent**	**# Questions**
Acculturation	Parent	4
Behavior change/goal setting/monitoring	Parent	6
Daily Physical Activity	Parent	2
Parenting around eating	Parent	45
Healthy Snacks and Drinks	Parent	2
Daily Serving of Fruits and Vegetables	Parent	2
Monitoring Sugar and Fiber	Parent	1
Meal Planning	Parent	1
Portion Control and Plating	Parent	2
Sleep	Parent	6
Group Cohesion	Parent	8
Social Network	Parent	8
Parenting Self-efficacy	Parent	16
Eating Location	Parent	2
Community Center Use	Parent	3
Stress	Parent	10
Parent Depression	Parent	21
Built Environment	Parent	85
Time Spent with Child	Parent	2
Exclusive Breastfeeding	Parent	1
Preterm Birth	Parent	1
Fast Food	Parent	1
Cognitive Functioning	Child	Series of tasks

References

Bickel, G, Nord, M., Price, C., Hamilton, W., Cook, J. (2000). *Guide to Measuring Household Food Security, Revised 2000*.

Birch, LL, Fisher, JO, Grimm-Thomas, K, Markey, CN, Sawyer, R, Johnson, SL. (2001). Confirmatory factor analysis of the Child Feeding Questionnaire: a measure of parental attitudes, beliefs and practices about child feeding and obesity proneness. *Appetite, 36*(3): 201-210. doi: 10.1006/appe.2001.0398

Borzekowski, D, Robinson TN. (1999). Viewing the viewers: 10 video case studies of children's television viewing behaviors. *Journal of Broadcasting and Electronic Media, 43*: 506-528.

Dorsey, R, Graham, G. (2011). New HHS data standards for race, ethnicity, sex, primary language, and disability status. *JAMA, 306*(21): 2378-2379. doi: 10.1001/jama.2011.1789

Marin, G, Gamba, RJ. (1996). A new measurement of acculturation for Hispanics: The bidimensional acculturation scale for Hispanics (BAS). *Hisp J Behav Sci, 18*(3): 297-316.

Norris, AE, Ford, K, Bova, CA. (1996). Psychometrics of a brief acculturation scale for hispanics in a probability sample of urban Hispanic adolescents and young adults. *Hisp J Behav Sci, 18*(1): 29-38.

Robinson, TN. (1999). Reducing children's television viewing to prevent obesity: a randomized controlled trial. *JAMA, 282*(16): 1561-1567.

Robinson, TN, Matheson, DM, Kraemer, HC, Wilson, DM, Obarzanek, E, Thompson, NS, Alhassan, S, Spencer, TR, Haydel, KF, Fujimoto, M, Varady, A, Killen, JD. (2010). A randomized controlled trial of culturally tailored dance and reducing screen time to prevent weight gain in low-income African American girls: Stanford GEMS. *Arch Pediatr Adolesc Med, 164*(11): 995-1004. doi: 10.1001/archpediatrics.2010.197

Schmitz, KH, Harnack, L, Fulton, JE, Jacobs, DR, Jr., Gao, S, Lytle, LA, Van Coevering, P. (2004). Reliability and validity of a brief questionnaire to assess television viewing and computer use by middle school children. *J Sch Health, 74*(9): 370-377.

Schwarz, EB, Brown, JS, Creasman, JM, Stuebe, A, McClure, CK, Van Den Eeden, SK, Thom, D. (2010). Lactation and maternal risk of type 2 diabetes: a population-based study. *Am J Med, 123*(9): 863 e861-866. doi: 10.1016/j.amjmed.2010.03.016

US Census Bureau. (2010). Overview of Race and Hispanic Origin: 2010 *2010 Census Brief*.

The Blood Pressure protocol was approved by the COPTR Steering Committee on March 9, 2012.

4.6. Blood Pressure

4.6.1. Background and Rationale

Elevated blood pressure (BP) in overweight children and adolescents is an increasingly recognized epidemic (Appel, 2006; Muntner, 2004; J. Sorof, 2002; J. M. Sorof, 2004). Many overweight/obese youth with elevated BP already have other cardiovascular risk factors and evidence of end-organ damage (Hanevold, 2004; National High Blood Pressure Education Program Working Group on High Blood Pressure in, 2004; Sinaiko, 2002; J. M. Sorof, 2004). Children with elevated BP are likely to become adults with elevated BP, and therefore are at increased risk for cardiovascular and renal disease (Appel, 2006; Bao, 1995; Dekkers, 2002; Gillman, 1993; Lewington, 2002). These data indicate that children with obesity and elevated BP are at particularly high risk, and require intervention (National High Blood Pressure Education Program Working Group on High Blood Pressure in, 2004). Weight loss is a powerful tool to reduce BP in children and adults (Appel, 2006; Rocchini, 1988). A diet rich in fruits/vegetables, low-fat dairy, low-fat protein (e.g. DASH-like diet) and/or reduced sodium intake can also reduce BP, particularly in African-American adults (Appel, 2006; Couch, 2008; Falkner, 2000; He, 2006; Svetkey, 1999). Combined with calorie reduction, activity, and behavioral interventions, DASH diets facilitate simultaneous reduction of BMI and BP in adults (Elmer, 2006). However, the most effective methods to facilitate adoption of these lifestyle changes in children are not clear, and education alone (usual care) is often ineffective (Couch, 2008). This knowledge gap is particularly important because of the huge potential impact of small changes in BP (Appel, 2006). Therefore, blood pressure will be obtained for all participants from the two COPTR sites (Case Western Reserve University and Stanford University) testing interventions to treat overweight and obesity.

4.6.2. Objective

We will determine if interventions to reduce overweight and obesity reduce blood pressure. In addition we will use the 3-year longitudinal data to examine risk factors and correlates of blood pressure changes over time in children and adolescents.

4.6.3. Methods

An automated blood pressure measurement device (OMRON HEM-705-CP or OMRON HEM-705-CPN Digital Blood Pressure Monitor) and a standardized procedure for the measurement of blood pressure and pulse will be utilized, as specified in the Blood Pressure Manual of Procedures (MOP). The design and operation of the OMRON HEM-705-CP and the OMRON HEM-705-CPN Digital Blood Pressure Monitor are based upon the combined principles of compression of the brachial artery under an elastic, inflatable cuff and estimation of the systolic and diastolic blood pressure levels by oscillometric methods.

Blood pressure and pulse will be measured at four data collection time points – baseline, 12 months, 24 months and 36 months. All baseline blood pressure and pulse measurements will be collected prior to randomization. Blood pressure measurement must be conducted early in the visit and not following potentially stressful exam components such as the blood drawing. Before measurements commence participants

are offered the opportunity to visit a restroom or bathroom. The participant should not have smoked or had any caffeine within the last 30 minutes prior to the blood pressure determinations.

Blood pressure measurements will be taken using the right arm. Participants should sit quietly for 4-5 minutes before the first measurement is taken. Seated, resting blood pressure and pulse are measured three times at each evaluation visit. The first reading will be discarded and the average of the second and third measurements will be used in analysis.

The OMRON HEM-705-CP and the OMRON HEM-705-CPN are automated devices. The data collector determines and places the correct size cuff on the participant's arm, pushes the button on the device and waits for the output. All readings will be recorded to the nearest integer.

COPTR uses a “train the trainer” model. Each Field Site designates two or more “master trainers” who participate in central trainings organized by the RCU at the University of North Carolina at Chapel Hill, NC from April 16 to April 18, 2012. The designated master trainers are responsible for training and certifying the data collection staff at their center. For certification, the data collector is observed by the trainer. The participants must include five or more children requiring varying cuff sizes. The trainee must correctly select the appropriate cuff size and demonstrate consistent compliance with the MOP to be certified. No blood pressure and pulse measurements will be taken until after the data collector has been trained and certified.

References

Appel, LJ, Brands, MW, Daniels, SR, Karanja, N, Elmer, PJ, Sacks, FM, American Heart, A. (2006). Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. *Hypertension, 47*(2): 296-308. doi: 10.1161/01.HYP.0000202568.01167.B6

Bao, W, Threefoot, SA, Srinivasan, SR, Berenson, GS. (1995). Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: the Bogalusa Heart Study. *American Journal of Hypertension, 8*(7): 657-665. doi: 10.1016/0895-7061(95)00116-7

Couch, SC, Saelens, BE, Levin, L, Dart, K, Falciglia, G, Daniels, SR. (2008). The efficacy of a clinic-based behavioral nutrition intervention emphasizing a DASH-type diet for adolescents with elevated blood pressure. *J Pediatr, 152*(4): 494-501. doi: 10.1016/j.jpeds.2007.09.022

Dekkers, JC, Snieder, H, Van Den Oord, EJ, Treiber, FA. (2002). Moderators of blood pressure development from childhood to adulthood: a 10-year longitudinal study. *J Pediatr, 141*(6): 770-779. doi: 10.1067/mpd.2002.128113

Elmer, PJ, Obarzanek, E, Vollmer, WM, Simons-Morton, D, Stevens, VJ, Young, DR, Lin, PH, Champagne, C, Harsha, DW, Svetkey, LP, Ard, J, Brantley, PJ, Proschan, MA, Erlinger, TP, Appel, LJ, Group, PCR. (2006). Effects of comprehensive lifestyle modification on diet, weight, physical fitness, and blood pressure control: 18-month results of a randomized trial. *Ann Intern Med, 144*(7): 485-495.

Falkner, B, Sherif, K, Michel, S, Kushner, H. (2000). Dietary nutrients and blood pressure in urban minority adolescents at risk for hypertension. *Arch Pediatr Adolesc Med, 154*(9): 918-922.

Gillman, MW, Cook, NR, Rosner, B, Evans, DA, Keough, ME, Taylor, JO, Hennekens, CH. (1993). Identifying children at high risk for the development of essential hypertension. *J Pediatr, 122*(6): 837-846.

Hanevold, C, Waller, J, Daniels, S, Portman, R, Sorof, J, International Pediatric Hypertension, A. (2004). The effects of obesity, gender, and ethnic group on left ventricular hypertrophy and geometry in hypertensive children: a collaborative study of the International Pediatric Hypertension Association. *Pediatrics, 113*(2): 328-333.

He, FJ, MacGregor, GA. (2006). Importance of salt in determining blood pressure in children: meta-analysis of controlled trials. *Hypertension, 48*(5): 861-869. doi: 10.1161/01.HYP.0000245672.27270.4a

Lewington, S, Clarke, R, Qizilbash, N, Peto, R, Collins, R, Prospective Studies, C. (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet, 360*(9349): 1903-1913.

Muntner, P, He, J, Cutler, JA, Wildman, RP, Whelton, PK. (2004). Trends in blood pressure among children and adolescents. *Jama-Journal of the American Medical Association, 291*(17): 2107-2113.

National High Blood Pressure Education Program Working Group on High Blood Pressure in, C, Adolescents. (2004). The fourth report on the diagnosis,

evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics, 114*(2 Suppl 4th Report): 555-576.

Rocchini, AP, Katch, V, Anderson, J, Hinderliter, J, Becque, D, Martin, M, Marks, C. (1988). Blood pressure in obese adolescents: effect of weight loss. *Pediatrics, 82*(1): 16-23.

Sinaiko, AR, Steinberger, J, Moran, A, Prineas, RJ, Jacobs, DR, Jr. (2002). Relation of insulin resistance to blood pressure in childhood. *J Hypertens, 20*(3): 509-517.

Sorof, J, Daniels, S. (2002). Obesity hypertension in children: a problem of epidemic proportions. *Hypertension, 40*(4): 441-447.

Sorof, JM, Lai, D, Turner, J, Poffenbarger, T, Portman, RJ. (2004). Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics, 113*(3 Pt 1): 475-482.

Svetkey, LP, Simons-Morton, D, Vollmer, WM, Appel, LJ, Conlin, PR, Ryan, DH, Ard, J, Kennedy, BM. (1999). Effects of dietary patterns on blood pressure: subgroup analysis of the Dietary Approaches to Stop Hypertension (DASH) randomized clinical trial. *Arch Intern Med, 159*(3): 285-293.

4.7. Biomedical Measures

4.7.1. Background and Rationale

Hyperinsulinemia/insulin resistance is a risk factor for future Type 2 diabetes, and is associated with increased blood pressure, adverse lipid profiles and increased body fatness in children and adolescents (Freedman, 1999; Garcia-Webb, 1980; Gower, 1998), and weight loss is associated with improved insulin sensitivity among adolescents (Hoffman, 1995). Thus, insulin resistance serves both as a direct indicator of a significant risk factor and as a biochemical marker of metabolically-significant adiposity and changes in adiposity. Increased fasting insulin concentration is an appropriate marker of insulin resistance for this study.

Fasting insulin concentrations can also be combined with fasting glucose concentrations using a number of algorithms, including the HOMA and QUICKI, among others, to generate indices. However, all of these calculated measures of insulin resistance appear to be highly correlated with fasting insulin concentrations in non-diabetic subjects (Yeni-Komshian, 2000). The Stanford group has recently confirmed this with their own data from the 8-10 year old African-American girls in Stanford GEMS (correlations of .98-.99). However, because a fasting glucose will also be collected, the Field Sites will be able to examine each of these combination indices. Fasting glucose will also be collected to identify children with previously undiagnosed type 2 diabetes (fasting glucose ≥ 126 mg/dl) and to identify children who will be referred for further evaluation by their primary care medical provider (fasting glucose ≥ 110 mg/dl) according to Field Sites clinical monitoring protocol.

Adverse lipid profiles are risk factors for cardiovascular diseases and increased BMI is associated with increased total cholesterol, LDL-cholesterol, and triglycerides and lower HDL-cholesterol concentrations (J. T. Dwyer, 1998; T. Dwyer, 1996; Freedman, 1999; Laskarzewski, 1980; Zwiauer, 1990). Thus, lipid measures also serve both as direct indicators of a significant risk factor and as a biochemical marker of metabolically-significant adiposity and changes in adiposity.

High levels of C-reactive protein (CRP) is a marker for inflammation. CRP prospectively assesses the risk of atherosclerotic complications, may be a mediator of vascular injury and is strongly related to obesity (Groner, 2006; Serdula, 1993). In adults, higher BMI levels are associated with higher CRP concentrations. Some clinicians are starting to use CRP levels when assessing risk for cardiovascular disease. Using cross-sectional data from the National Health and Nutrition Examination survey 1999-2000, Ford found significant associations between CRP levels and BMI in children 3 to 17 years of age (Ford, 2003). CRP levels were also associated with age and systolic blood pressure, but BMI had the strongest association.

Unexplained elevated levels of alanine Aminotransferase (ALT) has been liked with adiposity and may be a marker for nonalcoholic fatty liver disease (NAFLD) in adolescents and adults (Carrillo-Iregui, 2010; Park, 2005). Researchers have found a close association between metabolic syndrome, insulin resistance, elevated ALT levels

and NAFLD in overweight/obese children and adolescents (Carrillo-Iregui, 2010; El-Koofy, 2012).

4.7.2. Objective

We will determine if the COPTR interventions to reduce overweight and obesity change cardiovascular risk factors measured in blood. In addition we will use the 3-year longitudinal data to examine the risk factors and their correlates over time.

4.7.3. Methods

Blood specimens are collected at baseline, 12 months and 36 months at the two Field Sites testing interventions to treat overweight and obesity – Case Western Reserve University and Stanford University. All baseline blood specimen samples are collected prior to randomization. All blood specimens are analyzed by the Northwest Lipid Metabolism and Diabetes Research Laboratories (NWRL). The biomedical measures analyzed in the index child are Hemoglobin A1c (HbA1c), Glucose, Total Cholesterol, LDL-cholesterol, HDL-cholesterol, Triglycerides, high-sensitivity C-reactive protein (hs-CRP), Insulin and Alanine Aminotransferase (ALT).

Fasting status will be collected prior to blood draw. A trained phlebotomist at each site is responsible for the blood collection. However, a data collector might have the responsibility for mailing the blood specimens to the NWRL. All specimen samples will be frozen to allow for batch shipment. The assays and quality control for each measurement is described below.

HbA1c

The measurement of the relative proportion of hemoglobin subclasses and calculation of the HbA1c levels are performed by an NGSP-certified auto-analyzer (G-8 Tosoh, Biosciences, Inc.) using non-porous ion exchange high performance chromatography to achieve rapid and precise separation of stable HBa1c from other hemoglobin fractions. The system calibration is maintained using two point calibration reagents. A set of quality control samples are analyzed twice daily. The acceptance allowance for quality control is + 0.1% variance from the target value for the low level, and + 0.2% variance from the target value for the high level. The inter-assay CVs for the low and high quality control samples are 0.9% and 0.6%, respectively.

Glucose

Analysis of fasting and post glucose intake samples is performed enzymatically on a Roche Hitachi Modular P chemistry autoanalyzer. This instrument executes the glucose hexokinase method described by Schmidt (Schmidt, 1961), Bergmeyer (Bergmeyer, 1974) and Peterson and Young (Peterson, 1968) and recognized as the most specific method for the determination of glucose. Quality control samples with normal and high glucose levels are used for monitoring glucose assay performance. The inter-assay CV is <3%. Lyophilized samples at two different glucose concentrations are used to monitor possible analytical drift.

Lipid Profile
Measurements of total plasma cholesterol in plasma, cholesterol in the lipoprotein fractions and triglycerides are performed enzymatically on the Roche Modular P autoanalyzer using methods standardized to the Centers for Disease Control and Prevention Reference Methods. Determination of HDL-cholesterol is performed after precipitation of apo B-containing particles by dextran sulfate Mg2+. LDL-cholesterol is calculated by the Friedewald equation. This approach for measuring LDL-cholesterol is clinically reliable if the measurements of total cholesterol, HDL-cholesterol and triglycerides are performed with a high level of accuracy and precision. However, the Friedewald equation for the estimation of LDL-cholesterol is inaccurate when triglycerides are >400 mg/dl. In this case, a complete lipoprotein separation by ultracentrifugation which allows quantitation of the individual lipoprotein classes is performed using the Lipid Research Clinics Beta Quantification procedure.

Quality control materials (BCL-Low, BCL-High (Biocell Laboratories) and L1-Medium (In-house prepared fresh frozen pool) are used at the beginning and at the end of each run.

The inter-assay CVs are consistently <1.5% for total cholesterol and triglycerides and <2% for HDL cholesterol.

Long-term Drift: A large quantity of two lyophilized quality control materials was acquired from Bio Rad for lipids. Values for each analyte were assigned by analyzing the samples daily for at least two weeks to achieve a minimum of 50 values. The mean of all the values constitutes the target value for each analyte. These materials are stored at -70°C and analyzed monthly to monitor for analysis drift. Actions are taken if the values are consistently above or below the 2 SD limit on two consecutive months.

C-Reactive Protein
Levels of C-reactive protein (CRP) in plasma are measured immunochemically on a nephelometer autoanalyzer (BNII). The reagents are obtained from Siemens Inc. This high sensitivity method is based on polystyrene particles coated with monoclonal antibodies specific to CRP which form immunocomplexes with CRP in plasma samples.The intensity of the scattered light in the nephelometer is directly proportional to the concentration of CRP which is determined versus dilutions of a standard of a known CRP concentration. The method is standardized against the IFCC/BCR/CAP reference preparation.

Insulin
The Insulin assay is a two site immuno-enzymometeric assay performed using Tosoh 2000 auto-analyzer. The assay is calibrated to WHO IRP 66/304 standard. The assay has a sensitivity level of 0.5 uU/mL and the standard curve linearity is up to 330 uU/mL. A set of high, medium and low insulin level controls are included in each batch of samples to monitor assay performance. The inter assay CVs for Low, Medium and High insulin level controls are 2.8%, 2.5% and 2.0% respectively. The assay has high specificity as cross- reactivity with Human C-peptide, intact Proinsulin, split (32, 33)

Proinsulin and Des (64,65) proinsulin is 0%, 2 %, 2.6% and 39.8 % respectively. A Reference Interval for apparently healthy donors has been established at <17.0 uU/mL. The laboratory has participated in external proficiency evaluation program by the College of American Pathologists (CAP). Additionally, the laboratory has participated in the ADA sponsored Insulin Standardization workshops in 2007 and 2011. In the 2007 insulin standardization workshop this assay was reported as top performer with high sensitivity and specificity. Most recently, in 2011, ADA Insulin standard prepared and target level assigned by IDMS reference method was distributed to the laboratories. The ADA criteria of individual laboratory performance was set at up to 15.5% measurement bias from the assigned target level. Using the current insulin assay our laboratory achieved a bias less than 8.5%.

Alanine Aminotransferase (ALT)
This assay is performed on a Roche Double Modular P Analytics automated analyzer using Roche Diagnostics reagents. L-alanine reacts with alphaketoglutarate in the presence of ALT to form pyruvate and Lglutamate. NADH is then added to the pyruvate in the presence ofLDH to form L-lactate and NAD+. The rate of NADH oxidation to form NAD+ is directly proportional to the rate of pyruvate formation indicating ALT activity. The rate of decrease in absorbance at 340nm due to the formation of NAD is directly proportional to the rate of pyruvate formation and proportional to the ALT activity of the sample. The normal reference ranges for adults are: 17–67U/L (Male) and 13–50U/L (Female).

References

Bergmeyer, HV, E. Bernt, F. Schmidt, H. Stork. (1974). D-Glucose: determination with hexokinase and glucose-6-phosphate dehydrogenase. In F. H. Schmidt, Bergmeyer, H.V. (Ed.), *Methods of Enzymatic Analysis* (2nd english ed. ed., pp. 1196-1201). New York: Academic.

Carrillo-Iregui, A, Lopez-Mitnik, G, Cossio, S, Garibay-Nieto, N, Arheart, KL, Messiah, SE. (2010). Relationship between aminotransferases levels and components of the metabolic syndrome among multiethnic adolescents. *J Pediatr Endocrinol Metab, 23*(12): 1253-1261.

Dwyer, JT, Stone, EJ, Yang, M, Feldman, H, Webber, LS, Must, A, Perry, CL, Nader, PR, Parcel, GS. (1998). Predictors of overweight and overfatness in a multiethnic pediatric population. Child and Adolescent Trial for Cardiovascular Health Collaborative Research Group. *Am J Clin Nutr, 67*(4): 602-610.

Dwyer, T, Blizzard, CL. (1996). Defining obesity in children by biological endpoint rather than population distribution. *Int J Obes Relat Metab Disord, 20*(5): 472-480.

El-Koofy, NM, Anwar, GM, El-Raziky, MS, El-Hennawy, AM, El-Mougy, FM, El-Karaksy, HM, Hassanin, FM, Helmy, HM. (2012). The association of metabolic syndrome, insulin resistance and non-alcoholic fatty liver disease in overweight/obese children. *Saudi J Gastroenterol, 18*(1): 44-49. doi: 10.4103/1319-3767.91738

Ford, ES, National, H, Nutrition Examination, S. (2003). C-reactive protein concentration and cardiovascular disease risk factors in children: findings from the National Health and Nutrition Examination Survey 1999-2000. *Circulation, 108*(9): 1053-1058. doi: 10.1161/01.CIR.0000080913.81393.B8

Freedman, DS, Dietz, WH, Srinivasan, SR, Berenson, GS. (1999). The relation of overweight to cardiovascular risk factors among children and adolescents: The Bogalusa heart study. *Pediatrics, 103*(6): 1175-1182.

Garcia-Webb, P, Bonser, A, Wearne, KL, Gracey, M. (1980). Obesity and insulin secretion in fasting high school students. *Diabetologia, 19*(3): 194-197.

Gower, BA, Nagy, TR, Trowbridge, CA, Dezenberg, C, Goran, MI. (1998). Fat distribution and insulin response in prepubertal African American and white children. *Am J Clin Nutr, 67*(5): 821-827.

Groner, JA, Joshi, M, Bauer, JA. (2006). Pediatric precursors of adult cardiovascular disease: noninvasive assessment of early vascular changes in children and adolescents. *Pediatrics, 118*(4): 1683-1691. doi: 10.1542/peds.2005-2992

Hoffman, RP, Stumbo, PJ, Janz, KF, Nielsen, DH. (1995). Altered insulin resistance is associated with increased dietary weight loss in obese children. *Horm Res, 44*(1): 17-22.

Laskarzewski, P, Morrison, JA, Mellies, MJ, Kelly, K, Gartside, PS, Khoury, P, Glueck, CJ. (1980). Relationships of measurements of body mass to plasma lipoproteins in schoolchildren and adults. *Am J Epidemiol, 111*(4): 395-406.

Park, HS, Han, JH, Choi, KM, Kim, SM. (2005). Relation between elevated serum alanine aminotransferase and metabolic syndrome in Korean adolescents. *Am J Clin Nutr, 82*(5): 1046-1051.

Peterson, JI, Young, DS. (1968). Evaluation of the hexokinase-glucose-6-phosphate dehydrogenase method of determination of glucose in urine. *Anal Biochem, 23*(2): 301-316.

Schmidt, FH. (1961). Die enzymatische Bestimmung von Glucose und Fructose nebeneinander. *Klin. Wschr., 39*: 1244-1247.

Serdula, MK, Ivery, D, Coates, RJ, Freedman, DS, Williamson, DF, Byers, T. (1993). Do obese children become obese adults? A review of the literature. *Prev Med, 22*(2): 167-177. doi: 10.1006/pmed.1993.1014

Yeni-Komshian, H, Carantoni, M, Abbasi, F, Reaven, GM. (2000). Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy nondiabetic volunteers. *Diabetes Care, 23*(2): 171-175.

Zwiauer, K, Widhalm, K, Kerbl, B. (1990). Relationship between body fat distribution and blood lipids in obese adolescents. *Int J Obes, 14*(3): 271-277.