Coursework Description

Sample #1

Plan for formal coursework

Master in Public Health Degree Program at ██████ **School of Public Health**
As a formal coursework to strengthen my clinical research skills, I have already completed an intensive, 7-week, whole day summer course; **Program of Clinical Effectiveness** offered through the ████████████████████████████. The program in Clinical Effectiveness provides 15 credits towards a Masters in Public Health (MPH). With the support from AAN fellowship, I would further pursue the Degree Program for MPH as a part-time student, using an additional summer. **The MPH Degree Program at** ████ is a demanding, interdisciplinary program emphasizing student-directed learning, problem-solving, and the acquisition of fundamental public health skills (biostatistics, epidemiology, ethics in medical research, environmental health sciences, health services administration, and social and behavioral sciences). In addition to completing a core curriculum, I will pursue my professional objectives through a choice of seven career-oriented concentrations, which offer specialty electives and areas of interest. A **Concentration on Clinical Effectiveness** offers students the comprehensive curriculum suitable clinical researcher which includes clinical epidemiology and biostatistics, cost-effectiveness analysis, medical decision analysis, health services research, quality improvement in health care, and measurement of health-related quality of life. As the culmination of the program, I will give presentations demonstrating the application of their knowledge in a real world context. My proposing project was also cultured through meetings with senior investigators and professors, feed back in office hours and small-group workshops at the summer program.
I plan to attend this course as a Summer-only student (spends additional two summers to complete the degree program) to balance the commitment to the proposing project.

The **Clinical Research Program (CRP)** at Children's Hospital Boston will also provide me continuous training and internal support for my clinical research from a number of hospital and department-based resources. The CRP provides methodological support, education, and collaborative assistance to investigators for the design, conduct, and analysis of clinical research. The CRP provides consultations for protocol design, study design, statistical analysis, and database management.

The CRP also houses the **Clinical Translational Study Unit (CTSU)**. This center, funded by the National Institutes of Health, is housed in the main hospital building and supports both inpatient and outpatient research, and also includes a core laboratory, Informatics, Biostatistics, Nutrition, and a Phenotype/Genotype Core Program. The CTSU supports 140 active protocols at present. The patient follow up clinic in my TTTS study will be also held in the CTSU.

I will also able to consult with the Departmental Biostatistician in Department of Neurology, ████████████████████. This biostatistician has specific expertise in neuroscience research. I will be able to consult with him on my research projects during the year, particularly with regard to analysis of results and application of statistical methods to my data.

In summary, during the next two years, I will receive (1) formal comprehensive coursework in methods of clinical research, (2) computer support in data analysis, and (3) guidance to ethical practices in conducting research involving children. As evident from the above description, I am training in an academic environment committed to outstanding clinical research and the cultivation of young investigators.

Research Plan

Sample #1

Hypotheses and Specific Aims

<u>T</u>win <u>T</u>win <u>T</u>ransfusion <u>S</u>yndrome (TTTS) is a systemic fetal disease which has high mortality and morbidity, including long-term neurodevelopmental impairment. The fetal pathology that causes such long-term impairment is poorly understood, and clinical measures to predict neurodevelopmental outcomes are needed.

Hypothesis: In the third trimester, brain injury associated with TTTS is a progressive neuropathology *in utero* that results in accumulating microstructural injury and aberrant development. Quantitative fetal magnetic resonance imaging (MRI) can detect such changes between TTTS and control fetuses.
<u>**Specific Aim 1:**</u> We will determine if the trajectories of fetal brain development in TTTS are different from those in age-matched control fetuses, in the third trimester, as determined by quantitative fetal brain MR measures of regional brain volumes, white matter integrity (fractional anisotropy [FA] and apparent diffusion coefficient [ADC]), and gyral curvature.

Hypothesis: The fetal MRI measures in TTTS reflecting microstructural injury and aberrant development persist after birth and affect neurodevelopmental outcomes in TTTS infants and young children (1 and 2 years).
<u>**Specific Aim 2:**</u> We will identify the predictive validity of the above measures in fetal MRI in association with one- and two-year neurodevelopmental outcomes of TTTS infants, as determined by developmental scales in various domains (general development [Bayley Scales of Infant Development III (BSID-III)], language development [REEL-3 and REEL-3 Vocabulary tests, Rosetti Language Screening], executive function, behavioral development [Vineland Adaptive Behavior Scales II (VABS-II), Toddler Behavior questionnaire], and autistic features [MCHAT Autism Screening test]).

Background and Significance

Clinical impact of fetal brain injury in TTTS: Fetal brain injury is increasingly recognized as a major etiology of childhood neurodevelopmental disabilities, including both cerebral palsy (CP) and non-CP neurodevelopmental impairment. In the US, currently about 800,000 children and adults suffer from CP and about 10,000 neonates are born yearly with CP [1, 2]. Disabilities in learning and behavior are prevalent in CP, but they may also present separately as non-CP neurodevelopmental impairments without obvious motor deficits. The economical and emotional burden on affected families and society is enormous. Seventy to eighty percent of CP cases are considered to have their origin in the fetal period [2, 3]. For the majority of these cases, limited knowledge is available regarding the evolution of the injury, specifically its impact on brain development, and the correlation of these to prognosis.

Twinning has a large risk of fetal brain injury compared with singleton pregnancy [4]. <u>T</u>win-<u>t</u>win <u>t</u>ransfusion syndrome (TTTS) is second only to prematurity as a major etiology of CP and non-CP impairments to twins. TTTS is diagnosed by obstetrical ultrasound typically around 16-20 weeks of gestation (wkGA) as discordant fetal growth and/or amniotic volume size, and features of circulatory failure in one or both twins. TTTS occurs in 20% of monochorionic (identical) twin pregnancies (yearly 7,500 cases in the US) [5]. It is associated with persistent and progressive systemic fetal disease resulting a high risk of perinatal mortality (30 %) and morbidity, in particular neurodevelopmental disability in survivors (18 %) [6-8]. Limited knowledge is available regarding injury evolution in TTTS, its impact on brain development, and its correlation to prognosis, largely due to the lack of efficient methods of measuring fetal brain injury and developmental progress. Such measures need to be established as a first step.

Fetal MRI findings and neuropathology of TTTS: Previous fetal neuroimaging studies have found brain lesions that originate in fetal life, including hydranencephaly, porencephaly, polymicrogyria, periventricular leukomalacia, basal ganglia damage, germinal matrix hemorrhage, ventriculomegaly and intracranial hemorrhage. They are varied based on the timing and mechanism of injury [9-11]. These studies demonstrated the diagnostic validity of conventional fetal MRI to be superior to that of ultrasound. In addition to such destructive lesions, secondary malformations (polymicrogyria, dysmatured sulcation) can also results from the injuries.

Neurodevelopmental impairments are associated with the severity of such lesions. However, even in surviving children without apparent destructive or malformatory lesions on conventional MRI, long-term follow up studies revealed a high prevalence of such impairments, especially non-CP impairments [7, 8]. Such subtle abnormalities are likely to be characterized as cumulative microstructural injury and/or developmental aberration. Therefore, we hypothesize that, during the period of cerebral development in the third trimester when white matter integrity and gyration are drastically changing, brain growth and changes in histological integration and gyration in the fetal brain with TTTS is different compared with that of a normal control fetus. Furthermore, we hypothesize that these changes will correlate with infantile and early childhood (1 and 2 years) neurodevelopmental outcomes of

affected children.

Innovation of this study: This study will implement novel methodology to quantify developmental and injurious pathology in fetal brain, which would provide new insight into the evolution of brain pathology in TTTS. By establishing such fetal measures and their predictive validity for postnatal neurological outcome, we may (1) contribute to the obstetricians' ability to determine which fetus is at higher risk, and (2) provide valuable parameters for obstetricians regarding when and how they should pursue further intervention. These measures may also be applicable to other forms of brain injury or malformation *in utero* such as fetal growth restriction, gestational diabetes and congenital heart disease.

Preliminary Data (Biometric fetal brain MRI analysis in TTTS)

Higher incidence of cerebral lesions: Retrospective review of fetal MRIs captured in 33 pregnancies and 65 fetuses with TTTS (ranged from 15+5/7 to 32+1/7 wkGA) referred to our center revealed abnormalities in 10 pregnancies (29.4%, 8 donors, 2 recipients) higher than in previous report [10]. The major abnormality was bilateral or unilateral ventriculomegaly (7) following marked asymmetry in ventricular size (2), and Dandy-Walker malformation (1). No hemorrhagic or porencephalic changes were seen. One case had exacerbating ventriculomegaly reflecting progressive nature of brain injury in TTTS.

"Normal" twin brains were not normal: We further performed biometric analyses in 17 twin pairs whose 18 brain MRIs were diagnosed as normal by gross visual review (study age ranged from 15+5/7 to 29 wkGA). The donor twins had significantly thinner cerebral mantle than their co-twins (recipient) and age-matched control singleton fetuses. This finding remained true after the cerebral mantle thickness was corrected with cerebral biparietal diameter suggesting specific impact on cerebral mantle development. The trajectories of cerebral mantle growth were significantly different between donor, recipient and control fetuses suggesting persistent developmental aberration. These abnormalities may reflect microstructural injuries and/or growth restriction of developing intermediate zone (future white matter) and cortical plate. Such "subtle" abnormalities were underestimated by gross visual MRI review.

Methodology

We propose a prospective prenatal-to-postnatal cohort study (1) to describe quantitative MRI measures of fetal brain in TTTS and control fetuses at a minimum of 3 time points, (i) time of referral (15-24 wkGA), (ii) at 25-28 wkGA, and (iii) at 30-32 wkGA; and (2) to test the predictive validity of these measures for postnatal neurodevelopmental outcome at 1 and 2 years of age.

Resources and Environment: Recruitment, fetal imaging studies, and postnatal follow up will be held at regional multidisciplinary comprehensive fetal center, the Advanced Fetal Care Center (AFCC), the Departments of Radiology and Neurology at Children's Hospital Boston (CHB) (Boston, MA) in collaboration with the Obstetrics clinic at Brigham and Women's Hospital over 2 year period. Post-acquisition analyses will be performed at The Center for Fetal-Neonatal Neuroimaging and Developmental Science (Center) at CHB. Led by Dr. Ellen Grant (study mentor), the Center has over the past decade developed, studied and published the following quantitative MRI methodologies and analytic systems in the fetal and pediatric brain.

Recruitment: Typical enrollment occurs at 15-20 wkGA when TTTS is diagnosed. We aim to recruit 20 pregnant mothers with TTTS and 15 control mothers with healthy twin fetuses (30 fetuses) for this study.

Inclusion and exclusion criteria: The **TTTS subject** is a monochorionic twin pregnant mother whose fetus has TTTS – obstetrical US diagnosis with (i) proven monochorionicity, (ii) discordance in amniotic fluid volume and/or size (with larger twin in the polyhydramniotic sac), and (iii) same-sex twins with a single placental mass [6]. Mothers with contraindication to MRI (with pacemaker, metal in body), claustrophobia, and medical instability to pursue MRI study will be excluded. Healthy monochorionic twin pregnant mothers without any known medical complications will be recruited as **control subjects** matching gestational age at the time of MRI study.

Fetal MRI acquisition: We will obtain 3 fetal MRI studies at the above-mentioned gestational ages. These studies are timed to capture the trajectory of rapid fetal brain growth and development in the third trimester. The third trimester is a period of active process in each developmental domain, such as growth, white-matter maturation and gyrification. According to the Safety Committee of the Society for Magnetic Resonance Imaging and American College of Radiology, MR procedures are considered to be safe and standard of care for

Fig. 1 Regional volumetrics (Nishida et al, 2006)
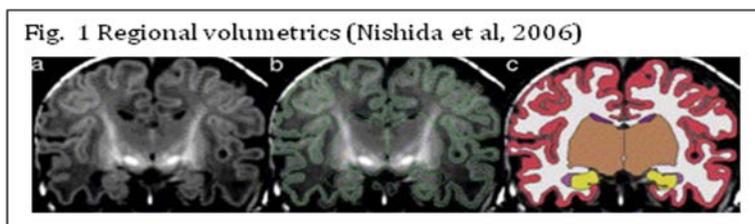
use in pregnant women and fetuses [12].

Post-acquisition analyses: The original electronic imaging data in PACS (Picture Archiving and Communication System) will be copied to the Linux system at the Center for further computation.

(1) Volumetric and biometric analyses: In the coronal fetal brain MRI plains, regional structures will be segmented and measured for each area. Regional structural volumes will be computed by reconstruction of consecutive planes (Fig. 1)[13]. Volumetric measures in the neonatal brain are a valid measure to predict early neurodevelopmental outcome in growth restricted fetus [14]. Recently, their validity was also reported in the normal fetus [15] and the fetus with congenital heart disease [16].

(2) White matter integrity: Diffusion MRI sequences can quantify the mobility of water molecules in areas of interest by measuring FA and ADC. In third-trimester white matter, such diffusivity is progressively restricted as the tissue integrity increases reflecting integrating axons and the initiation of myelination (Fig. 2). Microstructural injury and poor development of white matter can be measured as abnormal values of FA and ADC in comparison with norms [17].

Fig. 2 *Ex vivo* fetal reconstructed tractography (Takahashi & Grant, unpublished)
18 wkGA 24 wkGA

(3) Cortical development (cortical surface area and gyral curvature): Rapid expansion of cortical surface and complex scheduled gyrus formation is the hallmarks of brain development in the third trimester. Brain surface will be reconstructed through image analysis with a triangular-shaped mesh. The curvature value at each angle of these triangles will be measured and registered. The curvature profile of two brains will be compared by histogram of whole brain curvature measurements and color-based registration of the "strong" and "weak" curvature on the reconstructed fetal brain surface (Fig. 3)[18, 19].

Fig. 3 Curvature analysis process
a. Grey/white junction surface reconstruction
b. Projections of principal curvature Functions k1 (top) and k2 (bottom) across entire brain surfaces
31 wkGA   39 wkGA   7y   39 y
k2   k1
30 wkGA   40 wkGA   Adult
From Pienaar et al, 2008

Neurodevelopmental Evaluation: Postnatal neurological examination will be studied in a standardized format. Detailed developmental evaluations will be performed at 1 and 2 years of age. Special test batteries will be utilized to look for impairments affected by subtle white matter injuries (language, executive impairments and autistic features): BSID-III, language development (REEL-3 and REEL-3 Vocabulary tests, Rosetti Language Screening), executive function, behavioral development (Vineland Adaptive Behavior Scales II [VABS-II], Toddler Behavior questionnaire) and autistic features (MCHAT Autism Screening test). BSID-III is well established and the most widely used gold standard developmental test, with a series of measurements used primarily to assess motor (fine and gross), language (receptive and expressive), and cognitive development of infants and toddlers, ages 0-3 (likely becomes valid after 6 months of age).[20] The cut-off in BSID-III is a test score less than 2SD (<70).

Analysis Plan
Aim 1: Two-sample t-test: To test for group differences in each fetal MRI measure, we will perform a two-sample t-test. The test will subsequently be performed for three study ages.
Aim 2: Linear regression analysis: To assess the correlation between fetal MRI measures and postnatal neurodevelopmental outcome on each summary scale (BSID-III, REEL-3, VABS-II, and MCHAT), separate regression models will be fit for each summary scale. Such regression model will be fit for each gestational age group data. Sensitivity and specificity of abnormal fetal MRI measures to predict developmental abnormalities will be established, using the dichotomized BSID-III scale (<70) as the gold standard. Cut-off for each fetal MRI measure will be determined to maximize area under the receiver operating characteristic (ROC) curve.
Sample size calculation: **Aim 1:** Fetal whole brain volume by MRI is in the range of 50-100mL (24-28wkGA)[15] with an SD of 10[13]. In a two-sample t-test power estimation with 20 TTTS and 30 control subjects, we expect to detect a mean volume difference of 9 mL between two groups with a power of 0.86. The significance level is set at 0.05. **Aim 2:** BSID-III has a continuous value, specific to each domain. The cognitive composite score (subdomain of BSID) has mean values of 100, SD of 15 [20]. To fit a linear regression model for the attention scale, with 20 TTTS and 30 control subjects, we expect to be able to detect a difference of 0.6 SD between the slopes of the two groups, with a power of 0.82. This calculation was based on the test that the regression coefficient for the interaction terms was different from zero using the standard likelihood ratio test. The significance level is set at 0.05.
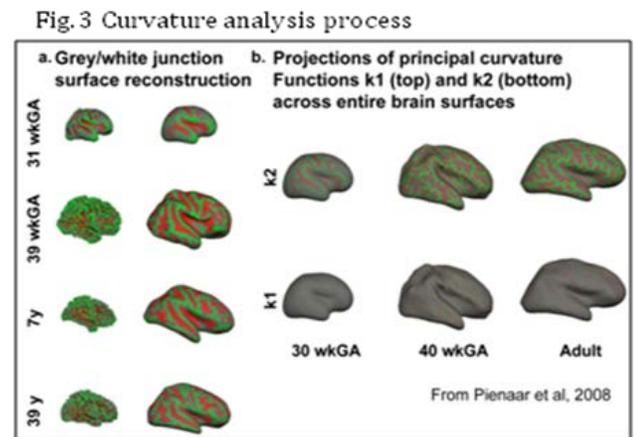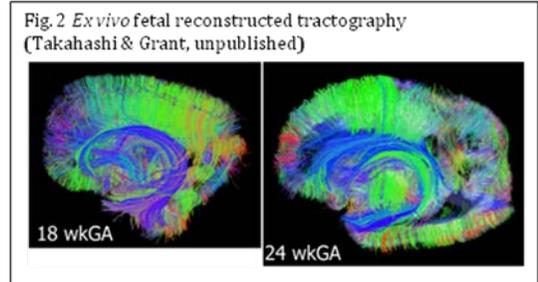
Reference

1.   Bhasin TK, Brocksen S, Avchen RN, Van Naarden Braun K. Prevalence of four developmental disabilities among children aged 8 years--Metropolitan Atlanta Developmental Disabilities Surveillance Program, 1996 and 2000. *MMWR Surveill Summ.* Jan 27 2006;55(1):1-9.
2.   Cerebral Palsy: Hope Through Research. NIH Publication 2009. http://www.ninds.nih.gov/disorders/cerebral_palsy/detail_cerebral_palsy.htm#154443104.
3.   Nelson KB. Causative factors in cerebral palsy. *Clin Obstet Gynecol.* Dec 2008;51(4):749-762.
4.   Luu TM, Vohr B. Twinning on the brain: the effect on neurodevelopmental outcomes. *Am J Med Genet C Semin Med Genet.* May 15 2009;151C(2):142-147.
5.   Harkness UF, Crombleholme TM. Twin-twin transfusion syndrome: where do we go from here? *Semin Perinatol.* Oct 2005;29(5):296-304.
6.   Habli M, Lim FY, Crombleholme T. Twin-to-twin transfusion syndrome: a comprehensive update. *Clin Perinatol.* Jun 2009;36(2):391-416, x.
7.   Graef C, Ellenrieder B, Hecher K, Hackeloer BJ, Huber A, Bartmann P. Long-term neurodevelopmental outcome of 167 children after intrauterine laser treatment for severe twin-twin transfusion syndrome. *Am J Obstet Gynecol.* Feb 2006;194(2):303-308.
8.   Lopriore E, Middeldorp JM, Sueters M, Oepkes D, Vandenbussche FP, Walther FJ. Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. *Am J Obstet Gynecol.* Mar 2007;196(3):231 e231-234.
9.   Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. *Am J Obstet Gynecol.* May 2006;194(5):1215-1220.
10.   Quarello E, Molho M, Ville Y. Incidence, mechanisms, and patterns of fetal cerebral lesions in twin-to-twin transfusion syndrome. *J Matern Fetal Neonatal Med.* Aug 2007;20(8):589-597.
11.   Jelin AC, Norton ME, Bartha AI, Fick AL, Glenn OA. Intracranial magnetic resonance imaging findings in the surviving fetus after spontaneous monochorionic cotwin demise. *Am J Obstet Gynecol.* Oct 2008;199(4):398 e391-395.
12.   Kanal E, Barkovich AJ, Bell C, et al. ACR guidance document for safe MR practices: 2007. *AJR Am J Roentgenol.* Jun 2007;188(6):1447-1474.
13.   Nishida M, Makris N, Kennedy DN, et al. Detailed semiautomated MRI based morphometry of the neonatal brain: preliminary results. *Neuroimage.* Sep 2006;32(3):1041-1049.
14.   Tolsa CB, Zimine S, Warfield SK, et al. Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. *Pediatr Res.* Jul 2004;56(1):132-138.
15.   Grossman R, Hoffman C, Mardor Y, Biegon A. Quantitative MRI measurements of human fetal brain development in utero. *Neuroimage.* Nov 1 2006;33(2):463-470.
16.   Limperopoulos C, Tworetzky W, McElhinney DB, et al. Brain volume and metabolism in fetuses with congenital heart disease: evaluation with quantitative magnetic resonance imaging and spectroscopy. *Circulation.* Jan 5;121(1):26-33.
17.   Ment LR, Hirtz D, Huppi PS. Imaging biomarkers of outcome in the developing preterm brain. *Lancet Neurol.* Nov 2009;8(11):1042-1055.
18.   Yu P, Grant PE, Qi Y, et al. Cortical surface shape analysis based on spherical wavelets. *IEEE transactions on medical imaging.* Apr 2007;26(4):582-597.
19.   Pienaar R, Fischl B, Caviness V, Makris N, Grant PE. A Methodology for Analyzing Curvature in the Developing Brain from Preterm to Adult. *Int J Imaging Syst Technol.* 2008;18:42–68.
20.   Skovgaard AM, Houmann T, Landorph SL, Christiansen E. Assessment and classification of psychopathology in epidemiological research of children 0-3 years of age: a review of the literature. *Eur Child Adolesc Psychiatry.* Dec 2004;13(6):337-346.

Coursework Description

Sample #2

Coursework and Additional Training During Award Period

Career development activities will follow 5 categories of application: 1) active mentorship from Drs. ▮▮▮▮ and De ▮▮ ; 2) formal educational seminars and journal clubs and laboratory training through the ▮▮▮▮▮ ▮▮▮▮▮▮▮▮▮▮ ; 3) coursework activities, 4) complementary professional responsibilities; and 5) the proposed and ensuing research projects leading to application for independent funding.

Mentorship: Regular, weekly scheduled meetings with Drs. ▮▮▮▮ and De ▮▮ will provide a forum for guidance and advisory in study implementation and career development.

Formal Educational and Laboratory Training: A curriculum of didactic and experiential training will be pursued through participation with the established post-graduate fellowship program offered through the ▮▮▮▮. Fellows with the program work collaboratively with a large and experienced interdisciplinary group of more than 15 scientists with an extensive record of expertise, publication and NIH-funded research in the areas of human body composition, energy expenditure, ingestive behavior molecular biology and genetics, and the hormonal and molecular effects of obesity. The ▮▮▮▮ studies virtually all aspects of obesity ranging from molecular biology to epidemiology. Investigators include molecular biologists, physicians, psychologists, statisticians, physiologists, nutritionists, and engineers. Through this participation, I will develop a background and expertise in the study of body composition, energy expenditure and metabolism that will drive increasingly independent inquiry and investigation in areas of nutrition and body composition applied to pediatric neuromuscular disease.

Career Development			
SKILL ACQUISITION	**MENTOR**	**COURSES**	**CAREER PROGRESS**
Developing and refining methods for assessment of energy expenditure and caloric needs	**Gallagher**	Educational seminars and laboratory training through the NYONRC	Translate and implement tools and methods to estimate energy expenditure and caloric requirements for application among subjects with neuromuscular disease.
Understanding methods for assessment of body composition in children with neuromuscular disease.	**Gallagher, De Vivo**	Educational seminars and laboratory training through the NYONRC	Determine the appropriate use of individual measures of body composition as applied to the study of phenotypically varied subject populations with neuromuscular disease.
Understanding of methods for assessment of nutrition and metabolic changes related to obesity	**Gallagher**	Human Nutrition M8207

Human Nutrition M8205 | Understanding of nutritional physiology and, approaches and challenges to the study of nutritional status and disregulation among patients with neuromuscular diseases |

Coursework: I will complete a Master's Degree in Biostatistics-Clinical Research Methods in May of 2011, prior to the award period. I will enroll in additional didactic courses through the ▮▮▮▮▮▮▮▮▮▮▮ to expand my background in nutritional physiology and clinical practice. I will enroll in the following courses: 1) Human Nutrition M8207: Clinical Nutrition. This course covers the physiological aspects of clinical disorders, including symptoms, risk factors, biological pathology, and clinical management, as well as the role of nutrition in their prevention and treatment; and 2) Human Nutrition M8205: Biochemical and Physiological Bases of Nutrition. This is a full year, two semester course covers the aspects of carbohydrate, lipid, protein, and energy metabolism, and the roles of vitamins (fat and water-soluble) and minerals in human nutrition that are relevant to the understanding of human nutrition at a cellular, organ and system level.

Professional Responsibilities: My professional responsibilities attending the pediatric neuromuscular center complements my work on this project, as I manage multidisciplinary care for children with SMA and other neuromuscular diseases for whom this research will be directly or indirectly applicable. My role as an increasingly senior, integral member of the clinical research team at the ▮▮▮▮▮ ▮▮▮▮▮▮▮▮▮ meshes with activities supported by this award. As my experience grows over the course of this career developmental award, I expect to assume an increasingly senior and lead investigator role on several of the ongoing and future clinical research projects at the center.

Research Projects: The proposed project will serve as the basis for multiple potential research projects addressing questions of nutritional care, clinical management and testing, and dietary interventions. It is anticipated that the results, observations and experience gained from this initial work will spur application for independent funding (R-01) to further enhance and expand the scope of investigation.

Computer and Statistical Support

▮▮▮▮▮▮▮ offers extensive tutorial and statistical assistance through active collaboration with statisticians through the department of ▮▮▮▮▮▮▮▮▮▮▮▮. I have extensive computer and statistical

computing access, including a personal PC computer with SAS and SPSS statistical computing packages, Microsoft Excel, and graphical programs for poster design for conference presentations.

Training in Responsible Conduct of Research

I will enroll in the course Responsible Conduct of Research and Related Policy Issues at ███████ (Course number G4010) taught by ████████, Professor, Department of Pathology and Cell Biology and ████████, Director of Research Development, Department of Medicine. This course explores a variety of ethical and policy issues that arise during the conduct of basic, translational, and clinical biomedical scientific research. Topics addressed include: (1) research misconduct; (2) "every day" ethical issues faced by biomedical scientists; (3) the use of laboratory animals in scientific research; (4) human research participants and scientific research; (5) authorship practices in scientific publications; (6) conflicts of interest arising from scientists acting as policy consultants and experts; (7) data sharing and data secrecy; (8) mentoring; (9) research with stem cells, and (10) scientists as citizens. Course sessions include lectures, discussion periods, and analyses of case studies.

Table 1: Proposed timeline for career development activities during career development award

Months of Award	Start	6	12	18	24
RESEARCH					
IRB Approvals, Study implementation		xxxxxx	xxxxxxxxxxx	xxxxxxxxxxx	xxxxxxxxxxx
Data Collection				xxxxxxxxxxx	xxxxxxxxxxx	xxxxxx
Apply for R01 to expand and continue the cohort			xxxxxx	xxxxxxxxxxx	xxxxxx
CAREER DEVELOPMENT					
Weekly meeting with Drs. Gallagher & De Vivo	xxxxxxxxxxx	xxxxxxxxxxx	xxxxxxxxxxx	xxxxxxxxxxx	xxxxxx
Biannual meetings with panels (NYORC, CUMC)	xxxxxxxxxxx	xxxxxxxxxxx	xxxxxxxxxxx	xxxxxxxxxxx	xxxxxx
Coursework		xxxxxxxxxxx	xxxxxxxxxxx	xxxxxxxxxxx	
MANUSCRIPT PREPARATION					
Papers				xxxxxxxxxxx	xxxxxx
Abstract submission to Families of SMA, American Academy of Neurology annual meetings			X		X

Research Plan

Sample #2

Body Composition and Energy Utilization in Spinal Muscular Atrophy

███████████████, M.D.

Spinal muscular atrophy (SMA) is a progressive, recessively-inherited disease characterized by weakness and muscle atrophy due to loss of spinal cord motor neurons. The disease presents across a wide spectrum of severity, ranging from extremely weak young infants with a severe clinical phenotype and prognosis (Werdnig-Hoffmann, or SMA type 1)(1, 2) to mildly affected, ambulatory children and adults (Kugelberg-Welander, SMA type 3)(3). There is no effective therapy. Nevertheless, recent years have seen dramatic improvements in the morbidity and mortality associated with SMA, particularly SMA type 1, due to advances in pulmonary and orthopedic care that have significantly altered the natural course of the disease(4). Despite widespread recognition of the importance of nutrition and diet in SMA(5), little information is available to guide nutritional management. The caloric needs, body composition and growth expectations for infants and young children with SMA are likewise poorly understood. This ignorance is of particular note given recent reports from patients with amyotrophic lateral sclerosis (ALS, like SMA a disease affecting the motor neuron)(6-8) and the mouse model of SMA(9) that suggest that improved nutrition may alter disease progression for the most severely affected children with this disease.

We propose to study energy expenditure, caloric intake and body composition among young children with SMA types 1 and 2, a population for whom questions of body composition, nutrition and caloric needs are particularly critical. A large available pool of patients at the ███, and clinical research resources available through the ███████████████████████████████████████ and the ████████████ ████████████████████████████ provide a unique opportunity to rigorously explore these questions in a large and well-characterized cohort of patients with SMA.

Aims

1) Assess and describe baseline and longitudinal resting energy expenditure (REE) and caloric intake, and baseline total daily energy expenditure (TDEE), among infants and young children with SMA types 1 and 2;

2) Establish baseline and longitudinal body composition and anthropometric measures (height and weight) among infants and children with SMA.

Background

In a recent review of standard of care for clinical management in SMA (5), **body composition and nutrition were recognized as areas of critical importance but almost completely lacking in data and peer-reviewed study**. Management recommendations have been extrapolated from children with spinal cord injury who suffer from diminished lean and increased fat percentage compared to unaffected individuals(10). Based on this experience, patients with SMA should maintain lower body weight relative to height compared to age and gender-matched peers.

Since publication of the standard of care document in 2007, potentially conflicting data have emerged regarding body composition in SMA. Messina et al (2008) reported a high frequency of swallowing dysfunction and subnormal weight among patients with SMA type II, suggesting that such patients are at risk of malnutrition(11). The strength of this conclusion is, of course, limited by the fact that weight is, at best, an indirect measure of body composition of limited utility in diseases marked by muscle atrophy such as SMA. We recently described a high rate of adiposity above the 50th percentile and obesity in a mixed cohort of type 2 and 3 subjects using modern body composition imaging techniques (dual energy x-ray aborptiometry [DXA]) and comparative data from healthy children(12). While only our study directly assessed body composition, these observations suggest that **children with SMA are potentially at risk for both obesity and malnutrition, depending on the balance of activity level and caloric intake**. These studies highlight the importance of direct assessment of body composition rather than indirect measures such as weight or application of body mass index (BMI, kg weight / height in m2) percentiles based on studies of healthy children. This need is particularly important for the management of infants and young children with SMA for whom questions of optimal body weight and growth expectations are especially common and acute.

Ultimately, body mass is a function of the relationship between caloric intake and energy expenditure. Unfortunately, the dietary habits of children with SMA remain poorly described. Furthermore, REE **and TDEE, and thus daily caloric needs of patients with SMA, are unknown.** There is no analogous disease state that informs expectations of REE and TDEE in growing children with SMA. However, adults with ALS (another disease of the motor neuron), have elevated REE and caloric needs(7, 8); furthermore, low BMI is associated with increased mortality(6). Muscle fasciculations, less prominent in SMA save for children with SMA type 1, may underlay part of the increased energy expenditure seen in ALS, although other factors common to both diseases, such as increased work of breathing, increased thermogenesis of food and increased energy expenditure to maintain sitting posture, may also impact REE in ALS.

Recent data from a mouse model of SMA type 1 suggests that nutritional supplementation may modulate disease severity and improve survival. Early treatment with a deacetylase inhibitor, trichostatin A (TSA), supplemented by nutritional support (Enfamil lipil delivered by feeding tube and rodent chow moistened with high calorie syrup) improved survival compared to SMA mice treated with TSA alone, and maintained weight gain, motor function (righting time) and structurally intact motor units well after discontinuation of the drug on post-natal day 20[9]. **Although the energy expenditure (and caloric needs) of these animals is unknown, this observation suggests that nutritional supplementation may modulate the muscle wasting seen in SMA.** While extrapolations from an engineered mouse model must be made with extreme caution, the observed relationship between increased caloric intake and survival may mirror observations in children with SMA type 1, for whom the observed increased survival in recent years may be in part attributable to improved nutritional support using gastrostomy feedings[4].

Improved understanding of the caloric needs of this population, and the relationship between disease severity and energy expenditure, would advance the current nutritional management that is, at present, based loosely on recommendations for healthy children. The determination of energy expenditure and caloric needs in SMA is a potentially important aspect to care, particularly as applied to infants with SMA type 1. If energy expenditure is elevated, as is seen in ALS[7, 8, 13], nutritional supplementation may be a viable intervention to improve outcome among SMA type 1 patients in a manner similar to that noted in the SMA mouse model.

Methods

Approach: We will measure REE, TDEE, caloric intake and body composition among infants and young children with SMA types 1 and 2.

Subjects: We will study 6 subjects under 3 years of age with genetically confirmed SMA Type 1, 6 subjects with SMA type 2 (classified using conventional definitions)[5, 14], and 12 age and gender-matched controls.

Schedule of Events: The study will involve 4 study visits over a 12 month interval. The study implementation will be performed in collaboration with researchers from the NYONRC and the ██████████████████████ ██████████████████████

Sample Size Considerations: Difference in REE between SMA type 1 and control subjects will be the primary analysis. While there is no relevant, direct data from patients with SMA, among boys with Duchenne muscular dystrophy REE was 238 ± 23 kJ/Kg of fat-free mass[15]. Among healthy one month-old infants, resting energy expenditure

Schedule of events	Week 0	Week 13	Week 26	Week 52
Assessment of Eligibility	X			
Signed Consent	X			
Medical History	X	X	X	X
Measurement of weight and height	X	X	X	X
CHOP-INTEND / HFMS	X*	X*	X*	X*
Hood calorimetry	X	X	X	X
Administration of doubly-labeled water	X*			
Dietary history by nutritionist	X	X	X	X
Body Composition Measurements				
DXA	X	X	X	X
BIA	X	X	X	X

(1) Urine will be collected pre-administration and at 1 and 7 days post-administration for delivery to the testing center; this will not require an additional study visit.

* Testing will be performed on SMA cohorts only

was 62 ± 9 kcal/kg/d[16] Based upon an assumed $\sigma_{REE} = 12.5\%$ of calculated REE, we will aim to be able to detect a 20% difference in REE (adjusted for muscle mass) between children with SMA Type 1 and age and gender-matched control subjects, with 80% power at the 0.05 significance level. These parameters yield a sample size of 5 experimental (SMA type 1) subjects and 5 controls. In order to also account for attrition, we will plan to recruit 6 subjects and 6 matched controls. We will enroll a similar number of children with SMA type 2 for secondary analyses.

General Methods:

Measurement of REE: Indirect hood calorimetry will be performed using a portable metabolic cart (ParvoMedics TrueMax 2400 Metabolic Monitor, ParvoMedics Inc., Sandy, UT) to measure REE in the resting, recumbent, alert state after an overnight fast using established methods[17].

Determination of Total Daily Energy Expenditure (TDEE): TDEE will be measured using the doubly-labeled water (DLW) method[18], which estimates integral CO_2 production by measuring the difference in elimination rates of deuterium and ^{18}O from labeled body water. DLW administration will occur on a metabolic ward using trained personnel. Urine samples will be collected before dosing. On post-dose Day 1 and day 7 after equilibration with total body water, additional urine specimen (containing water enriched in ^2H and ^{18}O) will be obtained for analysis of ^2H and ^{18}O; TDEE will be calculated according to standardized methods[19]. Due to expense, DLW will be performed on SMA type 1 and type 2 cohorts at baseline only.

Measurement of body composition: Body composition will be measured using DXA[20], selected as "gold-standard" for the purposes of data analysis, water dilution[21, 22] and Bioelectrical Impedance Analysis[23] methods.

Measurement of caloric intake: A 7-day dietary record will be obtained and analyzed using a computerized nutrient analysis system, the NDS-R version 2007 (██████████████████████████████████████)[24]. We expect the majority

of subjects, particularly SMA type 1, to receive standing formula therapy for which a 7-day dietary record will be unnecessary.

Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders(CHOP-INTEND): The CHOP-INTEND, a measure with demonstrated reliability and reproducibility, and developed specifically to assess motor skills in infants with SMA type 1, will be used to characterize clinical function among subjects with SMA type 1(25, 26).

The Hammersmith Functional Motor Scale(HFMS): The HFMS, an established and accepted measure of clinical function in SMA(27) that has good inter-rater reliability(27, 28) and correlates well with biomarkers of disease severity(29) will be used to allow stratification by clinical function among SMA type 2 subjects.

Experimental Methods:

Aim 1: Assess and describe baseline and longitudinal REE, TDEE and caloric intake among infants and young children with SMA types 1 and 2.

We hypothesize that infants with SMA type 1 may have increased energy expenditure compared to expected caloric needs based on their (reduced) lean mass and minimal activity. We also hypothesize that factors that underlay the increased energy expenditure in SMA type 1, such as fasciculations and increased work of breathing, are less prominent in milder forms of the disease. REE among children with SMA type 2 under such a model would more closely resemble that of healthy children, and TDEE would be reduced, commensurate with low activity. Establishment of energy expenditure across the disease spectrum of SMA would have tangible immediate benefits to care by improving and individualizing the approach to nutritional care in this heterogeneous and phenotypically variable population. Nutritional insufficiency can result in catabolism of functional muscle to maintain energy balance. Conversely, overfeeding can lead to excess weight, imposing additional stress on weak muscles, increases fatigue, and impairs nocturnal ventilation. As discussed above, dietary recommendations and estimates of body composition based on experience in healthy children are not applicable to this population.

Statistical Analysis: REE among both SMA cohorts will be compared to control subjects using a paired Student's t-test. TDEE for SMA type 1 and SMA type 2 will be determined and compared using the DLW "gold standard"(19). TDEE for each subject cohort will also be compared with expected TDEE based on the Harris-Benedict equation(30). The Harris-Benedict equation is widely used by dieticians and was developed to estimate TDEE among healthy individuals based on imputed resting metabolic rate and activity. It is expected that this formula is not valid in the SMA population; however, the potential for and degree of overestimation (through reduced activity and diminished muscle mass) or underestimation (due to failure to account for disease-related excess energy expenditure) of energy needs based on extrapolation from standard nutrition practice is unknown.

Aim 2: Establish body composition and anthropometric measures among infants and children with SMA

Body composition and anthropometric measures will be measured using DXA and BIA at baseline, 3, 6 and 12 months to describe baseline body composition, longitudinal growth and body composition changes over the course of a 1 year interval. The first few years of life are a period during which rapid physical and neurological development is to a greater or lesser degree counterbalanced by disease progression; the growth and body composition changes that occur among children with SMA type 1 and SMA type 2 are presently unknown.

Statistical Analysis: Muscle and fat mass will be compared to established normative values for fat (FMI) and fat-free (lean, FFMI) mass indexes(31) using an adapted protocol derived from a large cohort of healthy children studied at the NYONRC whose demographic and anthropometric data closely resembles that of the NHANES III dataset that is the basis for pediatric growth expectations (growth curves) in the United States. Fat and Fat-free mass among subjects with SMA types 1 and 2 will be compared to control subjects using a paired Student's t-test. We expect lean mass to be markedly reduced across the disease cohort; the degree and longitudinal change in lean mass relative to healthy peers is unknown. We expect children with SMA type 1 to have reduced fat mass while those with SMA type 2 to have increased fat mass, mirroring differences in energy expenditure in these populations.

Anticipated Outcomes and Implications for Future Research

This study will advance our understanding of body composition and energy needs among young children with SMA, and will have immediate clinical impact on the medical and nutritional care of patients with this disease. Results will spur not just further study of body composition and nutrition in SMA, but also inform approaches to the study of body composition in other pediatric neurological and neuromuscular diseases. Description of the longitudinal changes in lean (fat-free) mass seen in young children with SMA, in particular, may have important implications for the development of biomarkers of treatment effect as clinical trials are considered in the near future. The demonstration that patients with SMA have increased energy expenditure would have potential bearing on the nutritional care of these children, particularly those children and infants with SMA type 1 for whom caloric supplementation may be a viable therapeutic intervention.

Bibliography and References Cited:

1.   Werdnig G. Zwei frühinfantile hereditäre Fälle von progressiver Muskelatrophie unter dem Bilde der Dystrophie, aber auf neurotischer Grundlage. Arch Psychiatrie Nervenkrankheiten. 1891;22:437-80.
2.   Hoffmann J. Über chronische spinale Muskelatrophie im Kindesalter, auf familiärer Basis. Deutsche Z Nervenheilkunde. 1893;3:427.
3.   Kugelberg E, Welander L. Heredofamilial juvenile muscular atrophy simulating muscular dystrophy. AMA Arch Neurol Psychiatry. 1956;75(5):500-9.
4.   Oskoui M, Levy G, Garland CJ, Gray JM, O'Hagen J, De Vivo DC, et al. The changing natural history of spinal muscular atrophy type 1. Neurology. 2007;69(20):1931-6.
5.   Wang CH, Finkel RS, Bertini ES, Schroth M, Simonds A, Wong B, et al. Consensus statement for standard of care in spinal muscular atrophy. J Child Neurol. 2007;22(8):1027-49.
6.   Desport JC, Preux PM, Truong TC, Vallat JM, Sautereau D, Couratier P. Nutritional status is a prognostic factor for survival in ALS patients. Neurology. 1999;53(5):1059-63.
7.   Desport JC, Torny F, Lacoste M, Preux PM, Couratier P. Hypermetabolism in ALS: correlations with clinical and paraclinical parameters. Neurodegener Dis. 2005;2(3-4):202-7.
8.   Bouteloup C, Desport JC, Clavelou P, Guy N, Derumeaux-Burel H, Ferrier A, et al. Hypermetabolism in ALS patients: an early and persistent phenomenon. J Neurol. 2009;Epub ahead of print.
9.   Narver HL, Kong L, Burnett BGC, D.W., Bosch-Marcé M, Taye AA, Eckhaus MA, et al. Sustained improvement of spinal muscular atrophy mice treated with trichostatin A plus nutrition. Ann Neurol. 2008;64(4):465-70.
10.   Liusuwan A, Abresch R, McDonald C. Altered body composition affects resting energy expenditure and interpretation of body mass index in children with spinal cord injury. J Spinal Cord Med. 2004;27((Suppl 11)):S24-S8.
11.   Messina S, Pane M, De Rose P, Vasta I, Sorleti D, Aloysius A, et al. Feeding problems and malnutrition in spinal muscular atrophy type II. Neuromuscul Disord. 2008;18(5):389-93.
12.   Sproule DM, Montes J, Montgomery M, Battista V, Koenigsberger D, Shen W, et al. Increased fat mass and high incidence of overweight despite low body mass index in patients with spinal muscular atrophy. Neuromuscul Disord. 2009;19(6):391-6.
13.   Desport JC, Preux PM, Magy L, Boirie Y, Vallat JM, Beaufrère B, et al. Factors correlated with hypermetabolism in patients with amyotrophic lateral sclerosis. Am J Clin Nutr. 2001;74(3):328-34.
14.   Russman BS. Spinal muscular atrophy: clinical classification and disease heterogeneity. J Child Neurol. 2007;22(8):946-51.
15.   Zanardi M, Tagliabue A, Orcesi S, Berardinelli A, Uggetti C, A P. Body composition and energy expenditure in Duchenne muscular dystrophy. Eur J Clin Nutr. 2003 Feb;57(2):273-8.
16.   Bauer J, Werner C, Gerss J. Metabolic rate analysis of healthy preterm and full-term infants during the first weeks of life. Am J Clin Nutr. 2009;90(1517-1524).
17.   E. F. The theoretical bases of indirect calorimetry: a review. Metabolism. 1988;37(3):287-301.
18.   Schoeller DA. Measurement of energy expenditure in free-living humans by using doubly labeled water. J Nutr. 1988;118(11):1278-89.
19.   Silva AM, Wang J, Pierson RNJ, Wang Z, Heymsfield SB, Sardinha LB, et al. Extracellular water: greater expansion with age in African Americans. J Appl Physiol. 2005;99(1):261-70.
20.   Sopher AB, Thornton JC, Wang J, Pierson RNJ, Heymsfield SB, Horlick M. Measurement of percentage of body fat in 411 children and adolescents: a comparison of dual-energy X-ray absorptiometry with a four-compartment model. Pediatrics. 2004;113(5):1285-90.
21.   Schoeller DA. Hydrometry. In: Heymsfield SB RA, and Lohman TG, editor. Human Body Composition. Champaign, IL: Human Kinetics; 1996. p. 25-44.
22.   Silva AM, Wang J, Pierson RNJ, Wang Z, Heymsfield SB, Sardinha LB, et al. Extracellular water: greater expansion with age in African Americans. J Appl Physiol. 2005;99(1):261-7.
23.   Desport JC, Preux PM, Bouteloup-Demange C, Clavelou P, Beaufrère B, Bonnet C, et al. Validation of bioelectrical impedance analysis in patients with amyotrophic lateral sclerosis. Am J Clin Nutr. 2003;77:1179-85.
24.   Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. J Am Diet Assoc. 1988;88(10):1268-71.
25.   Glanzman AM, Mazzone E, Main M, Pelliccioni M, Wood J, Swoboda KJ, et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): test development and reliability. Neuromuscul Disord. 2010;20(3):155-61.
26.   Finkel RS, Glanzman A, Main M, Bertini E, Mercuri E. The CHOP INTEND: A reliable motor scale for infants with neuromuscular disease. Neuromuscul Disord. 2006;16(9-10):668.

27.   Main M, Kairon H, Mercuri E, Muntoni F. The Hammersmith functional motor scale for children with spinal muscular atrophy: a scale to test ability and monitor progress in children with limited ambulation. Eur J Paediatr Neurol. 2003;7(4):155-9.

28.   Mercuri E, Messina S, Battini R, Berardinelli A, Boffi P, Bono R, et al. Reliability of the Hammersmith functional motor scale for spinal muscular atrophy in a multicentric study. Neuromuscul Disord. 2006;16(2):93-8.

29.   Tiziano FD, Bertini E, Messina S, Angelozzi C, Pane M, D'Amico A, et al. The Hammersmith functional score correlates with the SMN2 copy number: a multicentric study. Neuromuscul Disord. 2007;17(5):400-3.

30.   Harris J, Benedict F. A biometric study of basal metabolism in man. Proc Sci USA 1918;12(4):370-3.

31.   Freedman D, Wang J, Maynard L, Thornton J, Mei Z, Pierson R, et al. Relation of BMI to fat and fat-free mass among children and adolescents. Int J Obes (Lond). 2005;29(1):1-8.