A Double-Blind, Controlled, Randomized Clinical Trial of the Effect of Early L-Carnitine Supplementation on Neurodevelopmental Outcomes in Very Preterm Infants

Background/Significance:
Prematurity is a known risk factor for neurodevelopmental problems.[1]Risk factors for poor outcome include hypoxemia, ischemia, acidosis, hypoglycemia and infections.[2]One factor associated with improved neurodevelopmental outcome in preterm infants is growth. In a study of infants receiving intensive neonatal nutritional support, better weight gain and head circumference growth during the initial NICU stay were associated with improvements in long-term neurodevelopment.[3]

Neuroprotective effects of carnitine:
The susceptibility of the newborn brain to damage is determined by various factors, including severity and duration of an insult, membrane lipid composition, the number and duration of excitatory N-methyl-D-aspartate receptors in the brain, and the presence of antioxidants.[4]Antioxidants play an important role in protecting vulnerable cells from hypoxic-ischemic damage. One such antioxidant, carnitine, is an important nutrient which is synthesized in the liver and kidney during the breakdown of endogenous proteins from the resultant lysine and methionine. Carnitine enhances energy production by transporting long-chain fatty acids into mitochondria for beta-oxidation. Carnitine maintains adequate free coenzyme-A for metabolic pathways and protects cells against accumulation of toxic acyl-coenzyme-A compounds. In vitro models have shown that carnitine protects against neuronal damage. The mechanism(s) contributing to cell death in hypoxic-ischemic brain injury were simulated in 1-day old rat cerebellar granular cell cultures by exposing these cells to either glutamate or the glutamate-receptor agonist, kainic acid (KA).[5] The administration of L-carnitine decreased neuronal cell death in both glutamate- and KA-treated cells.

In vivo animal models have also demonstrated the neuroprotective effect of carnitine. Sprague–Dawley rats exposed to hypobaric hypoxia for 14 consecutive days showed spatial memory impairments, which were reduced in rats treated with acetyl-L-carnitine (ALCAR) compared with controls.[6] Similarly, in a rat model of Alzheimer's Disease, spatial memory impairments were associated with tau hyperphosphorylation by GSK-3β. The administration of ALCAR decreased the spatial memory impairment of the rats by antagonizing GSK-3β activation.[7] In addition, ALCAR arrested microtubule-associated protein tau hyperphosphorylation at multiple Alzheimer's disease sites and enhanced the expression of several memory-associated proteins in the rat hippocampus. In a rat model of Parkinson's disease, acetyl-L-carnitine was shown to improve motor performance and reduce the level of lipid peroxides in rat brains as compared to controls.[8] Treatment with L-carnitine in newborn rats during exposure to hypoxic-ischemic injury was associated with reduced neurologic injury, as demonstrated by less brain weight loss and less neuronal death.[9] Carnitine treatment in these studies was administered over a short period, up to two weeks.

Carnitine has also been studied in neurologic disorders in human subjects. A randomized controlled trial of carnitine supplementation in pediatric patients with Rett Syndrome indicated that carnitine was associated with improvement in report of well-being and improvement in the Hand Apraxia Scale.[10]A randomized controlled trial of carnitine in patients with diabetic neuropathy reported that carnitine was effective and well-tolerated in improving neurophysiological parameters and in reducing pain over a 1-year period.[11]

Carnitine and the preterm infant:
Due to a deficiency in precursors and decreased enzyme activity, neonates have a reduced ability to synthesize carnitine and become deficient without external supplementation. Since placental transfer of carnitine occurs during the third trimester, premature infants have lower stores and are at increased risk of carnitine deficiency.[12] After birth, carnitine levels in very preterm infants decline more rapidly over the first one to two weeks of life, compared with infants born at later gestational ages.[13] These factors combine to make exogenous sources of carnitine important in order to prevent deficiency in the preterm infant. Breast milk contains carnitine, and both cow's milk and soy-based infant formulas are supplemented with carnitine.[14] However, parenteral nutrition is not routinely supplemented with carnitine, and the most stressed infants often receive parenteral nutrition for prolonged periods. Our group

previously conducted a national survey of neonatal intensive care units and found that carnitine was routinely supplemented in approximately one-third of the units.[15]

Supplementation with L-carnitine in preterm infants has been shown to enhance lipid tolerance and fatty acid oxidation and to increase nitrogen balance.[16–24] Helms et al. and Bonner et al. reported better early weight gain (at two weeks of life) in infants supplemented with carnitine.[23,24]However, several studies investigating the effect of carnitine supplementation on long-term weight gain in preterm infants did not show a beneficial effect.[25–27]We have previously reported the results of a double-blind parallel placebo-controlled randomized clinical trial to investigate the effect of L-carnitine supplementation on weight gain in very preterm infants.[28] Infants were randomized to receive 50 umol/kg/day of L-carnitine or placebo. No significant difference was seen in average daily weight gain from birth until 36 weeks' PMA or until hospital discharge. However, we observed decreased weight loss in infants during the time period when they received supplemental carnitine with parenteral nutrition (data not previously reported). Similar to our findings, Crill and colleagues demonstrated a shorter time to regain birthweight in carnitine-supplemented infants compared with controls.[29]These improvements were noted within a two week time period.

In general, the literature shows the effect of carnitine on weight gain in premature infants may be related to a dosing effect. The studies that failed to show an effect on weight gain and apnea of prematurity utilized supra-physiologic dosages of carnitine (156 – 625 umol/kg/day), whereas Helms, Bonner, and we utilized supplementation dosages of carnitine within the physiologic range (50 umol/kg/day or 8 mg/kg/day). This dosage was calculated based upon daily in utero accretion and with the estimated intake of carnitine from 150 ml/kg/day of pooled breast milk.[30–32] This suggests that physiologic dosing may strike a balance between deficiency and over-supplementation.

Carnitine and systemic effects in the preterm infant

In addition to early weight gain, differences in three additional outcomes were noted in our previous study:rates of proven infection, hearing screen failure and PDA ligation(unpublished data). The study was not powered to detect these additional outcomes, but previous work indicates that there may be a physiologic basis for the differences noted. Infants treated with carnitine had a significantly lower rate of proven infection than control infants, a finding which may be related to previous work investigating the impact of carnitine on lymphocyte counts in immunocompromised patients.[33] The rate of PDA ligation was greater in infants treated with carnitine. We discuss the potential basis and implications of this finding in the "Risks and Discomforts" section of this proposal. The rate of hearing screen failure was markedly reduced in carnitine-treated infants. This finding is congruent with previous work by our group, which showed that carnitine is protective against ototoxicity.[34]

Nutrition and brain growth

During the early window in which carnitine supplementation appears to have a positive impact on weight gain, the brain of the premature infant is also developing. One study examined the impact of nucleotide-supplemented formula on rates of weight gain and head growth in formula-fed infants born at >37 weeks gestation. Infants fed the nucleotide-supplemented formula had greater weight at 8 weeks and a greater increase in occipitofrontal head circumference and weight from birth to 8 weeks than those fed control formula.[35] Thus, it seems reasonable to infer that the increased weight gain and decreased weight loss seen in previous studies with carnitine might be linked with an increased rate of head and brain growth.

The rate of brain growth in premature infants has been associated with cognitive ability. Studies have shown the association between premature birth and relatively smaller cerebral volume later in life, as well as altered white matter microstructure.[36,37] In children born before 30 weeks' gestational age, the rate of cerebral cortical growth has been shown to predict cognitive ability in later childhood.[38] Thus, improvement in rate of brain growth is likely associated with improved neurodevelopmental outcomes.

Additional work has focused on the long-term impact of dietary supplementation beyond weight gain and head growth. Replenishment of deficient nutrients during the critical period of early brain growth can improve neurodevelopmental outcomes. A trial comparing preterm infants weighing less than 1850 gm at birth were randomized to receive standard infant formula versus enriched preterm formula. The survivors

underwent intelligence testing at 7 1/2 - 8 years of age. A higher proportion of term formula infants were found to have low verbal IQ, and the term formula group had a higher incidence of cerebral palsy.[39] More specifically, single-nutrient replenishment has been linked to neurodevelopmental status. A mono-centric cohort study of premature infants less than 28 weeks gestational age investigated the relationship between neurological development at a corrected age of one year and the cumulative intakes of proteins, carbohydrates, lipids and energy during the first 28 days of life. In multivariate analysis, developmental quotient at one year was significantly associated with cumulative lipid intake at 14 days of life.[40]

In summary, in vitro and in vivo models have demonstrated neuroprotective effects of L-carnitine supplementation against brain injury. Carnitine supplementation in preterm infants at physiologic dosages also enhances early weight gain at a critical period of brain development. We postulate that carnitine supplementation in preterm infants may protect against neuronal damage and enhance brain growth, and thus improve neurodevelopmental outcomes in childhood. The effect of carnitine supplementation on neurodevelopmental outcomes in premature infants remains unexplored.

Hypothesis:
We hypothesize that preterm infants supplemented with early physiologic doses of L-carnitine while on parenteral nutrition will have improved short-term growth parameters and significantly higher neurobehavioral scores when compared with control infants.

Primary Aims:
To determine whether carnitine supplementation is associated with:

1. Decreased percent weight loss within the first two weeks of life and decreased length of time to regain birthweight.
2. Improved performance on the NICU Network Neurobehavioral Scale (NNNS) at 40 weeks post-menstrual age (+/- 2 weeks).

Secondary Aims:
To determine whether carnitine supplementation is associated with:

1. Increased rate of growth of head circumference within the first month of life and increased food efficiency (weight gain/calorie intake) during the supplementation period.
2. Normalization of the serum carnitine profile at two weeks of life, compared with infants born at a post-menstrual age 2 weeks later.
3. Enhanced brain maturation as measured by amplitude-integrated EEG (aEEG) measurements obtained during the hospital course
4. Fewer abnormal findings, better brain growth, white matter development and brain metabolism on brain magnetic resonance imaging (MRI) at 36-40 weeks' corrected age (prior to hospital discharge)

Because of the potential for systemic effects of L-carnitine supplementation, we will also monitor those variables which showed significant difference in our prior study: rates of proven infection, hearing screen failure and PDA ligation.

Primary Outcomes:

1. Length of time to regain birthweight,
2. Percent weight loss in the first two weeks of life,
3. Neurodevelopmental scores on the NICU Network Neurobehavioral Scale (NNNS) at 40 weeks post-menstrual age (+/- 2 weeks)

Methods/Design:
This is a prospective, randomized controlled clinical trial involving infants with gestational age (GA) <32 wks who are born at either the Jack D. Weiler Hospital or the Wakefield Division of Montefiore and admitted to theirNeonatal Intensive Care Unit. Informed consent will be obtained from the parents of eligible patients as early as possible within 72 hours of birth. Infants whose parents have signed an informed consent form will be randomized to receive either carnitine supplementation (50 µmol/kg/day) versus placebo within 72-96 hours of birth.

Inclusion criteria:

- Infants born <32weeks' gestation

Exclusion criteria:

- Presence of
  - potentially life-threatening congenital anomaly
  - known hereditary metabolic disorder
  - known chromosomal abnormality
  - teratogen exposure with symptomatic substance withdrawal
  - congenital viral infections
  - microcephaly
  - Grade IV intraventricular hemorrhage (IVH) or seizures documented within first 72 hours of birth
- Critically-ill with life expectancy less than 72 hours
- Unable to obtain consent within 72 hours of birth
- Enrolled in another intervention trial

Participant recruitment: Parents or guardians of infants who meet study criteria will be approached regarding the study as early as possible within 72 hours of the infant's birth. Informed consent will be obtained by study investigators and coordinators. Permission will be requested to re-contact the families after completion of the supplementation study for follow-up.

Randomization: As previously described by Pande et al., enrolled patients will be randomized to either the treatment group (L-carnitine, 50 µmol/kg/day) or placebo group (5% glucose, similar volume as the L-carnitine group).[28] Patients who meet study entry criteria and have signed informed parental/guardian consent will be enrolled in the study. Because gestational age and small for gestational age (SGA) status will impact our outcome measures, we will ensure they are equally distributed in the arms of the study by stratifying based upon these variables. We will analyze the two study arms using an intent-to-treat analysis. Therefore, enrolled patients will be stratified into fourgroups: gestational age 23 to 26 6/7 weeks, gestational age 27 to 29 6/7weeks, gestational age 30 to 31 6/7 weeks and small for gestational age (SGA). Patients within each stratum will be randomized by the pharmacist using a computerized block-generation with sets of 4. In the case of multiple births, all infants must meet study criteria, and the infants will be randomized as a set. One infant of the multiple-birth set will be randomly chosen for inclusion in the analysis. A recruitment log of all screened infants will be maintained. Clinicians and the nursing staff will be unaware of the arm of the treatment protocol to which the patient is assigned. Codes will be unblinded only after all patients have reached the study end-point, or at the request of the Data Safety Monitoring Board.

Study supplementation: Infants randomized to the treatment group will receive 50 µmol/kg/day of L-carnitine intravenously for 2 weeks, and infants randomized to placebo will received 5% glucose, similar volume to the L-carnitine group. At two weeks, sufficient carnitine is provided in enteral feeds of either breast milk or infant formula. If no intravenous access is available before the supplementation endpoint, the equivalent dose of enteral study supplement (L-carnitine or placebo) will be administered.[41] Parenteral and enteral nutrition will be provided according to standard NICU protocol.

Of note,as many as 10% of the infants in the study will likely continue to rely primarily on parenteral nutrition beyond the proposed two-week supplementation period. Enteral feeds may be withheld from these infants due to underlying illnesses such as sepsis or gastrointestinal disorders like necrotizing enterocolitis. Due to the presence of underlying illness, these infants are at an even higher risk for developing developmental delays.[42]Therefore, in study patients who are not receiving adequate enteral nutrition (100 cc/kg/day of enteral intake) after 2-weeks of study supplements, carnitine supplementation will be continued until these infant are receiving adequate enteral feeds; at this point, their physiologic carnitine requirements will be met by enteral nutrition alone.

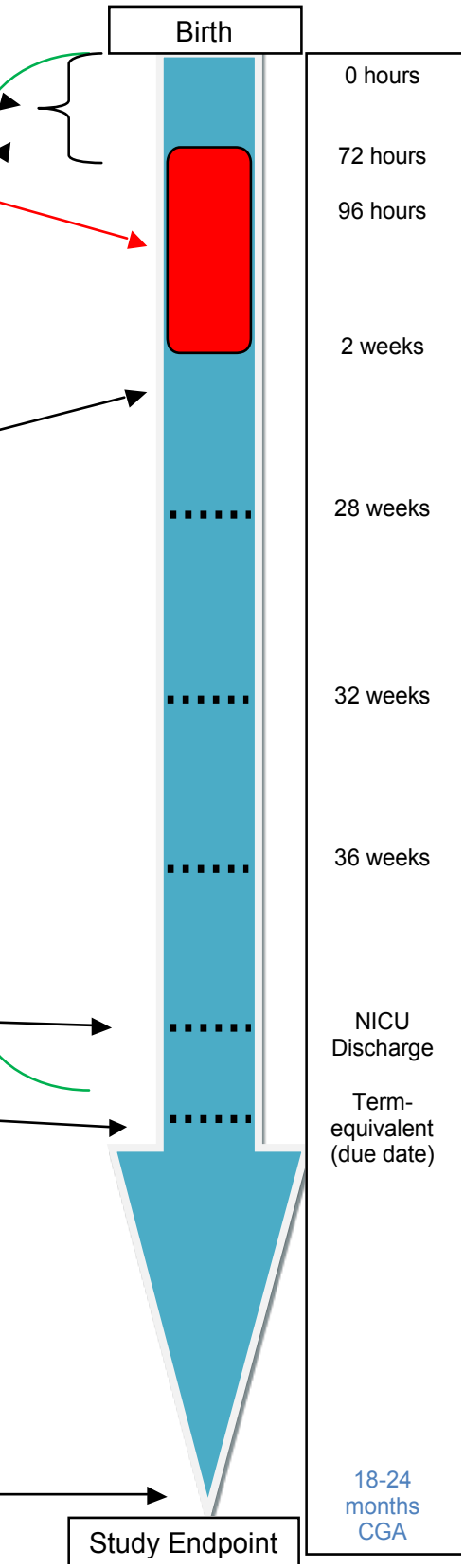
Timeline of Assessments:

a. Within 72 hours of birth
   i. Patient enrollment and initiation of study protocol

   ii. Initial carnitine panel drawn
   iii. Initial aEEG reading obtained within 2-3 days of study initiation
   iv. Study Supplement begins within 72-96 hours of birth
b. Two weeks after study initiation
   i. Study Supplement concludes (in a small sub-set of patients, study supplementation will be continued until enteral feeds reach 100 cc/kg/day)
   ii. Follow-up carnitine panel drawn (in addition, as blood transfusions may impact carnitine levels, serum carnitine profiles will be drawn prior to blood transfusion administered between the first and second weeks of life)
c. aEEG readings will be obtained within 2-3 days of study initiation and at the following post-menstrual ages: 28 weeks (if infant <27 weeks at birth), 32 weeks', 36 weeks', and at 40 weeks' (if infant is still admitted to the NICU).
d. 36-38 weeks' corrected age
   i. Cranial MRI
e. 40 weeks GA (+/- 2weeks)
   i. Neurodevelopmental assessment
f. Neurodevelopmental assessment at 18-24 months corrected age
   i. Bayley Scales of Infant and Toddler Development III (BSID-III)
   ii. Modified Checklist for Autism in Toddlers (M-CHAT)
   iii. Child Behavior Checklist (CBCL)

Individual Infant Timeline:

1. Study Enrollment (within 72 hours of birth)
2. Study supplement given (within 24 hours of enrollment for total 2 weeks or until enteral intake is at 100 cc/kg/day)
3. Data collection throughout the NICU course (Growth parameters, fluid and nutrition intake and important hospital events), also includes interview with mother to record demographic information and maternal characteristics
4. Blood carnitine panels – drawn at study enrollment and at the end of the intervention (and again if a blood transfusion is administered within this period)
5. Amplitude-integrated EEG readings – within 2-3 of study enrollment and at 28, 32, 36 and 40 weeks' post-menstrual age (dashed lines - - - -)
6. Hearing screens – OAE and BAER during the NICU course
7. Brain MRI at 36-40 weeks' GA
8. NNNS at term-equivalent follow-up visit
9. Neurodevelopment assessment at 18-24 months corrected age



Serum carnitine profiles: Blood samples (dried blood spots) will be collected at study enrollment and again at two weeks of lifefor measurement of acylcarnitine profiles as well as total and free carnitine levels. Collected samples will be batched and shipped to Baylor College of Medicine. As blood transfusions may impact carnitine levels, these studies will be drawn prior to a blood transfusion required between the first and second weeks of life.

Study specimens will be stored and used for future research purposes provided parents give consent for the storage and future use of the samples. Clinical information will be linked to the study specimens.

Data collection: After study enrollment, medical history and baseline data will be collected for each infant enrolled in the study. Prospective data will be recorded on a weekly basis throughout the NICU stay and after discharge at specific time points. Data will be recorded on standardized case report forms (CRFs) organized into the following categories:

1. Patient demographics: date of birth, birth weight, gestational age as determined by best obstetric estimate or Ballard score, SGA or LGA status, Apgar scores, birth history, delivery room management, gender, ethnicity.
2. Maternal characteristics: maternal age, maternal language, ethnicity, presence of prenatal care, type of insurance coverage, maternal educational status, presence of maternal depression, teratogen exposure (alcohol, tobacco or illicit drug use during pregnancy), antenatal steroid use, hypertension/pre-ecclampsia, gestational diabetes/Type I or II diabetes, and maternal infections.
3. Management of fluid and nutrition: daily fluid intake and urine output, glucose infusion rate, total caloric intake, total protein intake, and food efficiency (weight gain divided by caloric intake) while on study supplement.
4. Hospital course:
    a. Growth parameters: weight, length and head circumference at birth, daily weight until birthweight regained and then weekly, weekly length, and head circumference two times per week for four weeks)
    b. Cardiovascular: need for vasopressors,presence of PDA (including medical or surgical treatment; platelet count and mean fluid intake at time of treatment)
    c. Gastrointestinal: feeding intolerance, NEC, presence of gastrointestinal reflux,
    d. Neurological: results of aEEG readings, results of head ultrasounds including presence of germinal matrix/intraventricular hemorrhage and periventricular leukomalacia, cranial MRI results
    e. Opthalmological: grade of retinopathy of prematurity and treatment or ophthalmologic diagnosis,
    f. Hematologic: transfusion history including dates
    g. Infectious disease: presence of concurrent presumed and proven infection,
    h. Other: performance of any surgical procedures, duration of intravenous access, use of medications, and hearing screen results (OAE and/or ABR) with observed thresholds of hearing frequencies.
    i. Clinical Risk Index for Babies (CRIB) scores will be calculated for each infant.
    j. We will also monitor for possible short-term side effects.
5. Discharge information: weight, length, head circumference, and diagnoses at time of discharge, routinely-ordered discharge brain MRI findings
6. Neurodevelopmental assessment:
    a. Study patients will be followed at 40 weeks PMA (+/- 2 weeks) to assess neurodevelopmental outcomes among survivors (including incidence of cerebral palsy, neurobehavioral delay, blindness and deafness). Patient information such as weight, length, head circumference, and inter-current illness will be recorded. Infants will be tested using the NICU Network Neurobehavioral Scale, and their scores recorded.
    b. Study patients will be followed at 18-24 months corrected age to assess neurodevelopmental outcomes. During this follow-up visit, we will administer the following:
        i. Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III)
        ii. Modified Checklist for Autism in Toddlers (M-CHAT)
        iii. Child Behavior Checklist (CBCL)

Amplitude-integrated EEG measurements (aEEG)

It has previously been reported that background aEEG activity is concordant with the neurological examination and with background patterns on full-scale EEG.[43,44] Furthermore, in preterm infants, aEEG appears to have a predictive value for developmental outcomes at 3 years of age.[45] We will, therefore, use aEEG recordings for neurophysiological assessments in our study population. Sedation is not required for this procedure.

We will perform 3-hour aEEG recordings within 2-3 days of initiation of carnitine or placebo supplementation and then at the following PMA: 28 weeks', 32 weeks', 36 weeks', and at 40 weeks' PMA if infant is still admitted to the NICU. aEEG readings will be recorded from the surface of the brain using surface disc electrodes and a Cerebral Function Monitor (CFM) System (Olympic Brainz Monitor; Natus Medical Inc., San Carlos, CA). The Brainz monitor is a bedside brain monitor designed for continuous use in the NICU environment. With its 2-channel capability, this system offers clinicians the ability to monitor brain function at the bedside displaying both real time EEG activity and a time-compressed trend of EEG called amplitude integrated EEG (aEEG) from both cerebral hemispheres. The EEG recordings are stored as downloadable digital files for offline analysis and archiving. The quality of the recording will be monitored by continuous impedance tracing. The tracings will be assessed for both quantitative and qualitative characteristics observed in the whole tracing. We will assess the following: (1) continuity, (2) sleep-wake cycling, (3) voltage and span or bandwidth of the signals, and (4) presence or absence of seizure activity.

These aEEG recordings are non-invasive and do not create additional risk for the patient. These aEEG readings will be performed by study personnel and will be provided at no additional cost to the patient.

MRI Brain Imaging

Imaging of the brain at term-equivalent is the standard of care for the evaluation of preterm infants prior to being discharged home. In our Neonatal Intensive Care Units, we routinely obtain brain MRI at ≥ 36 weeks' corrected age (prior to discharge home). The MRI examination is performed in sleeping infants after they have been fed; therefore, sedation is not required for the procedure. MRI will be performed per routine protocol at 36 weeks' corrected age, prior to discharge home. Brain growth will be assessed on anatomical images, through manual segmentation of the basal ganglia, cerebellum and whole brain. White matter development will be assessed on diffusion tensor imaging (DTI), using the tract-based spatial statistics method. The DTI measures fractional anisotropy and mean diffusivity, which have previously been shown to be abnormal in ex-preterm children; these will be compared in multiple white matter regions (cerebral peduncle, posterior limb of the internal capsule, corona radiata, corpus callosum, and optic radiations).[37,46,47]

Brain metabolism will be assessed using proton MR spectroscopy, obtained from both white matter (centrum semiovale) and gray matter (basal ganglia) structures. The relative concentrations of the brain metabolites choline, creatine and n-acetylaspartate (NAA) will be compared between control and carnitine-supplemented children. Each metabolite reflects specific cellular and biochemical processes. NAA is a neuronal marker and decreases with any disease that adversely affects neuronal integrity. Choline is a constituent of cell membranes and an intermediate of phospholipid metabolism. Choline levels on MR spectroscopy increase with increased membrane turnover, and are taken as a measure of cell proliferation. Creatine plays a role in maintaining energy-dependent systems in brain cells by serving as a reserve for high energy phosphates. Because the creatine peak remains fairly stable even in face of disease, it may be used as a control value.[48] In children born preterm, the NAA/Choline ratio at term-equivalent age correlates with later cognitive development.[43]

These measurements will be obtained through brain MRIs routinely obtained in preterm infants prior to discharge. There will be no change in the routine protocol in order to obtain these outcomes. The reading and analyses of these MRIs will be performed at no additional cost to the study subject.

Neurobehavioral Assessment:

Infants discharged from the Weiler and Montefiore North Division NICUs routinely follow-up with our neonatologists at term-equivalent age. At this visit, the infants routinely receive a neurobehavioral

assessment. We have chosen to administer the NICU Network Neurobehavioral Scale due to its prevalence of use in neonatal research and its strong psychometric properties.[49]

The NICU Network Neurobehavioral Scale (NNNS) was developed by Barry M. Lester, Ph.D., & Edward Z. Tronick, Ph.D.[50] The scale is intended for use with use with healthy and at-risk preterm infants and full term infants at risk because of prenatal substance exposure or other conditions. The NNNS can be utilized in either a clinical or research setting in order to obtain a comprehensive assessment of neurobehavioral functioning. There is a focus on neurologic, stress, and withdrawal signs. The tool may be used with infants through 6 weeks of age and may be used as early as 30 weeks for preterm infants. The exam assesses 115 items in three categories:

- Neurological items: active and passive muscle tone, primitive reflexes, and central nervous system integrity
- Behavioral state, sensory, and interactive responses
- Stress/abstinence items

The examiner assesses the items. Summary scores are generated for stress, withdrawal, and the major domains of neurobehavioral performance. The NNNS is administered in a standardized format which allows objective assessment of the infant. Normed scores are available for at-risk and healthy infants. Because the assessment is relatively new, there are no long-term studies looking at predictive validity. However, the test is based upon a variety of prior tools, where long-term predictive validity has been shown.[51] The test takes approximately 20 minutes to administer. (Ordering information: http://www.brookespublishing.com/store/books/lester-7659/index.htm)

We will assess neurodevelopmental outcomes at 18-24 months corrected age using the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III). The BSID-III is a validated instrument used for assessing the development of Cognitive, Language (Receptive and Expressive), Motor (Fine and Gross), Social-Emotional and Adaptive skills in infants and toddlers. This normative assessment yields scaled scores, composite scores, percentile ranks, and developmental age equivalents, all of which provide important information about a child's level of functioning relative to his or her same-age peers. While Cognitive, Language and Motor skills are assessed through direct interaction with the child, Social-Emotional and Adaptive skills are obtained from parent report. Because the BSID-III is commonly used for identifying children who are in need of early intervention due to developmental delays, this instrument will be sensitive to detecting the types of developmental issues we often see in very young children born preterm. Additionally, at the follow-up visit at 18-24 months corrected age, we will administer two parental questionnaires: the Modified Checklist for Autism in Toddlers (M-CHAT) and the Child Behavior Checklist (CBCL). M-CHAT is a parent-report checklist developed to screen children for early signs of autistic features. The CBCL is a standardized, well-validated parent-report questionnaire developed to assess various maladaptive behavioral and emotional problems in young children.

Record confidentiality:Records relating to the study will be kept in a secure location, to which only study personnel will have access. Records will be kept in a locked file cabinet in the neonatology offices and in a password-protected computer.

Statistical Analyses:
Statistical analysis will be performed with the assistance of a statistician.

Sample size:
We plan to recruit a total of 150 infants for our study over approximately 36months' time. This number is based upon an expected attrition of approximately 50 infants over the course of the study and a desired sample size of 100 infants. The inclusion of 100 infants in our study (50 in each study arm) will allow us to detect a moderate difference in our primary outcomes at a significance level of 0.05 with a power of 0.8. (0.5 SD; $\alpha = .05$; power = .80).

Power Calculation**:**

Primary Outcomes	Mean	Standard Deviation	0.5 x Standard Deviation
Percent weight loss*	10.8%	6.2	3.1
Time to regain birthweight*	11.3 days	4.3	2.2
NNNS Scores**			
Habituation	7.91	1.14	0.57
Attention	5.30	1.04	0.52
Arousal	4.16	0.81	0.41
Regulation	5.00	0.82	0.41
Handling	0.27	0.27	0.14
Quality of Movement	3.81	0.78	0.39
Excitability	4.23	2.10	1.05
Lethargy	6.32	3.24	1.62
Nonoptimal Reflexes	4.32	1.73	0.87
Asymmetry	1.93	1.33	0.67
Hypertonicity	0.07	0.26	0.13
Hypotonicity	0.55	0.76	0.38
Stress Abstinence	0.15	0.05	0.03

*Data available from Jack D. Weiler NICU retrospective analysis (2010)
**Tronick, E. Z., K. Olson, et al. (2004). "Normative neurobehavioral performance of healthy infants on the Neonatal Intensive Care Unit Network Neurobehavioral Scale." Pediatrics 113(3 Pt 2): 676-678.

Baseline Comparability:
Distribution of all variables will be examined for normality. Mean (continuous variables), or median (ordinal data and not normally distributed data) will be used as measures of central tendency. Range, percentiles, variance and standard deviation will be used as measures of dispersion. Standard error of the mean and 95% confidence intervals will be calculated to estimate the true populations mean for the placebo and the carnitine supplemented group. Frequency distribution will be presented as grouped, as well as relative frequency. Bivariate distributions will be presented using contingency tables. Descriptive statistics of infant's characteristics at baseline will be compared between carnitine supplemented and placebo group using t-tests for continuous variables (birthweight, age at enrollment) and chi-square test for categorical variables (race, sex, prenatal care, multiple gestation, type of delivery).

Outcome Measures:
The effect of L-carnitine supplementation on outcome measures in enrolled infants will be examined for significance by comparing the two groups using unpaired data analysis with the assistance of a statistician. Continuous data (percent weight loss, time to regain birthweight, NNNS score, BSID-III scores) will be compared between the “placebo” and the “carnitine supplemented” groups by unpaired student t-test (a 2 tailed P value <0.05 will be considered to indicate statistical significance) or Wilcoxon’s rank sum test for non-parametric parameters. Unmatched nominal data will be compared using Fisher’s Exact Test or Chi-square test as required (e.g.: presence or absence of normalization of the carnitine profile; or presence or absence of pathological findings on the discharge MRI). Logistic regression models will be used to adjust for potentially confounding factors including gestational age, birth weight, race, gender, Apgar scores.

Risks and Discomforts:
A brief description of the risks and discomforts associated with this study is included in the protocol consent form and will be discussed in detail with each family at the time of study enrollment.

Carnitine is routinely administered with parenteral nutrition in approximately one-third of the neonatal intensive care units in the United States, although not in the Jack D. Weiler Hospital or Montefiore North Division NICU’s. Carnitine is FDA-approved, and there have been no reports of severe toxicity from

overdose. Data available from the adult literature reports side effects of mild gastrointestinal symptoms such as gastritis, vomiting, diarrhea or abdominal cramping; body odor; and seizures, in patients with or without a known seizure disorder. These side effects are dose-dependent and might be avoided by slow consumption of the solution or greater dilution. One investigator reported transient elevation of triglycerides and increased platelet aggregation in uremic patients with severe renal insufficiency who received high doses of carnitine supplementation.[52]

There is a theoretical risk of reduced closure of patent ductus arteriosus (PDA) in infants treated with indomethacin who receive carnitine supplementation as opposed to placebo.[28] In our previous study, an increase in PDA ligation among infants treated with indomethacin was seen. The observation may have been due to chance. It is also possible that an interaction between carnitine and indomethacin occurred. Indomethacin enhances the process of apoptosis, which mediates PDA closure.[53] Carnitine has been shown to reduce apoptosis in such models as ischemia-reperfusion injury.[54] However, it should also be noted that the group of carnitine-supplemented infants with the increased rate of PDA ligation had lower birthweight and had received more fluids prior to intervention, compared to the infants in the placebo group. Increased fluid intake has been shown to be associated with failure of medical management of PDA.[55]If this effect was in fact associated with decreased apoptosis, it reinforces our hypothesis regarding neuronal protection, a benefit we feel outweighs a theoretical risk of reduced PDA closure.

Despite this incidental finding, there have been no other reports of adverse effects in neonates receiving supplementation doses (approximately 50 µmol/kg/day) although approximately one-third of NICU's in the United States supplement carnitine routinely in parenteral nutrition. In addition, there have been at least 13 clinical trials of carnitine in premature infants which did not report side effects.

We have established a Data and Safety Monitoring Board. The DSMB will meet approximately every 6 months to review safety data. Meetings will be convened via conference calls. Emergency meetings may be convened at any time by the board in the event that questions/concerns regarding patient safety have been raised or if an unexpected AE/SAE occurs that is possibly related to the study. A report will be circulated to the board members approximately 2 weeks before the board meeting in order to provide them with adequate time to review the materials.

Infants in our study will be monitored closely in order to detect unforeseen side effects. All AEs will be collected and recorded as part of the study. For each, the PI will include the following assessments:

- Anticipated/unanticipated
- Relationship to the study intervention
- Outcome

The DSMB will review all adverse events as described in the Charter. All unanticipated problems will be reported to the DSMB and IRB within 5 days of the PI becoming aware of the problems.

With the upcoming revisions in the IRB AE reporting policy, we have decided to not follow the current IRB adverse event reporting policy dated 2008; instead we will report AEs to the IRB as follows:

1. Unanticipated Problems defined as anyevent,deviation,orproblem thatmeetsallofthefollowingcriteria:
   - unanticipated; AND
   - possibly,probablyordefinitelyrelatedto studyparticipation;AND
   - fatal,life-threatening,orseriousORsuggestsgreaterriskofharmtostudy participant(s)or othersthanwaspreviouslyknownorrecognized
2. DeathofaparticipantifitoccurswithIn30 daysofadministrationofthestudymedication
3. AProtocolDeviation or an incident, experience,oroutcomethatmayplacetheparticipantorothersatgreatermedical, physiological,socialriskoreconomicriskthanwaspreviouslyknownorrecognized
4. AnydeviationfromIRBorInstitutionalPolicyorProcedurewhichhasthepotentialto adverselyimpactoneormoresubjectsortheoverallintegrityofdatacollected
5. AnyreportingthattheIRBcitesasaconditionofapprovaloftheprotocol
6. Complaintfromaparticipantorotherindividualwhenthecomplaintindicatesunexpectedrisksorthecomplaintcannotberesolvedbytheresearchteam

7. DeviationfromtheIRBInformedConsentPolicy
8. Systematicdatacollectionerrors
9. Breachofconfidentiality

It is well-known that the very preterm, low birth weight infants included in this study have increased mortality compared to full-term infants. Review of our own Weiler NICU data in 2010 revealed a high mortality rate ininfants less than 30 weeks gestational age: approximately 75% for infants of gestational age of 23 weeks, 24% for gestational age 24-26 weeks, and 5% for infants of gestational age 27 – 29 weeks. Thus, we expect similar rates in infants enrolled in our study.

An additional amount of blood will be drawn as part of the study protocol. The estimated amount of blood is approximately 200 – 300 µl two to three times over the course of the study. The blood draw will be obtained with other routine bloodwork whenever possible in order to avoid additional discomfort to the infant.

Benefits:
Carnitine has been shown to have anti-oxidant effects, enhance cellular function and prevent apoptosis. Supplementation has been associated with protection of neurons against hypoxic-ischemic injury in both in vitro and in vivo models. In preterm infants, carnitine supplementation is associated with decreased time to regain birthweight. Thus, potential benefits of carnitine supplementation in premature infants include hypoxic-ischemic neuronal injury protection and enhanced brain growth, leading to improved neurodevelopmental outcomes.

Problems with neurodevelopment are a well-known morbidity for infants born very preterm. Thus, it is important from a public health and scientific standpoint to determine whether carnitine supplementation has an impact on this outcome. The potential identification of an important physiological benefit of carnitine could lead to changing current recommendations for nutritional supplementation for preterm infants to include carnitine.

Financial Compensation:
No financial compensation is planned. Carnitine supplementation will not be charged to the patient or insurance company.

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