THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Wills A-M, Hubbard J, Macklin EA, et al. Hypercaloric enteral nutrition in patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet* 2014; published online Feb 28. http://dx.doi.org/10.1016/S0140-6736(14)60222-1.

Supplementary Appendix

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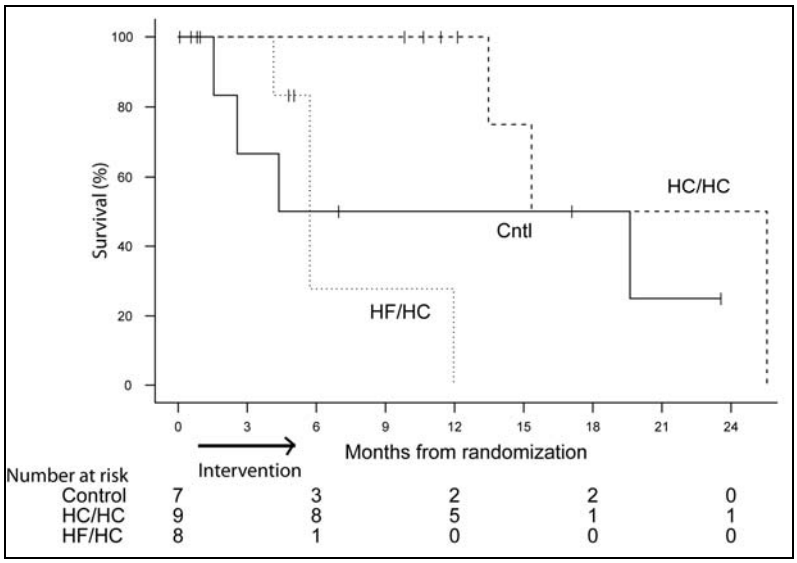
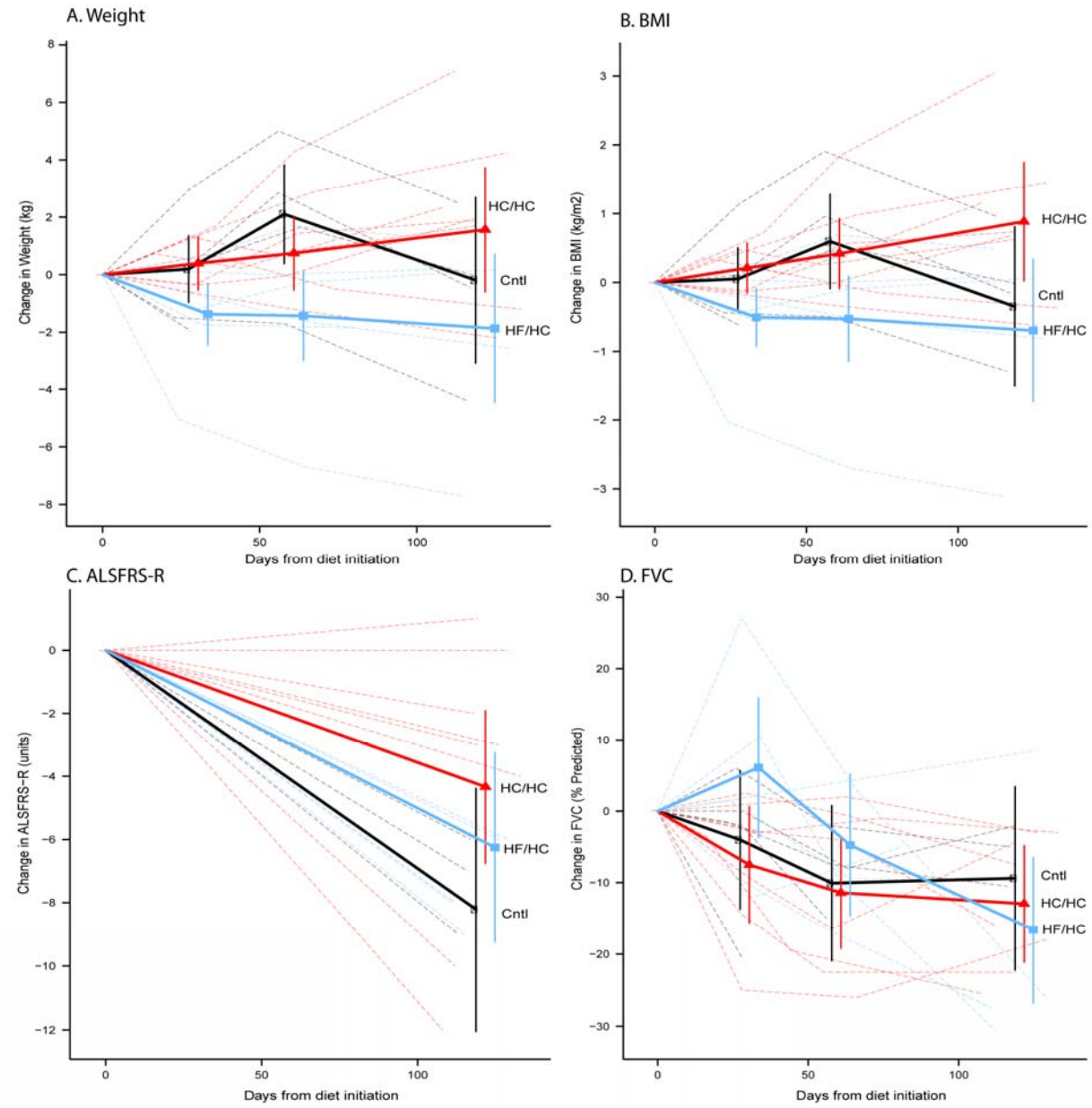


Supplementary Figure 1: Kaplan-Meier curves for ventilator-free survival. HC/HC=high-carbohydrate/high-calorie diet; Cntl=Control diet; HF/HC= high-fat/ high-calorie diet. The estimated median survival in the HC/HC arm was 20•5 months vs•12 months in the controls and 5•7 months in the HF/HC arm. However, this is unreliable due to the fact that participants were no longer on study diet after month five and due to the high degree of censoring. The log-rank test for the difference across all treatments was p=0•070. The log-rank test for the difference between the HC/HC and Cntl arms was p=0•22.



Supplementary Figure 2: Change in secondary outcome measures from baseline. The high-carbohydrate/high-calorie (HC/HC) is shown in red; control diet (Cntl) is shown in black; the high-fat/ high-calorie diet (HF/HC) is shown in blue. Shown are adjusted means and 95% confidence intervals superimposed on unadjusted spaghetti plots for weight (in kg, Panel A), BMI (in kg/m^2, Panel B), ALSFRS-R (in units, Panel C), and forced vital capacity (in percent predicted, Panel D). None of these secondary outcomes were statistically significant.

Supplementary Table 1: Full Inclusion/Exclusion Criteria

To be eligible for enrollment into this study, research participants must meet the following eligibility criteria within 21 days prior to the Baseline visit:

1. Participants with familial or sporadic ALS diagnosed as suspected, possible, laboratory-supported probable, probable, or definite according to the World Federation of Neurology El Escorial criteria ; subjects may have either limb-onset or bulbar-onset disease.
2. Male or female subjects aged 18 years or older.
3. Subjects must be capable of providing informed consent and complying with trial procedures.
4. Subjects must have competent caregiver able to assist with tube feeding.
5. Subjects must have already tolerated tube feeding through either a gastrostomy tube or jejunostomy tube with a minimum tube size of French 8.
6. Subjects must require non-invasive ventilation for less than 10 hours/day, and in the judgment of the Investigator, be able to complete this study.
7. Subjects may be taking riluzole at a stable dose for the previous 60 days.
8. Women must not be able to become pregnant (e.g. post menopausal, surgically sterile, or using adequate birth control methods) for the duration of the study. Adequate contraception includes: abstinence, hormonal contraception (oral contraception, implanted contraception, injected contraception or other hormonal (patch or contraceptive ring, for example) contraception), intrauterine device (IUD) in place for ≥ 3 months, barrier method in conjunction with spermicide, or another adequate method (as determined by steering committee member review). Women of childbearing potential must have a negative pregnancy test at screening and be non-lactating.

Exclusion Criteria

1. Clinical evidence of unstable medical or psychiatric illness in the investigator's judgment.
2. Concurrent enrollment in a clinical trial of an investigational agent. Phase IV studies and open-label extensions of completed trials are allowed if the subject has been on a stable dose for at least 60 days.
3. History of hepatitis including non-alcoholic steatohepatitis (NASH), cholecystectomy, prior biliary disease such as gallstones, diabetes, prior myocardial infarction or stroke.

4. Laboratory values: Screening alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than 2.0 times the upper limit of normal or total bilirubin greater than 1.5 times the upper limit of normal.
5. Allergy to soy, fish, or milk products.
6. Prior intolerance of Jevity 1.0, Jevity 1.5 or Oxepa.
7. Absence of adequate social support and cooperation, or personal motivation (in the judgment of the investigator) to complete the study satisfactorily.

Major Protocol Deviations:

1. One participant in the Jevity 1.5 arm received incorrect tube feeds (Jevity 1.0) for 2 days before the error was identified and corrected.

Checklist of Items for Reporting Trials of Nonpharmacologic Treatments*

Section	Item	Standard CONSORT Description	Extension for Nonpharmacologic Trials	Reported on Page No.
Title and abstract†	1	How participants were allocated to interventions (e.g., "random allocation," "randomized," or "randomly assigned")	In the abstract, description of the experimental treatment, comparator, care providers, centers, and blinding status	1
Introduction				
Background	2	Scientific background and explanation of rationale		1
Methods				
Participants†	3	Eligibility criteria for participants and the settings and locations where the data were collected	When applicable, eligibility criteria for centers and those performing the interventions	2
Interventions†	4	Precise details of the interventions intended for each group and how and when they were actually administered	Precise details of both the experimental treatment and comparator	2-3
	4A		Description of the different components of the interventions and, when applicable, descriptions of the procedure for tailoring the interventions to individual participants	3
	4B		Details of how the interventions were standardized	3
	4C		Details of how adherence of care providers with the protocol was assessed or enhanced	3
Objectives	5	Specific objectives and hypotheses		2
Outcomes	6	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (e.g., multiple observations, training of assessors)		3
Sample size†	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	When applicable, details of whether and how the clustering by care providers or centers was addressed	4
Randomization–sequence generation†	8	Method used to generate the random allocation sequence, including details of any restriction (e.g., blocking, stratification)	When applicable, how care providers were allocated to each trial group	2

Allocation concealment	9	Method used to implement the random allocation sequence (e.g., numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned		2
Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups		2
Blinding (masking)†	11A	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment	Whether or not those administering co-interventions were blinded to group assignment	2
	11B		If blinded, method of blinding and description of the similarity of interventions†	2
Statistical methods†	12	Statistical methods used to compare groups for primary outcome(s); methods for additional analyses, such as subgroup analyses and adjusted analyses	When applicable, details of whether and how the clustering by care providers or centers was addressed	4
Results				
Participant flow†	13	Flow of participants through each stage (a diagram is strongly recommended)--- specifically, for each group, report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome; describe deviations from study as planned, together with reasons	The number of care providers or centers performing the intervention in each group and the number of patients treated by each care provider or in each center	Figure 1
Implementation of intervention†	New item		Details of the experimental treatment and comparator as they were implemented	2-3
Recruitment	14	Dates defining the periods of recruitment and follow-up		2
Baseline data†	15	Baseline demographic and clinical characteristics of each group	When applicable, a description of care providers (case volume, qualification, expertise, etc.) and centers (volume) in each group	Table 1
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether analysis was by “intention-to-treat”; state the results in absolute numbers when feasible (e.g., 10/20, not 50%)		Figure 1

Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group and the estimated effect size and its precision (e.g., 95% confidence interval)		4-6, Table 3
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those prespecified and those exploratory		6
Adverse events	19	All important adverse events or side effects in each intervention group		5, Table 2
Discussion				
Interpretation†	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision, and the dangers associated with multiplicity of analyses and outcomes	In addition, take into account the choice of the comparator, lack of or partial blinding, and unequal expertise of care providers or centers in each group	6,7
Generalizability†	21	Generalizability (external validity) of the trial findings	Generalizability (external validity) of the trial findings according to the intervention, comparators, patients, and care providers and centers involved in the trial	6
Overall evidence	22	General interpretation of the results in the context of current evidence		7

*Additions or modifications to the CONSORT checklist. CONSORT = Consolidated Standards of Reporting Trials.
†This item was modified in the 2007 revised version of the CONSORT checklist.

Trial of High fat/High Calorie Diet versus Optimal Nutrition in Amyotrophic Lateral Sclerosis

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Supported By: **Muscular Dystrophy Association**

Sponsor of IND: Not Applicable

Protocol Date: **September 2, 2011**

Protocol Version: **Version 6.0**

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Appendix 10: World Federation of Neurology El Escorial criteria

INVESTIGATOR'S AGREEMENT

I have read the attached protocol entitled, "Trial of High Fat/High Calorie Diet versus Optimal Nutrition in Amyotrophic Lateral Sclerosis," dated **September 2, 2011** (**Version 6.0**) and agree to abide by all described protocol procedures. I agree to comply with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects, the International Conference on Harmonisation Tripartite Guidelines on Good Clinical Practice, applicable U.S. Food and Drug Administration (FDA) regulations and guidelines identified in 21 CFR Parts 11, 50, 56, and 312.7, the applicable provisions of sections 402(i) and 402(j) of the U.S. Public Health Service Acts (PHS Act) [42 U.S.C. §§ 282 (i) and (j)], amended by Title VII of the FDA Amendments Act of 2007 (Public Law No. 110-85, 121 Stat.904), local Institutional Review Board (IRB) guidelines and policies, and the U.S. Health Insurance Portability and Accountability Act (HIPAA).

Principal Investigator Signature: _____ Date: _____

Print Principal Investigator Name:

_____

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Co- Principal Investigator Signature: _____ Date: _____

Print Co- Principal Investigator Name:

_____

PRÉCIS

Title

Trial of High Fat/High Calorie Diet versus Optimal Nutrition in Amyotrophic Lateral Sclerosis

Objectives

Primary Objectives

The primary objectives of this study are to determine the safety and tolerability of high fat/high calorie versus high calorie versus normal tube feed diets in subjects with ALS.

Secondary Objectives

The secondary objectives are to measure biomarkers of body composition and lipid metabolism before and during diet intervention on the three diets, including lipid levels, weight, body mass, body composition and preliminary effects on disease progression.

Background and Rationale

Amyotrophic lateral sclerosis is a progressive neurodegenerative disorder affecting upper and lower motor neurons. Survival is typically 2 to 5 years from symptom onset; death is usually from respiratory paralysis. Standard therapy is with Riluzole 100 mg/day, a FDA approved treatment for ALS that has a small effect on survival [1, 2]. There is a strong need for more effective therapies in ALS.

Progressive weight loss is a common symptom of ALS and correlates with disease progression and time to death [3]. Persons with ALS have a hypermetabolic state with increased energy expenditure in the setting of reduced caloric intake [4, 5]. Kasarskis et al. first recommended increasing calorie intake in patients with ALS based on a retrospective review of ALS subjects close to the time of death, showing that these subjects consumed only 84% of the recommended daily allowance of calories [3, 6]. However the type of caloric intake and exact amount has not been adequately studied (reviewed in [7]).

Several studies have now shown that a high fat diet can dramatically slow disease progression in the mutant SOD1 mouse model. Specifically, a high fat diet consisting of 38% carbohydrates, 47% fats and 15% protein (by calorie content) increased the median survival time of G93A SOD1 mice from 140 to 270 days [8]. Therefore, the primary question we wish to address with this trial is whether a high fat/high calorie diet will be safely tolerated by people with ALS. The long-term goal is to determine whether such a diet can slow ALS progression.

Study Design and Outcomes

We propose a phase II double-blind placebo-controlled clinical trial to study the safety, tolerability and feasibility of a high fat/high calorie diet versus high calorie diet versus normal diet. Secondary outcome measures include lipid levels, weight, body mass, body composition and preliminary effects on disease progression, on the three diets. Ten subjects from approximately 10-20 ALS centers who are already receiving percutaneous nutrition will be randomized to each of the three treatment arms and followed for five months. Energy needs for each subject will be calculated based on measured energy expenditure using indirect calorimetry and basal metabolic rate (BMR). The control diet will be treated with optimal calorie

replacement while both intervention arms will be provided a high calorie diet 1.25 times their energy needs. Subjects and the evaluating investigators will be blinded to treatment assignment. Primary outcome measures will be adverse events and compliance rates. Subjects will remain in the study 4 weeks after the end of the dietary intervention, allowing time to observe adverse events in respect to the primary endpoint of safety.

Secondary outcome measures: We will test biomarkers of body composition and lipid metabolism before and during diet intervention. Serum levels of total cholesterol, HDL, non-HDL cholesterol, calculated LDL, triglycerides, and oxidized cholesterol will be measured at enrollment in a fasting state and postprandial to test whether there is an appropriate rise in levels after the dietary intervention. Subjects will also be assessed at enrollment and at study end for BMI, fat mass (FM) and fat-free mass (FFM), using dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA) and skinfold-thickness measurements (SKF). Finally, we plan to examine preliminary effects of the three diets on measures of disease progression. We will examine trends in disease measures on the three diets including weight loss, the ALS Functional Rating Scale-Revised (ALSFRS-R), forced vital capacity (FVC), grip strength and survival.

The use of an electronic data capture system will allow for rapid decision making with regard to safety and study continutation. For all stages of the study, adverse event monitoring will be performed on a regular basis, in addition to the outcome measures.

Interventions and Duration

Administration of Intervention

The intervention tube feed diet will be administered at the baseline visit and given to subjects to take home. Continued supply of the tube feed will be shipped to subjects at month 3 and given to subjects at the 1 and 2 month visits. The control arm consists of Jevity 1.0, a standard tube feed, which has a caloric density of 1 calorie/milliliter. The intervention tube feeds have caloric densities of 1.5 calories/milliliter, which will allow the three study arms to remain blinded in terms of tube feed volume. Subjects and caregivers will be instructed how to administer the tube feed either in boluses or in a continuous infusion based on gravity.

Duration of Participation

The total study length from first enrolled subject will be approximately 28 months. Each subject will be followed for approximately 5 months including 4 months while they receive the tube feed intervention.

Subject Recruitment, Sample Size and Population

Participants in this study will be subjects with familial or sporadic ALS diagnosed as suspected, possible, laboratory-supported probable, probable, or definite, according to the World Federation of Neurology El Escorial criteria [9, 10]. Subjects must already have tolerated tube feeding through either a gastrostomy tube or jejunostomy tube. Diagnostic and Inclusionary/Exclusionary criteria will be clearly outlined in the protocol. A total of 30 subjects will participate in the study at approximately 10-20 centers across the US.

Randomization

A total of 30 eligible subjects from approximately 10-20 centers will be randomized (1:1:1) using a web-based computer-generated system. The randomization plan will be provided by the MGH Biostatistical center and randomization will be blocked by center. Randomization will be blocked to ensure that the treatment groups are balanced within a site after a certain number of subjects have been enrolled at that site.

LIST OF ABBREVIATIONS

AAN	American Academy of Neurology
AE	Adverse Event/Experience
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scores – revised
ALT	Alanine aminotransferase/ serum glutamic pyruvic transaminase/SGPT
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate aminotransferase/ serum glutamic oxaloacetic transaminase/ SGOT
βhCG	Beta-Human Chorionic Gonadotropic
BIA	Bioelectrical Impedance Analysis
BIPAP	Bilevel Positive Airway Pressure
BMI	Body Mass Index
BMP	Basic Metabolic Panel
BMR	Basal Metabolic Rate
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CE	Clinical evaluator
CK	Creatine Kinase/ Creatine Phosphokinase
CRF	Case report form
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CNS	Central nervous system
CO_2	Carbon Dioxide
COSTART	Coding Symbol Thesaurus for Adverse Event Reporting
CRC	Clinical Research Center
CTA	Clinical Trial Application
CTCAE	Common Terminology Criteria for Coding Adverse Events
CTSC	Clinical and Translational Science Center
CV	Curriculum vitae
DM	Data Management
DSMB	Data Safety Monitoring Board
DXA	Dual-energy Xray Absorptiometry
eCRF	Electronic Case Report Form
EDC	Electronic data capture
EKG	Electrocardiogram
FALS	Familial amyotrophic lateral sclerosis
FDA	Food and Drug Administration
FFM	Fat Free Mass
FM	Fat Mass
FVC	Forced vital capacity
GCP	Good Clinical Practice
GCRC	General Clinical Research Center
GIQLI	Gastrointestinal Quality of Life Index
HDL	High-Density Lipoprotein
HPLC	High performance liquid chromatography

HRC	Human Resource Committee
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IGF-1	Insulin-like growth factor
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
LDL	Low-Density Lipoprotein
LFT	Liver function test
MDA	Muscular Dystrophy Association
MGH	Massachusetts General Hospital
MND	Motor neuron disease
MOP	Manual of Operations
MRC	Medical Research Council
MREE	Measured Resting Energy Expenditure
MREM	Millirem
MVI	Multi-Vitamin Infusion
NASH	Non-Alcoholic Steatohepatitis
NCTU	Neurology Clinical Trials Unit
NEALS	Northeast Amyotrophic Lateral Sclerosis
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NP	Nurse Practitioner
ODBC	Open Database Connectivity
OHRP	Office for Human Research Protections
PA	Physician's Assistant
PAV	Permanent assisted ventilation
PEG	Percutaneous Endoscopic Gastrostomy
PDF	Portable Document Format
$P_{ET_{CO2}}$	End Tidal carbon dioxide partial pressure
PI	Principal Investigator
pO_2	Partial Pressure of Oxygen
qd	Quaque die / once-a-day
RIG	Radiological Inserted Gastrostomy
RN	Registered Nurse
RQ	Respiratory Quotient
RS2	Randomization system 2
SAE	Serious adverse event
SALS	Sporadic amyotrophic lateral sclerosis
SDF	Source Document Form
SKF	Skinfold Thickness
SOD1	Superoxide dismutase-1
SUNY	State University of New York
T	Telephone data
TDEE	Total Daily Energy Expenditure
TG	Triglycerides
V_1	Apparent volume of the central compartment

VC	Vital capacity
WBC	White blood cells
WHO	World Health Organization

1. STUDY OBJECTIVES

1.1 Specific Aims

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that usually leads to death in 2 to 5 years. Rapid weight loss is a hallmark of the disease, due to inadequate caloric intake and, in 80% of patients, a hypermetabolic state. Patients are often instructed to increase their calorie intake; however, the ideal amount and type of increased caloric diet has not been assessed in a scientific manner in humans. Results from retrospective case-control analyses and animal models suggest that a high fat diet may reduce the risk of ALS and slow the rate of ALS progression. We therefore propose a phase II double-blind placebo-controlled clinical trial to study the safety, tolerability and feasibility of a high fat/high calorie diet versus high calorie diet versus normal diet. Secondary outcome measures include lipid levels, weight, body mass, body composition and preliminary effects on disease progression, on the three diets.

Aim 1: To compare safety and tolerability in three subject groups randomized to high fat/high calorie, high calorie, or control diets. Twenty subjects receiving percutaneous nutrition will be randomized to each treatment arm and followed for five months. Energy needs will be calculated based on measured energy expenditure using indirect calorimetry and basal metabolic rate (BMR). The control diet will be treated with optimal calorie replacement while both intervention arms will be provided a high calorie diet. Subjects and the evaluating investigators will be blinded to treatment assignment. Endpoints will be adverse events and compliance rates.

Aim 2: To measure biomarkers of body composition and lipid metabolism before and during diet intervention. Serum levels of total cholesterol, HDL, non-HDL cholesterol, LDL, triglycerides, and oxidized cholesterol will be measured at baseline in a fasting state and 2-4 hours postprandial. Fasting blood levels will be repeated at 2 and 4 months. Subjects will also be assessed at enrollment and at study end for BMI, fat mass (FM), fat-free mass (FFM), dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA) and skinfold-thickness measurements (SKF). We will test the effects of diet on lipid metabolism and body composition.

Aim 3: To examine preliminary effects of the three diets on measures of disease progression. The study is not powered to detect clinical efficacy. We will however examine trends in several disease measures including weight loss, the ALS Functional Rating Scale-Revised (ALSFRS-R), forced vital capacity (FVC), grip strength and survival.

1.2 Study Overview

Approximately 30 ALS subjects requiring percutaneous nutrition from approximately 10-20 centers will be randomized (1:1:1) to a high fat/high calorie diet, high calorie diet, or control diet for a total of 4 months. Safety and tolerability will be the primary outcomes. Biomarkers of lipid metabolism and body composition will be measured and will constitute secondary outcome measures. Adverse events will be monitored throughout the study and assessed by an independent data safety monitoring board (DSMB) every 3-6 months. Changes in vital capacity, ALSFRS-R score and grip strength will be collected. Although the study is not powered to look at the effects of the diet interventions on disease progression, we will examine trends in these tertiary outcome measures.

This randomized controlled pilot study will provide information needed to design a phase III efficacy study on the safety, feasibility and effect on measures of disease progression of a high fat/high caloric diet. Data from this study will contribute to dietary recommendations in an ALS practice parameter. This study addresses an important management question that arises daily in ALS clinics.

2 BACKGROUND AND SIGNIFICANCE

2.1 Background and Rationale

2.1.1 Clinical Features and Epidemiology of ALS

Amyotrophic lateral sclerosis is a progressive neurodegenerative disorder affecting upper and lower motor neurons. Survival is typically 2 to 5 years from symptom onset; death is usually from respiratory paralysis. The incidence of ALS in the U.S. increases from less than 1/100,000 under age 50 to 15/100,000 in those aged 60-69 [11]. Standard therapy is with Riluzole 100 mg/day, a FDA approved treatment for ALS that has a small effect on survival [1, 2]. There is therefore a strong need for more effective therapies in ALS.

Essential features of ALS are progressive signs and symptoms of lower motor neuron dysfunction (atrophy, cramps, and fasciculations) associated with corticospinal tract signs (spasticity, enhanced and pathological reflexes) in the absence of sensory findings [12]**.** There is relative sparing of muscles of eye movement and the urinary sphincters. The course is relentless with decline in strength, respiratory function and overall function with time during the active phase of the disease[13]. Natural history studies have determined that age at onset, site of onset, delay from first symptom to entering ALS clinic, and rate of change in respiratory function are significant covariates of survival [14-17]**.** No study has correlated activity of any surrogate marker with the extent of motor neuron loss as detected by post-mortem examination.

2.1.2 Overview of ALS Pathogenesis

The majority of ALS cases are sporadic (SALS); 10% are familial (FALS). Over 100 mutations of the cytosolic copper-zinc superoxide dismutase 1 (SOD1) have been identified in cases of familial ALS but these account for only 2% of all ALS cases (reviewed in [18]). Forced

expression of high levels of a mutant SOD1 transgene causes progressive motor neuron disease in mice and rats [19]. Six additional genetic defects have now been reported to cause FALS [20-25].

Several hypotheses have been proposed to explain the etiology of ALS in the 90% of cases that are not inherited. These include: oxidative damage, toxicity from abnormal protein folding and intracellular aggregates [5] atypical enteroviral or retroviral infections [26-28] autoantibody-provoked calcium influxes [29, 30], motor neuron excitotoxicity [31], microglial inflammation and elevated levels of COX-2 [32-34]. Environmental risk factors for ALS have been identified in numerous epidemiologic studies. In Guam and several other areas in the Western Pacific, cycasin and the non-protein amino acid b -N-methylamino- L-alanine (BMAA), both of which are found in the cycad nut, cause a disease which appears very similar to ALS[35, 36]. A spastic paraparesis without lower motor neuron involvement has been linked to the chickling pea *Lathyrus sativa* in Bangladesh, China, Ethiopia and India [37] and to Cassava flour in East Africa [38].

2.1.3 Abnormal metabolism in ALS

Progressive weight loss is a common symptom of ALS and correlates with disease progression and time to death [3]. Weight loss is not due to dysphagia alone and is accompanied by loss of both muscle and fat [3]. Persons with ALS have a hypermetabolic state with increased energy expenditure in the setting of reduced caloric intake. Desport et al. found that persons with ALS expend 14% more energy than controls at rest [4, 5]. Sherman et al. found that patients who used non-invasive ventilation required on average 113% more calories/day than their calculated energy needs [39]. Kasarskis et al. found that the measured resting energy expenditure in ALS increased as the disease progressed [3]. Retrospective analysis suggests that subjects who developed ALS were less likely to have been obese pre-morbidly [40] although a recent large prospective epidemiologic study found that an increased BMI was not a significant protective factor [41].

Epidemiologic data suggests that fat intake may reduce the risk of ALS. A recent prospective epidemiologic study of 891,920 subjects found no significant protection from butter intake, although there was a trend (p=0.05 and p=0.06) towards reduced ALS risk for each higher quintile of fatty meat and fried food [41]. Okamoto et al. found in a Japanese case-control retrospective study that the odds ratios for the highest tertile of intake compared to the lowest were 0.41 (95% CI 0.21-0.80) for total fat, 0.30 (95% CI 0.16-0.5) for saturated fatty acids (SFAs), 0.35 (95% CI 0.18-0.69) for monounsaturated fatty acids (MUFAs) and 0.58 (95%CI 0.40-0.96) for polyunsaturated fatty acids (PUFAs) [42]. Veldink in a case-control retrospective study found an odds ratio of 0.4 (95% C.I.0.2-0.7) for developing ALS in the highest tertile of PUFA intake, but not total fat intake [43]. Contrary to this, Nelson et al. reported that in a case-control retrospective epidemiologic study of dietary intake there was a non-significant trend towards increased risk of ALS in subjects who reported a high fat diet, however this was not adjusted for tobacco use [44].

Lipid metabolism may also be abnormal in ALS. Fergani et al. showed that transgenic mutant superoxide dismutase (SOD1) mice have lower postprandial levels of triglycerides, VLDL and LDL, which correlates with weight loss in these mice [45]. Most recently, Dupuis et al. found that ALS subjects with a high (>2.99) LDL to HDL ratio had a median survival12 months longer than those with a low LDL/HDL ratio, suggesting that lipid profiles might be used as a separate prognostic marker [46]. However, the primary difference in lipid profiles was due

to elevated HDL (mean 81 mg/dL) in the poor prognostic group, which can be seen with increased work of breathing and weight loss [47, 48]. Indeed the poor prognostic group had a reduced BMI compared to the high LDL/HDL group. This study did not include dietary intake data, nor were repeat lipid measurements over time obtained to test whether the abnormal lipid profiles were due to or preceded disease progression and weight loss.

2.1.4 High Fat Dietary Intervention as a Potential ALS therapy

Kasarskis et al. first recommended increasing calorie intake in patients with ALS based on a retrospective review of ALS subjects close to the time of death, showing that these subjects consumed only 84% of the recommended daily allowance of calories [3, 6]. However the type of caloric intake and exact amount has not been adequately studied (reviewed in [7]). Nau et al recommended that caloric intake should slightly exceed patients' needs [49]. Stanich performed a small trial of a protein supplement (Meritene containing 18 gm of protein and 275 Kcal) in 20 subjects with ALS for 6 months and found no effect on disease progression or loss of muscle mass, although this was a small, non-placebo controlled study without careful analysis of calorie intake [50].

Several studies have now shown that a high fat diet can dramatically slow disease progression in the mutant SOD1 mouse model. Specifically, a high fat diet consisting of 38% carbohydrates, 47% fats and 15% protein (by calorie content) increased the median survival time of G93A SOD1 mice from 140 to 270 days ([8] and Figure 1a). A high fat diet consisting of

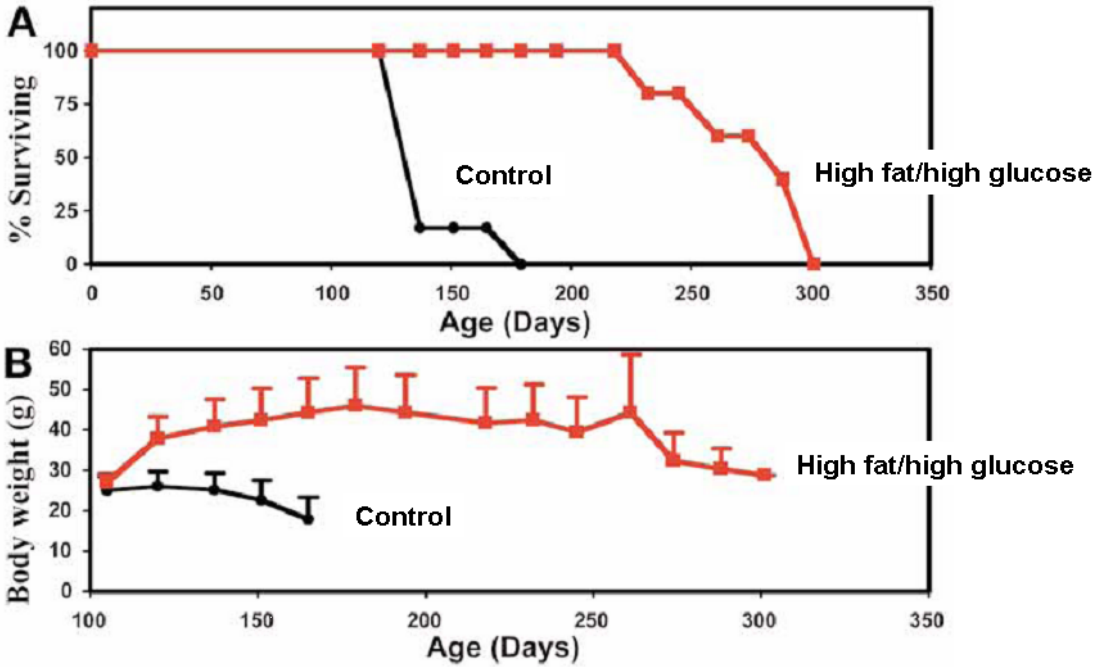


Figure 1(a): From Mattson, 2007: survival of G93A SOD1 mice on a high fat/high glucose diet consisting of 47% fat compared to a normal control mouse diet consisting of 17% fat. 12 mice were litter-matched and gender-matched and started on the diet at 6 weeks. Survival was defined as time to grade 4 paralysis. Median survival in the high fat/high glucose diet was 270 days compared to 140 days in the control group. **(b):** Average weight and S.D. for the mice in each diet group.

21% butter fat and 0.15% cholesterol (by weight) increased the mean survival of G86R SOD1 mice by 20 days in a second study [51]. A ketogenic diet consisting of 60% fat, 20%

carbohydrate and 20% protein did not result in a significant increase in lifespan, however this study only enrolled 11 mice total [52]. Conversely, calorie restriction in the mutant SOD1 mouse model significantly reduces survival [53, 54].

The mechanism by which high fat prolongs survival in the mutant SOD1 mouse is unknown. Phospholipids and cholesterol are essential for axonal membrane assembly and cholesterol biosynthesis is reduced in peripheral nerves during degeneration and regeneration (reviewed in [55]). In experimental models of peripheral nerve injury, there is a dramatic increase in the expression of LDL receptors which allow the regenerating nerve to import cholesterol into the cell, possibly bound to ApoE, for purposes of axonal repair [56-58]. Exogenous LDL, but not HDL, can rescue axonal growth after it has been suppressed by HMGcoA inhibitors in cultured sympathetic neurons [59]. Fergani et al. 2007 recently found increased LDL receptor mRNA levels in liver and muscle, along with reduced LDL but not HDL levels in presymptomatic and diseased mutant SOD1 mice [45]. This may suggest a mechanism by which elevated circulating LDL contributes to prolonged survival in ALS, through survival of peripheral motor neurons. The same group also found that a diet consisting of 21% butter fat normalized serum cholesterol levels which were reduced in the mutant SOD1 mice fed a regular diet [45, 51].

We therefore plan to modify the dietary fat content for people with ALS. Oral nutritional supplements have been studied in other diseases of chronic wasting. Oral supplements have been shown to improve weight gain, mortality and to shorten hospital stays, especially in those whose BMI is less than 20 kg/m2 (reviewed in [60]). Beattie et al. found that supplementation of 1500 kcal/day (Ensure) improved grip strength and reduced infection rates in subjects after gastrointestinal surgery[61]. Rabeneck et al. also found an increase in grip strength, although not statistically significant, in HIV infected men randomized to 960 extra Kcal/day [62]. These studies did not carefully examine the dietary components of their supplements, nor did they calculate the overall calorie intake of their subjects.

In summary, there is strong epidemiologic data showing that weight loss is a common symptom of ALS both in humans and in mice and may contribute to disease progression. There is also data suggesting that increased fat intake and elevated cholesterol might reduce the risk of ALS and the rate disease progression. Finally, data from animal studies strongly suggests that increasing dietary intake of fat ameliorates disease progression over and above increasing calories alone (see animal data below). The primary question we wish to address with this trial is whether a high fat/high calorie diet will be safely tolerated by people with ALS. The long-term goal is to determine whether such a diet can slow ALS progression.

2.1.5 Rationale for Choosing Oxepa

The commercially available tube feed formula from Abbott pharmaceuticals “Oxepa” contains 55% calories from fat and 28% calories from carbohydrate, which is a similar to the composition of the high fat/high protein diet used by Mattson in SOD1 mice (see Table 1 and Figure 3). We have analyzed the fatty acid breakdown of this diet and found that aside from saturated fats, it contains similar amounts of mono- and polyunsaturated fatty acids found in the rodent high fat diet.

Increasing calories through fat may have several beneficial effects. First, the high fat diet is less likely to increase blood glucose levels, which may complicate infections in ALS patients including pneumonia, bronchopneumonia and pressure ulcers. Oxepa has been shown to reduce the incidence of pressure ulcers associated with ICU ventilation [63]. It has also been shown to

improve survival and decrease the rate of respiratory complications in three trials involving Acute Respiratory Distress Syndrome (ARDS, [64-66]. Second, we predict that the high fat diet arm will result in less carbon dioxide (CO_2) production and a lower respiratory quotient than the high calorie arm, similar to that seen in COPD patients fed high fat diets [67]. Oxepa contains significantly more omega-3 essential fatty acids (10.15 versus 2.4 gm/L) than Jevity 1.5 or Jevity 1.0, as well as the polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Jevity 1.0 and Jevity 1.5 contain no EPA or DHA, however these tube feeds are commonly used in ALS patients for long-term nutrition.

2.1.6 Experimental Therapeutics in ALS

Riluzole, a drug that has multiple mechanisms of action including inhibition of release of glutamate at pre-synaptic terminals, is the only drug to have been reported in two controlled studies to extend survival by three months (about 11%) in ALS, although without a concomitant improvement in strength [68]. This is currently the only FDA approved agent for use in ALS. Trials of CNTF [69], gabapentin [70, 71], BDNF [72], Xaliproden [73], topiramate [74], Celebrex [75], creatine [76, 77] and subcutaneous IGF-1 [78] were shown to be ineffective in treating ALS. Talampanel and lithium are currently being tested in clinical trials.

The development of transgenic mice and rats expressing mutant forms of SOD1 [19, 79] has provided a valuable animal model of the disease for understanding pathways that can lead to motor neuron cell death. In an effort to find treatments for ALS, over 160 different therapeutic trials have been published in ALS mice (reviewed in [80]). Most, at least from the published literature, have extremely marginal effects, on average 12-13 days (10%) difference in onset or survival. In spite of the pathologic similarity of their motor neuron cell death, the transgenic mice have not been predictive of human trial outcomes. There are several possible explanations. First, the transgenic mouse was engineered to have early disease onset and a rapid course by greatly overexpressing the human mutant SOD1 gene. Therapeutic efficacy in this model is therefore not directly translatable to human disease, where the mutant gene product is not artificially overexpressed. In addition, although FALS is similar to sporadic ALS with regard to a wide range of cytotoxic events, including evidence for excitotoxicity (e.g. loss of glutamate transport protein), oxidative injury, markers/genes reflecting programmed cell death, and neuroinflammation, total equivalency between sporadic and familial disease has not been demonstrated. In particular, newly identified TDP-43 protein inclusions which are present in sporadic ALS spinal cords have not been found in mutant mice or spinal cords of patients with SOD1 mutations [81, 82]. The lack of a clear predictive relationship between efficacy demonstrated in the transgenic mouse model and human disease raises the question of whether every drug considered for human trial should first be evaluated in the mouse.

2.1.7 Significance

Despite recent critical advances in understanding the pathogenesis of ALS, this remains an untreatable and uniformly lethal disease. There are several reasons to pursue a dietary intervention in ALS subjects despite prior disappointments in ALS clinical trials. First, the effects of a high fat diet in the SOD1 mice were much more robust (mean increased lifespan between 20 to 140 days) than any published drug study to date. Second, there is strong evidence

in humans that weight loss contributes to disease progression. Third, there are no current standard guidelines for nutrition in ALS subjects requiring tube feeds. Finally, regardless of the outcome of this trial, we will gain important information regarding metabolic abnormalities in ALS, which may help decipher the underlying biology of motor neuron diseases and lead to further development of effective treatments.

2.2 Supporting Data

2.2.1 Human Subject Data

Retrospective analysis of all MGH ALS clinic patients who have received percutaneous feeding tubes in the last 2 years reveals a mean survival time of 13.5 months and median survival of 8.3 months (Figure 2). This is in contrast to the previously reported survival time for subjects undergoing radiological inserted gastrostomy (RIG) or percutaneous endoscopic gastrostomy (PEG) of 4.5-8 months [83]. One key difference is that the previous study reported survival in England rather than the U.S. We note that almost 50% of our patients are alive at 2 years and therefore we believe that our subjects should be able to complete the 5 months of study.

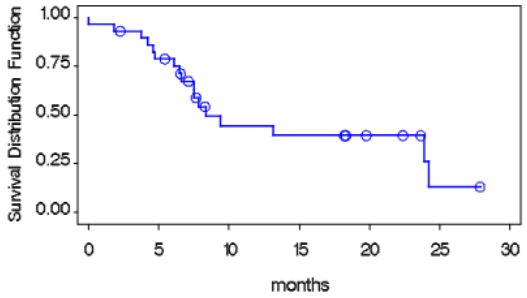


Figure 2: Survival after placement of percutaneous feeding tubes in ALS patients at MGH in the past 2 years. Circles mark censored observations.

Using data from 84 early ALS subjects enrolled in the phase IIa trial of Arimoclomol in ALS [84], we confirmed the decline in BMI seen in prior ALS studies and also demonstrated that total cholesterol declines in parallel to declining BMI (Figure 3 a&b). Despite the large standard

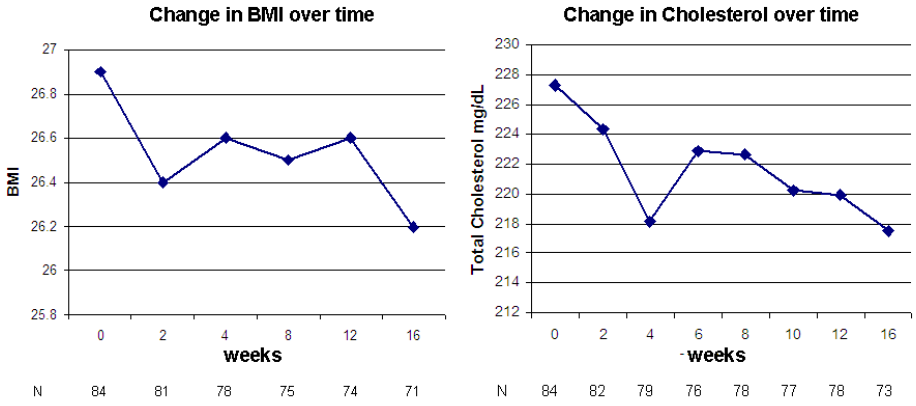


Figure 3(a): Measured BMI in 84 ALS subjects over the Phase IIa Arimoclomol trial in ALS. Change over the 16 weeks was not significant due to the large sample variance. N signifies the number of subjects tested at each time point. **(b)** Measured total serum cholesterol in 84 subjects over the Phase IIa Arimoclomol trial in ALS. This change was significant (p=0.02) over the 16 week trial period.

deviation in total cholesterol, (38.4 mg/dl) there was a significant decline in total cholesterol by 16 weeks, (p=0.02). Because HDL, LDL and triglycerides were not measured, we are unable to determine using this data whether the decline in total cholesterol and BMI was associated with an increase in HDL and decrease in LDL, as we would hypothesize based on the recent article of Dupuis [46].

Animal Studies. *High fat diet significantly prolongs survival in the G93A SOD1mouse.*

Dr. Mark Mattson at the NIH has kindly provided unpublished data comparing various diets in male G93A SOD1 mouse. Survival based on five diets is provided in Figure 4a while the effects of these same diets on weight are found in Figure 4b. The most effective diets by far

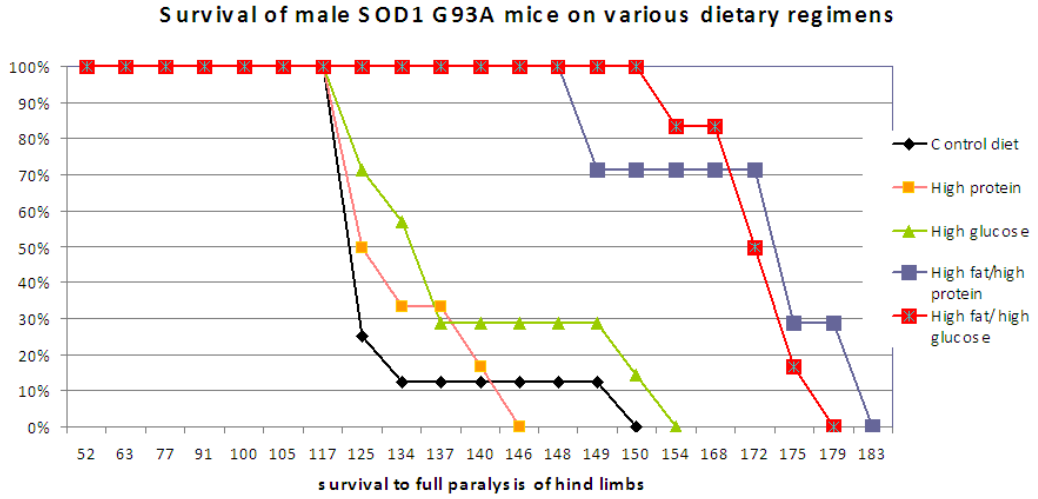


Figure 4(a): Survival of male G93A SOD1 mutant mice on 5 different diets. Survival was defined as the time to grade 4 paralysis. All mice were started at 7.5 weeks and litters were divided into each diet arm. All survival data was collected in a blinded manner. Control diet: N=8, median survival =125 days; High protein diet N=6, median survival=125 days; High glucose diet N=7, median survival =135 days; High fat/high protein diet N=7, median survival =173 days; High fat/ high glucose diet N=6, median survival=172 days.

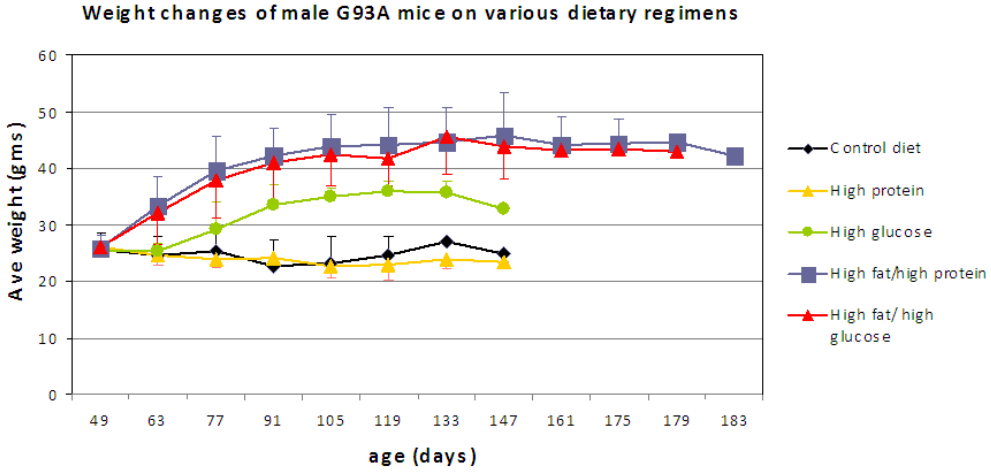


Figure 4(b): Mean measured weights of the male G93A SOD1 mice on each diet. Error bars show standard deviations. Mice fed the high fat/high protein and high fat/high glucose diets consumed 3% more mouse chow by weight, however the primary difference in caloric intake was due to the increased calorie density of the high fat diets (125%-130%). This led to an increase in weight of on average 20 gm/mouse.

were the two high fat diets, which contained 54.6% and 46.6% calories from fat, consisting of soybean oil, butter and lard (Figure 5). The variation in protein and carbohydrate content between the two high fat diets did not affect survival. As shown, the control diet produced a

median survival of 134 days, while the high fat diets produced a median survival of 172 and 173 days. The greater life extension observed in the follow-up study [8] may have been due to the fact that females were included in that study and all animals were started on the diet at an earlier age.

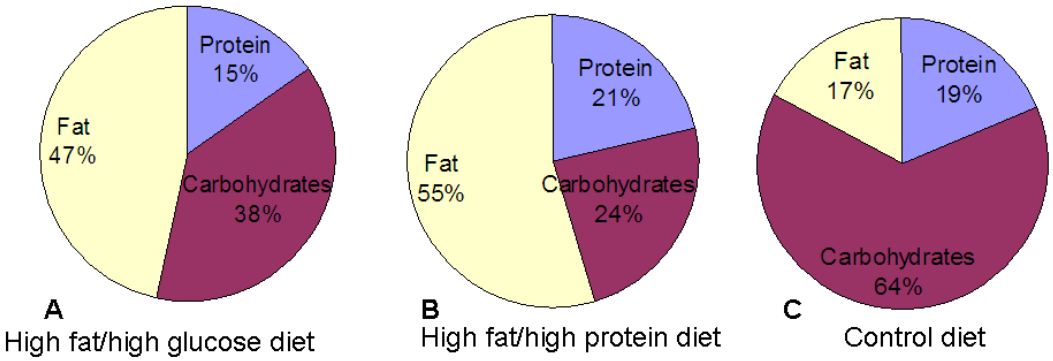


Figure 5(a): Diet composition of the high fat/high glucose diet used in Figures 1& 2 (and in Mattson, 2007). For every 100 grams of mouse chow, 7 grams of soybean oil,14.84 grams of butter and 2 grams of lard were added. This resulted in a calorie content of 464.5 kcal/100gm. **(b)** Diet composition of the high fat/high protein diet from figure 1. For every 100 grams of mouse chow, 9.7 grams of soybean oil,14.84 grams of butter and 5 grams of lard were added. This resulted in a calorie content of 492.2 kcal/100gm. **(c)** Diet composition of the standard control diet used in figure 1 (Mattson, 2007). The only source of fat in this diet is from 7 grams of soybean oil. The total calorie content was 377.4 kcal/100gm.

By comparison, the control rodent diet used in these studies has only 17.4% calories by fat, which is lower than the typical human diet. It is worth noting that the high fat diets contained 1.25 to 1.3 times more calories per gram than the control diet (464.5 kcal/100gm and 492.4 kcal/100gm versus 377.4 kcal/100gm, Figure 5), which primarily explains the increase in weight seen in the high fat diet animals (Figure 4b). The high glucose and high protein diets by comparison had the same caloric density as the control diet. We therefore selected a high fat diet that is 1.25 times the total calories of the control diet to reproduce the high fat animal diets. In order to test whether it is the effect of the elevated fat content or the result of increasing the total number of calories consumed, we are including an arm of the study that will have 1.25 times the number of calories of the control arm, without modifying the source of calories.

2.2.2 Risks of Gastrostomy or Jejunostomy Tubes

Subjects will be enrolled if they have already tolerated percutaneous tube feeds, and will not have feeding tube placement performed as part of the protocol. However, if the subjects experience tube malfunction for any reason (clogging, infection, or the tube falls out), subjects will be able to be seen quickly for unscheduled visits and study staff will help to facilitate their replacement if needed. No clear data currently exists regarding the extent to which gastrostomy tube placement prolongs survival, although current practice parameters suggest that gastrostomy should be considered before pulmonary function is clearly compromised [85]. Studies to determine the effect of gastrostomy on survival are in progress; however, it seems clear that quality of life is improved with appropriate placement [6, 86-88].

2.2.3 Risks of Jevity 1.5/High Calorie Diet

Weight gain is an anticipated risk and would not be considered an adverse event during the trial. Diarrhea is a common side effect of all tube feeds and is commonly treated by adding fiber to the tube feed. Subjects may also experience fullness, nausea or discomfort, which may require the tube feed rate to be reduced. We estimate that the maximum amount of tube feed that any one subject will receive will be approximately 2956 cc/day, which could be comfortably given at a rate of 123cc/hour, which is only slightly greater than our usual maximum tube feed rate of 120cc/hour.

The risk of increasing CO_2 production (increasing the respiratory quotient) through overfeeding has not been previously assessed in patients with respiratory failure due to neurological weakness. However, in ventilated COPD patients, excessive CO_2 production was seen when subjects received 1.5 or more times their resting energy needs [89]. In the same study, in patients receiving 1.3 times their resting energy expenditure, increasing the carbohydrate to fat ratio did not affect CO_2 production (VCO_2). Therefore by feeding subjects 1.25 times the resting energy expenditure, we hope to avoid excessive CO_2 production. Indirect calorimetry to measure CO_2 production will be performed at the 1 and 2 month visits, or earlier in an unscheduled visit in subjects who complain of shortness of breath. In patients with respiratory failure due to neurologic weakness from myasthenia and Guillian-Barre, it has been shown that the ventilatory drive from increased carbon dioxide production remains intact [90]. Therefore we believe ALS patients should be able to report an increased ventilatory drive. We are including a dyspnea questionnaire, FVC, end-tidal CO_2 and pulse oximetry to screen for impaired respiratory function. Subjects who complain of increased respiratory difficulty, have reduced oxygen saturation, increased end-tidal CO_2 or reduced FVC will be evaluated and started on non-invasive ventilatory support as per the AAN Practice Parameters. Because ALS subjects are expected to experience a decline in their respiratory function, any non-acute changes in respiratory function would not be considered an adverse event. An expert pulmonologist is available at the coordinating center for consultation regarding management of increased carbon dioxide production.

2.2.4 Risks of Oxepa

An increase in weight in either the high fat/high calorie or the high calorie diet arms is expected and would not be considered an adverse event. As described in the preliminary animal data, a substantial increase in weight was seen in the two high fat diet arms and correlated with the increase in survival. We will therefore warn subjects that they are likely to gain weight and that we will not consider this an adverse event.

Abbott pharmaceuticals reports no significant increase in adverse effects from Oxepa or Jevity 1.0 or 1.5 compared to other tube feeds, including post-marketing reports. Two thirds of all non-protein calories in Oxepa come from fat, which is slightly less than the 3:1 fat to carbohydrate ratio recommended in patients with advanced COPD [91]. By using Oxepa, we hypothesize that we will be able to increase calorie intake without significantly increasing CO_2 production. However, it is possible that subjects who receive excess calories from Oxepa may have an increase in their respiratory quotient.

Because of the high fat content of Oxepa, we anticipate that patients may experience gastrointestinal side effects such as nausea, fullness or diarrhea. Importantly, Oxepa has not been compared to a diet low in fat to assess relative rates of gastrointestinal side effects. In the

first major study of 146 patients with ARDS assigned to Oxepa versus a control high fat diet consisting of 97% fat calories from corn oil, no difference in gastrointestinal side effects was seen (10/70 versus 12/76) [65]. Diarrhea was seen in 7 of the Oxepa subjects compared to 5 of the control subjects. A trial which compared 55 ARDS patients on Oxepa to 48 patients on a high fat diet consisting mostly of canola oil [64] reported diarrhea in 9 subjects in the Oxepa group versus 7 subjects in the control group. In one published study of Oxepa in non-ICU hemodialysis patients, 3/21 subjects reported diarrhea, however this did not result in study drop-out[92]. No increased incidence of biliary stones or hepatic steatosis was reported in these trials, however in one ICU study, 2/70 subjects in the Oxepa arm and 1/76 subject on the high fat control diet developed pancreatitis [65]. Other reported side effects included thrombocythemia (2/70 subjects) and rash (1/76 subjects).

We anticipate that increasing lipid levels through increased fat intake might possibly increase the risk of cardiovascular disease, and we are therefore testing EKGs at screening to be able to compare if subjects develop symptoms suggestive of cardiac disease. We are also planning to evaluate whether c-reactive protein increases in subjects fed a high fat diet. We are intentionally excluding patients with severe cardiovascular disease such as prior myocardial infarction or stroke. Interestingly, there were fewer cardiac adverse events in the Oxepa arm than the control tube feed diet reported in the trial of 146 patients (0/70 versus 5/76, p=0.06) [65]. An expert endocrinologist specializing in lipid disorders is consulting for this study.

Peak Riluzole serum levels are reduced by meals containing fat, although steady-state levels are not significantly affected. Therefore patients are generally instructed to take Riluzole either 1 hour before or 2 hours after meals. In order to assess whether the increased fat content of Oxepa will affect Riluzole absorption, we will have a pharmacologist Dr. David Greenblatt at Tufts University measure trough Riluzole levels at Screening, 1 month and 4 month visits.

2.2.5 Risks Vitamin E

Oxepa contains 320 IU of vitamin E/liter while the Jevity products contain only 45 IU/liter. Vitamin E supplementation has been tested in ALS and no significant improvement was seen from treatment with 500 mg bid for 12 months [93] and from 5,000 mg/day for 18 months [94]. This was despite the fact that high dose long term vitamin E supplementation was associated with reduced risk of ALS in a large prospective study [95].
Vitamin E may slightly increase the overall risk of death (RR 1.04, 95%C.I. 1.01-1.07) based on a sub-analysis of 26 studies from a large meta-analysis of 68 antioxidant studies [96]. However, no dose-effect was reported and the mean dose used in these studies was 569 IU. In the overall meta-analysis of 55 included studies, the effect of vitamin E was not significant (O.R. 1.01 C.I. 0.98-1.05) In a meta-analysis of vitamin E trials in ALS, no increased rates of death, heart disease or cancer were seen [97]. Subjects will not be allowed to take additional supplements containing vitamin E, except for a daily multivitamin.

2.2.6 Risks of Vitamin A

Oxepa contains 11,910 IU of vitamin A per liter, compared with the Jevity products which contain the recommended daily allowance of 5,000 IU per liter. Although this is less than the lowest toxic dose of 25,000 IU/day, we plan to test liver function tests regularly during the trial. Toxicity from long-term supplementation is rarely seen below 100,000 IU/day[98]. If liver function tests become elevated to greater than two times the normal limits, the investigational

tube feed will be suspended for one month. If liver function tests normalize, the subjects will be rechallenged with the study diet and their liver function tests followed closely. Oxepa also contains additional Vitamin C (850 mg/L versus 300 mg/L in Jevity) to reduce the risk of Vitamin A toxicity. Subjects will not be allowed to take additional supplements containing vitamin A, except for a daily multivitamin.

Importantly, Riluzole carries a risk of liver toxicity; therefore subjects will be required to be on a stable dose of Riluzole for at least 60 days prior to enrollment and will have their liver function tests checked at their baseline visit. If the liver function tests are already greater than twice normal in any parameter, they will not be enrolled.

In the same large meta-analysis of antioxidant studies, a sub-analysis of 5 studies that were considered low bias found an increase in the overall risk of death from Vitamin A (1.16 95%C.I. 1.10-1.24) [96]. The mean dose used in these studies was 20,219 IU and a slight dose-effect was reported (RR, 1.000006; 95% CI, 1.000002-1.000009). However in the overall meta-analysis of 16 included studies, the effect of vitamin A was not significant (O.R. 1.05 C.I. 0.93-1.19). Finally, excess vitamin A over 10,000 IU increases the risk of birth defects in women who are in their first trimester of pregnancy [99]. Therefore subjects who are pregnant or who may become pregnant during the course of the trial will not be enrolled. Subjects who are of childbearing age will be required to use adequate birth control to prevent pregnancy.

2.2.7 Risks of Dual-energy Xray Absorptiometry (DXA)

Radiation

As a result of this study, subjects will be exposed to two DXA scans, one at the baseline and one at the final visit. The radiation exposure associated with the whole body DXA scan is approximately 0.8mrem[100]. The total radiation exposure associated with this study is 1.6 mrem. This does not pose excessive risk to subjects. The average natural background including cosmic rays and radiation from naturally radioactive materials is approximately 300 millirem per year in the US (source: US Nuclear Regulatory Commission). Female participants of child-bearing age will be tested for pregnancy using a serum βhCG at the screening evaluation prior to enrollment, and using a urine pregnancy test immediately prior to DXA scanning at the 4 Month study visit. Subjects will be instructed to use at least 2 forms of birth control during the study.

2.2.8 Potential Benefits

There is unlikely to be a direct benefit to subjects participating in this trial. There is a future benefit of furthering ALS research and possibly contributing to finding an effective treatment for ALS. We will minimize risks of both the study diet and the mode of delivery. These include education of clinical trial staff, subjects and their caregivers on the potential risks of study diet and how to minimize these risks, inclusion of experts in lipid disorders and pulmonary medicine and a requirement for each site to have a bionutritionist involved in the study.

3. STUDY DESIGN

3.1 Study Design Overview (Figure 5)

This is a phase II safety and tolerability study involving 10-20 research centers across the U.S. As shown in Figure 5, thirty patients who require percutaneous nutrition will be randomly assigned to one of three study diets:

- Jevity 1.0 to replace their measured energy expenditure
- Jevity 1.5 containing 1.25 times their calculated caloric requirements
- Oxepa calculated to provide 1.25 times their caloric needs

At the screening visit, subjects will provide informed consent. No procedures will be done prior to consent. Energy expenditure will be assessed using indirect calorimetry and used to calculate their daily calorie requirements. In addition, their baseline metabolic and lipid profiles will be assessed as well as their baseline forced vital capacity and neurologic status. Subjects will be told to take their current prescribed tube feed diet and to weigh themselves at home. Subjects will then be given the Self Weighing Log, the Diet Diary and the Bouchard Activity Questionnaire. Subjects will be told to weigh themselves at home on a biweekly basis followed by weekly basis using the Self-Weighing Log (Appendix 1) and to contact the site if they lose 3 or more pounds on their home scale. The study site will contact the subject 4 to 7 days prior to the Baseline visit to measure weight loss using their Self Weighing Log. The site will report any weight loss of 3 or more pounds to the Coordination Center Bionutritionist at least 4 days before the Baseline visit. If the subject is unable to weigh themselves at home then it is acceptable for them to not complete the Self-Weighing Log.

Prior to the Baseline visit, subjects will be randomized 1:1:1 blocked by center using a web-based computer-generated system provided by the MGH Biostatistical center. Prior to dispensing tube feed, if the total volume of required tube feed is greater than can be tolerated using Jevity 1.0, the Coordination Center Bionutritionist may convert the subject to the 1.5 calorie Jevity without changing the treatment assignment (replacement calories arm). At the Baseline visit, subjects will be instructed how to use the formula and given their first tube feeding, followed by a postprandial blood draw to measure changes in lipid levels. If participants continue to take calories by mouth, they will be asked to document their additional oral intake on the Tube Feed Log. Subjects will be instructed to continue measuring their weight at home on a biweekly basis followed by weekly basis using the Self-Weighing Log and to inform their study site if they experience 3 or more pounds of weight loss. The site will contact the subjects by telephone on day 14 (+/- 3 days) to assess weight loss using the Self Weighing Log. If subjects lose 3 or more pounds, the study site will automatically increase the subject's diet by 1 can per day and will contact the Coordination Center Bionutritionist who may further adjust the subject's diet.

One and two month in-person visits will assess adverse events, vital signs, FVC and PET_{CO2}, safety labs and dietary compliance. Indirect calorimetry will be repeated and if there is any weight loss, the diet will be increased to compensate for this change. If there is weight loss but no difference in the calculated daily energy requirements, the diet will be increased by the amount required to recover lost weight. Adverse events and medication changes will be assessed

by telephone at 3 months. At the end of 4 months, subjects will resume their prior diets, and metabolic and lipid profiles will be reassessed. There will be a final telephone visit at 5 months to assess adverse events. Additional unscheduled visits may be made to evaluate any adverse symptoms.

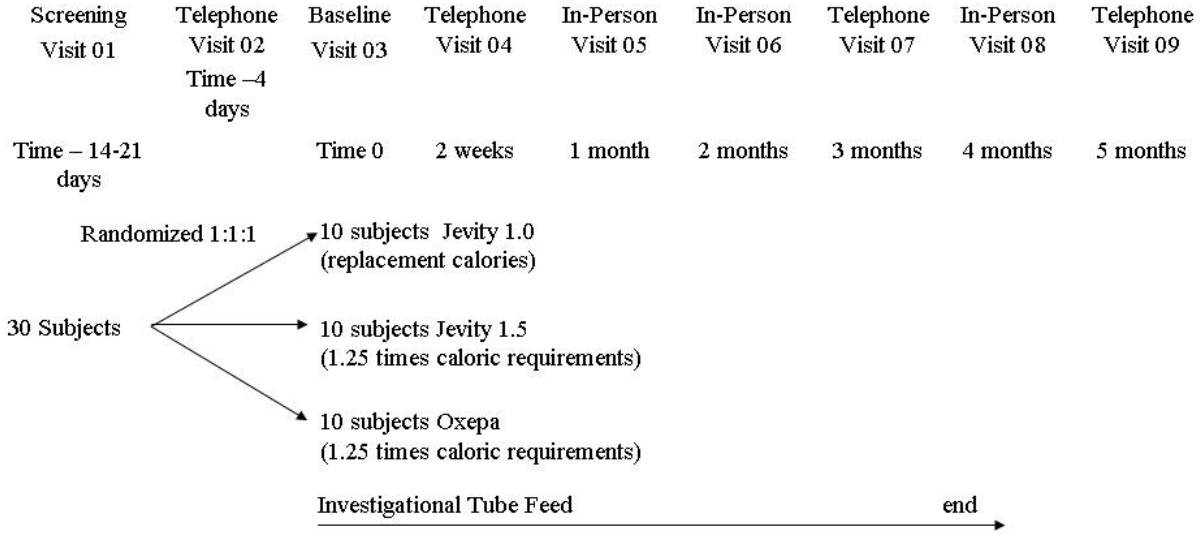
Primary outcome measures:

Safety and tolerability are the two primary outcome measures. Safety will be determined by the independent drug safety monitoring board (DSMB) in consultation with the study investigators. Adverse events (AE) will be reported and reviewed on a regular basis by the DSMB. Serious adverse events (SAE) will be reported to the site IRB, MGH IRB and the DSMB in a timely manner. If an excess number of AE or SAE are seen, the DSMB can recommend study discontinuation or modification. We calculate that our sample size will give us 95% probability of detecting an adverse event rate of 20% and a 75% probability of detecting an adverse event rate of 10% [101].

In the tolerability analyses, a subject will be regarded as a treatment success if he/she completes 4 months of the study while being compliant with >80% of the prescribed diet. This will be calculated by having participants bring unused tube feed back to the centers at the 1, 2 and 4 month visits and using the self-reported tube feed log. Subjects will be regarded as a treatment failure if they fail to complete month 4 of the study on the originally assigned treatment for any reason. We will consider a diet tolerable if the proportion of treatment failures for a diet is less than 40% with 80% confidence. With 10 subjects this would occur if less than or equal to 5 subjects fail to complete the 4 month study. With 10 subjects we will have more than an 80% chance of declaring a dosage tolerable if the true treatment failure rate is 20%.

3.2 Study Flow

Figure 5: Study Flow



3.3 Setting

The study will be conducted at approximately 10-20 sites coordinated by the Neurology Clinical Trials Unit of Massachusetts General Hospital.

Each site will employ a site investigator; an alternate physician-investigator who will be available at all times when the site PI is unavailable, a bionutritionist, a study coordinator and a clinical evaluator. As consultants for the overall trial, a bionutritionist, endocrinologist and pulmonologist at MGH will be available for clinical consultation for individual subjects.

4. SUBJECT SELECTION AND ENROLLMENT

4.1 *Inclusion Criteria*

To be eligible for enrollment into this study, research participants must meet the following eligibility criteria within 21 days prior to the Baseline visit:

1. Participants with familial or sporadic ALS diagnosed as suspected, possible, laboratory-supported probable, probable, or definite according to the World Federation of Neurology El Escorial criteria [9, 10]; subjects may have either limb-onset or bulbar-onset disease.
2. Male or female subjects aged 18 years or older.
3. Subjects must be capable of providing informed consent and complying with trial procedures.
4. Subjects must have competent caregiver able to assist with tube feeding.
5. Subjects must have already tolerated tube feeding through either a gastrostomy tube or jejunostomy tube with a minimum tube size of French 8.
6. Subjects must require non-invasive ventilation for less than 10 hours/day, and in the judgment of the Investigator, be able to complete this study.
7. Subjects may be taking riluzole at a stable dose for the previous 60 days.
8. Women must not be able to become pregnant (e.g. post menopausal, surgically sterile, or using adequate birth control methods) for the duration of the study. Adequate contraception includes: abstinence, hormonal contraception (oral contraception, implanted contraception, injected contraception or other hormonal (patch or contraceptive ring, for example) contraception), intrauterine device (IUD) in place for ≥ 3 months, barrier method in conjunction with spermicide, or another adequate method (as determined by steering committee member review). Women of childbearing potential must have a negative pregnancy test at screening and be non-lactating.

4.2 *Exclusion Criteria*

1. Clinical evidence of unstable medical or psychiatric illness in the investigator's judgment.
2. Concurrent enrollment in a clinical trial of an investigational agent. Phase IV studies and open-label extensions of completed trials are allowed if the subject has been on a stable dose for at least 60 days.
3. History of hepatitis including non-alcoholic steatohepatitis (NASH), cholecystectomy, prior biliary disease such as gallstones, diabetes, prior myocardial infarction or stroke.

4. Laboratory values: Screening alanine aminotransferase (ALT) or aspartate aminotransferase (AST) greater than 2.0 times the upper limit of normal or total bilirubin greater than 1.5 times the upper limit of normal.
5. Allergy to soy, fish, or milk products.
6. Prior intolerance of Jevity 1.0, Jevity 1.5 or Oxepa.
7. Absence of adequate social support and cooperation, or personal motivation (in the judgment of the investigator) to complete the study satisfactorily.

Riluzole. The use of riluzole will be permitted during the study. Subjects taking riluzole must be on a stable dosage for 60 days prior to screening and have stable liver function tests at enrollment. Subjects are not allowed to start taking riluzole during the trial due to its possible hepatotoxicity. About 60 percent of patients with ALS in the United States are currently taking riluzole [74]. Allowing subjects the choice of taking riluzole reduces dropout rate and ensures that the investigators know which subjects are taking riluzole.

4.3 Enrollment Procedures

4.3.1 Subject Availability and Recruitment

Each of the centers will randomize approximately 3 to 8 subjects per year over about 2 years. Enrollment will be closed as soon as 30 subjects have been randomized to treatment assignment. If a subject drops out prior to receiving the first dose of study tube feed, they may be replaced. The clinical trial will be advertised in local and national ALS newsletters and by letters to local neuromuscular physicians and general neurologists. Based on the high number of ALS patients at each enrolling site, we do not anticipate difficulty in recruitment. Should there still be a lag in accrual of subjects, we would increase advertising efforts, followed by lengthening the time for accrual.

4.3.2 Documentation of Screening and Eligibility

Please refer to section *6.2.1 Screening and Informed Consent* for details regarding screening procedures and documentation of ineligibility and nonparticipation of eligible subjects.

4.3.3 Informed Consent

The Coordination Center must receive written confirmation of IRB approval and a copy of the IRB approved consent forms from each site prior to initiation of enrollment at that center. The site investigator or IRB and Sponsor approved designee at the site will explain the protocol and obtain informed consent from all subjects prior to initiation of any research evaluations. The site investigator will determine study eligibility as determined by the inclusion and exclusion criteria. If the study subject agrees, provides informed consent, and signs the IRB approved informed consent form, the study visits are scheduled. If the subject is unable to sign the consent, they may provide verbal or typed consent and have a witness sign the consent. One copy of the signed informed consent form will be given to the subject, and another copy may be maintained in the subject's medical record. The informed consent details the potential benefits of participating in the research as well as the potential risks of the experimental interventions. A copy of the informed consent form can be found as Appendix 2.

4.3.4 Randomization and Randomization Number Assignment

The randomization will be blocked by site in a manner that will allow for a balanced distribution of each diet arm at each site. The randomization scheme will be independently developed by the Biostatistics Center at MGH and will indicate the treatment assignment for each subject (randomization) ID number. For each site, a randomization schedule will be provided to the coordinating center chief bionutritionist that will indicate the treatment assignment. The Randomization ID will also be used to identify the subject's Source Documents, electronic case report forms (eCRFs), laboratory tests, and all communications. The team of biostatisticians in the Biostatistics Center will develop the randomization plan under the guidance of the chief biostatistician. All Coordination Center clinical trial staff, with the exception of the chief bionutritionist, will remain blinded to all treatment assignments. Subjects, investigators, study monitors, site coordinators, site bionutritionists and site clinical evaluators will also be blinded to treatment group assignment throughout the study. If subjects drop out of the study after randomization but before the baseline visit and first tube feed, subjects will be replaced and will be excluded from the analysis of tolerability. Adequate randomization numbers are available to randomize 60 subjects per site if needed.

5. STUDY INTERVENTIONS

5.1 Study Diet – Interventions, Administration, and Duration

5.1.1 Study Diet

The amount of replacement calories required by each subject (total daily energy expenditure or TDEE) will be calculated based on each subject's measured resting energy expenditure (MREE) or based on the daily dietary intake required to maintain weight between Screening and Baseline visits, whichever is greater. To estimate resting energy expenditure we will use two methods of measurement at the screening visit: the Harris-Benedict equation [102] based on the basal metabolic rate, and measured energy expenditure based on indirect calorimetry using the Weir equation[103]. In cases where the formulae disagree, the higher value will be used. All results will be multiplied by 1.2 as a standard estimate of their activity factor. Subjects will return their Bouchard Activity Log and Diet Diary at the Baseline visit and if their activity quotient is greater than 120%, their overall prescribed diet will be adjusted accordingly. Subjects who use non-invasive ventilation will have their results multiplied by an additional 1.15 to account for use of non-invasive ventilation. Protein requirements will be calculated using the equation of Elia [104]. Fluid requirements will be calculated using the equation of Thomas [105]. This information will then be used to design each subject's individual diet.

Control Diet: subjects will be given an exact amount of Jevity 1.0 (or Jevity 1.5) to replace 100% of their calculated energy requirements.

High Calorie Diet: subjects will be given 125% of their calculated energy requirements using the Jevity 1.5 tube feed.

High Fat/ High Calorie Diet: subjects will be given 125% of their calculated energy requirements using Oxepa.

The number of milliliters to be taken each day will be written down and converted into the number of cans/day of tube feed that should be consumed each day. As described above, weight loss in subjects who are > 90% compliant with their tube feed formula will prompt an increase in the prescribed tube feeds as determined by the Coordination Center Bionutritionist. No subject will be allowed to lose weight during the study.

5.1.2 Study Diet Administration

The subject will receive the first bolus of study diet by gastrostomy or jejunostomy tube at the clinical research center at baseline and will be observed for any abdominal discomfort. Laboratory studies will be drawn 2-4 hours after the initial dose. All subsequent doses will be administered in the home by the subject and/or the caregiver, with the exception of the doses given at the hospital during study visits.

Throughout the study, trained healthcare personnel will be available to answer questions and offer ongoing instruction as needed. Written instructions for storage, measuring, and administration of study diet will be provided to all subjects.

Tube feeds can be stored at room temperature until they are opened. Once opened, they can be kept at room temperature for up to 4 hours. Subjects will be instructed to aliquot the amount that will be used immediately and to refrigerate the remainder within 4 hours. The tube feeds can be refrigerated for up to 48 hours.

5.1.3 Dosing Schedule

The first dose of study diet for each subject will be administered in the clinical research center and the subject will be observed for two hours during the clinical assessments. The day that a subject starts treatment with tube feed will be designated as DAY 0. All visits must be scheduled from Day 0, rather than the last assessment. Subjects will be instructed to take the appropriate amount of tube feed either by bolus or continuous infusion in one of the following schedules:

Bolus Tube Feed Schedule for *# of* cans of tube feed per day (these times are flexible):

Time	Step 1: 30 mL water flush	Step 2: full strength ***Formula***	Step 3: 30 mL water flush
7:30am	30 mL water	# mL ***Formula***	30 mL water
9:30 – 10 :30 am	30 mL water	# mL ***Formula***	30 mL water
11:30 am	30 mL water	# mL ***Formula***	30 mL water
1:30 pm	30 mL water	# mL ***Formula***	30 mL water
3:30 pm	30 mL water	# mL ***Formula***	30 mL water
5:30 pm	30 mL water	# mL ***Formula***	30 mL water
7:30 pm	30 mL water	# mL ***Formula***	30 mL water
8:30 - 9 pm	30 mL water	# mL ***Formula***	30 mL water

Continuous Feeding Administration Schedule

Day	Strength	Rate (mL/hr)	Volume (mL per day)
1	Full	25	600
2	Full	50	1200
3	Full	#	#

**If subjects have been tolerating tube feeds at target rate, may start at final rate.

Cans of tube feed will be dispensed to each participant along with instructions as to their administration. Subjects will be given 1 month's supply of tube feed on day 0 at the clinical research center. If they cannot bring the tube feeds home with them, the tube feeds will be shipped directly to their home. They will receive monthly supplies at the 1 and 2 month in-person visits. They will receive the next month's supply by mail before the 3 month telephone visit. They will continue to receive tube feeds either in person at hospital visits or by mail until they have finished the 4 month study.

5.2 Handling of Study Interventions

5.2.1 Tube Feed Distribution to Sites

Cans of Oxepa, Jevity 1.0 and Jevity 1.5 will be re-labeled "A, B, or C" and distributed by the MGH CRC Bionutrition Office to the research sites (see Appendix 3). Both the MGH CRC Bionutrition Office and the participating research sites will keep detailed documentation of the amount and dates that tube feeds are shipped to the clinical sites. The individual research sites will keep a log of dispensed tube feed identified by subject randomization ID, subject initials and date dispensed. The MGH chief bionutritionist will be unblinded to treatment assignment and will calculate the daily dose of tube feed according to each subject's total daily energy expenditure and randomization arm. Written instructions on how to measure and administer the study diets will be provided to all site treating bionutritionists and the chief bionutritionist from MGH will be available at all times for consultation. The Site Monitor will meet with each site's bionutritionists to ensure that study procedures are followed.
Oxepa, Jevity 1.0 and Jevity 1.5 vary from cream to beige in color and are not significantly different in appearance to result in unblinding.

5.2.2 Tube Feed Dispensing, Labeling and Storage

Each site's treating bionutritionist will check the condition of the tube feeds upon receipt and enter this data into the Proof of Receipt letter (packing slip) and accountability record. All tube feeds dispensed by the investigator will be accounted for throughout the study and tube feeds will be maintained in a secure area at each site. Unopened tube feeds can be stored at room temperature. Study tube feeds will be dispensed to the subjects in person at the Baseline, 1 and 2 month visits, and mailed to the subjects at 11 weeks between study visits 4 and 6. Supply of tube feeds shall be shipped with adequate anticipation to allow for shipping delays. A sufficient quantity will be shipped to allow for delays in re-supply or errors in administration that result in wasting of tube feeds. Subjects will be instructed to return all unopened cans or bottles of tube

feed at their in-person visits and to document any wasted amounts of tube feed in their tube feed log.

5.2.3 Compliance and Return of Tube Feeds (Adherence Assessment)

The primary method to check compliance for study diet will be to record the amount of tube feed dispensed and the amount returned at each visit in the eCRF. Subjects will be instructed to return all unused tube feeds at in-person visits months 1, 2 and 4 following the Baseline visit. Subjects will be instructed to keep a tube feed log (Appendix 4) which documents any wasted tube feeds and will bring this to each visit. Historically, subjects with ALS have a very high compliance rate (over 95%) with study medications. During each visit, the study coordinator will count and record the amount of unused cans returned.

Study tube feeds will be distributed as needed for the next four-week interval. In addition, blood samples for lipid levels will be obtained at months 1 and 2 to help determine if subjects are complying with a high fat diet. Serum lipid results will not be available to the Coordination Center or site staff until study completion. These determinations will enhance our monitoring of compliance as the high fat/high calorie diet arm would be expected to have higher serum levels of triglycerides.

5.3 Concomitant Medications

Throughout the study, investigators may prescribe any other concomitant medications or treatments deemed necessary to provide adequate supportive care providing that they are licensed in the US. All concomitant medications received by a subject will be recorded on the appropriate source documents and in the Electronic Data Capture (EDC) System.

5.3.1 Exclusionary Medications

Subjects should not take experimental agents or additional supplements of Vitamin E or A, other than what is found in a daily multivitamin, during the study. Subjects will not be allowed to take cholesterol lowering medications such as Lipitor, Pravachol, Zocor, Niacin, Vytorin, Zetia, Cholestyramine, or Lopid while participating in the study. Standard ALS care is to discontinue HMGCoA reductase inhibitors due to a rare ALS–like syndrome that has been reported during statin use [106].

5.4 Rate Changes

Changes to the rate of investigational tube feed administration will be at the discretion of the treating investigator and bionutritionist at each site. Rate changes that would reduce the amount of total daily tube feed administered should be discussed with the Coordination Center.

5.4.1 Rate Reduction

The investigator may temporarily reduce the rate at which tube feeds are given for symptoms of gastric discomfort such as distension, nausea, or severe diarrhea. After the rate reduction, the investigator may wish to slowly increase the rate at which the tube feed is given as described in

5.4.2 Study Administration

In case a subject loses their gastrostomy or jejunostomy access, the site investigator will facilitate the placement of a temporary tube and the replacement of the feeding tube and document the number of hours or days that the subject was unable to take the study diet. In this case subjects may be able to continue to take nutrition by mouth and will document their intake in their diet diaries. The site will contact the Coordination Center within 1 working day of any dosage change.

5.4.3 Study Diet Suspension

For adverse events thought to be related to the study diet, the site investigator may discontinue the study diet and the subject may resume their prior diet. If a serious adverse event requires hospitalization or is life threatening, the study diet will be suspended. Additionally, a study diet suspension may occur at any time at the Site Investigator's discretion. The site will notify the Coordination Center within 24 hours of any study diet suspension**.**

5.4.4 Diet Re-challenge

The Site Investigator may choose to re-challenge a subject if the adverse event resolves. If the tube feeds were suspended for another reason (such as loss of G or J tube), the study diet can be restarted at the discretion of the site investigator. The site will notify the Coordination Center prior to re-challenging a subject.

5.4.5 Study Diet Discontinuation

Certain conditions require that study diet be permanently discontinued for safety reasons. In this case, the subject will continue to be followed until the adverse event has resolved or has stabilized for at least 1 month. Additionally, if a subject chooses to permanently discontinue study diet for tolerability reasons, they will still be followed until the 5 month final safety evaluation. The site will notify the Coordination Center within 24 hours of learning about a serious adverse event and/or study diet discontinuation.

5.5 Code Break Procedures

An emergency unblinding procedure will allow site investigators the option of disclosing the treatment assignment for an individual subject if clinical circumstances require such an unblinding. The chief bionutritionist, study biostatistician and DSMB chair will have randomization numbers and treatment assignments and can release this information to the site investigators if unblinding becomes necessary. Rarely is such an extreme action taken. Experimental medications can usually be suspended in a subject experiencing adverse effects without the need for unblinding. In the event that emergency disclosure of treatment assignment is required, the site investigator should contact the Coordination Center prior to unblinding. The study statistician and chief bionutritionist and DSMB chair will also notify the Coordination Center of any emergency unblinding within 24 hours of occurrence.

6 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Assessments

The Schedule of Assessments outlines all study procedures (Figure 6)

Activity	Screening Visit Day – 12-21 days	Telephone Visit – 7 to -4 days	Baseline Visit Day 0	2 Week Telephone Visit 14 Days (+/- 3 days)	1 Month Visit 28 Days (+/- 6 days)	2 Month Visit 56 Days (+/- 6 days)	3 Month Telephone Visit 84 Days (+/- 3 days)	4 Month (final) Visit 112 Days (+/- 6 days)	5 Month Telephone Visit 140 Days (+/- 3 days)
Written Informed Consent	X								
Inclusion/Exclusion review	X								
Randomization	X								
Medical History/ Demographics	X								
Concomitant Medications	X		X		X	X	X	X	X
Investigational Diet Review			X[4]		X	X	X	X	
Bouchard Activity Log & Diet Diary	X[2]		X						
BIPAP log	X		X		X	X	X	X	X
Study Diet Accountability Tube Feed Log Review			X[3]		X	X	X	X	
Self-Weighing Log Review	X[2]	X	X	X	X	X	X	X	
Adverse Events Review	X	X	X	X	X	X	X	X	X
Vital Signs, Weight, Height, pO_2	X		X		X	X		X	
Forced Vital Capacity (FVC)	X		X		X	X		X	
Electrocardiogram (EKG)	X								
Physical Exam	X				X	X		X	
Neurological Exam	X							X	
Grip Strength	X							X	
**Safety Labs	X				X	X[F]		X	
Optional Future Research Blood Sample	X		X[F & PP]		X	X[F & PP]		X	
***MGH Core Lab Blood Sample	X		X[F & PP]		X	X[F & PP]		X	
Optional NIH Lab Blood	X				X	X[F]		X	

Sample									
Trough Riluzole Levels Blood Sample	X[1]				X			X[1]	
Pregnancy Test	X (serum)							X (urine)	
Indirect Calorimetry, PET_{CO2}	X				X	X		X	
Basal Metabolic Rate Calculation	X								
Fat Mass, Fat Free Mass (DXA, BIA, SKF Measurements)			X					X	
ALS Functional Rating Scale-Revised (ALSFRS-R)			X					X	
GIQLI & Multi Dyspnea Questionnaire			X		X	X	X	X	X
D-xylose test[5]			X						
Sudan III staining of stool[5]			X						

* 1 month is defined as 28 calendar days. All visits are scheduled from the Baseline visit (date of first dose of investigational diet)
** Safety Labs include: Basic Metabolic Panel (Na, K, Cl, CO_2, BUN, Creatinine, Calcium, Glucose), Liver Function Tests (AST, ALT, Total Bilirubin, Direct Bilirubin, Total Protein,, Alk Phos), Complete Blood Count (WBC, Hct, Hgb, RBC, Plt) and Creatine Kinase (CK).
***MGH Core Lab Blood Samples include; (Lipids panel, CRP, TSH, IGF-1, Albumin, Pre-albumin and Acetone (ketones)).
F and PP: At Baseline and Month 2 a blood draw will be done fasting and then again post-prandial. F, indicates that the blood test will be done fasting. PP, indicates that the blood draw will be done post-prandial.
1. A blood sample for trough riluzole levels will be collected if patient was previously taking riluzole.
2. Self-weighing, Diet Diary, and Bouchard's Activity Log with instructions for their use will be given to the patient at this visit to complete at home.
3. The first tube feed log will be dispensed at this visit.
4. Study diet will be dispensed
5. If subjects consent to participate in these optional tests but are unable to perform the procedures at the Baseline visit, the malabsorption studies may be performed at any of the in-person visits.

6.2 Timing of Evaluations

Subjects will be screened at the initial visit 12-21 days prior to enrollment. At this visit the total daily energy expenditure will be calculated by indirect calorimetry and, if subjects qualify for the study after the screening visit, subjects will be randomized to one of the three treatment arms. The study diet will be calculated at the coordinating center prior to the baseline visit when subjects will receive their first dose of study diet. All subsequent study visits will be within a 6 day window of the 1, 2 and 4 month targets. We will make every effort to coordinate the subjects' doctor visits with study visits. Subjects will receive their re-supply of study diet at least 1 week prior to the 3 month telephone call. All visit windows are consecutive calendar days and are calculated from the day the subject starts the investigational diet (Baseline Visit). All subsequent visits must be scheduled from Day 0 (Baseline Visit), not the date of the subjects last assessment.

6.2.1 Screening Visit and Informed Consent (V01)

The Coordination Center will require a copy of each site's written Institutional Review Board (IRB) approval of the protocol and approved consent form prior to site initiation. At the screening visit, potential subjects will be informed about study procedures and will then sign an informed consent form (Appendix 2). Screening procedures will take place within12 to 21 days of the baseline (randomization) visit. Subjects will have been instructed to fast for twelve hours prior to their study visit in order to measure their baseline resting energy expenditure. If subjects are taking Riluzole, they will be instructed to hold their morning dose and bring it to the study visit. Blood will be drawn for Riluzole trough levels and for fasting cholesterol levels. They will then be told to take their morning dose of Riluzole.
The inclusion/exclusion criteria will be reviewed and a medical history and full physical examination including neurological exam will be completed. At all study visits the concomitant medication, adverse event log and BIPAP log will be updated.

Vital signs and FVC will be determined. Pulse oximetry to measure oxygen saturation (pO_2) and end-tidal carbon dioxide (PET_{CO2}) will be measured using a metabolic cart. An electrocardiogram (EKG) will be performed to have a baseline. Height, weight and concomitant medications will be recorded. Hand held dynamometry for grip strength will be performed. A diet diary (Appendix 5) and Bouchard's Activity Log (Appendix 6) with instructions for their use will be given to the patient at this visit for them to complete at home.

Safety laboratory tests will be performed including basic metabolic panel, liver function tests (ALT, AST, total and direct bilirubin,) complete blood count, creatine kinase (CK) and a serum pregnancy test for women of child-bearing potential. A urine pregnancy test will be completed at the 4 month visit, prior to the follow-up DXA scan. Serum levels of total cholesterol, HDL, LDL, triglycerides, albumin, pre-albumin, c-reactive protein, IGF-1, TSH and ketones will be measured using the MGH core research laboratory. Collaborating trial centers will ship their blood samples to the MGH NCTU either overnight using a cool pack or in batches on dry ice to facilitate uniform blood measurements. The Coordination Center will provide sample collection tubes, cryovials, labels, and instructions for sample collections. The sites will record the date and time of each sample collection.

For samples which must be frozen immediately, blood will be collected in a silica clot activator tube. Serum will be separated by centrifugation at 1750 G for 10 minutes. Immediately after centrifugation, serum will be aliquoted into polypropylene screw cap tubes, and frozen within 15 minutes. Samples will be sent on dry ice to the MGH NCTU in batches, where they will be frozen at –80ºC until analysis. One ml aliquots of serum will be sent from the NCTU to Dr. David Greenblatt at Tufts University for measurement of trough Riluzole levels at the Screening, Month 1 and Month 4 visits. For the optional future research studies into the cause or mechanism of ALS, samples will be stored at the NCTU Biorepository indefinitely. One to two ml aliquots of serum will be sent to Dr. Mark Mattson at the NIA/NIH for analysis of oxidized lipids and metabolic markers, this blood test is also optional.

Indirect calorimetry will be performed using a portable metabolic cart (VMAX 29N Spectra metabolic cart, SensorMedics, Yorba Linda, CA). Indirect Calorimetry will be performed in a fasting state after the subject has rested for 30 minutes in the supine position. The measured energy expenditure will then be calculated using the Weir equation[103]. The total amount of replacement calories will then be calculated based on the measured energy expenditure and used in calculating the study diet.

All inclusion and exclusion criteria and safety laboratory tests will be reviewed by the site investigator prior to randomization and scheduling the baseline visit. The site study staff will electronically register the subject in the study and the subject will be assigned a randomization number. A log will be kept at the site to record all subjects screened for entry into the study. This information will also be captured electronically. Demographic characteristics of all subjects who are screened will be recorded whether or not they qualify for entry into the study. The reason for non-qualification will be recorded for all subjects who are not eligible. The reason for non-participation will also be recorded for subjects who are eligible but choose not to participate in the trial. If a subject fails initial screening for the study, the subject can be re-screened later in the study if the Site Investigator determines it is appropriate to do so. At any time during the study, repeat laboratory tests can be obtained if the Site Investigator or central laboratory thinks that a laboratory test result is in error. Additional laboratory tests, such as liver function tests, may also be obtained at any time during the study, at the discretion of the Site Investigator.

Subjects will be told to take their current prescribed tube feed diet. Subjects will then be given the Self Weighing Log (Appendix 1) in addition to the Diet Diary and the Bouchard Activity Questionnaire. Subjects will be told to weigh themselves at home on a biweekly basis, followed by weekly basis using the Self-Weighing Log and to contact the center if they lose 3 or more pounds on their home scale. The site and subject may modify their tube feeds as necessary to maintain weight. If the subject is unable to weigh themselves at home then it is acceptable for them to not complete the Self-Weighing Log.

Telephone Visit V02 (-7 to -4 days prior to Baseline Visit)

The study site will contact the subject at least 4 days prior to their Baseline visit to check for adverse events and weight loss using the Self Weighing Log. Subjects will be asked to compare their current weight to their home weight value on the day of their Screening Visit and if there is a change of 3 or more pounds, to contact the study site. If the subjects have lost weight on their pre-randomization diet, the site or subject may modify the diet as needed to maintain weight stability. Sites will then contact the Coordination Center Bionutritionist at least 4 days before the

Baseline visit to discuss any weight loss indicated on the Self Weighing Log and the type and amount of tube feed consumed.

6.2.2 On Study Evaluations

Baseline Visit V03 (Day 0)

This visit will occur 12-21 days after the Screening visit. The baseline (day 0) visit includes study diet dispensing and the first dose of the study diet. Subjects will have been randomized between the Screening and Baseline visits to one of the three treatment arms (Control Jevity 1.0, High calorie Jevity 1.5, or High Fat/High Calorie Oxepa) in a 1:1:1 allocation blocked by center. The Coordinating Center bionutritionist will have contacted the treating site bionutritionist with instructions for the study diet ('A,' 'B' or 'C') and amount of study diet that will be administered.

The first dose of study diet will then be administered in a one time bolus. Blood will be drawn fasting for the optional future research studies. Serum levels of total cholesterol will be drawn fasting and then again approximately 2-4 hours (ideally 3 hours) after the first bolus tube feeding. Subjects and their families/caregiver will be taught maintenance and care of the gastrostomy or jejunostomy tube, warning signs and symptoms, storage of study tube feeds. Subjects will be given the tube feed log and Self Weighing Log to complete before their next visit.

At this visit the vital signs, FVC, and pO_2 will be repeated. The concomitant medication, adverse events and BIPAP logs will be updated. The GIQLI (Appendix7) and Multidimensional Dyspnea Profile questionnaires (Appendix 8) will be completed. All completed GIQLI will be faxed to the coordination center along with the Bouchard Activity Log. The ALSFRS-Revised questionnaire (Appendix 9) will also be performed at this visit. Fat mass (FM) and fat-free mass (FFM) will be measured using whole body Dual-Energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA) and skinfold-thickness measurements (SKF). Dual Energy X-ray Absorptiometry will be performed at each site using a Hologic Discovery A densitometer (or equivalent) to determine total body and regional percent fat and lean body mass. The technique has a precision error (1 SD) of 3% for fat and 1.5% for lean body mass. Bioelectrical impedance analysis will be measured using the Quantum II BIA analyzer (or equivalent). Skinfold-thickness measurements will be taken of the arm (biceps, triceps) back (sub-scapular) and waist (suprailiac) using skinfold calipers (Lange). The Self Weighing Log, Activity Log and Diet Diary will be collected and faxed directly to the Coordinating Center Bionutritionist who will tally the results.

Optional malabsorption study:

If subjects consent to participate in an optional study of malabsorption; fecal fat staining and d-xylose malabsorption tests will be administered at the baseline visit. If subjects consent to participate in the optional study of malabsorption but are unable to perform the procedures at the baseline visit, the malabsorption studies may be performed at any of the in-person visits. For the fecal fat staining test, subjects will be instructed to bring a spot stool sample to the visit for Sudan III staining of lipids (a plastic container for the stool sample will be provided to the subjects). For the d-xylose malabsorption test, twenty five grams of d-xylose will be administered orally at least 2 hours prior to the subjects' first dose of tube feeds. Blood will be

drawn at 2 hours to measure blood d-xylose levels. Urine will be captured over the period of 5 hours after the oral load and blood and urine will be shipped to Quest Diagnostics for analysis. If the optional study procedures are performed at either the Baseline or Month 2 visit, the post-prandial cholesterol levels will not be measured at those visits. Sites will then ship all samples overnight on dry ice to Quest Diagnostics for processing (note that stool samples must be shipped frozen, blood and urine may be shipped at room temperature overnight if subjects do not provide stool sample).

Telephone Visit V04 (2 Weeks)

All subjects will be called on Day 14 (+/- 3 days) to check for adverse events and weight loss using the Self Weighing Log. Subjects will be asked to compare their current weight to their home weight on the day of the Baseline Visit and if there is a change of 3 or more pounds, to contact the site. If there is weight loss of 3 or more pounds, sites should instruct the subject to increase the prescribed dose by 1 can per day and should contact the Coordination Center Bionutritionist for further instruction.

Study Visit V05 (Month 1)

The visit window is Day 28 +/-6 days. Subjects will have been instructed to fast for 12 hours prior to the study visit in order to measure their baseline resting energy expenditure. If subjects are taking Riluzole, they will be instructed to hold their morning dose and bring it to the study visit. Blood will be drawn for Riluzole trough levels and for fasting cholesterol levels. They will then be told to take their morning dose of Riluzole.

At the one month study visit, participants will be seen for assessment of vital signs, pO_2, $P_{ET_{CO2}}$, FVC, weight and any changes to concomitant medications and medical history. An abbreviated physical exam including cardiovascular, pulmonary and skin will be performed. The GIQLI and Multidimensional Dyspnea Profile questionnaires will be completed. Significant adverse events in gastrointestinal and respiratory symptoms as elicited on these questionnaires will also be written in the adverse event log. The concomitant medication, adverse event and BIPAP logs will be updated. Safety laboratory studies will be drawn and site investigators will be notified by their clinical laboratories if there are any changes in the chemistry and liver functions tests. Optional future research study blood samples and optional NIA/NIH blood laboratory tests for oxidized lipids and metabolic markers will be drawn.

Indirect calorimetry will be repeated at this visit and the total daily energy expenditure (TDEE) will be re-calculated. If there is weight loss or a change in the measured resting energy expenditure (MREE), the coordinating center and chief bionutritionist will be contacted. If there is a decline in weight and a change in the calculated MREE, the tube feed diet will be changed according to the protocol guidelines. If there is weight loss despite no change in the MREE, the investigational diet will be increased by 1 can/day and the coordination center will be notified.

Study diet accountability and compliance will be assessed at this and at all in-person study visits. Subjects will return any unused and unopened tube feed at this study visit in order to assess compliance. The Self Weighing Log will be reviewed with the subject along with the tube

feed log to verify compliance with the study diet to compare it with the number of unused cans/bottles of tube feed returned at this visit. Subjects will be given a new Tube Feed Log and Self Weighing Log to complete before their next visit.

Study Visit V06 (Month 2)

The visit window is Day 56 +/- 6 days. Subjects will be instructed to fast for 12 hours prior to the study visit. Participants will be seen for assessment of vital signs, pO_2 and $PET_{CO2,}$ FVC, weight and any changes to medical history. An abbreviated physical exam including cardiovascular, pulmonary and skin will be performed. Blood will be drawn fasting for safety laboratory studies, optional future research study blood samples, and optional NIA/NIH blood laboratory tests for oxidized lipids and metabolic markers. Serum levels of total cholesterol will be drawn in a fasting state and then again postprandial 2-4 hours (ideally 3 hours) after a bolus feeding on-site. (If the optional malabsorption study procedures are performed at the Month 2 visit, the post-prandial cholesterol levels will not be measured at this visit.) The GIQLI and Multidimensional Dyspnea Profile questionnaires will be completed. The concomitant medication, adverse event and BIPAP logs will be updated. Safety laboratory studies will be repeated and site investigators will be notified by their clinical laboratories if there are any changes in the chemistry and liver functions tests.

Indirect calorimetry will be repeated at this visit and the total daily energy expenditure (TDEE) will be re-calculated. If there is a change in the calculated TDEE, or if there is a decline in weight, the tube feed diet will be changed according to the protocol guidelines. If there is a change in the measured resting energy expenditure (MREE), the coordinating center and chief bionutritionist will be contacted to re-calculate the investigational study diet. If there is weight loss despite no change in the MREE, the investigational diet will be increased by 1 can/day and the coordination center will be notified.

Study diet accountability and compliance will be assessed and subjects will return any unused and unopened tube feed at this study visit in order to assess compliance. The Self Weighing Log will be reviewed with the subject along with the Tube Feed Log to verify compliance with the study diet and compared with the number of unused cans of tube feed returned at this visit. Subjects will be given a new Tube Feed Log (2 months worth) and Self Weighing Log to complete before their next visit.

Telephone Visits V07 and V09 (Months 3 and 5)

All subjects will be called at months 3 and 5; Day 84 and Day 140 (the visit window is +/- 3 days). The concomitant medication, adverse event and BIPAP logs will be updated. At month 3, the study coordinators will verify compliance with the investigational study diet and answer any questions that may have arisen regarding the study diet. At both telephone visits, adverse events will be queried including gastrointestinal and respiratory symptoms. Symptoms of increased alveolar CO_2 such as shortness of breath, air hunger, increased daytime sleepiness will be queried using the Multidimensional Dyspnea Profile questionnaire. Gastrointestinal side-effects will be assessed using the GastroIntestinal Quality of Life Index (GIQLI).

Study Visit V08 (Final Visit, Month 4)

The visit window is +/- 6 days. Subjects will have been instructed to fast for 12 hours before the study visit. If subjects are taking Riluzole, they will be instructed to hold their morning dose

and bring it to the study visit. Blood will be drawn for Riluzole trough levels and for fasting cholesterol levels. They will then be told to take their morning dose of Riluzole.

At the 4 month study visit, all assessments performed at the screening and baseline visits will be repeated including vital signs, FVC, pO_2, $P_{ET}CO_2$, Indirect Calorimetry, grip strength, DXA, BIA, SKF, GIQLI, Multidimensional Dyspnea Profile and ALSFRS-R questionnaires. A full physical examination including neurological exam will be completed. Adverse events, concomitant medication and BIPAP logs will be updated. A urine pregnancy test will be repeated prior to the DXA scan. Subjects will have one last blood draw (fasting) for trough Riluzole levels, safety labs, lipid panel, optional oxidized lipids and metabolic markers and for optional future studies. The Self Weighing Log will be reviewed and study diet accountability and compliance will be assessed by having subjects return any unused and unopened tube feeds and by reviewing the Tube Feed Log. Subjects will be instructed to resume their prior (pre-study) tube feed diets. There will be one final telephone visit 1 month after the study diet ends (Month 5) in order to document resolution of adverse events.

Missed Visits

When a subject fails to appear for a scheduled visit, the site should contact the subject and a partial evaluation may be performed over the telephone including any changes to medical history, concomitant medication review, GIQLI questionnaire, Multidimensional Dyspnea Profile, Adverse Event review, BIPAP log and (if appropriate) ALSFRS-R. The site should stress that $P_{ET}CO_2$ and indirect calorimetry safety labs are critical, and would require an in-person visit. If the subject is not able to come to any of the in-person visits, the site may evaluate the subject at a home visit (see 6.2.3).

6.2.3 Home Visits

If subjects are unable to return to the study site for evaluations but wish to continue on their study diet, sites have the option to perform home study visits. If the subject continues in the study with home visits, adjustments to the subjects' tube feed dose will be made according to the subjects' weights (using their home scale or portable scale). If the subject is unable to weight themselves at home, then they will be withdrawn from the study and the diet will be considered at treatment failure.

The following activities can be performed at home visits: Vital signs, pO_2, FVC, weight and any changes to medical history, physical exam and or neurological exam, GIQLI, ALSFRS-R and Multidimensional Dyspnea Profile questionnaires, grip strength, concomitant medications, adverse events and BIPAP logs, study diet accountability and compliance can be assessed including the Tube Feed Log and Self Weighing Log. Safety laboratory studies should be obtained. Optional future research study blood samples and optional NIA/NIH blood laboratory tests for oxidized lipids and metabolic markers may be drawn if the site is able to freeze blood samples within 1 hour of the blood draw. Skinfold thickness can be measured at the sites' discretion.

6.2.4 Treatment Withdrawals and Loss to Follow-up

A subject has the right to refuse study treatment and study visits at any time and for any reason. A subject can also be withdrawn from treatment for intolerable adverse events. All efforts should be made to follow the subject for resolution of the adverse event and for survival. The site investigator must notify the Coordination Center within 24 hours of any subject permanently discontinuing study diet, documenting the reasons for discontinuation in the EDC and on the appropriate source documents.

If a subject discontinues the study diet at any time, they will be allowed to resume their prior pre-study diet and they will be considered a treatment failure in the tolerability analysis. In all circumstances, whether or not the subject agrees to continue in-person visits, the subject will be asked for permission to contact them every 4 weeks via telephone after their Baseline (Day 0) visit until the end of the five months. Follow-up of subjects who have discontinued study diet will continue in the intention to treat analysis. For the primary safety analysis, telephone visits will be used to document adverse events, changes in medical history and medications. All attempts will be made to follow these subjects for all outcome measures. The analysis of data from subjects who stopped treatment and/or refused study visits is discussed in the data analysis section.

The subject can agree or decline to return for study visits at any time during the course of the trial. For secondary outcome measures which will be analyzed by intent to treat, the importance of participating in outcomes measure assessments even after diet discontinuation will be stressed. If they are willing to continue to be followed in the study, they will undergo the same schedule of activities as planned at their subsequent visit, including indirect calorimetry and safety labs. If they are unwilling to undergo in-person visits but consent to monthly telephone visits, these telephone visits will document ALSFRS-R, length of time on non-invasive or permanent assisted ventilation and survival status. Within the limits of consent and participation, every effort will be made to collect survival and ALSFRS-R data on subjects.

Replacement of Subjects

If a subject withdraws from the trial after randomization but before they are administered their first dose of study tube feed, that subject can be replaced in order to ensure adequate sample size for the primary outcome measures of safety and tolerability.

6.2.5 Final Evaluations and Post Intervention Phone Call

Study diet will be discontinued at the month 4 study visit and subjects will resume their prior diets. The site staff will contact the subject or caregiver 28 days (+/- 3 days) following the month 4 visit. The purpose of this telephone call will be to ascertain whether any adverse events that were ongoing at the last clinic visit have resolved, and to collect any other adverse events occurring since the last visit. The subject's primary care doctor will be notified by phone and writing (if consent has been obtained) of any adverse events which continue beyond the 28 day follow-up period. Referrals will be made to appropriate specialists if needed.

6.2.6 Optional End of Study Phone Call

Subjects will be asked to consent to a final telephone contact at the end of the study (when all subjects have completed the study and the treatment assignments have been unblinded). At that visit, the site will determine the vital status (living or deceased) of the subject. If the subject is

deceased or has undergone tracheostomy, the site will obtain the date of death or tracheostomy. The coordination center will inform the sites of any results/findings from the study which can be shared with the subjects. The coordination center may also inform the sites of the treatment assignments of each subject if requested. Subjects may then be informed of their diet assignment at this final telephone visit. No additional adverse event information will be obtained at this visit.

6.3 Special Instructions and Definitions of Evaluations

6.3.1 Informed Consent

The informed consent process is described in Sections 4.3.3 and section 6.6.1.

6.3.2 Documentation of ALS

To be eligible for participation in this trial, participants must have familial or sporadic ALS diagnosed as suspected, possible, laboratory supported probable, probable or definite according to the World Federation of Neurology El Escorial criteria [10] (Appendix 10). Each Site Investigator will be responsible for documenting this diagnosis, based on the specific El Escorial criteria. These criteria will be clearly designated on source documentation and in the electronic case report forms.

6.3.3 Protocol Violations

Study visits occurring outside of the visit windows will be considered a minor protocol violation. Missed visits and any procedures not performed (not attempted) for reasons other than illness or progressive disability will be reported as major protocol violations. Procedures or visits not performed due to illness or disability and procedures that were attempted but failed will not be reported as protocol violations. Study diet compliance will be reported for tolerability analysis but will not be considered a protocol violation.

6.3.4 Clinical Outcome Measures

Primary Outcome Measures

Safety and Tolerability: Safety is evaluated using vital signs pO_2 and $PET_{CO2,}$ clinical laboratory determinations, reporting of adverse events, deaths and other serious adverse events, and treatment discontinuations due to adverse events. Tolerability will be determined by the ability to complete the study on the assigned experimental diet.

Adverse Events

Adverse events will be documented at each study visit and each telephone visit. Information on adverse effects of study medication and on inter-current events will be determined at each visit by direct questioning of the participants, clinical examination, review of concomitant medications, vital signs and weight, and safety laboratory test results. Gastrointestinal and pulmonary side effects will be queried using the GIQLI and Multidimensional Dyspnea Profile questionnaires. Tolerability will be determined by the ability to complete the study on the

assigned treatment. Of particular interest are side effects that would not be tolerable in a diet that might have to be administered life long. Given the severity of the disease fairly significant side effects might be tolerated.

Vital Signs

Vital signs will be obtained after the volunteer has been in a seated position for at least 3 minutes. Systolic and diastolic blood pressure, and pulse rate (radial artery) will be obtained at specified visits. Oxygen saturation will be measured using portable pulse oximetry and $P_{ET}CO_2$ will be measured using a metabolic cart equivalent to the VMAX 29N Spectra metabolic cart, (SensorMedics, Yorba Linda, CA).

EKG

A standard 12-lead EKG will be performed at the Screening Visit. A copy of the tracing will be kept on site as part of the source documents.

Physical and Neurological Examinations

A physical examination will be performed and recorded at the Screening and Months 1, 2 and 4 visits and will include the following systems: cardiovascular, lungs, abdomen, extremities, and skin. In addition, a complete neurological examination will be performed at screening and month 4. A physical examination will also be performed at any unscheduled visits.

Clinical Laboratory Assessments

The clinical measurements are presented in the following table. Safety laboratory tests will be performed on-site while laboratory tests that will be performed at the coordinating center (MGH core labs) will be sent in batches on dry ice by overnight mail. Core laboratory results will not be available to site investigators to prevent unblinding. For the optional study of malabsorption tests, sites will ship the blood, urine and stool samples to Quest Diagnostics. The stool samples need to be shipped on dry ice.

Safety laboratory tests	Coordinating Center laboratory tests	Optional Study of Malabsorption tests
Basic Metabolic Panel (Na, K, Cl, CO_2, BUN, Creatinine, Calcium, Glucose)	Lipid Panel (cholesterol, Triglycerides, direct HDL, calculated LDL)	D-xylose test
Liver Function Tests (AST, ALT, Total Bilirubin, Direct Bilirubin, Total Protein, , Alk Phos)	CRP (c-reactive protein)	Sudan III staining of stool
Complete Blood Count (WBC, Hct, Hgb, RBC, Plt)	TSH, IGF-1	
HCG for women of childbearing potential	Albumin, Pre-albumin	
Creatine Kinase (CK)	Acetone (ketones)	

Secondary Outcome Measures

Secondary outcome measures include changes in weight, fat mass, fat free mass, lipid levels and serum markers of nutritional status. Tertiary outcome measures include survival, defined as time to death, tracheostomy or the initiation of permanent assisted ventilation (PAV), change in grip

strength, forced vital capacity and ALSFRS-R. Given that the goal of this study is to determine safety and tolerability of the two intervention diets, we have not powered the study to detect changes in disease progression.

Body Mass Index Height and weight will be measured and recorded at screening. Height will be obtained from a standing measurement when possible, from a prior measurement in our center if the patient is unable to stand, or ascertained by history and confirmed by measured knee height, when no prior height measurement is available.

Fat mass (FM), fat-free mass (FFM) Fat mass and fat-free mass will be measured using dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), and skinfold-thickness measurements (SKF). Dual-energy x-ray absorptiometry will be completed using a Hologic QDR-Discovery A (Hologic Inc., Waltham, MA) or equivalent to determine total body and regional percent fat and lean body mass. The technique has a precision error (1SD) of 3% for fat and 1.5% for lean body mass. Total scan time equals 2 minutes. Bioelectrical impedance analysis will be measured using a BIA analyzer, (similar to the Quantum II, RJL Systems, Clinton Township, MI)[107]. Finally, multiple skin fold measurements will be taken (biceps, triceps, subscapular, suprailiac) in addition to bicep and abdominal circumferences.

Vital Capacity (VC) The vital capacity (VC) (percent of predicted normal) will be determined using the slow VC method. The VC can be measured using conventional spirometers that have had a calibration check prior to volunteer testing. A printout from the spirometer of all VC trials will be retained. All VC Evaluators must be NEALS certified. Three VC trials are required for each testing session, however up to 5 trials may be performed if the variability between the highest and second highest VC is 10% or greater for the first 3 trials. Only the 3 best trials are recorded on the CRF.

Non-Invasive Ventilatory Support Length of time using non invasive positive airway pressure ventilatory support will be documented at every visit using the BIPAP log including the telephone visits. A dramatic increase in the ventilatory requirements after initiation of the study diet will result in an unplanned study visit to measure FVC and RQ. In general, several small prospective and retrospective studies suggest that use of non invasive positive airway pressure ventilatory support prolongs survival in ALS patients [108] [109]. For this reason, investigators will be asked to follow the AAN Practice Parameters for the initiation of non-invasive ventilatory support (see below).

ALSFRS-R The ALSFRS-R is a quickly administered (five minute) ordinal rating scale (ratings 0-4) used to determine patients' assessment of their capability and independence in 12 functional activities/questions. All 12 activities are relevant in ALS. Initial validity was established by documenting that in ALS patients, change in ALSFRS-R scores correlated with change in strength over time, was closely associated with quality of life measures, and predicted survival [110-112]. The test-retest reliability is greater than 0.88 for all test items. The advantages of the ALSFRS-R are that the categories are relevant to ALS, it is a sensitive and reliable tool for assessing activities of daily living function in patients with ALS, and it is quickly administered. In a recent trial employing the ALSFRS as a secondary outcome measure, placebo treated patients showed a decline of 0.92 units per month, with a standard error of 0.08 [74]. With appropriate training the ALSFRS-R can be administered with high inter-rater reliability and test-

retest reliability. The ALSFRS-R can be administered by phone, again with good inter-rater and test-retest reliability [113]. The equivalency of phone vs. in person testing, and the equivalency of subject vs. caregiver responses have also recently been established [114]. Therefore, if necessary, the ALSFRS-R may be given to the subject over the phone.

Survival We will ascertain the status of all subjects at the end of the study and every subject will be included in the analysis whether or not they elected to stop treatment before the end of the study. Ventilation will be defined as permanent when noninvasive or invasive ventilation is used for more than 22 hours in a 24-hour period for 14 consecutive days. In our experience, time to death or time to permanent assisted ventilation (PAV) are similar because less than 10% of our patients elect PAV to prolong their lives. Given that one of our primary goals is to evaluate whether overfeeding ALS subjects results in an increase in CO_2 production leading to increased non-invasive ventilatory dependence, time to PAV may be an important measure of safety. If the fraction of subjects progressing to PAV or death is different between treatments, we will evaluate if this is reflected in a higher RQ in those subjects requiring PAV. The analysis of survival will use a log-rank test stratified by riluzole use. Several factors influence survival in patients with ALS, including age at diagnosis and site of onset [115-118]. In this study, we will not formally stratify for such variables, but will expect that randomization will result in equal distributions of sex and site of distribution, as has occurred in prior studies. We expect that the randomization will balance ancillary treatments among the treatment groups.

Hand Grip Using the Jamar grip dynamometer, bilateral hand grip strength will be measured.

Training and Validation Evaluators will be NEALS certified by SUNY Upstate Medical University in ALSFRS-R, FVC and Hand Grip assessment. It is strongly preferred that a single evaluator perform all measures throughout the study; however if more than one evaluator is used at a single site, inter-rater reliability criteria will need to be met.

7. MANAGEMENT OF ADVERSE EXPERIENCES

Descriptions, monitoring and management plans for the most frequent and anticipated adverse events will be reviewed at the Investigator Meeting and during subsequent training sessions for new sites. This information will also be provided to all sites in an excel file database of expected AE's including CTCAE codes. Adverse event monitoring and management plans will be updated in the MOP as needed throughout the course of the study. Expected adverse events from the high/calorie Jevity 1.5 are listed alphabetical order below in section 7.1. Expected adverse events from Oxepa are listed in alphabetical order in section 7.2. Adverse Events related to phlebotomy are listed in 7.3. Adverse Events that require discontinuation of study diet are described in section 8: *Criteria for Intervention Discontinuation.*

7.1 Adverse Experiences Related to Jevity 1.5/ High Calorie Diet

Increased Respiratory Quotient

ALS subjects routinely experience progression in their respiratory compromise and therefore increased shortness of breath or hyperpnea will not in and of itself constitute an AE. The risk of

increasing CO_2 production (increasing the respiratory quotient) through overfeeding has not been previously assessed in patients with respiratory failure due to neurological weakness. By feeding subjects only 1.25 times the resting energy expenditure, we hope to avoid excessive CO_2 production. A Pulmonologist consultant is assisting with this portion of the trial. Repeat FVC, PET_{CO2} and indirect calorimetry to measure CO_2 production will be performed at the 1 and 2 month visits, or earlier in an unscheduled visit in subjects who complain of dyspnea. Symptoms of increased alveolar CO_2 can include feelings of shortness of breath, air hunger, increased daytime sleepiness, and will be queried using the Multidimensional Dyspnea Profile questionnaire (Appendix 8). If subjects are not currently using non-invasive ventilatory support, Bilevel Positive Airway Pressure (BIPAP) will be instituted if the subjects meet any of the following criteria[119]:

- Maximal inspiratory pressure is < 60 cm H2O
- The forced vital capacity is < 50% of predicted or
- The PET_{CO2} (effectively equivalent to an arterial blood gas $PaCO_2$ level) is $\geq$ 45 mm Hg

Subjects who are already receiving BIPAP will be allowed to increase their use of the machine as needed, and will have the daily use queried and documented at each telephone and in-person visit.

Weight gain

An increase in weight in either the high fat/high calorie or the high calorie diet arms is expected and would not be considered an adverse event. As described in the preliminary animal data, a substantial increase in weight was seen in the two high fat diet arms and correlated with the increase in survival. We will therefore warn subjects that they are likely to gain weight and that we will not consider this an adverse event.

Diabetes

We do not anticipate that subjects will develop insulin resistance due to 4 months of overfeeding, however we will perform safety labs including a fasting serum glucose test at the screening, 1 and 2 month visits.

- If a subject has fasting blood glucose between 100 and 125 mg/dL, safety labs will be repeated after one month instead of after two months.
- If a subject has elevated fasting serum glucose at any point during the study above 126 mg/dL, the subject will be removed from the study intervention and a follow-up study will be scheduled after 1 month to verify that the blood glucose has returned to normal. If the fasting blood glucose is still elevated above 100 mg/dL, a 2 hour glucose tolerance test will be scheduled and the subjects' primary care provider will be contacted.
- If subjects are receiving continuous tube feeds and have a random glucose result above 200 mg/dL, the tube feed will be held overnight and the subject will return to the clinic to have a fasting blood glucose checked. If this is elevated above 126 mg/dL, the subject will be withdrawn from the study, and the subjects' primary care provider will be contacted.

7.2 Adverse Experiences Related to Oxepa

Weight gain, increased Respiratory Quotient and diabetes are theoretical risks that will be addressed as above. In addition, prior studies of Oxepa included diarrhea, pancreatitis, and thrombocytopenia. We would also anticipate a possible risk of fatty liver, atherosclerosis, and hepatotoxicity from Vitamin A.

Diarrhea

General measures to reduce rates of diarrhea will be used including instructing the subjects and their care givers in strict hand hygiene, carefully documenting the amount of time that a bottle or can of tube feed has been open, aliquoting and refrigerating unused tube feeds after opening. Diarrhea has been reported in 8-16% of intensive care patients [64, 65] and only 1/70 Oxepa-fed subject was unable to reach the caloric goal due to gastrointestinal side effects described as abdominal distension, high residuals or diarrhea. A study of 21 outpatient renal dialysis patients receiving Oxepa described 3 subjects with diarrhea, however diarrhea did not result in study drop-out. This is compared to a reported rate of 12-68% of diarrhea in subjects receiving other forms of enteral nutrition (depending on the definition of 'diarrhea'). We will screen for diarrhea using the GIQLI which includes a severity rating. For those subjects who complain of pain with diarrhea, the rate of tube feeds will be reduced temporarily and then re-challenged if there is improvement. If subjects are receiving their tube feeds as boluses, the rate will be changed to a continuous pump infusion.

For those subjects who on the GIQLI report diarrhea "all of the time" or "most of the time," we will evaluate the subjects for dehydration including asking the subjects if they have had symptoms of dry mouth, skin pallor, weight loss, cool extremities, dizziness, rapid heartbeat or infrequent urination. Safety labs will also evaluate renal function to assess dehydration. If there is evidence of dehydration but the subjects are willing to continue in the study, we will increase the amount of free water boluses the subject is taking and have them return for an unscheduled visit after 2-7 days, depending on the severity of the diarrhea. If the diarrhea leads to electrolyte abnormalities, such as low potassium, treatment will be stopped and subjects will be considered a treatment failure.

If there is no evidence of dehydration, the rate at which the tube feeds is given will be modified and changed to a continuous pump infusion if the subjects had been receiving bolus feedings. symptomatic treatment for diarrhea may be instituted, including adding fiber to the tube feeds, such as psyllium (Metamucil, Citrocel) or guar gum, followed by loperamide 4-8 mg/day in divided doses, but excluding bile salt binding agents such as cholestyramine which lower fat absorption [120, 121] [122]. A detailed management plan is provided to the sites in the manual of operations.

Pancreatitis

Pancreatitis was reported in 2/70 subjects in the Oxepa arm and 1/76 subject on the high fat control diet in one study [65]. Pancreatitis was not secondary to gallstones in this study. Pancreatitis is almost always painful (90-95% of patients) presenting as upper abdominal pain, sometimes radiating to the back [123]. Subjects will be queried for abdominal pain at every visit

using the GIQLI, and an abdominal exam will be performed at every in-person visit. If subjects report moderate to severe abdominal pain, they will be sent to the emergency department of the clinical site. If the subjects report mild abdominal pain or tenderness, an unscheduled visit will be added and subjects will have an abdominal exam, LFT's and amylase and lipase will be drawn. If these are abnormal, the study diet will be discontinued and subjects will be considered a treatment failure. Subjects with diagnosed pancreatitis will be sent to the emergency department of the clinical site.

Cholelithiasis

Cholelithiasis has not been reported in prior studies of Oxepa, however subjects with a prior history of biliary disease will be excluded from the study. As in the diagnosis of pancreatitis, subjects will be tested for liver function tests at each in-person visit and additional unscheduled visits to evaluate liver function tests and the abdominal exam will be scheduled if subjects report abdominal pain or tenderness on the GIQLI.

Hepatotoxicity

Although liver function abnormalities have not been reported in prior studies of Oxepa, we anticipate that a high fat diet may result in non-alcoholic steatohepatitis (NASH), and have included safety laboratory tests of LFT's in all in-person visits. Oxepa contains 320 IU of vitamin E, which is lower than the dose of Vitamins C and E (1000 mg and 1000 IU) which was shown to reduce fibrosis in NASH [124]. Fatty liver usually results in an elevation of ALT more than AST and rarely results in elevations greater than 5 times the upper normal limit. If subjects experience liver function abnormalities greater than 2 times the upper limit of normal, the diet will be suspended and liver function tests rechecked at the following visit. If liver function tests have normalized, the diet will be re-introduced and follow-up liver function tests performed the following month.

The only FDA-approved drug for ALS, Riluzole, carries a significant risk of liver toxicity; therefore subjects will be required to be on a stable dose of Riluzole for at least 60 days prior to enrollment and will have their liver function tests checked at the screening visit. If the liver function tests are already greater than twice normal in any parameter, they will not be enrolled.

In addition, Oxepa contains 11,910 IU of vitamin A per liter which may increase the risk of hepatotoxicity. Although this is less than the lowest toxic dose of 25,000 IU/day, we plan to test liver function tests regularly during the trial. Toxicity from long-term supplementation is rarely seen below 100,000 IU/day [98]. Oxepa also contains additional Vitamin C (850 mg/L versus 300 mg/L in Jevity) to reduce the risk of Vitamin A toxicity. Subjects will not be allowed to take additional supplements containing vitamin A, except for a daily multivitamin.

Birth Defects

Oxepa contains 11,910 IU of vitamin A per liter. Excess vitamin A over 10,000 IU is known to increase the risk of birth defects in women who are in their first trimester of pregnancy [99]. Subjects will also be subjected to a small amount of whole-body radiation in the two DXA scans. Therefore subjects who are pregnant or who may become pregnant during the course of the trial

will not be enrolled. Subjects who are of childbearing age will be required to use adequate birth control to prevent pregnancy.

Cardiovascular Disease

We anticipate that increasing lipid levels through increased fat intake might possibly increase the risk of cardiovascular disease; however cardiac events in prior studies have been rare. In the trial of 55 ARDS subjects fed Oxepa, one subject in the Oxepa group experienced an arrhythmia (type unknown) and one in the control group experienced "fibrillation." There were no cardiac adverse events in the Oxepa arm of a study of 70 ARDS patients, compared to 5 cardiac events in 76 patients fed the control diet including 2 cardiac arrests and 2 "fibrillation" events (p=0.06) [65]. Therefore although we plan to minimize risks to subjects by excluding patients with severe cardiovascular disease such prior myocardial infarction or stroke, we do not expect an excess number of cardiac events. A cardiologist and endocrinologist specializing in lipids are consulting for this study. We plan to check the baseline EKG on all subjects and to repeat the EKG at unscheduled visits if there are symptoms suggestive of cardiac disease. If there is a change on any follow-up EKG during the trial, we will refer subjects to a cardiologist not affiliated with the study and discontinue the study diet if appropriate. If subjects report any acute cardiac symptoms during the trial, we recommend that they be evaluated by their local emergency room and we will withdraw subjects from the study diet who develop cardiac disease as treatment failures.

Thrombocytopenia

Two out of 70 ARDS subjects on Oxepa experienced thrombocythemia compared to 0 in the control group. We plan to measure platelets at the screening visit and every 2 months during the study. Subjects will be warned to watch for any rashes, including petechial rashes, and easy bruising and will be seen urgently for unscheduled appointments. If thrombocythemia occurs, subjects will be withdrawn from the study diet and referred to a hematologist.

7.3 Adverse Experiences Related to Phlebotomy

Risks associated with phlebotomy include local discomfort, site irritation, or infection. Serious adverse events are rare. Subjects will be instructed on signs and symptoms of infection and how to contact study staff in this event.

7.4 Adverse Experiences Related to Xylose

There is a small risk of nausea, vomiting or diarrhea from taking the Xylose. There is a risk of feeling hungry, light-headed or dizzy from not eating during the Xylose test.

8. CRITERIA FOR INTERVENTION SUSPENSION, RECHALLENGE, OR PERMANENT DISCONTINUATION

8.1 Intervention Suspension Related to Jevity and Oxepa Use

Study diet will be suspended under the following circumstances:

- If the subject is being treated for severe dehydration from diarrhea
- If the subject is diagnosed with pancreatitis
- If the subject is diagnosed with symptomatic cholelithiasis
- If the subject is diagnosed with acute coronary syndrome
- If the subject is diagnosed with peripheral vascular disease
- If the subject is diagnosed with stroke
- If the subject is diagnosed with thrombocytopenia

Subjects may be rechallenged with study diet under the following circumstances:

- Upon resolution of dehydration at the discretion of the site investigator
- Upon resolution of symptomatic cholelithiasis

Subjects can not be rechallenged with study diet under the following circumstances:

- If the subject is diagnosed with pancreatitis
- If the subject is diagnosed with acute coronary syndrome
- If the subject is diagnosed with peripheral vascular disease
- If the subject is diagnosed with stroke
- If the subject is diagnosed with thrombocytopenia

9. STATISTICAL CONSIDERATIONS

9.1 General Design, Data Analysis and Power

The Senior Biostatistician at Massachusetts General Hospital will be primarily responsible for the biostatistical component of this study.

9.1.1 Safety and Tolerability Study

In accordance with the intent-to-treat principle, all subjects randomized will be kept in their originally assigned treatment group for analysis. All randomized subjects will be considered evaluable for tolerability and safety. Subjects lost to follow-up will be considered treatment failures at the time they drop out of the study. The data will be reviewed by the Steering Committee, using the considerations here as guidelines. Their recommendation would then be reviewed by the DSMB.

Safety data will be reported to the DSMB on a monthly basis. The DSMB will analyze the frequency of adverse events on a 3-6 month basis or sooner if there is a high number of adverse events. The treatment groups will be compared with respect to occurrence of each adverse event. Total number of adverse events and abnormal laboratory tests will be compared between groups using Fisher's exact test. Withdrawal, abnormal laboratory tests, vital signs and use of concomitant medications will be assessed to characterize the safety profile of the two intervention diets. With 10 subjects there is a 89% chance of seeing at least one occurrence of an adverse event that occurs 20% of the time, and a 90% chance of seeing at least one occurrence

of an event that occurs 30% of the time[101]. Descriptive statistics denoting the changes from baseline to the final assessment visit with respect to key laboratory parameters and vital signs will also be provided.

In the tolerability analyses, subjects will be regarded as a treatment failure if they fail to complete the Month 4 visit of the study on the originally assigned treatment for any reason. If subjects have side effects, such as nausea or diarrhea, but are still able to comply with the diet, we will consider the side effects to be tolerable. If subjects lose weight despite optimal nutritional intervention, we will withdraw them from the study and consider them treatment failures. Compliance data will be determined for each visit and by treatment group. The time to subject refusal will be compared between treatment groups using survival analysis in order to better determine tolerability. We will consider a dosage tolerable if the proportion of treatment failures for a dosage is less than 40% with 80% confidence. With 10 subjects this would occur if 5 or fewer subjects fail to complete the 4 month study. With 10 subjects we will have more than a 68% chance of declaring a dosage tolerable if the true treatment failure rate is 20%.

9.1.2 Secondary Outcomes:

The secondary outcome measures of biomarkers of body composition and lipid metabolism will be analyzed by 'intent to treat.' Change over time in BMI, fat mass, fat-free mass, albumin, pre-albumin, and lipid levels will be compared between the 3 treatment groups using longitudinal regression where treatment, age, gender, duration of disease, and bulbar onset will be used as covariates. We hypothesize that both intervention arms will have higher levels of total cholesterol, LDL, and triglycerides compared to the non-intervention arm. If any of the results are significantly different across the three arms, we will then do a pairwise analysis. We will adjust for multiple testing using a strict Bonferroni adjustment.

Using the standard deviation of the trial of Arimoclomol in ALS, 10 subjects in each arm would have only 33% power to detect a 2 point change in BMI between the 3 arms at a one-sided significance level of 0.1 [84]. However, using the standard deviation reported in Dupuis et al. [46] for BMI, we calculate that a total of 10 subjects in each arm would have 60% power to detect a 2 point difference in BMI between the treatment and control arms at a one sided p=0.1 significance level [125, 126]. Using the standard deviation for total cholesterol measured in the trial of Arimoclomol in ALS, we calculate that the sample size of 10 would have 86% power to detect a difference of 40 mg/dL in total cholesterol at a one-sided significance level of 0.1. Using the standard deviations for lipid measurements from Dupuis, 2008[46], a sample size of 10 would have over 80% probability of detecting a difference of 40 mg/dL in LDL levels [125] at a one-sided significance level of 0.1.

9.1.3 Tertiary Outcomes:

Although the study is not powered to look at the effects of the diet interventions on disease progression, we will examine trends in measures of disease progression. The preliminary analysis of survival, ALSFRS-R and vital capacity will be by "intention to treat." We will ascertain the ALSFRS-R and vital status of all subjects at the end of the study and every subject will be included in the analysis whether or not they elected to stop treatment before the end of the study.

A sample size of 10 subjects will provide only the ability to detect a positive trend in survival at a one-sided significance level of 0.1 if the hazard ratio is 6 or greater [126-128]. Given the small sample size, survival analysis will not be stratified for gender and for riluzole use.

We will use a random effects model for longitudinal data including baseline covariates such as age, gender, race, family history of ALS, mean time since onset of ALS, age at disease onset, bulbar onset, weight, BMI, ALSFRS-R score at inclusion, and riluzole use. The basic idea of this model is that each subject has his or her own trajectory with a random slope, intercept and curvature the average value of which may depend on treatment. We will look carefully at the residuals for this model to be sure that it fits the data. If there appear to be problems with fit, we will use a robust approach of assessing significance where the p-value for any treatment contrast is calculated using a permutation test.

We have calculated the variance of the mean rate of decline in ALSFRS-R and VC in subjects from the celecoxib treat ALS trial who received a percutaneous endoscopic gastrostomy during the trial (N=34)[75]. Using this variance, our study will be able to detect a trend towards reduced disease progression, using a one sided p value of 0.1, if there is difference in slope of decline of 0.94 of the ALSFRS-R between the control group and a treatment group over the 4-month period [126, 127].

The analysis of vital capacity and dynamometry will use inverse probability of censoring weighted (IPCW) log-rank tests, which account for informative censoring due to death [129]. Using the variance calculated from the celecoxib treat ALS trial, our study would be able to detect a difference of 3.4 in the slope of rate of change of the vital capacity between the treatment and control groups.

Stopping for Safety

Decisions to stop the trial early for safety will be made by the DSMB, according to but not limited by rules outlined in the DSMB charter. The suggestion from the study Principal Investigator and the study Statistician is that there will be no early stopping rule for futility or efficacy in this phase II trial. However there will be a rule for stopping if the study diet appears to increase mortality defined as death or permanent assisted ventilation. As outlined in the DSMB Charter, survival in each arm compared to the control group will be calculated using the logrank test. We will use an alpha spending rule to look for increased mortality compared to control diet using a 1-sided p value of 0.1 for either of the intervention arms according to the following table:

DSMB Meeting Date	Expected Number of Events	Z statistic	Cumulative Alpha	P-value to stop
Month 9	3.6	3.089	0.001	0.001004
Month 12	7.3	2.060	0.020	0.019699
Month 15	10.9	1.630	0.058	0.051551
Month 18	14.5	1.390	0.100	0.082264

Thus, if at the first DSMB meeting at month 3, the p-value is less than 0.001004 for the logrank test comparing the survival distributions of one of the treatments versus control diet (Jevity 1.0), then this treatment arm will be stopped. One study arm (one dietary intervention) may be stopped early without stopping the study. The overall probability of stopping one arm of the study early if there is no effect on mortality is 10%.

The first meeting of the DSMB will occur three months after the start of accrual or as soon as 30 subjects have been enrolled, whichever occurs sooner, and will continue every 3-6 months throughout the study.

Accrual Targets: The study should take five months to begin fully accruing at a rate of 2-4 subjects per month.

Treatment Discontinuation Rate Targets: Subjects who discontinue treatment will be considered treatment failures in the tolerability analysis. However if there is an elevated discontinuation or study drop-out rate, it will reduce the power to detect adverse events for the safety analysis primary outcome. The DSMB will analyze enrollment and discontinuation rates at pre-specified intervals and if it appears that fewer than 10 subjects per arm will complete the 4 month study, they may recommend stopping for futility. Ten subjects or 50% of the target enrollment will give us 89% probability of detecting an adverse event rate of 20% [101].

Analysis of Data on Women and Minorities. Our proposal for the analyses of these data follows NIH Guidelines. We intend to use the data from the trial to determine whether women or minorities respond differently to treatment. This will be accomplished by testing for a treatment gender and treatment ethnicity interaction in our efficacy and safety analyses. Although the power of these analyses may be low for the primary outcome the power will be adequate for the secondary outcomes.

9.2 Outcomes

Section 9.1, above, contains detailed information regarding Outcomes.

9.3 Sample Size and Accrual

Section 9.1, above, contains detailed information regarding Sample Size and Accrual.

9.4 Data and Safety Monitoring

9.4.1 Monitoring and Reporting Process

The Principal Investigator is responsible for oversight of the data safety and will work with the Co-PI to monitor the proposed study. The PI and the Steering Committee will work with the appointed DSMB to evaluate safety on an ongoing basis. The stopping rules for safety and tolerability are described in detail in Section **9.1.3.** In summary, the trial will be stopped for safety if there are significantly increased numbers of adverse events in any arm at each of interim safety analyses. The trial will be stopped for futility if it does not reach accrual targets or if the dropout rate reduces the power to show safety. Stopping for tolerability will be based upon alpha spending rules with analyses at 6 and 12 months.

The DSMB will teleconference at least every 3 months and at the end of the study. In addition, the P.I. and members of the Steering Committee may call ad hoc meetings. The PI will be responsible for overseeing the preparation of reports to the DSMB as well as interim communications to the co-investigators, should more frequent notification of safety issues be required. Blinded summary reports will be generated monthly by the Statistical Consultant and the Coordination Center and forwarded to the DSMB for review. Blinded and, if requested, unblinded DSMB reports including all adverse events and laboratory data stratified by group will be generated prior to each 3 month DSMB meeting. As necessary, the DSMB can review the frequencies of clinical and laboratory abnormalities. The DSMB will be responsible for determining safety-stopping rules. The PI will provide suggested safety stopping rules for consideration by the DSMB. Recommendations for modification or termination of the trial based on safety data will be made by the DSMB to the PI.

9.5 Data Analyses

Section 9.1, above, includes detailed information regarding Data Analyses

10. DATA COLLECTION, SITE MONITORING, AND ADVERSE EXPERIENCE MONITORING

10.1 Records to be Kept and Project Organization

The MGH Neurology Clinical Trials Unit (NCTU) Coordination Center and the Biostatistics Center at the MGH will conduct the project coordination, data management, biostatistics, regulatory compliance, adverse event reporting, study monitoring, quality assurance issues and budgetary and contract components. The outcome measure training will be conducted by SUNY Upstate Medical University. The project coordination staff will work in collaboration with the PI and Co-PIs to accomplish all the tasks necessary for preparation and implementation of the project, including the drafting of the study protocol, development of the model consent document, assisting in communications with the Steering Committee, DSMB, developing project timelines, compiling required documents from sites, preparing the detailed manual of operations, and overseeing enrollment and enrollment projections. The project coordination staff will reply to site protocol inquiries on a daily basis, track adverse events, study diet discontinuations and study compliance.

The Muscular Dystrophy Association Clinical Research Network will facilitate communication between sites using Internet, telephone conference calls, fax and periodic mailings as its means of communications. A Web portal will be established to provide the study personnel with an easily accessible repository of documents, which is essential for sharing information across multiple clinical trial sites. The secure, password-protected portal will contain role-based resources for study personnel. For example, the portal provides personnel lists with contact information, the complete study protocol, model informed consent forms and other study-related documents, a list of subject accrual figures that is updated continuously to allow anyone in the group to monitor study progress, a list of frequently asked questions for quick reference, and other resources that the clinical investigators and coordinators might find useful.

The Coordination Center will use the MGH core laboratories as the central laboratory for the study. This laboratory has provided its current certificate of the laboratory and the curriculum vitae of the central laboratory director to the Study PI. In addition, a table listing the ranges of values considered normal for the determinations each laboratory is performing for the trial has been provided.

10.2 Role of Data Management

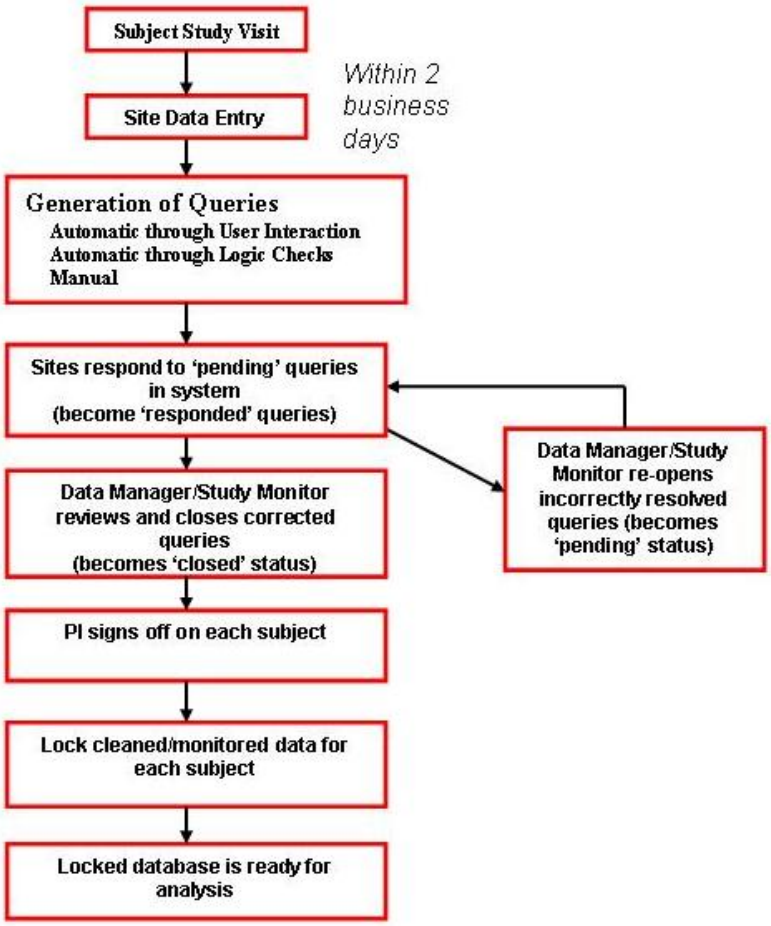
10.2.1 Data Flow and Management Overview

The Data Management staff at the MGH NCTU will be responsible for all aspects of data acquisition and processing, from the design of instruments used to collect data through the delivery of an accurate and timely database to the Biostatistics Center. In conjunction with the PI and the Biostatistician, the data management staff at MGH will design the electronic case report forms (eCRFs) that will capture all the data collected as part of the protocol. Interactive computer modules for real-time capture of all important study events including enrollments, serious adverse events (SAEs), reportable incidents and premature withdrawals will be created. The Electronic Data Capture and Data Management System (EDC&DM) will be tested and validated to ensure accuracy, reliability and consistent intended performance. The system conforms to the 21 CFR Part 11 and other guidance documents on computerized systems in clinical trials. The system will allow the study sites to single-enter the data via an Internet browser-based interface. The entered data will be saved in a Microsoft SQL Server database located on a server maintained by Partners Healthcare Systems IS department. Necessary documentation on the validation procedure will be maintained by the Coordination Center. A full time data manager will be responsible for the data acquisition and management at the MGH Coordination Center.

The flow of data and the data clarification process is summarized in Figure 7. The site personnel are instructed to enter information within 48 hours of the visit. An edit checking and data clarification process will be put in place to ensure accuracy and completeness of the database. Logic and range checks as well as more sophisticated rules will be built into the Web-based forms (eCRFs) to provide immediate error checking of the data entered. The system will automatically create electronic queries on behalf of the Data Manager if saved electronic Case Report Forms (eCRFs) contain data that are out of range, out of window, missing or not calculated correctly. The Data Manager identifies the errors in the EDC system by using electronic logic checks and the Study Monitor identifies errors by direct visualization and comparison of data entered into the system with the source documents. Any inconsistent or questionable data points are queried to the sites and followed up on by both the Study Monitor and Data Manager as needed. Once the site addresses the query, the Data Manager or the Study Monitor can verify that the response is satisfactory, and that the value in the data field has been corrected. The Data Manager or Study Monitor can then "close" the query in the database. The sites and Study Monitor will only have access to the queries concerning their subjects. The Data Manager will be able to see the queries for the entire subject population. The Sites will have the ability to write a comment for a form or field. Each comment has its own history, which is recorded in a log. The log records the information that has been entered in the comment, who entered it, and when it was entered. The comment field is treated as a regular data field; hence all changes to the comments are tracked in the audit trail. We propose to use the National

Cancer Institute's (NCI) Common Terminology Criteria for Coding Adverse Events (CTCAE) version 3.0. This is a descriptive terminology, organized by body system and including specific criteria for grading severity of Adverse Events. This system will allow study coordinators to quickly search for the most relevant term for each event and will give specific criteria governing the reporting of severity for each term. With this system, the event will be coded at the site and subsequently checked by the Data Manager. The system will have predefined roles and system Administrators will assign them to the system users. Depending on the role assigned, the users will differ not only in their rights to enter or view certain data, but also in their rights to access certain forms and views.

Figure 7. Data Flow and Clarification



10.2.2 Database Security

The MS SQL Server database is located on a secure database server. This server is located in a restricted area of the Partners Healthcare server farm and physical access to it is limited to authorized personnel only. Both database and Web servers are located on the Partners Healthcare network behind the firewall. Access to the data at the clinical site will be restricted and monitored through the system's software with its required log-on, security procedures and audit trail. The data will not be altered, browsed, queried, or reported via external software applications that do not enter through the protective system software. There will be a cumulative record that indicates, for any point in time, the names of authorized personnel, their titles, and a description of their access privileges. The record will be in the study documentation accessible at the site. Controls will be in place to prevent, detect, and mitigate effects of computer viruses on study data and software. The application utilizes SSL (Secure Sockets Layer) technology and 128-bit encryption to comply with requirements of 21 CFR Part 11 for Open Systems. Backups of the database will be performed nightly using the services provided by the MGH network. All PC's run virus protection software full-time and are updated with the latest virus detection strings regularly; the Windows NT server does this as well and has the additional security of

scanning all e-mail for viruses before a user can even access them. All accounts are password protected and passwords must be changed on a regular basis.

In addition, the EDC system will have an extra level of password security. At study initiation, the Data Manager will set default passwords for the relevant study personnel at the MGH NCTU and at the study sites. When a new user logs in with the assigned username and default password for the first time, he or she will be forced to change the password to a unique one (at least six characters long), known only to the user. An ongoing paper log will be kept showing when usernames and passwords are set up, for whom, in what user capacity and when usernames are disabled. In case an employee forgets her/his password or a new user is added, they will submit a password request form via email to the Data Manager, who will issue a new default password. They must then go through the Change Password process. The passwords will expire every three months, when users will be required to go through the Change Password process. To avoid password-based software attacks, the system will lock a user for 1 minute if an incorrect password is provided 3 times in a row. A user will also be able to change the password at will if he or she feels that it may have been compromised.

10.2.3 Data Lock Process

The application will have the ability to lock the database to prevent any modification of data once the study is closed. Once this option is activated, every user will have Read-Only access to the data. Throughout the study, the Study Monitors will be verifying the source documents against the database. The Study Monitors will review source documents and electronic case report forms for accuracy and completeness as described in the study monitoring plan. The Data Manager can only lock the database once the following steps occur: the Site Investigator has signed off on each subject, the Study Monitor has verified the subject's data, and all queries have been resolved. The database will be transferred to the Biostatistics Center by unloading the relational MS SQL Server database to a SAS format for statistical analysis. The database will also be accessible to biostatisticians for reporting and statistical analysis during the trial.

10.3 Quality Assurance

Study Monitoring. Study monitors, supervised by the PI and Co-PIs, will visit each study site annually to review source documentation materials, informed consent forms, and confirm entered data and that queries have been accurately completed. Study Monitors will also verify that Adverse Events and protocol violations have been reported appropriately to the Coordination Center and their local IRB as required. The Study Monitor will also review clinical facilities resources and procedures for evaluating study subjects and study diet dispensing. Subsequently, the Study Monitor will provide reports of protocol compliance to PI and the Steering Committee. Completed informed consent forms from each subject must be available in the subjects file and verified for proper documentation.

10.4 Adverse Experiences Monitoring and Reporting

An adverse event (AE) is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device whether or not considered related to the drug product or device.

The adverse event (AE) definitions and reporting procedures provided in this protocol comply with all applicable U.S. FDA and ICH guidelines and regulations. The Site Investigator will carefully monitor each subject throughout the study for possible adverse events. All AEs will be documented on eCRFs designed specifically for this purpose. It is also important to report all AEs, especially those that result in permanent discontinuation of the investigational drug being studied, whether serious or non-serious.

For the purposes of this study, symptoms of progression/worsening of ALS, including 'normal' progression, will be recorded as adverse events. The following measures of disease progression will not be recorded as adverse events; even if they worsen (they are being recorded and analyzed separately): vital capacity results, non-invasive ventilatory requirements, grip strength results, and ALSFRS-R ratings.

Subjects will be monitored for adverse events from the time they sign consent until completion of their participation in the study. Relationship of adverse experiences to the experimental intervention will be assessed at each in-person and telephone study visit by recording all voluntary complaints of subjects and by assessment of the clinical features of ALS. Attention will be directed to clinical adverse experiences associated with prior Oxepa studies, as well as any evidence of unexpected worsening of the underlying ALS. Laboratory surveillance tests will be obtained as outlined above.

A serious adverse event is defined as an adverse event that meets any of the following criteria:

- Results in death

- Is life threatening: that is, poses an immediate risk of death as the event occurred
This serious criterion applies if the study subject, in the view of the Site Investigator, is at substantial risk of dying from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization.
Hospitalization for an elective procedure (including planned tracheostomy) or a routinely scheduled treatment is not an SAE by this criterion because a "procedure" or a "treatment" is not an untoward medical occurrence.

- Results in persistent or significant disability or incapacity
This serious criterion applies if the "disability" caused by the reported AE results in a substantial disruption of the subjects' ability to carry out normal life functions.

- Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female)

- Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.

An event is considered "life-threatening" if it places the subject, in the view of the Investigator, at immediate risk of death from the reaction as it occurred; i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical adverse event may meet criteria for "seriousness" but is not an adverse experience, and will therefore, not be considered an SAE. An example of this would include a social admission (subject admitted for other reasons than medical, e.g., lives far from the hospital, has no place to sleep).

An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with expected risks of the study intervention.

All serious adverse events (SAE) (including death and hospitalization), premature withdrawal, dosage changes and emergency treatment disclosures will be reported to the Coordination Center within one working day and SAE forms completed within 24 hours of their occurrence. The DSMB will review all SAE reports. Serious adverse events that occur during this study will be recorded in the subject's chart and on the CRF. All unexpected and related SAEs will be reported to the site IRB and Coordination Center. The Coordination Center will then report to the DSMB,, FDA if necessary and to all sites. . Death due to progression of disease, as expected in ALS, will not be reported in an expedited manner except in cases where the outcome of death is deemed related to study treatment.

Sites will be instructed to submit these to their IRBs as well. Any SAE that results in the study being put on hold at any site will be reported to all sites IRB, the DSMB, and the MDA. All unexpected and related SAEs will be reported to the FDA if necessary, in an expedited manner, as required by FDA guidelines. Any adverse experiences will be followed for resolution.

There will be ongoing monitoring of non-serious Adverse Experiences through both the EDC system and on-site monitoring visits, to ensure adequate reporting of such events. The MGH Coordination Center will report all AEs to the DSMB monthly and to the MGH IRB annually, and sites will be instructed to report AEs as required by their local IRBs. We have included in the Steering Committee experts in lipid disorders, cardiology and pulmonology to advise us on how to minimize adverse events from the study diets.

10.4.1 Evaluating and Recording of Adverse Events

At each visit all adverse events that are observed, elicited by the Site Investigator, or reported by the subject will be recorded in the appropriate section of the Adverse Event log, entered into the EDC and evaluated by the Site Investigator.

Minimum information required for each AE includes type of event, duration (start and end dates), severity, seriousness, causality to study intervention, action taken, and outcome.

The Site Investigator will grade the severity of AEs. The severity grading will be based on the unique clinical descriptions of severity for each AE in the CTCAE system, which follows this general guideline:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

The relationship of the AE to the study intervention should be specified by the Site Investigator, using the following definitions:

1. Not Related: Concomitant illness, accident or event with no reasonable association with treatment.
2. Unlikely: The reaction has little or no temporal sequence from administration of the study intervention, and/or a more likely alternative etiology exists.
3. Possibly Related: The reaction follows a reasonably temporal sequence from administration of the intervention and follows a known response pattern to the suspected intervention; the reaction could have been produced by the study intervention or could have been produced by the volunteer's clinical state or by other modes of therapy administered to the volunteer.
4. Probably Related: The reaction follows a reasonable temporal sequence from administration of study intervention; is confirmed by discontinuation of the study intervention or by rechallenge; and cannot be reasonably explained by the known characteristics of the volunteer's clinical state.
5. Definitely Related: The reaction follows a reasonable temporal sequence from administration of study medication; that follows a known or expected response pattern to the study medication; and that is confirmed by improvement on stopping or reducing the dosage of the study medication, and reappearance of the reaction on repeated exposure.

If discernible at the time of completing the AE log entry, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Site Investigator and recorded on the AE log. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the Site Investigator to be a component of a

specific disease or syndrome, then it should be recorded as a separate AE on the AE log. Clinically significant laboratory abnormalities are those that are identified as such by the Site Investigator and/or those that require intervention. The only exception to this will be ALS progression symptoms as previously noted.

11 HUMAN SUBJECTS

11.1 Institutional Review Board (IRB) Review and Informed Consent

The PI has obtained approval from the Partners Human Research Committee (HRC, or Partners IRB) for the NCTU to serve as the Coordination Center for this trial. Each of the participating sites will also be required to obtain IRB approval of the protocol and consent form and send copies of these to the Coordination Center. The PI and Project Manager will review any alterations of the consent documents made by individual sites, to assure that all essential elements remain. Any subsequent modifications of the protocol or consent documents will be reviewed and approved by the MGH HRC and all site IRBs. Signed consent will be obtained from each subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and this will be documented.

The PI and all key personnel involved in the study will have completed the Collaborative IRB training initiative, a mandatory tutorial on the responsible conduct of human subject research, or will have completed a comparable, institution-approved tutorial regarding the protection of human subjects. An independent Data Safety Monitoring Board will review the study data for safety concerns. The PI will maintain minutes of the DSMB meetings and provide these to all the sites for submission to their IRBs. The PI will be responsible to report adverse events experienced by study subjects to the MGH IRB, the DSMB, MDA and to all site investigators.

11.2 Subject Confidentiality

Confidentiality will be maintained, as all subject research data will be coded with subject ID number and initials. Blood samples will be labeled using an alphanumeric code consisting of the 6 digit subject ID, 3 digit site ID and sequential three digit number 001, 002, 003, etc. for each site, prior to shipping to the MGH NCTU. The Coordination Center files will be kept in a secure, double-locked area. The electronic database used during the trial will be secure. To date no breach of our security barriers has occurred, and we actively maintain a high level of security to assess the confidentiality of our databases. Only key personnel in this proposal will have access to the data and the codes. Subject results will never be discussed in any form in the presence of other subjects in the study or with non-laboratory personnel. A subject will be referred to by his/her randomization ID number only. The primary risk of participating in the study is the potential adverse effects from the experimental intervention.. The PI, co-PI, Steering Committee, and Data Safety and Monitoring Board will monitor safety and risks throughout the study. In the rare event that an investigator needs immediate knowledge of the subject's treatment assignment, the individual treatment assignment generated by the Biostatistics Center will be available.

11.3 Inclusion of Women

The gender distribution for subjects with sporadic ALS is approximately 60% male and 40% female. The study goal is to recruit men and women with ALS in a 3:2 ratio. The MGH patient population includes 53% men and 47% women. Subjects at participating sites will be recruited from those site-specific areas and their surrounding communities. Advertising the study with several ALS and MND foundations and patient support groups will aid in the recruitment process and, in particular, with the recruitment of female subjects.

The sites participating in the study have demonstrated the ability to enroll females in prior NEALS and industry-sponsored studies. In the NEALS multi-center topiramate trial in ALS, 36% of the subjects were female. Similarly in the NEALS clinical trial of creatine in ALS, and in the clinical trial of celebrex in ALS, 39% and 36% of the subjects were female, respectively. Each site has provided detailed enrollment goals and all sites documented ability to enroll 40% females. Copies of these documents are kept at the MGH Coordination Center. Based on this information, we do not anticipate difficulty enrolling the number of females for this study that represents the percentage of females with ALS in the total population.

11.4 Inclusion of Minorities

ALS is a relatively rare disease and there is mixed data on its incidence in minority groups. Most epidemiologic studies of ALS have investigated homogeneous populations. One of the only mortality studies conducted within a large, multi-ethnic population by Annegers et al. (1991) found that age- and sex-adjusted mortality did not differ among ethnic groups [130] Some studies suggest that ALS may be less frequent in non-white individuals. An incidence study in the state of Washington found rates in white males to be 1.8 per 100,000 compared to 0.74 per 100,000 per year for black males, although the difference was not statistically significant due to the small numbers of incident cases [131]. Dr. Kasarskis recently determined the rates of ALS in whites, blacks and other racial groups in a cohort of US soldiers serving during the Gulf War I era. They concluded that the rate for ALS/MND in “Other racial groups” (mainly Hispanic) was significantly elevated compared to whites in this young, predominantly male cohort whereas the overall rate for blacks was 33% lower than the specific rates estimates [132].

Approximately 17% of the national population is of a non-white racial background. For the purposes of recruitment in this study we will assume a racial distribution similar to the overall US population. Ethnically, 12.5% of the national population is Hispanic or Latino (http://www.census.gov). Thus, the study goal is to enroll at least 12.5% of the subjects from this ethnic group. Approximately 13% of our nation’s population is Black, 0.3% is Hawaiian/Pacific Islander, 4.2% is Asian, and approximately 1.5% is American Indian. Our goal is to enroll subjects with ALS that are representative of the nationwide demographics. The racial composition of MGH is 82% White, 3% Black, 1% Asian, and 12% unknown. The ethnic population of MGH is 3% Hispanic and 97% non-Hispanic. For geographical reasons, much of the local minority population does not have the MGH as its primary care facility in Boston.

To reach the recruitment goal, efforts will be focused on enrollment of individuals with ALS who are members of underrepresented minorities. Recruitment of members of minority groups

will be closely monitored. Subjects will be recruited from the clinic population of the participating sites and through contact with support groups and healthcare providers from surrounding areas. IRB approval will be obtained for all planned letters, newsletters and web advertisements.

Potential study subjects will not be excluded from this study for reasons of race or gender and efforts will be made to enroll in representative numbers with respect to both gender and race. In particular, no racial discrimination will be made in subject enrollment. The participation of minority subjects will be actively encouraged throughout the study.

The study PI will emphasize the significance of a balanced recruitment effort with each site investigator, as well as conduct reviews of recruitment rates.

11.5 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the IRB (or Human Research Committee), the MDA, the Office for Human Research Protections (OHRP), or other government agencies as part of their duties to ensure that subjects are protected.

12 PUBLICATION OF RESEARCH FINDINGS

The Steering Committee, chaired by Drs. Wills and Cudkowicz, will be responsible for publications of results from this trial. The Steering Committee will comply with NINDS guidelines on publication of NIH funded clinical trials. Their responsibilities will include the following:

- Analyze and interpret data gathered in this study, and write publications from these data.
- Submit manuscripts to selected journals and address peer reviewers' comments.
- Submit abstracts to selected meetings and present data at the meetings.
- Determine authorship on the basis of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (International Committee of Medical Journal Editors,1997).

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