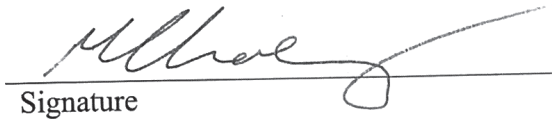
Clinical Study Protocol

Title	**A Randomized, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Physiological Effects on Liver Structure and Function of an Amino Acid Food Product, AXA1957, in Adolescent Subjects with Fatty Liver**
Protocol Number	AXA1957-002
Date (version)	13-Dec-2019 – Version 3.0
Sponsor Address	Axcella Health Inc. 840 Memorial Drive, 3rd Floor Cambridge, MA 02139

SPONSOR PROTOCOL APPROVAL PAGE



Signature

Manu Chakravarthy, M.D., Ph.D.
Sponsor Responsible Person

Chief Medical Officer
Title

13-Dec-2019
Date

INVESTIGATOR'S SIGNATURE OF AGREEMENT PAGE

Protocol Title: A Randomized, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Physiological Effects on Liver Structure and Function of an Amino Acid Food Product, AXA1957, in Adolescent Subjects with Fatty Liver

Protocol Number: AXA1957-002

I, the undersigned, have reviewed this protocol, including appendices and I will conduct the study as described in compliance with this protocol, current Good Clinical Practice (GCP) guidelines and relevant International Conference on Harmonization (ICH) guidelines.

Once the protocol has been approved by the Investigational Review Board (IRB)/Research Ethics Committee (REC), I will not modify this protocol without obtaining prior approval of Axcella Health (Sponsor) and of the IRB/REC. I will submit the protocol modifications and/or any informed consent form(s) (ICF) modifications to Axcella Health and the IRB/REC, and approval will be obtained before any modifications are implemented. except where necessary to eliminate an immediate hazard(s) to the study subjects. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, ICF(s), recruitment materials, and all subject materials will be submitted to the IRB/REC for review and approval. Approval of both the protocol and the consent form(s) must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB/REC before the changes are implemented to the study. All changes to the consent form(s) will be IRB/REC approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form(s).

I understand that all information obtained during the conduct of the study regarding the subjects' state of health will be regarded as confidential. No subjects' names will be disclosed. All subjects will be identified by assigned numbers on all Case Report Forms (CFRs), laboratory samples or source documents forwarded to the Sponsor. Clinical information may be reviewed by the Sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.

Information developed in this clinical study may be disclosed by Axcella Health, to other clinical investigators, regulatory agencies, or other health authority or government agencies as required.

Principal Investigator/Title (printed name):

_____

Institution: _____

Address: _____

Telephone number: _____

Signature: _____

Date: _____

SPONSOR CONTACT INFORMATION

Sponsor Responsible Person and Study Director:	Margaret Koziel, MD 840 Memorial Drive, 3rd floor, Cambridge, MA 02139 USA Telephone: +1 857 320 2238 E-mail:mkoziel@axcellahealth.com
Chief Investigator:	Saul N. Faust, MBBS MRCPCH PhD FHEA Professor of Paediatric Immunology & Infectious Diseases, and Director NIHR Clinical Research Facility University of Southampton, C Level, West Wing, Mailpoint 218, University Hospital Southampton NHS Foundation Trust, Tremona Road, Southampton, SO16 6YD. Email: s.faust@soton.ac.uk

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Study Synopsis

Protocol Number	AXA1957-002
Protocol Title	A Randomized, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Physiological Effects on Liver Structure and Function of an Amino Acid Food Product, AXA1957, in Adolescent Subjects with Fatty Liver.
Study Objectives	To assess the safety, tolerability, and parameters of liver health in adolescent subjects with fatty liver when administered AXA1957, an amino acid food product.
Study Assessments	Safety and tolerability will be assessed by: • Reported clinical adverse events (AEs) • Physical examinations, including changes in body weight and body composition such as lean mass and fat mass • Vital sign assessments • Electrocardiograms (ECGs) • Clinical laboratory tests including changes in standard haematology, chemistry, and lipid panels Liver structure and function will be assessed by: • Multiparametric magnetic resonance imaging (MRI) assessments of liver structure (fat content and inflammation changes) • Blood tests of liver function, including markers of inflammation and fibrosis
Number of Study Sites	Up to 7 sites in the UK and US
Study Population	Male and female subjects between 12 and 17 years of age, inclusive.
Number of Subjects	Sufficient number of subjects will be screened to have approximately 30 subjects complete the study. Note: Subjects withdrawing early in Part 1 may be replaced. Subjects will not be replaced in Part 2.

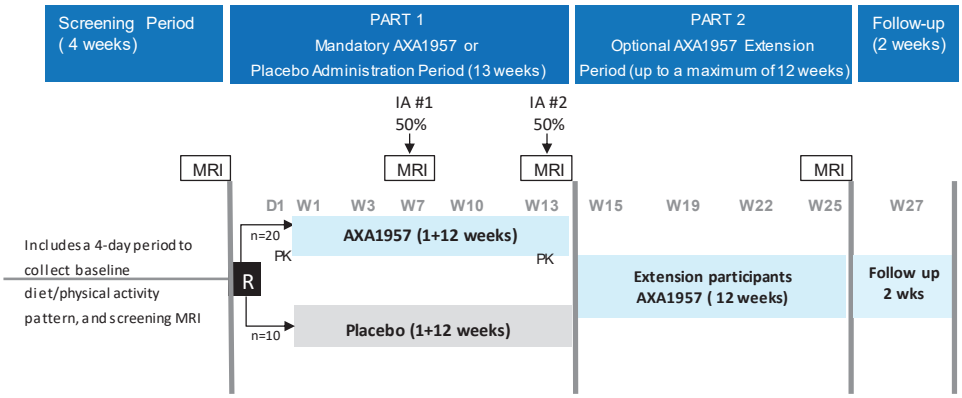
Summary of Study Design	This is a randomized, placebo-controlled study conducted in 2 Parts: a mandatory 13-wk administration period (Part 1) and an optional extension period up to a maximum of 12 additional weeks (Part 2); see Figure 1 below. **Figure 1: Study Schematic**  The total duration of the study from Screening to the end of Part 2 follow-up is anticipated to be approximately 31 weeks. It is anticipated that there may be a total of approximately up to 12 study visits during the entire study (Screening, Parts 1 and 2). In Part 1, following up to a 4-week screening period, eligible subjects will be randomized in a 2:1 ratio to receive either AXA1957 or placebo for a 13-week administration period. Subjects will be centrally randomized with gender (male/female) as the stratification factor to AXA1957 or placebo groups. Randomization will occur via an interactive web response system (IWRS) after eligibility is confirmed and prior to the Day 1 Visit. Study food product amounts will be gradually escalated through the first week of study participation to assess tolerability issues to the food product during the initial week, and to enable subjects and their caregivers to get accustomed to the twice daily regimen. Safety and tolerability will be monitored throughout the study. All subjects will be provided diet and physical activity recommendations consistent with Guidance and Lifestyle Recommendations for Adolescents with NAFLD; see Appendix 1. Following the mandatory Part 1 period, all subjects including those randomized to the placebo-arm in Part 1, will have an option to continue in the study in Part 2 for up to an additional maximum of 12 weeks on the AXA1957 food product. All subjects who opt to enter Part 2 will be administered AXA1957 at the same amount and regimen as in Part 1 (i.e. up to 2 stick packs twice daily starting from Week 14) and will continue to be provided the standard lifestyle guidance. Subjects who choose not to participate in Part 2, will undergo a safety follow up visit approximately 2-weeks after Visit 6 per the procedures in Schedule of Assessments (SOA)

	Table 1. There will be a 2-week follow up period after subjects complete Part 2. If subjects drop out at any time for any reason during either Part 1 or 2 of the study, including the follow up period, their last visit should capture all the assessments as the end-of-study assessments as shown in Table 1. **Screening Period:** Once written informed consent/assent is obtained, screening procedures will be completed per the SOA in Table 1. Screening period may be up to 4 weeks before starting Part 1. During this period, subjects will be asked to complete a diet and exercise diary over 4 days, which should include two weekdays and 2 weekend days. Subjects will record to the best of their ability all the typical foods/beverages they eat/drink on those days, and their usual physical activity routines to establish a baseline lifestyle pattern. During the entire screening period subjects should continue their normal habitual diet, physical activity patterns and routines (e.g., school, extracurricular activities, including their usual sports activities), and their prescribed standard of care therapies, if applicable. Subjects are also be required to undergo a screening multiparametric MRI scan within approximately 7 days before potential randomization. Subjects will be instructed to fast for at least 4-hours prior to their scheduled MRI; subjects will have to meet the screening MRI criteria to be eligible for randomization. Subjects who screen fail due to ALT may be re-screened one time. **PART 1: MANDATORY ADMINISTRATION PERIOD (13 WEEKS)** **Visit 1 / Day 1 and up to Week 1:** Eligible subjects will be randomized via the IWRS and will receive either placebo or AXA1957 with instructions (to both subjects and their caregivers) on how to prepare and consume the study food product, including timing of administration. On Day 1, subjects will arrive to the study site following an overnight fast of approximately 8 hours. Prior to study food product administration, subjects will undergo fasting blood draws and other assessments per the SOA in Table 1. The first administration of the study food product will be at the study site supervised by the study staff. After the study food product administration, subjects will have one additional blood draw for plasma amino acid concentration approximately 1-2 hours after the study food product administration and then discharged from the study site. During the Day 1 visit, all subjects will be provided Guidance and Lifestyle Recommendations for Adolescents with NAFLD (See Appendix 1). Qualified site staff will explain these recommendations and answer any questions the study participant and/or their family may have.

	During the first week of study food product administration in both AXA1957 and placebo groups, subjects will follow the administration schedule below: - Days 1 to 3: 1 stick pack twice daily (total of 2 stick packs daily) - Days 4 through the remainder of the study: 2 stick packs twice daily (total of 4 stick packs daily) Twice daily regimen of the food product should occur within 30 minutes (i.e. 30 ±5 minutes) **before** meals (e.g., before breakfast and dinner or before lunch and dinner, if breakfast is not a usual part of their daily routine). Any adjustments to this regimen or amount may be considered on a case-by-case basis (see additional details under Study Food Product Section below). Site staff will conduct a phone call on Day 3 to assess tolerability, and if tolerated, remind the subject to increase the food product to 2 stick packs twice a day (4 stick packs daily) for the remainder of the study administration period. **Visit 2 (Week 1) to Visit 6 (Week 13):** All procedures during Visits 2 through 6 will be performed per the SOA in Table 1. Subjects will be asked to bring all their study food product stick packs (full and empty) to each clinic visit for site staff to perform accountability. Subjects are also required to bring their study food product administration and lifestyle diaries for review at each clinic visit. During weeks where there are no clinic visits, qualified study staff will make approximately weekly phone calls to subjects to ensure compliance with the study food product administration/regimen, answer any questions, and check-in with subjects/caregivers. **PART 2: Optional Extension Period (12 Weeks) For All Subjects** **Visit 6 (Week 13) to Visit 10 (Week 25):** After the completion of the mandatory 13-week period (Visit 6), all subjects from Part 1 have the option to enter Part 2 where subjects (including those that received placebo in Part 1) will receive up to a maximum of 12 additional weeks of AXA1957 food product. The determination of whether to continue in the optional period is based upon the following: • The subject is willing and able to participate in the study for up to 12 additional weeks, and • There are no negative signals as determined by the clinical

	judgement of the study medical monitor, investigator, dietician, and/or Sponsor after carefully considering safety, tolerability, health status, body weight, body composition, MRI, and/or blood parameters. Subjects who do not participate in Part 2 will undergo an end-of-study follow up visit approximately 2-weeks after Visit 6 per procedures in the SOA in Table 1. Subjects who participate in Part 2 will be provided a supply of AXA1957 study food product at Visit 6 and will be instructed to start consuming 2 stick packs twice daily (4 stick packs daily). There will be at least 4 study visits in Part 2, and all procedures during those visits will be performed per the SOA in Table 1. Safety and tolerability of the study food product will continue to be assessed as was in Part 1. If subjects drop out at any time for any reason during the study, their last visit should capture all the assessments as the end-of-study assessments as shown in Table 1. Subjects will not be replaced in Part 2. **Follow up Visit** Subjects who participate in Part 1 only will return to the study site for a follow-up visit approximately 2 weeks after Visit 6 and will follow the SOA procedures per Table 1. Subjects who opt to participate in Part 2 will return to the study site for follow-up visit approximately 2 weeks after Visit 10 and will follow the SOA procedures per Table 1. **Early Termination Visit** Subjects who discontinue the study early for any reason are expected to complete the Early Termination Visit and will follow the SOA procedures per Table 1.
Study Dietary and Physical Activity Requirements	During each study visit, subjects will meet with a study dietician or other qualified study staff who will reinforce the lifestyle recommendations in Appendix 1 to subjects and their caregivers. In between the study visits, subjects may receive phone calls approximately once a week where qualified study staff may continue to reinforce these lifestyle recommendations.
Study Food Products	AXA1957 is composed of naturally occurring amino acids, which are normal ingredients of food or those readily available as dietary supplements. The placebo is a balanced food product formulated as a dry powder that will be reconstituted with ~6 oz (~180 mL) of water to form an orange flavored drink that is color, taste, and calorie matched to AXA1957.

	Study food products are provided in dry powder form in stick packs, which are then mixed in ~6 oz (~180 mL) of water, and then consumed twice daily approximately 30 min (i.e., 30 ±5 minutes) **before** meals (e.g., before breakfast and dinner or before lunch and dinner, if breakfast is not a usual part of their daily routine) for the entire duration of the study. Note: While subjects are encouraged to consume the study food product at defined times as indicated above to establish a clear routine which improves adherence to study procedures, **it is more important in this study to ensure that subjects consume their assigned products twice a day**. To that end, the following flexibility is allowed within the administration periods: • The twice daily (BID) administrations can occur at any time during the day if the two administrations are at least 4 hours apart, and the study food products are not consumed immediately before, with, or immediately after a main meal. • If subjects experience any clinically significant gastrointestinal (GI) discomfort while taking their study food product prior to meals, administrations may occur approximately 30-60 minutes after the meal, and only after consulting with the investigator, and Medical Monitor or Sponsor. • Every attempt should be made to encourage subjects to stay on their assigned amount and regimen for the full duration of the study. However, if the study investigator determines any tolerability issues of clinical significance, the amount of AXA1957 or placebo may be reduced for an individual subject to an amount and/or regimen that was previously tolerated, but only after discussion with the Medical Monitor or Sponsor. For instance: o If the 2-stick pack twice daily amount is not tolerated, then the amount for *that individual subject* may be reduced to 1 stick pack twice daily ; o Any subject who is not able to tolerate the study food product or placebo at any amount (e.g., even at 1 stick pack twice daily) should be discontinued from the study and undergo the End-of Study visit procedures. Compliance check-in and accountability review with both the study food product and lifestyle recommendations will be performed by study staff at every visit during both Parts 1 and 2.
Inclusion Criteria	Subjects must meet all the following criteria to be eligible to participate in the study: 1. Male and female adolescent subjects between 12 and 17 years of age, inclusive. 2. Female subjects must be at least 2-years post-menarche as determined by clinical history.

	3. Willing and able to provide written informed consent from parent(s) or legal guardian, as required. 4. Willing and able to provide written assent from subject, as required. 5. Stated willingness and ability of parent/guardian and the subject to take the study food product twice a day and adhere with all study procedures and requirements. 6. Body weight ≥ 60 kg at time of screening and subjects should be within ±5% of their body weight over the last 30 days prior to Screening. 7. Subjects should be at risk for fatty liver, which may be assessed by any <u>one</u> (<u>1</u>) of the following: a. Historical liver biopsy that was obtained up to 6 months prior to Screening (local pathology interpretation may be used) with Steatosis greater than Grade 1, if applicable as part of standard of care. **Note*:*** For the historical liver biopsy to satisfy the fatty liver requirement, the biopsy must have been obtained with no NAFLD or NASH treatment, medications that can cause steatosis or NASH, or other interventional agents/procedures for the treatment of NAFLD/NASH within 3 months prior to when the liver biopsy was obtained; OR b. Fasting ALT ≥ 50 U/L in boys, ≥45 U/L in girls, and ≤ 150 U/L in both genders at the Screening visit labs; OR c. Fibroscan with Control Attenuation Parameter (CAP) ≥ 280 dB/m within 90 days of Randomization; OR d. Any of the following right upper quadrant (RUQ) ultrasound sonographic features suggestive of hepatic steatosis within 90 days of Randomization, for e.g.: liver uniformly heterogeneous; echogenic diffusely with bright hepatic echoes within the first 2-3 cm of depth with increased hepatorenal echogenicity (i.e., liver with areas of significantly increased echogenicity relative to the renal parenchyma); attenuation of image quickly within 4-5 cm of depth making deeper structures difficult to decipher; thick subcutaneous depth (> 2 cm); vascular blurring of portal or hepatic vein. 8. MRI Proton Density Fat Fraction (PDFF) >10% **<u>and</u>** corrected T1 (cT1) > 820 msec using multiparametric MR imaging of the liver. **Note:** Screening MRI should occur approximately within 7 days prior to randomization and must NOT occur until each of the above entry criteria #1 through #7 have been confirmed.

	9. Subjects may have either prediabetes or type 2 diabetes (T2DM). Prediabetes is established by one of the following: a. Fasting Blood Glucose is ≥100 mg/dL (5.5 mmol/L) but <126 mg/dL (7.0 mmol/L) or HbA1c ≥5.7% but <6.4% based on the Screening visit labs; OR b. Historical evidence (i.e. within 3 months of Randomization) of impaired fasting glucose/glucose intolerance per the standard of care guidelines and usual procedures of the study center; OR c. Subject is already on a stable regimen for approximately 60 days prior to Randomization for the treatment of prediabetes (e.g., diet/lifestyle, metformin, etc.). 10. Subjects with Investigator-confirmed T2DM should have HbA1c ≤9.5% at Screening and must be stable on their usual diabetic medications for approximately 60 days prior to Randomization and should not be anticipating clinically significant dose adjustments of the T2DM medications for the anticipated duration of the study. 11. Subjects taking vitamins and/or other dietary/herbal supplements are permitted as long as ALL the following are met: a. Vitamin(s) or other supplement(s) are not on the list of prohibited medications (see Section 6.1.1), and b. Subject has been on stable doses and regimens for at least 2 months prior to Randomization; *however, if vitamin E is at doses ≥400 IU/day, it must be discontinued prior to Randomization if subject otherwise qualifies into study*, and c. There are no anticipated dose adjustments or changes (i.e. stopping or adding) of vitamins/supplements for the duration of the entire study, including the optional extension period. 12. Heterosexually active female participants of childbearing potential (i.e. post-pubertal and /or post-menarchal, not surgically sterile [defined as tubal ligation, hysterectomy, or bilateral oophorectomy]) must agree to <u>abstain</u> from sexual intercourse for the entire duration of the study (i.e., from time ICF is signed to end-of-study follow up) as any hormone-based contraception approaches are not allowed in the study (e.g., oral contraceptive pills are excluded medications for this study), and barrier approaches (e.g., condom with spermicide, diaphragm with spermicide) alone are not considered adequate to prevent pregnancy. Sexual activity will be ascertained at each study visit, and every female participant in the study must complete a required pregnancy test at each study visit.
Exclusion Criteria	Subjects are not eligible to participate in the study if any of the following criteria are met:

	1. History of any acute or chronic liver disease, other than non-alcoholic fatty liver disease, such as drug-induced, autoimmune, or any viral hepatitis, hemochromatosis, Wilson's, primary sclerosing cholangitis. Clinical / medical history or appropriate laboratory /serological assessments, if necessary, may be relied upon to rule out these other causes of liver disease. 2. Known positive for human immunodeficiency virus (HIV) 3. Presence of Stage 4 fibrosis in the liver 4. Non-compensated liver disease with any one of the following hematologic, biochemical, and serological criteria at Screening: a. Hemoglobin < 10 g/dL b. White blood cell < 3,500 cells/mm c. Neutrophil count < 1,500 cells/mm^3 of blood d. Platelets < 130,000 cells/mm^3 of blood e. Direct bilirubin > 1.0 mg/dL (>17.1 μmol/L) f. Total bilirubin ≥ 3 mg/dL (≥51.3 μmol/L, unless Gilbert's Syndrome). However, subjects with Gilbert's syndrome with direct bilirubin above upper limits of normal (ULN) in addition to total bilirubin ≥ 3 mg/dL (≥51.3 μmol/L), or with evidence of hemolysis contributing to elevated total bilirubin, should be excluded. g. Albumin < 3.2 g/dL (<48.1 μmol/L) h. International normalized ratio (INR) > 1.4 5. Any active/clinically unstable GI (e.g., motility disorders, celiac disease, malabsorption syndromes), cardiovascular (e.g., hypertension and hyperlipidemia requiring pharmacologic treatment), endocrine (e.g., Cushing's syndrome congenital adrenal hyperplasia, polyglandular endocrinopathies, hypo/hyperthyroidism requiring pharmacologic treatment, genetic/secondary causes of obesity), flaring autoimmune diseases (juvenile idiopathic arthritis, adolescent lupus, juvenile dermatomyositis) or psychiatric conditions (clinically significant depression, ADHD, psychosis). **Note 1:** Subjects with asthma may be enrolled at the discretion of the Investigator if their condition is stable (i.e. no exacerbations within 4-6 weeks prior to Randomization and no clinically meaningful changes in their asthma management regimens are anticipated for the duration of the study). **Note 2**: Subjects who are on a stable dose of anti-depressant medication(s) or medication(s) for ADHD for at least 6 months prior to Randomization and whose Investigator considers them to be psychiatrically stable may be enrolled. 6. Known history of Inborn Errors of Metabolism and/or genetic deficiencies that impact amino acid metabolism or transport [e.g., urea cycle disorders, carnitine deficiency, carnitine palmitoyl

	transferase (CPT) I or II deficiency, beta-oxidation defects, pyruvate carboxylase deficiency, porphyria, etc.] 7. Current acute illness/health condition requiring ongoing medical treatment. Subjects with minor illness/injury that the investigator considers unlikely to significantly impact study participation or study assessments/endpoints should be discussed with the study Medical Monitor and/or Sponsor to determine whether the subject may be included, if all other eligibility criteria are met. 8. Any form of diabetes other than T2DM (e.g., Type 1, MODY). 9. Uncontrolled T2DM, which is defined as: a. HbA1c >9.5 % on their current T2DM regimen at Screening and must be stable on their usual diabetic medications for approximately 60 days prior to Randomization and should not be anticipating clinically significant dose adjustments of the T2DM medications for the anticipated duration of the study, and/or b. Requiring >10% insulin dose adjustments within 60 days prior to Randomization, and/or c. Requiring a complex medical regimen, e.g., needing 2 or more oral anti-diabetics (OAD)s, and/or with a history of severe hypoglycemia on their OAD regimens. 10. Uncontrolled hypertension (i.e. systolic blood pressure >130 mmHg and/or diastolic blood pressure >80 mmHg) at Screening. 11. Uncontrolled hyperlipidemia (i.e., triglycerides >350 mg/dL and/or low-density lipoprotein (LDL) >160 mg/dL) at Screening. 12. Impaired renal function (i.e., glomerular filtration rate ≤ 90 mL/min/1.73 m^2) at Screening. 13. History of bariatric surgery or planning to undergo bariatric surgery during the study duration (including Part 2). However, prior laparoscopic-band, intra-gastric balloon, or other weight loss device which was removed >12 months prior to Screening is not exclusionary. 14. Any contraindications to an MRI scan, including: a. Presence of non-removable ferromagnetic implants, pacemakers, aneurysm clips or other foreign bodies, or b. Unable to have or complete the MRI exam due to body weight exceeding scanner table limit or girth exceeding scanner bore diameter, or c. Clinically significant claustrophobia. Subjects experiencing mild anxiety due to transient claustrophobic symptoms may be treated with anxiolytics, if appropriate and per study site SOPs, for mitigation of those symptoms, if necessary. 15. Known sensitivity and/or clinically significant food intolerance/allergies to proteins (such as whey, soy, casein, collagen, gelatin, amino acids, etc.), gluten, fats, carbohydrates

	(e.g., lactose), nuts or any ingredient in the study food product formulations. 16. History of total parenteral nutrition (TPN) 6 months prior to Screening. 17. Current or planned use of any dietary supplement intended for general metabolic health, weight maintenance, weight loss, OR those containing proteins, amino acids, ketones, or fish oils, from the time the ICF is signed through the end of the study. **Note:** Common examples of excluded protein, amino acid, and other dietary supplements include, but are not limited to: ketones, protein powders, shakes, bars, gels containing whey, soy, casein, collagen, amino acids, and their derivatives/metabolites, N-acetylcysteine, L-carnitine, etc. A few examples of supplements used for weight loss include green tea, green coffee bean extracts, garcinia cambogia, any ketogenic products, etc. 18. Currently on or planning to be on any extreme or unbalanced diet such as Ketogenic, Atkins, Paleo, etc.) from the time the ICF is signed through the end of the study. 19. Use within 2 months prior to or during Screening, or during the study, of systemic glucocorticoids, any biologics (e.g., monoclonal antibodies), hormones of any kind (e.g., estrogens, progesterone, testosterone), valproic acid, ursodeoxycholic acid, any anti-obesity compounds (e.g., orlistat, locaserin, Qsymia), GLP-1 agonists, TZDs, or any other medications known to cause hepatic steatosis, or steatohepatitis, or use of any other known or potential hepatotoxins. 20. Use of any other excluded medications listed in Section 6.1.1 of the protocol not otherwise specified in the entry criteria. 21. Use of any illicit substances, including alcohol, and nicotine products 22. If female, ongoing pregnancy (defined as positive serum and/or urine pregnancy test), and/or planned pregnancy, breast-feeding. 23. Male subjects with female partners of child-bearing potential who are unwilling or unable to adhere to contraception requirements. 24. History of any malignancy, other than nonmelanoma skin cancer, within 4 years prior to Screening. 25. Subjects who are currently enrolled in a clinical trial or who received an investigational study drug/medication, or any dietary supplement(s), food product(s), or participated in a lifestyle / nutritional interventional study/program within 60 days prior to Screening. 26. Any other condition that, in the opinion of the investigator, renders or places the subject at risk for inclusion in the study, or that would impede compliance, or hinder completion of the study.

Statistical Methods	All analyses will be performed, and all tables, figures, and data listings will be prepared using SAS version 9.4 or higher. Summary statistics for continuous variables will include the mean, standard deviation, median, minimum and maximum value; categorical variables will be presented as counts and percentages. Some subgroup analyses (such as liver fat or ALT assessments) may be performed within each of the gender groups given the known and well-established gender differences in these parameters. Safety and tolerability will be evaluated using descriptive statistics and listings of AEs, physical examination, including body composition (lean and visceral fat mass) changes, clinical laboratory test values, including plasma amino acid levels, vital signs, weight, body temperature, ECGs and other safety parameters. Analyses of AEs will be performed for those events that are considered study food product-emergent, where study food product-emergent is defined as any AE with onset (or worsening of a pre-existing condition) after the first administration of the study food product. Interim analyses in addition to the final analysis are planned for this study. Up to two interim analyses are planned in Part 1: one after approximately 50% of subjects complete Visit 4 (Week 7), and another after approximately 50% of subjects complete Visit 6 (Week 13). Subjects will be centrally randomized into AXA and Placebo with a block size of 3, stratified by gender through an IWRS system.

Table 1. Schedule of Assessments Table

Study Period	**Screening Period**	**PART 1** Mandatory AXA1957 or Placebo Administration Period (13 weeks)						**PART 2**[17] Optional AXA1957 Extension Period (up to a maximum of 12 weeks)				**Follow-up Period** (2 weeks)	Early Term[1]
	Up to 28 days prior to Day 1	Visit 1 **Day 1**	Visit 2 Wk 1 (+3 days)	Visit 3 Wk 3 (+/- 5 days)	Visit 4 **Wk 7** (+/- 5 days)	Visit 5 Wk 10 (+/- 5 days)	Visit 6 **Wk 13** (+/- 5 days)	Visit 7[14] Wk 15 (+/-5 days)	Visit 8 **Wk 19** (+/- 5 days)	Visit 9 Wk 22 (+/-5 days)	Visit 10 **Wk 25** (+/- 5 days)	Visit 11 Wk 27 (+/-3 days)	
Informed consent/Assent	X												
Confirm eligibility	X	X											
Randomization[16]		X											
Demographics and Medical History	X												
Con. Medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination[2]	X	X		X	X	X	X	X	X	X	X	X	X
Height, weight and waist circumference	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[3]	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test[4]	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG	X						X				X		X
MRI Scan[5]	X				X		X				X		
Fibroscan or RUQ ultrasound	X												
Serum chemistry[11]	X	X			X		X		X		X	X	X
Haematology/coagulation	X	X			X		X		X		X	X	X
HbA1c[11]	X	X			X		X				X		X
Blood samples for metabolic, inflammation and fibrosis biomarkers [7,11]		X			X		X		X		X		X
Fasting lipid profile[11]	X	X					X				X		X
OWL lipidomic profile[11]		X					X				X		X
Blood sample for PBMC isolation[6,11]		X					X				X		

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	Up to 28 days prior to Day 1	Visit 1 **Day 1**	Visit 2 Wk 1 (+3 days)	Visit 3 Wk 3 (+/- 5 days)	Visit 4 **Wk 7** (+/- 5 days)	Visit 5 Wk 10 (+/- 5 days)	Visit 6 **Wk 13** (+/- 5 days)	Visit 7 Wk 15 (+/-5 days)	Visit 8 **Wk 19** (+/- 5 days)	Visit 9 Wk 22 (+/-5 days)	Visit 10 **Wk 25** (+/- 5 days)	Follow-Up[14] (+/-3 days)	
Blood sample for amino acid concentrations[12]		X					X				X		X
Urinalysis (quantitative)	X	X					X				X		X
Lifestyle guidance & monitoring		X	X	X	X	X	X	X	X	X	X	X	X
4-day baseline diet and exercise diary[15]	X												
Diet/Exercise lifestyle Questionnaire[10]		X	X	X	X	X	X	X	X	X	X	X	X
Study food product administration diary[8]		X	X	X	X	X	X	X	X	X	X		
VAS[9]		X		X	X	X	X	X	X	X	X		
Dispense Study Food Product[13]		X		X	X	X	X[13]	X	X	X			
Study food product accountability		X	X	X	X	X	X	X	X	X	X		X
Record adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
Assess eligibility and willingness of subject to participate in Part 2							X						
Weekly Phone Calls[8]		X	X	X	X	X	X	X	X	X	X		

Schedule of Assessment Footnotes

[1]Subjects who discontinue early in Part 1 or Part 2 will be asked to complete all assessments as indicated. Subjects who discontinue early and who have had the multiparametric MRI scan within 1 week of the date of discontinuation will not be required to repeat the early termination MRI.

[2]PE will consist of an assessment of general appearance, skin, thorax/lungs, abdomen, lymph nodes, head, ears, eyes, nose, throat, neck, cardiovascular, musculoskeletal, and neurological systems.

[3]Vital signs include sitting systolic and diastolic blood pressure, heart rate, respirations and temperature. Blood pressure should be obtained after the subject has been sitting calmly for at least 5 minutes

[4]Serum pregnancy test at Screening visit and urine pregnancy test on all other visits.

[5]The screening MRI scan should occur approximately within 7 days prior to randomization and must NOT occur until each of the inclusion criteria #1 through #7 have been confirmed. Subjects are required to be fasting for at least 4 hours prior to all MRI scans. Scans should be scheduled based on scanner availability which may not correlate with the same day as the study clinic visit. MRIs at Visit 7 and 13 are not required to occur the same day as the study clinic visit, but must occur within the +/- 5 day visit window. Every attempt should be made to schedule scans at roughly the same time of the day to reduce diurnal fluctuation in daily liver lipid levels.

[6]PBMC isolation required on Day 1 and Day W13 and Week 25 (extension period).

[7]See Table 8– Clinical Laboratory Evaluations for a list of biomarkers to be collected at the indicated time points. A retain sample of plasma will be collected at each biomarker timepoint for possible future non-genetic exploratory analysis.

[8]The study dietician (or other qualified staff) will confirm that subjects are administering their assigned study food product accurately, and answer any questions on usual Lifestyle Recommendations for Adolescents with NAFLD (See Appendix 1).

[9]Subjects will complete a hunger and satiety VAS at the indicated time points biweekly and must return completed VAS worksheets for review by study site qualified staff at each study visit.

[10]Subjects are expected to follow the study Guidance and Lifestyle Recommendations for Adolescents with NAFLD (Appendix 1). The Diet and Exercise questionnaire will be a tool for the study dietician (or other qualified staff) to help monitor adherence to the guidance throughout the study. The questionnaire is easy to use and requires a simple “yes/no” response.

[11] Subjects are required to fast approximately 8 hours. Prior to their clinic visits on days when blood draws are obtained. On those days, the morning administration of their assigned study food product should be held until blood draws are completed. Subjects may consume their assigned study food product after the blood draw. Site staff should inspect the venipuncture site(s) prior to subject’s discharge.

[12] On Day 1, Week 13, and Week 25, subjects will arrive at the study site after having fasted approximately 8 hours. They will have a fasted blood draw (T=0), will be administered their assigned study food product at the study site, and then approximately T=1-2 hours later, another blood sample will be collected for plasma amino acid concentrations. The exact clock times of the T=0 (pre-administration) and T=1-2 hr (post-administration) samples should be recorded in the source documents.

[13] AXA1957 will be provided to all subjects entering Part 2, there is no placebo.

[14]The Follow-Up Visit will occur 2 weeks after Visit 6 for subjects participating in Part 1 only. The Follow-Up Visit will occur 2 weeks after Visit 10 for those participating in Part 2.

[15] Diary must be filled out over 4 days which should include 2 weekdays and 2 weekend days. Subjects will record to the best of their ability all the typical foods/beverages they eat/drink on those days, and their usual physical activity routines to establish a baseline lifestyle pattern.

[16] Randomization will occur only after eligibility is confirmed (including MRI criteria).

[17] A gap between Part 1 and Part 2 of up to 1 week is permitted.

Abbreviations

AAP	American Academy of Pediatrics
Adipo-IR	Adipose tissue Insulin Resistance
AE	Adverse Event
ALT	Alanine Aminotransferase
Arg	Arginine
AST	Aspartate Aminotransferase
BCAA	Branched-Chain Amino Acids
BHB	beta- hydroxybutyrate
BID	Twice Daily
PBMC	Peripheral blood mononuclear cell
BMI	Body Mass Index
BW	Body weight
CAP	Control Attenuation Parameter
CBD	Cannabidiol
CRF	Case Report Form
cT1	Corrected T1
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
EOS	End of Study
GCP	Good Clinical Practice
GI	Gastrointestinal
GLP-1	Glucagon Like Peptide 1
Gln	Glutamine
HbA1c	Hemoglobin A1c
HDL	High-Density Lipoprotein
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostatic Model Assessment Insulin Resistance
Ile	Isoleucine
ICH	International Council for Harmonization
INR	International Normalized Ratio
IWRS	Interactive Web Response System

IRB	Institutional Review Board
L-car	L-carnitine
LDL	Low-Density Lipoprotein
Leu	Leucine
MODY	Maturity-Onset Diabetes of the Young
MRI	Magnetic Resonance Imaging
OWL	One Way Liver
NAC	N-acetylcysteine
NAFLD	Nonalcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Nonalcoholic Steatohepatitis
NEFA	Non-Esterified Fatty Acids
OAD	Oral Anti-Diabetic Drug
PBC	Primary biliary cholangitis
PBMC	Peripheral Blood Mononuclear Cells
PDFF	Protein Density Fat Fraction
PE	Physical Examination or Product Emergent
PK	Pharmacokinetic
PSC	Primary sclerosing cholangitis
RUQ	Right Upper Quadrant
SAE	Serious Adverse Event
SAS	Statistical Analysis System
Ser	Serine
SOA	Schedule of Assessments
T2D	Type 2 Diabetes
TG	Triglycerides
TPN	Total Parenteral Nutrition
TZD	Thiazolidinediones
ULN	Upper Limit of Normal
VAS	Visual Analog Scale

1 INTRODUCTION

1.1 Background and Rationale for the Proposed Study

From 1988 to 2010, the prevalence of nonalcoholic fatty liver disease (NAFLD) increased among children and adolescents (Anderson et al., 2015), becoming the most common liver disease in children (Welsh et al., 2013), with an overall prevalence of 13%; the highest rates were seen in obese children (38%) (Schwimmer et al., 2006). It is associated with increased risk of type 2 diabetes, end-stage liver disease, liver cancer, and cardiovascular disease (Chalasani et al., 2012). Pediatric guidelines recommend "lifestyle modification to improve diet" (Vos et al., 2017), but do not support one specific diet over another because of the limited available evidence.

The most important rational for doing this study in teenagers is that the metabolic changes that result from severe obesity start in childhood and teenage years, and it is this age group who potentially have most to benefit in the long term from reduction in liver damage that occurs on an ongoing basis. For individuals and society as a whole, this research is potentially more important in teenagers and young people than in the adult population.

Extensive prior literature suggests amino acids can have important physiological and health-related effects. There is evidence that liver health as well as metabolism may be impacted by specific combinations of amino acids. For example, branched chain amino acids (BCAAs), cysteine, and arginine have been implicated in enhancing liver health [(Tajiri & Shimizu, 2013), (Khoshbaten, et al., 2010), (Kakumitsu, Shijo, & Yokoyama, 1998)]. Investigations in subjects with type 2 diabetes (T2DM) showed that the usually reduced insulin response after carbohydrate ingestion can almost be tripled by co-ingestion of a free amino acid/protein mixture that contains whey protein hydrolysate, free leucine, and phenylalanine (van Loon, et al., 2003). Thus, selective supplementation of certain amino acids may have the potential to restore optimal physiological processes in the liver.

Axcella's amino acid food product, AXA1957, consists of specific combinations of natural amino acids that are naturally produced by the body, all of which are normal food ingredients and/or readily available as dietary supplements. The intention and objective of this study is to primarily understand the safety and tolerability profile of AXA1957 and its impact on normal liver structure and function for up to 25 weeks of administration in adolescent subjects with fatty liver.

1.2 Rationale for the Study Design and Amounts of Study Food Product Selected

The amount of amino acids to be administered in this study are within the known, well tolerated ranges for individual or mixtures of amino acids administered to humans in several published studies, including repeated administrations in people with T2DM and/or metabolic syndrome (Table 2). These amounts are also similar to readily available over-the-counter amino acid/dietary supplement formulations including amounts present in food (Table 2).

Table 2. Amounts of AXA1957 Components Present in Diet

Components within AXA1957	Amount Present in AXA1957 Maximum Daily Amount (g)	Common OTC Dietary Supplement Formulations Daily Amount (g)	Adult RDA (g, assuming 70 kg)	Mean in Diet (g)	99th Percentile in Diet (g)
Leucine (L)	6	5 NutraBio BCAA 5000 (GNC), BID suggested regimen	2.94 [a]	6.08 [a,b]	12.70 [a,b]
Isoleucine (I)	3	2.5 NutraBio BCAA 5000 (GNC), BID suggested regimen	1.33 [a]	3.55 [a,b]	7.28 [a,b]
Arginine (R)	8 (given as 9.67 g R-HCl)	5 GNC L-Arginine 5000MG, QD suggested regimen	NA	4.18 [a,b]	9.10 [a,b]
Glutamine (Q)	4	5 Pro Performance L-Glutamine (GNC), QD post-exercise suggested regimen	NA	6.85 [c]	NA
N-acetylcysteine (NAC)	2.6	up to 3.6 NutraBio Amino Therapy NAC (GNC), upper suggested dose, BID suggested regimen	NA	NA	NA
L-carnitine (L-car)	2	2 Beyond Raw Chemistry Labs L-Carnitine (GNC)	NA	0.07 [d]	NA
Serine (S)	15	2 Bulk Supplements L-Serine	NA	3.51 [a,b]	7.15 [a,b]

[a] Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). (2005). pg 680, 988-1027. https://doi.org/10.17226/10490
[b] All Individuals except pregnant and lactating
[c] Healthy women aged 30-55 (Lenders et al., 2009)
[d] Adult omnivores assuming 70kg (Rebouche and Engel, 1984)

It is well documented that children have an increased rate of protein deposition due to growth and this rate decreases with age ("Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)," 2005). The estimated average requirement (EAR) and recommended dietary allowance (RDA) for protein and individual essential amino acids are higher in younger populations than adults on a g/kg basis ("Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)," 2005). For BCAAs, it has been reported that the EAR, as estimated by the indicator amino acid oxidation (IAAO) method, is increased in children compared with adults (Mager, Wykes, Ball, & Pencharz, 2003; Riazi, Wykes, Ball, & Pencharz, 2003) and is further increased in children with mild to moderate chronic cholestatic liver disease (Mager, Wykes, Roberts, Ball, & Pencharz, 2006) compared with healthy adolescents. It is therefore likely that the proposed amounts of AXA1957 to be administered to adolescents will result in exposure that is comparable, if not lower than that achieved when given to adults.

The amino acid components of AXA1957 have had safe upper limits of intake (UL) or no observed adverse event levels (NOAEL) suggested in literature based on studies in human adults. An UL for Leucine has been suggested at 35 and 30 g/day in healthy young men and healthy elderly men respectively based on the maximum rate of oxidation as determined by IAAO and assuming a 70 kg body weight (Elango, Chapman, Rafii, Ball, & Pencharz, 2012; Rasmussen, Gilbert, Turki, Madden, & Elango, 2016). The Leu amount in AXA1957 is 9 g/day (see Table 3). An observed safe level (OSL) for Arginine has been suggested at 30 g/day (McNeal, Meininger, Wilborn, Tekwe, & Wu, 2018) while an OSL at 20 g/day and a NOAEL at 30 g/day in healthy adults has also been suggested (Shao & Hathcock, 2008). Arg amount in AXA1957 is 8 g/day (see Table 3). An OSL and NOAEL for Glutamine has been suggested at 14 g/day and >45 g/day respectively (Shao & Hathcock, 2008). Glutamine amount in AXA1957 is 4 g/day (see Table 3). An OSL of 2 g/day has been suggested for Carnitine (Hathcock & Shao, 2006). L-Car amount in AXA1957 is up to 2 g/day (see Table 3). OSL or NOAELs for the other components (such as Ser, Ile) are not currently available.

As noted above and detailed further in Table 2, all these ULs are at or above the amounts of the individual AAs within AXA1957. While comparable studies have not been performed to establish UL or NOAEL values in adolescent populations, the Sponsor is not aware of any evidence suggesting that adolescents would have a lower tolerance for amino acid exposure than adults. In fact, the increased protein requirements and the increased rate of protein deposition in children and adolescents suggests that a lower plasma exposure would be achieved in the proposed adolescent population.

Table 3. Summary of Studies Supporting the Administration of the Components within the AXA1957 Food Product

Components within AXA1957	Daily amounts within AXA1957	Amounts /duration/safety in relevant prior adult clinical studies	Available data on amounts/duration/safety in pediatric patients
Leucine (L), Isoleucine (I),	9g/day of L & I at a 2:1 ratio	12g/day of L/I/V at 2:1:1 ratio → 6/3/3 g/day each of LIV in patients with liver cirrhosis over a 2-year period (Muto et al., 2005). Adverse events reported in 12% of BCAA cohort in Muto study primarily gastrointestinal problems such as abdominal distension, diarrhea and constipation (Muto et al., 2005)	BCAA dosed 0.33-0.39 g/kg/day L/I/V at 1.7:1:1.5 ratio as part of enteral nutrition formula delivered nasogastrically for 8 weeks in 19 pediatric end-stage liver disease patients, 10 completed study aged 0.7-5.9 years, incidence of complications of liver disease was similar in both treatment arms - BCAA-enriched and isonitrogenous enteral nutrition (Chin et al., 1992; Ooi, Gilmour, Yap, & Mager, 2018)
	(i.e. 6g L & 3g I per day total); 0g V	30g/day of oral BCAA (13.5g L, 9g I) in patients with cirrhosis and prior hepatic encephalopathy over 56 weeks (NCT00955500). No safety/tolerability reported in NCT009555000	BCAA being dosed up to 27 g BID L/I/V at 1:1:1 ratio for 21 days in NCT01860404 (Status: Recruiting), safety information not yet available, in children and adults aged 11-34 years
Arginine (R)	8 g/day	9g/day R given to patients with T2D for 3 months to study microcirculation (NCT00902616). No safety/tolerability reported in NCT00902616	0.1 g/kg TID oral or IV infusion Arg-HCl up to 30 g/day max for 5 days in children and adults with sickle cell disease aged 3-21, no serious drug-related adverse events observed, although one AE and one SAE occurred that led to discontinuation of the study drug (Morris et al., 2013)
	(given as 9.67g R-HCl)	9g/day R given to T2D patients for 1 month to improve peripheral and hepatic insulin sensitivity. No	

Table 3. Summary of Studies Supporting the Administration of the Components within the AXA1957 Food Product			
Components within AXA1957	**Daily amounts within AXA1957**	**Amounts /duration/safety in relevant prior adult clinical studies**	**Available data on amounts/duration/safety in pediatric patients**
		safety/tolerability reported (Piatti et al., 2001).	
Glutamine (Q)	4 g/day	15g/day for 8 weeks used to treat patients with Irritable Bowel Syndrome (TID as 5g/dose) (NCT01414244). No AEs reported in the study	Approximately 0.3 g/kg/day up to 30 g/day max for 48 weeks in children and adults with sickle cell disease aged 5-58 years (NCT01179217). The rate of adverse events was higher in the placebo group than in the l-glutamine group (100% vs. 98.0%), as was the rate of serious adverse events (87.1% vs. 78.2%), low-grade nausea, noncardiac chest pain, fatigue, and musculoskeletal pain occurred more frequently in the l-glutamine group than in the placebo group (Niihara et al., 2018)
N-acetylcysteine (NAC)	2.6 g/day	NAC improves liver function in patients with non-alcoholic steatohepatitis (NASH) at 1.2 g/day for 3 months (Khoshbaten et al., 2010). No AEs in patients with NASH	Up to 2.7 g/day thrice daily for 4 weeks at high dose (12 weeks total) in children with autism aged 3-12 years. Minimal adverse effects were observed except for one subject in the active group who experienced worsening of baseline agitation and irritability requiring early

Table 3. Summary of Studies Supporting the Administration of the Components within the AXA1957 Food Product			
Components within AXA1957	**Daily amounts within AXA1957**	**Amounts /duration/safety in relevant prior adult clinical studies**	**Available data on amounts/duration/safety in pediatric patients**
		3-4g/day for 14 days in mild traumatic brain injury (Hoffer, Balaban, Slade, Tsao, & Hoffer, 2013). NAC produced no side effects in subjects with blast-induced traumatic brain injury	termination followed by symptom resolution. This participant exhibited the same behavioral worsening 6 weeks after being terminated from the study, which led to a medical evaluation that revealed severe constipation. Most adverse effects were gastrointestinal, consistent with previous reports, no statistical significance between NAC and placebo groups was detected from chi-square tests for individual side effects ($p = 0.198$) or all gastrointestinal adverse effects combined ($p = 0.199$) (Hardan et al., 2012)
L-carnitine (L-car)	2 g/day	2g/day supplement added to diet for 24 weeks improved liver function and histological manifestations of NASH (Malaguarnera et al., 2010). Carnitine was well tolerated in all patients, with mild AEs reported in 5/36 subjects: 1 nausea, 2 headaches, 2 abdominal pain	0.1 g/kg twice daily up to 4 g/day max in boys aged 6-13 years with ADHD. Two boys broke their arm due to their hyperactive behavior during the placebo period. One boy had a short unexplained period of fainting a few days after the onset of the placebo period following carnitine. One boy had an elevated plasma creatinine level during the last placebo period, but 2 weeks after the trial, a normal level was found. Physical examination of all boys by the pediatrician at the end of the three trial periods showed no abnormalities. One patient observed an unpleasant body

Table 3. Summary of Studies Supporting the Administration of the Components within the AXA1957 Food Product

Components within AXA1957	Daily amounts within AXA1957	Amounts /duration/safety in relevant prior adult clinical studies	Available data on amounts/duration/safety in pediatric patients
			odor during treatment with carnitine, a well-known side effect, likely due to the formation of trimethylamine (Oudheusden & Scholte, 2002)
Serine (S)	15 g/day	20g/day Ser was administered for 14 days in NAFLD patients (Mardinoglu et al., 2017). No AEs reported, S was well tolerated 400 mg/kg/day (28 g/day in 70 kg adult) well-tolerated in adult subjects with HSAN1 (Fridman et al., 2019)[a]	Case reports of serine in infants and juveniles with 3-PGDH deficiency at doses ranging from 100-700 mg/kg/day (7-49 g/day in 70 kg). No safety/tolerability information reported (Tabatabaie et al., 2011)

[a] Serine is a non-essential amino acid synthesized from glucose in the body It is readily interconverted with glycine and metabolized in the TCA cycle. A serine dose of 400 mg/kg/day in subjects with HSAN1 resulted in a non-significant relative increase in plasma levels relative to placebo, likely due to rapid metabolism in liver and muscle (Fridman et al., 2019).

Based on the proposed amounts in AXA1957 (i.e. all at or below established ULs), and an extensive prior published scientific and clinical literature that have demonstrated the safety and tolerability of each amino acid present within AXA1957 in both the adult and pediatric populations, it is anticipated that administration of AXA1957 in an adolescent population will also be safe and well tolerated.

In addition, the proposed design incorporates a judicious escalating administration scheme (i.e. 6.7g BID for 3 days, and only if well-tolerated, 13.5g BID for up to 24 weeks. Such a design ensures monitoring for any unanticipated tolerability issues, and to gradually attain sufficient exposure of the AAs to adequately evaluate the safety and tolerability of this food product in a fatty liver population. At any time during the study, the design also allows for a down-titrating of the amounts that were previously shown to be well tolerated (e.g., from 13.5g BID to 6.7g BID).

The safety and tolerability of AXA1957 is also being studied over a duration of 16 weeks in an adult population of NAFLD with or without diabetes (Study AXA1125-003). This study is currently ongoing. The primary goal of the study is examination of the safety and tolerability of 2 different AXA compositions (AXA1125 and AXA1957), in addition to an exploration of the effect of AXA compositions on metabolism, especially amino acid and lipid metabolism, as well as liver structure and function. As it is a single blind study, safety and tolerability data are being analyzed on an ongoing basis on all available adult subjects enrolled in the study. An interim analysis of all subjects in the trial as of October 15, 2019 was performed and the data demonstrate that both amounts of AXA1957 are generally safe and well-tolerated.

As of 15-October-2019, a total of 58 adult subjects have received at least one dose of AXA1957 at either 13.5 g BID or 20.3g BID, with 31 subjects who have completed at least 8 weeks of study product administration and 28 subjects who have completed 16 weeks of study product administration (Axcella, data on file as of October 15, 2019). These preliminary data show a total of 69 study product emergent AEs in 58 subjects assigned to AXA1957 at either 13.5 g BID or 20.3g BID regimen have been reported to date (Table 4). All AEs to date during AXA1957 administration have been mild or moderate. Of the 69 total AEs, 13 were considered to be possibly or probably related to study product. Three subjects had dosing interruptions or discontinuations during study: one subject with transient diarrhea and abdominal bloating who subsequently was diagnosed with nephrolithiasis and discontinued administration (20.3g BID); one subject with transient moderate abdominal pain and diarrhea, who interrupted treatment due to moderate abdominal pain, diarrhea and nausea and subsequently withdrew from the study (20.3g BID), and another who had moderate nausea and discontinued study product (13.5g BID). There were no serious adverse events reported in subjects administered AXA1957.

There was no difference in this interim analysis between the AXA arms in terms of the number of AEs, study product-emergent AEs, reported severity or pattern of adverse events (Table 4). Of the product-emergent AEs, the majority of those considered to be possibly or probably related to AXA1957 were related to the gastrointestinal system (9/13), such as diarrhea, nausea, abdominal pain and distension. There was no vomiting thought to be related to study product. In terms of severity, all reported AEs in the AXA1957 arms were mild (17/33, 51.5%) or moderate (16/33, 48.4%). The only adverse events of moderate severity reported in 2 or more subjects were nausea (N=2, both 13.5g BID) and abdominal pain (N=2, both 20.3g BID). There were no clinically significant changes in safety laboratory (chemistry and hematology) parameters, vital signs, EKGs, or physical exam findings. Taken together, AXA1957 at both amounts continues to be well tolerated in adults with NAFLD.

In sum, there is no pattern in the evaluation of safety and tolerability to suggest that there is a dose response relationship in terms of the pattern or severity of AEs in subjects administered AXA1957.

Table 4. Summary of Adverse Events in Study AXA1125-003 in the Safety Population (observed as of 15 October 2019; reported in 2 or more subjects)

Adverse Events	Placebo (N=15)	AXA 20.3g BID (N=29)	AXA1957 13.5g BID (N=29)
Total Number of Product Emergent (PE) AEs	17	33	36
Any product-emergent AE	8	15	18
Dosing Disc., Inter. or Reduc. due to a PE AE	1	2	1
Gastrointestinal Disorders	2 (13.3)	11 (37.9)	9 (31.0)
Diarrhea	1(6.6)	5 (17.2	3 (10.3)
Nausea	1 (6.7)	3 (10.3)	3 (10.3)
Abdominal Pain	0	3 (10.3)	1 (3.4)
Abdominal Distension	1 (6.7)	2 (6.9)	0
Metabolism and nutrition	0	2 (6.9)	3 (10.3)
Decreased appetite	0	1 (3.4)	2 (6.9)
Infections	1 (6.7)	4 (13.8)	5 (17.2)
Urinary Tract Infection	0	0	2 (6.9)
Nervous System Disorders	2 (13.3)	1 (3.4)	4 (13.8)
Headache	1 (6.7)	0	3 (10.3)
Musculoskeletal Disorders	1 (6.7)	2 (6.9)	1 (3.4)
Pain in Extremity	0	2 (6.9)	1 (3.4)

In terms of biological effects, an interim analysis was done on subjects in the AXA1125-003 trial who had at least one post baseline MRI, had sufficient information on dosing to evaluate compliance as being greater than 80% and had no major protocol deviations (N= 7 for placebo, 15 for AXA1957 20.3 g BID and 16 for AXA 1957 13.5g BID). Of these, 8,12, and 16 subjects had completed 16 weeks of product administration in the placebo, AXA1957 20.3g BID and AXA 1957 13.5g BID arms, respectively as of October 15, 2019.

Review of the interim data for AXA 20.3g BID and 13.5 g BID amounts suggested that there were clinically meaningful biological effects observed at both amounts. On some measures (i.e. proton density fat fraction (PDFF) and corrected T1 (cT1), imaging assessments of the degree of hepatic steatosis and liver injury, respectively), the lower amount of AXA1957 appears to perform better than the higher amount (Table 5). On other laboratory-based assessments (e.g., ALT, CK18, proC3, markers of liver injury and fibrogenesis), the differences appear to be less apparent between the two AXA1957 amounts (Table 5). This study is ongoing; nonetheless, review of the biological data on balance suggest that administration of the 13.5g BID amount is likely to have a qualitatively greater than or equivalent effect on metabolism and organ function as the 20.3g BID amount.

Table 5. Interim Analysis of the Effects of AXA1957 on Metabolism and Organ Structure and Function from Study 1125-003 (Week 16)

Measure	Placebo (N=7)	AXA 20.3g BID (N=15)	AXA1957 13.5g BID (N=16)
Relative change in PDFF (%)	-0.15	-5.37	-23.59
Absolute change in PDFF	0.05	-1.13	-4.52
cT1 (mSec change)	30.3	3.5	-64
CK-18 (% change)	-12.7	-11.14	-11.99
Pro-C3 (% change)	-5.68	-27.8	-7.59

Taken together, after review of all available safety, tolerability, and biological data to date with AXA1957 at both 8 and 16 weeks in adult subjects with NAFLD, the amount of AXA1957 in adolescents is proposed as 13.5g BID. Given the anticipated weights of the adolescents in this study, biologic effects as related to intake amount are expected to be similar. Also, based on the accumulated data to date on these food products by the Sponsor, the anticipated additional ongoing study with AXA1957 in adult NAFLD subjects, and the extensive published literature [(Børsheim et al., 2009; Coker et al., 2015; Dreyer et al., 2008; Hurt et al., 2014; Huynh & Tayek, 2002; Jabłecka et al., 2012; McClure et al., 2014; Scarnà et al., 2003; Scolapio, McGreevy, Tennyson, & Buett, 2001; Williams et al., 2004); see Table 3 above], on specific amino acids as well as mixtures of amino acids across a variety of adult and pediatric populations and disease conditions, it is anticipated that safety and tolerability profile of AXA1957 in the proposed adolescent population of fatty liver would be similar to, or better, than that observed in prior studies.

2 OBJECTIVES

2.1 Study Objective

To assess the safety, tolerability, and parameters of liver health in adolescent subjects with fatty liver when administered AXA1957, an amino acid food product.

2.2 Study Assessments

Safety and tolerability will be assessed by:

- Reported clinical adverse events (AEs)
- Physical examinations, including changes in body weight and body composition such as lean mass and fat mass
- Vital sign assessments
- Electrocardiograms (ECGs)
- Clinical laboratory tests including changes in standard haematology, chemistry, and lipid panels

Liver structure and function will be assessed by:

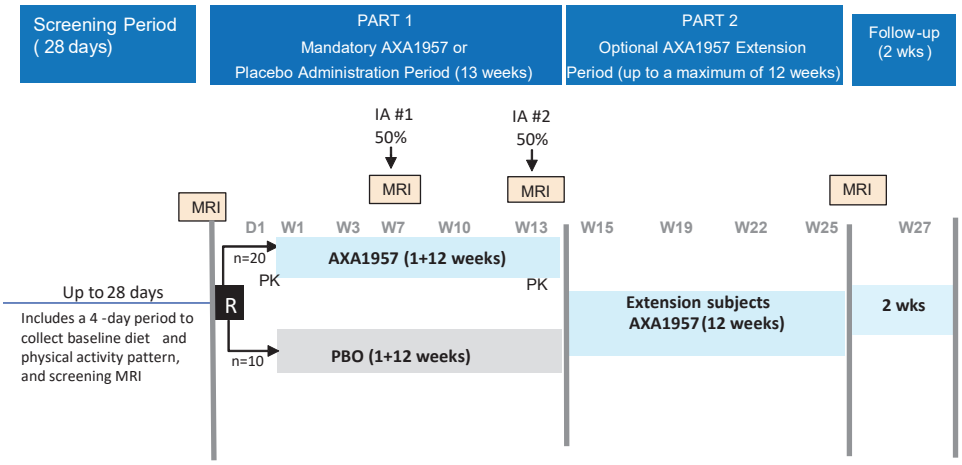
- Multiparametric magnetic resonance imaging (MRI) assessments of liver structure (fat content and inflammation changes)
- Blood tests of liver function, including markers of inflammation and fibrosis

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a randomized, placebo-controlled study conducted in 2 Parts: a mandatory 13-wk administration period (Part 1) and an optional extension period up to a maximum of 12 additional weeks (Part 2); see Figure 1 below.

Figure 1. Study Schematic



Total duration of the study from Screening to the end of Part 2 follow-up is anticipated to be approximately 31 weeks. It is anticipated that there may be a total of approximately up to twelve (12) study visits during the entire study (Screening, Parts 1 and 2).

In Part 1, following up to a 4-week screening period, eligible subjects will be randomized in a 2:1 ratio to receive either AXA1957 or placebo for a 13-week administration period.

Subjects will be centrally randomized with gender (male/female) as the stratification factor to AXA1957 or placebo groups. Randomization will occur via an interactive web response system (IWRS) after eligibility is confirmed and prior to the Day 1 Visit. Study food product amounts will be gradually escalated through the first week of study participation to assess tolerability issues to the food product during the initial week, and to enable subjects and their caregivers to get accustomed to the twice daily regimen. Safety and tolerability will be monitored throughout the study.

All subjects will be provided Guidance and Lifestyle Recommendations for Adolescents with NAFLD; see Appendix 1.

Following the mandatory Part 1 period, all subjects including those randomized to the placebo-arm in Part 1, will have an option to continue in the study in Part 2 for up to an additional maximum of 12 weeks on the AXA1957 food product. All subjects who opt to enter Part 2 will be administered AXA1957 at the same amount and regimen as in Part 1 (i.e. up to 2 stick packs twice daily starting from Week 14) and will continue to be provided the standard lifestyle guidance. Subjects who choose not to participate in Part 2, will undergo a safety follow up visit approximately 2-weeks after Visit 6 per the procedures in Schedule of Assessments (SOA) Table 1. There will be a 2-week follow up period after subjects complete Part 2.

If subjects drop out at any time for any reason during either Part 1 or 2 of the study, including the follow up period, their last visit should capture all the assessments as the end-of-study assessments as shown in Table 1.

3.2 Study Blinding

Part 1 is a single-blind design. Subjects will be blinded to study food product assignment (AXA1957 or placebo), but site personnel dispensing the product to subjects and the Sponsor will be unblinded. Part 2 is open label. All subjects will receive AXA1957 for an additional 12 weeks.

3.3 Study Dietary and Physical Activity Requirements

Diet and exercise regimens play an important role in subjects with NAFLD and can possibly impact study outcome measures. In order to control for this variable, all enrolled subjects will be provided study Guidance and Lifestyle Recommendations for Adolescents with NAFLD (See Appendix 1). All sites will provide the uniform guidance to ensure that subjects are receiving consistent diet and exercise instructions across different sites. The Sponsor will provide instructions to study sites on how to provide the guidance to enrolled subjects. Qualified site staff will provide the guidance and answer any questions subjects and/or their family have during all visits starting at Visit 1. During weeks where there are no study visits, the qualified site staff will call subjects to reinforce the guidance and address questions. Subjects will also be provided diaries to help track and monitor diet and exercise patterns throughout the study to the best of their ability.

3.4 Screening Period

Once written informed consent/assent is obtained, screening procedures will be completed per the SOA in Table 1. Screening period may be up to 4 weeks before starting Part 1. During this period, subjects will be asked to complete a diet and exercise diary over 4 days, which should include two weekdays and 2 weekend days. Subjects will record to the best of their ability all the typical foods/beverages they eat/drink on those days, and their usual physical activity routines to establish a baseline lifestyle pattern. During the entire screening period subjects should continue their normal habitual diet, physical activity patterns and routines (e.g., school, extracurricular activities, including their usual sports activities), and their prescribed standard of care therapies, if applicable

Subjects are also be required to undergo a screening multiparametric MRI scan within approximately 7 days before potential randomization. Subjects will be instructed to fast for at least 4 hrs. prior to their scheduled MRI subjects will have to meet the screening MRI criteria to be eligible for randomization.

Subjects who screen fail due to ALT may be re-screened one time.

Subjects who meet the criteria for inclusion in Version 2.0 of the Protocol may be rescreened for Version 3.0 of the Protocol. If more than 30 days have lapsed since laboratory assessments have been completed as part of Screening, these assessments should be repeated. If an ultrasound or Fibroscan was used to confirm the presence of NAFLD, these do not need to be repeated. If the baseline MRI was performed, this does not need to be repeated providing the interval between the MRI and randomization is less than 14 days and there has been no significant weight change (e.g.,

>5%). Other inclusion and exclusion criteria should be confirmed with the same time bounds as noted.

3.4.1 Randomization

Following completion of all Screening procedures, eligible subjects will be randomized in a 2: 1 ratio to receive either AXA1957 or Placebo and stratified by gender with a block of size of 3.

3.5 Part 1: Mandatory Administration Period

3.5.1 Visit 1 / Day 1 and up to Week 1

Eligible subjects will be randomized via the IWRS and will receive either placebo or AXA1957 with instructions (to both subjects and their caregivers) on how to prepare and consume the study food product, including timing of administration.

On Day 1, subjects will arrive to the study site following an overnight fast of approximately 8 hours. Prior to study product administration, subjects will undergo fasting blood draws and other assessments per the SOA in Table 1. The first administration of the study food product will be at the study site supervised by the study staff. After the study food product administration, subjects will have one additional blood draw for plasma amino acid concentration approximately 1-2 hours after the study product administration and then discharged from the study site.

During the Day 1 visit, all subjects will be provided Guidance and Lifestyle Recommendations for Adolescents with NAFLD (See Appendix 1). Qualified site staff will explain these recommendations and answer any questions the study participant and/or their family may have.

During the first week of study product administration in both AXA1957 and placebo groups, subjects will follow the administration schedule below:

- Days 1 to 3: 1 stick pack twice daily (total of 2 stick packs daily)
- Days 4 through the remainder of the study: 2 stick packs twice daily (total of 4 stick packs daily)

Twice daily regimen of the food product should occur within 30 minutes (i.e. 30 ±5 minutes) **before** meals (e.g., before breakfast and dinner or before lunch and dinner, if breakfast is not a usual part of their daily routine). Any adjustments to this regimen or amount may be considered on a case-by-case basis (see additional details under Study Food Product Section below).

Site staff will conduct a phone call on Day 3 to assess tolerability, and if tolerated, remind the subject to increase the food product to 2 stick packs twice a day (4 stick packs daily) for the remainder of the study administration period.

3.5.2 Visit 2 (Week 1), Visit 3 (Week 3), Visit 4 (Week 7), Visit 5 (Week 10) & Visit 6 (Week 13):

All procedures during Visits 2 through 6 will be performed per the SOA in Table 1. Subjects will be asked to bring all their study food product stick packs (full and empty) to each clinic visit for site staff to perform accountability. Subjects are also required to bring their study food product administration and lifestyle diaries for review at each clinic visit.

During weeks where there are no clinic visits, study staff will make approximately weekly phone calls to subjects to ensure compliance with the study food product administration/regimen, collect information regarding adverse events and concomitant medications, answer any questions, and check-in with subjects/caregivers. If the subject reports new medications, this should be carefully reviewed against the list of prohibited medications in Section 6.1.

3.6 Part 2: Optional Extension Period (12 weeks)

3.6.1 Visit 6 (Week 13) Visit 7 (Week 15), Visit 8 (Week 19), Visit 9 (Week 22) & Visit 10 (Week 25):

After the completion of the mandatory 13-week period (Visit 6), all subjects from Part 1 have the option to enter Part 2 where participating subjects (including those that received placebo in Part 1) will receive up to a maximum of 12 additional weeks of AXA1957 food product.

The determination of whether to continue in the optional period is based upon the following:

- The subject is willing and able to participate in the study for up to 12 additional weeks, and
- There are no negative signals as determined by the clinical judgement of the study medical monitor, investigator, dietician, and/or Sponsor after carefully considering safety, tolerability, health status, body weight, body composition, MRI, and/or blood parameters.

Subjects do not participate in Part 2 will undergo an end-of-study follow up visit approximately 2-weeks after Visit 6 per procedures in the SOA in Table 1.

Subjects who participate in Part 2 will be provided a supply of AXA1957 study food product at Visit 6 and will be instructed to start consuming 2 stick packs twice daily.

There will be at least 4 study visits in Part 2, and all procedures during those visits will be performed per the SOA in Table 1. Safety and tolerability of the study food product will continue to be assessed as was in Part 1.

If subjects drop out at any time for any reason during the study, their last visit should capture all the assessments as the end-of-study assessments as shown in Table 1. Subjects will not be replaced in Part 2.

3.7 Follow-up Visit

Subjects who participate in Part 1 only will return to the study site for a follow-up visit approximately 2-weeks after Visit 6 and will follow the SOA procedures per Table 1.

Subjects who opt to participate in Part 2 will return to the study site for a follow-up visit approximately 2-weeks after Visit 10 and will follow the SOA procedures per Table 1.

3.8 Early Discontinuation/Early Termination Visit

If subjects drop out at any time for any reason during either Part 1 or 2 of the study, including the follow up period, they are expected to complete an Early Termination Visit and will follow the SOA procedures per Table 1.

Subjects who discontinue early in Part 1 may be replaced; subjects who discontinue in Part 2 will not be replaced.

4 SELECTION OF STUDY POPULATION

Subjects who meet inclusion criteria and do not meet any of the exclusion criteria will be eligible for enrollment into the study.

4.1 Inclusion Criteria

Subjects must meet all the following criteria to be eligible to participate in the study:

1. Male and female adolescent subjects between 12 and 17 years of age, inclusive.
2. Female subjects must be at least 2-year post-menarche as determined by clinical history.
3. Willing and able to provide written informed consent from parent(s) or legal guardian, as required.
4. Willing and able to provide written assent from subject, as required.
5. Stated willingness and ability of parent/guardian and the subject to take the study food product twice a day and adhere with all study procedures and requirements.
6. Body weight ≥ 60 kg at time of screening and subjects should be within ±5% of their body weight over the last 30 days prior to Screening
7. Subjects should be at risk for fatty liver, which can be assessed by any one (1) of the following:
    a. Historical liver biopsy that was obtained up to 6 months prior to Screening (local pathology interpretation may be used) with Steatosis greater than Grade 1, if applicable as part of standard of care.

        Note: For the historical liver biopsy to satisfy the fatty liver requirement, the biopsy must have been obtained with no NAFLD or NASH treatment, medications that can cause steatosis or NASH, or other interventional agents/procedures for the treatment of NAFLD/NASH within 3 months prior to when the liver biopsy was obtained; OR

    b. Fasting ALT ≥ 50 U/L in boys, ≥45 U/L in girls, and ≤ 150 U/L in both genders in the Screening visit labs; OR

c. Fibroscan with Control Attenuation Parameter (CAP) ≥ 280 dB/m obtained within 90 days of Randomization; OR

d. Any of the following right upper quadrant ultrasound sonographic features suggestive of hepatic steatosis obtained within 90 days of Randomization, for e.g.: liver uniformly heterogeneous; echogenic diffusely with bright hepatic echoes within the first 2-3 cm of depth with increased hepatorenal echogenicity (i.e., liver with areas of significantly increased echogenicity relative to the renal parenchyma); attenuation of image quickly within 4-5 cm of depth making deeper structures difficult to decipher; thick subcutaneous depth (> 2 cm); vascular blurring of portal or hepatic vein.

8. MRI Proton Density Fat Fraction (PDFF) > 10% **and** corrected T1 (cT1) > 820msec using multiparametric MR imaging of the liver.

   Note: Screening MRI should occur approximately within 7 days prior to randomization and must NOT occur until each of the above entry criteria #1 through #7 have been confirmed.

9. Subjects may have either prediabetes or type 2 diabetes (T2DM). Prediabetes is established by one of the following:

   a. Fasting Blood Glucose is ≥100 mg/dL (5.5 mmol/L) but <126 mg/dL (7.0 mmol/L) or HbA1c ≥5.7% but < 6.4% based on the Screening visit labs; OR

   b. Historical evidence (i.e. within 3 months of Randomization) of impaired fasting glucose/glucose intolerance per the standard of care guidelines and usual procedures of the study center; OR

   c. Subject is already on a stable regimen for approximately 60 days prior to Randomization for the treatment of prediabetes (e.g., diet/lifestyle, metformin, etc.).

10. Subjects with Investigator-confirmed T2DM should have HbA1c ≤9.5% at Screening and must be stable on their usual diabetic medications for approximately 60 days prior to Randomization and should not be anticipating clinically significant dose adjustments of the T2DM medications for the anticipated duration of the study.

11. Subjects taking vitamin and/or other dietary/herbal supplements are permitted as long as ALL the following are met:

    a. Vitamin(s) or other supplement(s) are not on the list of prohibited medications (see Section 6.1.1), and

    b. Subject has been on stable doses and regimens for at least 2 months prior to Randomization; *however, if vitamin E is at doses ≥400 IU/day it must be discontinued prior to Randomization if the subject otherwise qualifies into study*, and

    c. There are no anticipated dose adjustments or changes (i.e. stopping or adding) of vitamins/supplements for the duration of the entire study, including the optional extension period.

12. Heterosexually active female participants of childbearing potential (i.e. post-pubertal and /or post-menarchal, not surgically sterile [defined as tubal ligation, hysterectomy, or bilateral oophorectomy]) must agree to abstain from sexual intercourse for the entire duration of the study (i.e., from time ICF is signed to end-of-study follow up) as any hormone-based contraception approaches are not allowed in the study (e.g., oral contraceptive pills are excluded medications for this study), and barrier approaches (e.g., condom with spermicide, diaphragm with spermicide) alone are not considered adequate to prevent pregnancy. Sexual activity will be ascertained at each study visit, and every female participant in the study must complete a required pregnancy test at each study visit.

4.2 Exclusion Criteria

Subjects are not eligible to participate in the study if any of the following criteria are met:

1. History of any acute or chronic liver disease, other than non-alcoholic fatty liver disease, such as drug-induced, autoimmune, or any viral hepatitis, hemochromatosis, Wilson's, primary sclerosing cholangitis. Clinical/medical history or appropriate laboratory /serological assessments, if necessary, may be relied upon to rule out these other causes of liver disease.
2. Known positive for human immunodeficiency virus (HIV)
3. Presence of Stage 4 fibrosis in the liver
4. Non-compensated liver disease with any one of the following hematologic, biochemical, and serological criteria at Screening:
    a. Hemoglobin < 10 g/dL
    b. White blood cell < 3,500 cells/mm
    c. Neutrophil count < 1,500 cells/mm3 of blood
    d. Platelets < 130,000 cells/mm3 of blood
    e. Direct bilirubin > 1.0 mg/dL (>17.1 μmol/L)
    f. Total bilirubin ≥ 3 mg/dL (≥51.3 μmol/L, unless Gilbert's Syndrome). However, subjects with Gilbert's syndrome with direct bilirubin above upper limits of normal (ULN) in addition to total bilirubin ≥ 3 mg/dL (≥51.3 μmol/L), or with evidence of hemolysis contributing to elevated total bilirubin, should be excluded.
    g. Albumin < 3.2 g/dL (<48.1 μmol/L)
    h. International normalized ratio (INR) > 1.4
5. Any active/clinically unstable GI (e.g., motility disorders, celiac disease, malabsorption syndromes), cardiovascular (e.g., hypertension and hyperlipidemia requiring pharmacologic treatment), endocrine (e.g., Cushing's syndrome congenital adrenal hyperplasia, polyglandular endocrinopathies, hypo/hyperthyroidism requiring pharmacologic treatment, genetic/secondary causes of obesity), flaring autoimmune diseases (juvenile idiopathic arthritis,

adolescent lupus, juvenile dermatomyositis) or psychiatric conditions (clinically significant depression, ADHD, psychosis).

Note 1: Subjects with asthma may be enrolled at the discretion of the Investigator if their condition is stable (i.e. no exacerbations within 4-6 weeks prior to Randomization and no clinically meaningful changes in their asthma management regimens are anticipated for the duration of the study).

Note 2: Subjects who are on a stable dose of anti-depressant medication(s) or medications(s) for ADHD for at least 6 months prior to Randomization and whose Investigator considers them to be psychiatrically stable may be enrolled.

6. Known history of Inborn Errors of Metabolism and/or genetic deficiencies that impact amino acid metabolism or transport [e.g., urea cycle disorders, carnitine deficiency, carnitine palmitoyl transferase (CPT) I or II deficiency, beta-oxidation defects, pyruvate carboxylase deficiency, porphyria, etc.].
7. Current acute illness/health condition requiring ongoing medical treatment. Subjects with minor illness/injury that the investigator considers unlikely to significantly impact study participation or study endpoints should be discussed with the study Medical Monitor and/or Sponsor to determine whether the subject may be included, if all other eligibility criteria are met.
8. Any form of diabetes other than T2DM (e.g., Type 1, MODY).
9. Uncontrolled T2DM, which is defined as:
    a. HbA1c >9.5 % on their current T2DM regimen at Screening and must be stable on their usual diabetic medications for approximately 60 days prior to Randomization and should not be anticipating clinically significant dose adjustments of the T2DM medications for the anticipated duration of the study, and/or
    b. Requiring >10% insulin dose adjustments within 60 days prior to Screening, and/or
    c. Requiring a complex medical regimen, e.g., needing 2 or more oral anti-diabetics (OAD)s, and/or with a history of severe hypoglycemia on their OAD regimens.
10. Uncontrolled hypertension (i.e. systolic blood pressure >130 mmHg and/or diastolic blood pressure >80 mmHg) at Screening.
11. Uncontrolled hyperlipidemia (i.e. triglycerides >350 mg/dL and/or low-density lipoprotein (LDL) >160 mg/dL) at Screening.
12. Impaired renal function (i.e. glomerular filtration rate ≤ 90 mL/min/1.73 m2) at Screening.
13. History of bariatric surgery or planning to undergo bariatric surgery during the study duration (including Part 2). However, prior laparoscopic-band, intra-gastric

balloon, or other weight loss device which was removed >12 months prior to Screening is not exclusionary.

14. Any contraindications to an MRI scan, including:

    a. Presence of non-removable ferromagnetic implants, pacemakers, aneurysm clips or other foreign bodies, or

    b. Unable to have or complete the MRI exam due to body weight exceeding scanner table limit or girth exceeding scanner bore diameter, or

    c. Clinically significant claustrophobia. Subjects experiencing mild anxiety due to transient claustrophobic symptoms may be treated with anxiolytics, if appropriate and per study site SOPs, for mitigation of those symptoms, if necessary.

15. Known sensitivity and/or clinically significant food intolerance/allergies to proteins (such as whey, soy, casein, collagen, gelatin, amino acids, etc.), gluten, fats, carbohydrates (e.g. lactose), nuts or any ingredient in the study food product formulations.

16. History of total parenteral nutrition (TPN) 6 months prior to Screening.

17. Current or planned use of any dietary supplement intended for general metabolic health, weight maintenance, weight loss, OR those containing proteins, amino acids, ketones, or fish oils, from the time the ICF is signed through the end of the study.

    Note: Common examples of excluded protein, amino acid, and other dietary supplements include, but are not limited to: ketones, protein powders, shakes, bars, gels containing whey, soy, casein, collagen, amino acids, and their derivatives/metabolites, N-acetylcysteine, L-carnitine, etc. A few examples of supplements used for weight loss include green tea, green coffee bean extracts, garcinia cambogia, any ketogenic products, etc.

18. Currently on or planning to be on any extreme or unbalanced diet such as Ketogenic, Atkins, Paleo , etc.) from the time the ICF is signed through the end of the study.

19. Use within 2 months prior to or during Screening, or during the study, of systemic glucocorticoids, any biologics (e.g., monoclonal antibodies), hormones of any kind (e.g., estrogens, progesterone, testosterone), valproic acid, ursodeoxycholic acid, any anti-obesity compounds (e.g., orlistat, locaserin, Qsymia), GLP-1 agonists, TZDs, or any other medications known to cause hepatic steatosis, or steatohepatitis, or use of any other known or potential hepatotoxins.

20. Use of any other excluded medications listed in Section 6.1.1 of the protocol not otherwise specified in the entry criteria.

21. Use of any illicit substances, including alcohol, and nicotine products

22. If female, ongoing pregnancy (defined as positive serum and/or urine pregnancy test) and/or planned pregnancy, breast-feeding.

23. Male subjects with female partners of child-bearing potential who are unwilling or unable to adhere to contraception requirements.
24. History of any malignancy, other than nonmelanoma skin cancer, within 4 years prior to Screening.
25. Subjects who are currently enrolled in a clinical trial or who received an investigational study drug/medication, or any dietary supplement(s), food product(s), or participated in a lifestyle / nutritional interventional study/program within 60 days prior to Screening.
26. Any other condition that, in the opinion of the investigator, renders or places the subject at risk for inclusion in the study, or that would impede compliance, or hinder completion of the study.

4.3 Number of Subjects Planned

Enough subjects will be screened to have at least 30 subjects complete the study.

Note: Subjects withdrawing early for any reason in Part 1 may be replaced. Subjects will not be replaced in Part 2.

4.4 Removal of Subjects from the Study

A subject may be discontinued from the study at any time if the subject, the Investigator, or the Sponsor believes that it is not in the subject's best interest to continue. The following is a list of potential reasons for discontinuation from the study:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Subject experiences an AE that, in the opinion of the Investigator, would be in the best interest of the subject to discontinue
- Treatment of a medical condition that requires use of a prohibited medication as listed in section 6.1.
- Pregnancy
- Protocol violation Lost to follow-up
- Sponsor request for early termination of the study.

If a subject is withdrawn due to an AE, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom is considered resolved, stabilized or unknown (e.g., lost to follow-up).

Subjects who prematurely discontinue for any reason will be asked to return to the clinic for an Early Termination visit following the last active study day. Week 13 and Week 25 study procedures will be conducted at the Early Discontinuations.

Subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the Investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents and eCRFs for early termination procedures.
Subjects who withdraw from the study may be replaced and subjects will not be replaced in Part 2.

5 STUDY FOOD PRODUCT INFORMATION

5.1 AXA1957 Composition and Formulation Overview

AXA1957 is a mixture of selected amino acids that are normally present in regular food and/or naturally produced by the body and/or readily available as dietary supplements and formulated as a dry powder that will be reconstituted with ~6 oz (~180 mL) of water to form an orange flavored drink. The composition of AXA1957 is presented in Table 6 and Table 7.

Table 6. Amino Acid Composition of AXA1957 per Stick Pack

Ingredients	%	Grams
L-Leucine	15%	1.00g
L-Isoleucine	7%	0.50g
L-Arginine	20%	1.34g
L-Glutamine	10%	0.67g
L-Serine	37%	2.50g
L-Carnitine	5%	0.33g
N-Acetylcysteine	6%	0.43g
Total AA	**100%**	**6.77g**
Total BCAA	**22%**	**1.50g**

Table 7. Excipient Composition of AXA1957

Ingredients/Excipients
Anhydrous Citric Acid
Sucralose Micronized
Xanthan Gum, Pre-Hydrated
Artificial Vanilla Custard Flavor
Natural Orange Flavor WONF
FD&C Yellow No. 6

5.2 Placebo Composition and Formulation Overview

The placebo is a well-balanced food product with a macronutrient composition of ~53% carbohydrate (maltodextrin), ~22% fat (sunflower oil), and ~25% protein (collagen hydrolysate) that is formulated as a dry powder to be reconstituted with ~6 oz (~180 mL) of water to form an orange flavored drink that is color-, taste-, excipient-, and calorie-matched to AXA1957.

5.3 Study Food Product Preparation and Administration

AXA1957 is composed of naturally occurring amino acids, which are normal ingredients of food or those readily available as dietary supplements.

Subjects will be provided detailed Study Food Product Administration Instructions by the study site. Subjects will also be provided with a 250mL Nalgene™ wide-mouth HDPE bottles with a screw-top lid (Thermo Scientific™ catalog #332189-0008), or similar container, that may be used for preparing and administering their assigned study food product.

Study food products are provided in dry powder form in stick packs, which are then mixed in ~6 oz (~180 mL) of water, and then consumed twice daily approximately 30 min (i.e. 30 ±5 minutes) **before** meals (e.g., before breakfast and dinner or before lunch and dinner, if breakfast is not a usual part of their daily routine) for the entire duration of the study.

Note: While subjects are encouraged to consume the study food product at defined times as indicated above to establish a clear routine which improves adherence to study procedures, **it is more important in this study to ensure that subjects consume their assigned products twice a day**. To that end, the following flexibility is allowed within the administration periods:

- The twice daily (BID) administrations can occur at any time during the day if the two administrations are at least 4 hours apart, and the study food products are not consumed immediately before, with, or immediately after a main meal.
- If subjects experience any clinically significant gastrointestinal (GI) discomfort while taking their study food product prior to meals, administrations may occur approximately 30-60 minutes after the meal, and only after consulting with the investigator and Medical Monitor or Sponsor.

5.3.1 Modification of Study Food Product Administration Amount for Tolerability

Every attempt should be made to encourage subjects to stay on their assigned amount and regimen for the full duration of the study. However, if the study investigator determines any tolerability issues of clinical significance, the amount of AXA1957 or placebo may be reduced for an individual subject to an amount and/or regimen that was previously tolerated, but only after discussion with the Medical Monitor or Sponsor. For instance:

- If the 2-stick pack twice daily amount is not tolerated, then the amount for that individual subject may be reduced to 1 stick pack twice daily;
- Any subject who is not able to tolerate the study food product or placebo at any amount (e.g., even at 1 stick pack twice daily amount) should be discontinued from the study and undergo the End-of Study visit procedures.

5.3.2 Missed Study Food Product Administration

If subjects miss their study food product administration before a meal then they should wait until the next meal to administer the product (e.g., if a subject forgets to take the study food product before breakfast, then wait and administer the product with lunch and dinner).

If subjects cannot wait until the next meal (e.g., a subject forgets to administer the study food product before dinner and will not be eating another meal that day) they may consume the study food product after a meal, but must wait at least 30 minutes AFTER the meal prior to consumption of the food product

5.4 Study Food Product Packaging, Labeling and Storage

AXA1957 and placebo will be supplied as pre-weighed powder in stick packs that will be reconstituted with water.

The individual labelled stick pack units are packaged into a secondary carton, containing a total of 42 stick packs per carton. The stick packs and exterior of the carton are labelled in accordance with local and national requirements, if applicable. The carton is sealed with tamper evident tape.

The AXA1957 and placebo study food products should be stored at 15-25°C (59°F-77°F) ambient temperature. The products are shipped at ambient temperature.

5.5 Method of Assigning Study Food Product to Subjects

Subjects will be screened sequentially and assigned a 5-digit subject number (3-digit site #- 2-digit subject #), as noted in the example below:

Site #100: 100-01, 100-02, 100-03 …

Site #200: 200-01, 200-02, 200-03 …

If a subject is re-screened they will need to be re-consented and a new subject number will be assigned the based on the numbering convention listed above.

5.6 Accountability, Disposal, Return, or Retention of Unused Study Food Product

The site staff and/or pharmacist will document receipt of study food product from the Sponsor or its designee and preparation of the product for administration to subjects. Product administration and returns will be documented on study food product accountability log(s). The site should maintain all unused product containers (cartons and stick packs) until final review of accountability is conducted by the study monitor, and instructions regarding return or disposal, as applicable, are provided.

5.7 Compliance with Study Food Product Administration

Subjects will be asked to return the cartons and any remaining stick packs at each visit. Compliance will be assessed by reviewing the number of returned stick packs, if any, at each visit. Any apparent discrepancies between quantity of stick packs returned and the number anticipated based on the administration schedule will be discussed with the subject to ensure an understanding of the administration instructions. Subjects will also record the amount of the product consumed in a daily administration diary. Repeated non-compliance with administration instructions may necessitate discontinuation from the study, based on the Investigator's and/or Sponsor's judgment.

6 CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the patient is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency.

If significant changes in antidiabetic medications are required during the study, the Investigator should discuss with study Medical Monitor and Sponsor whether the subjects should continue in the study or be withdrawn.

6.1.1 Concomitant Use of the Following is Prohibited <u>During the Study</u>:

- All prandial insulins (e.g. Lispro, Aspart, Glulisine, Regular, Humulin R, etc.)

  Note: Use of insulin mixtures (e.g. 70/30), Humulin N, NPH insulin, or other basal insulins (e.g. detemir, glargine, concentrated insulins such as U500 etc.) are permitted only if they are at stable doses and regimen for approximately 60 days prior to Screening, and no clinically significant dose adjustments in these insulin regimens are anticipated during the study duration. Minor adjustments as per the standard of care are permitted.

- Systemic glucocorticoids
- Amiodarone (even if it was used ≥2 months prior to Screening)
- Methotrexate
- Tetracyclines
- Tamoxifen
- Hormone-based contraception products of any kind and in any form (such as progesterone and/or estrogen containing pills, patches, creams, depots, implants, injections, devices, etc.), as well as any products or supplements containing estrogen, progesterone, or related products
- Any type of testosterone preparations (e.g., patches, gels, pills, depots, injections), including anabolic steroids
- Valproic acid
- Obeticholic acid (OCA)
- Ursodeoxycholic acid (Ursodiol® and Urso®)
- Anti-obesity medications and dietary supplements for weight loss (e.g., orlistat, locaserin, Qsymia, green tea extract, green coffee bean extract, garcinia cambogia, any ketogenic products, etc.)
- Dietary supplements containing ketones, fish oils, protein, and amino acids including, but not limited to: protein powders, shakes, bars, and gels containing whey, soy, collagen, gelatin, casein, amino acids or their derivatives/metabolites such as N-acetylcysteine, L-carnitine, and any ketogenic products.
- Vitamin E ≥400 IU/day
- GLP-1 analogs (e.g., exenatide, Byetta) or GLP-1 receptor agonists (e.g., liraglutide, dulaglutide, semaglutide, etc.)
- TZDs (e.g., pioglitazone, rosiglitazone)

- Other known hepatotoxins (acetaminophen is permitted as long as the dose is <600 mg/day)
- Any other investigational product.

The study Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy. The Medical Monitor, in consultation with the Study Sponsor, will make the final determination of which subjects are enrolled in the study and if a supplement is permitted to be initiated during the study (for example, vitamin D supplements will be permitted to treat laboratory-confirmed vitamin D deficiency).

7 STUDY ASSESSMENTS AND PROCEDURES

The schedule of assessments is summarized in Table 1. A description of study procedures and assessments is provided below.

7.1 Baseline and Disease Characteristics

Details regarding medical history will be collected during Screening. Prior and concomitant medications will also be recorded during this time.

7.2 Physical Examination, Vital Signs and Body Weight

Screening and Day 1/Baseline physical examination (PE) will consist of an assessment of general appearance, skin, thorax/lungs, abdomen, lymph nodes, head, ears, eyes, nose throat, neck, and cardiovascular, musculoskeletal, and neurological systems. Subsequent PE examinations will be abbreviated and will consist of general appearance, skin, thorax/lungs, cardiovascular system, and abdomen.

Physical examination findings will be documented in the subject's source documents. Any new PE finding that represents a new abnormal finding or a worsening from Baseline condition will be recorded as an AE. Vital sign measurements include blood pressure, pulse, body temperature, and body weight and will be measured at every study visit. Vital signs should be conducted after subjects have been sitting quietly in a resting position for at least 5 minutes. If needed, blood pressure at screening may be re-tested two additional times at >15-minute increments to confirm eligibility.

7.3 12-Lead Electrocardiogram

An ECG will be performed locally using validated machinery available locally to each clinical site. The ECG reports will be reviewed by the Investigator or qualified sub-Investigator and assessed as (i) normal, (ii) abnormal – not clinically significant, or (iii) abnormal clinically significant.

7.4 Lifestyle Guidance

During each study visit, subjects will meet with a study dietician or other qualified study staff who will reinforce the lifestyle recommendations in Appendix 1 to subjects and their caregivers. In between the study visits, subjects may receive phone calls approximately once a week where qualified study staff may continue to reinforce these lifestyle recommendations.

7.5 Multiparametric MRI

Subjects will undergo multiparametric MRI examination up to four times during the study to characterize and quantify liver tissue (e.g., liver fat/ inflammation) and body composition such as lean mass and fat mass. Some imaging centers may also offer the option to assess heart function. Multiparametric MR imaging will be conducted at the indicated time points in Table 1. The screening MRI scan should be performed only after all other eligibility criteria have been met and should occur approximately within 7 days prior to potential randomization. Scans should be scheduled based on scanner availability which may not correlate with the same day as the study clinic visit. MRIs at Visit 7 and 13 are not required to occur the same day as the study clinic visit, but must occur within the +/- 5 day visit window. Every attempt should be made to schedule scans at roughly the same time of the day to reduce diurnal fluctuation in daily liver lipid levels. . Subjects are required to be fasting for at least 4 hours prior to all MRI scans. Consequently, on the MRI scanning days, subjects may adjust their placebo or AXA1957 administration regimens accordingly.

Details of the multiparametric MRI assessments will be described in the Imaging Review Charter and Imaging Manuals.

7.6 Fibroscan or Right Upper Quadrant Ultrasound Assessment

A Fibroscan assessment measuring hepatic steatosis (via controlled attenuation parameter, CAP), or right upper quadrant ultrasound assessing sonographic features of hepatic steatosis, may be used to determine subject eligibility and to triage for the multiparametric MRI assessment provided it was obtained within 90 days of the Randomization.

7.7 Clinical Laboratory Assessments

Blood samples for clinical laboratory evaluations are listed in Table 8 below. Detailed blood collection procedure for will be described in the Laboratory Manual A retain sample of plasma will be collected at each biomarker timepoint for possible future non-genetic exploratory analysis.

Samples will be analyzed at central and specialty laboratories. Laboratory reports will be reviewed by the Investigator or designee and filed in the source document. Clinical laboratory findings that represent a worsening from a baseline value and are considered by the Investigator to be clinically significant will be recorded as an AE.

Subjects will be required to fast for approximately 8 hours prior to reporting to the study site for specific blood draws as indicated in Table 1.

Placement of a peripheral IV catheter for blood draws may be considered as per investigator discretion in accordance with institutional policies. If utilized, saline lock is recommended. In addition, care should be taken to ensure proper waste is drawn with blood collection and considered in the estimated blood volume collected during the study.

Topical anesthetics may also be used at the Investigator's discretion and/or the institution's policies.

Table 8. Laboratory Assessments

Chemistry (fasted)	Haematology (CBC)	Haematology (CBC)	Lipid panel (fasted)	Metabolites (fasted)	Metabolic Panel (fasted)	Biomarkers (fasted)	Biomarkers (fasted)	Other
• Albumin • Alkaline phosphatase (ALP) • ALT • AST • Blood urea nitrogen (BUN) • Calcium • Chloride • Creatinine • Gamma-glutamyl transferase (GGT) • Glucose • Phosphorus • Potassium • Sodium • Total Bilirubin • Total CO2 (measured as bicarbonate) • Total protein • Uric acid • eGFR	• Hematocrit • Hemoglobin • Mean corpuscular hemoglobin (MCH) • Mean corpuscular hemoglobin concentration (MCHC) • Mean corpuscular volume (MCV) • Platelet count • Red blood cell count	• White blood cell (WBC) count • WBC differential (% & absolute) • Basophils • Eosinophils • Lymphocytes • Monocytes • Neutrophils • PBMC isolation • Coagulation (INR, PT/PTT)	• Total cholesterol • High-density lipoprotein cholesterol (HDL-C) • Low-density lipoprotein (LDL-C) • Triglycerides • Non-HDL-C	• Lactate • Glycerol • Pyruvate	• Insulin • Beta-hydroxybutyrate (BHB) • Non-esterified fatty acids (NEFA) • Free amino acids • Hemoglobin A1c (HbA1c) • Fructosamine • Homeostasis model assessment of insulin resistance (HOMA-IR; calculated) • Adipose tissue insulin resistance (Adipo-IR; calculated) • Total Testosterone (T) • Dehydro-epiandrosterone (DHEA) • Estradiol (E2)	• Adiponectin • FGF-21 • IL-1beta • High sensitivity C-reactive protein (hsCRP) • MCP-1 • CK-18 (M30 and M65) • YKL-40 • Alpha-2 macroglobulin, • Enhanced liver fibrosis (ELF) Score (TIMP-1, PIIINP & hyaluronic acid) • Apolipoprotein B (ApoB) • Apolipoprotein CIII (Apo-CIII) • Lipoprotein a [Lp(a)] • OWL lipidomic panel	• N-terminal fragment of type III collagen (ProC3) • Internal epitope in the 7S domain of type IV collagen (ProC4) • Released N-terminal pro-peptide of type VI collagen (ProC6) • Internal epitope in the N-terminal pro-peptide of type 1 collagen (P1NP)	Serum Pregnancy Test at Screening; Urine Pregnancy test all other days.

Urinalysis (quantitative)	• pH • Specific gravity • Protein • Glucose • Ketones • Bilirubin • Blood • Nitrate • Urobilinogen • Leukocyte esterase • Urine urea nitrogen

7.8 Plasma Amino Acid Concentration Sampling

On Visit 1, Visit 6, and Visit 10, all subjects (including those on placebo) will be instructed to arrive at the study site after approximately 8 hours of overnight fast. They will have a fasted blood draw (T=0), will be administered their assigned study food product at the study site, and then approximately T=1-2 hours later, another blood sample will be collected for plasma amino acid concentrations. The exact clock times of the T=0 (pre-administration) and T=1-2hr (post-administration) samples should be recorded in the source documents.

Samples will be analyzed for administered amino acids (L-Leucine, L-Isoleucine, L-Arginine, L-Glutamine, L-Serine, L-Carnitine, Acetylcysteine) and other amino acids as appropriate). Detail will be described in the PK Analytical Plan.

7.9 Food and Activity Diaries & Dietary Guidance

7.9.1 Study Dietary and Exercise Guidance

Diet and exercise regimens play an important role in subjects with NAFLD and can possibly impact study outcome measures. In order to control for this variable, all enrolled subjects will be provided Guidance and Lifestyle Recommendations for Adolescents with NAFLD by qualified site staff (See Appendix 1). All sites will provide the uniform guidance to ensure that subjects are receiving consistent diet and exercise instructions across different sites. The Sponsor will provide instructions to study sites on how to provide the guidance to enrolled subjects. Qualified site staff will provide the guidance and answer any questions subjects and/or family have during all visits starting at Visit 1. During weeks where there are no study visits, the qualified site staff will call subjects to reinforce the guidance and address questions.

7.9.2 Four Day Diet and Exercise Diary

Subjects (with the help of their family) will be asked to complete a diet and exercise diary over a 4- day period which should include 2 weekdays and 2 weekend days prior to Visit 1. Subjects will carefully record all foods/beverages they eat/drink on a daily basis, and their usual physical activity routines to establish a baseline lifestyle pattern. Subjects should not alter their normal routines or diets during this time. The intent of the diary is to capture diet and exercise regimens over four typical days in their lives.

7.9.3 National Institute for Health and Care Excellence Quality Standard Life Style Questions

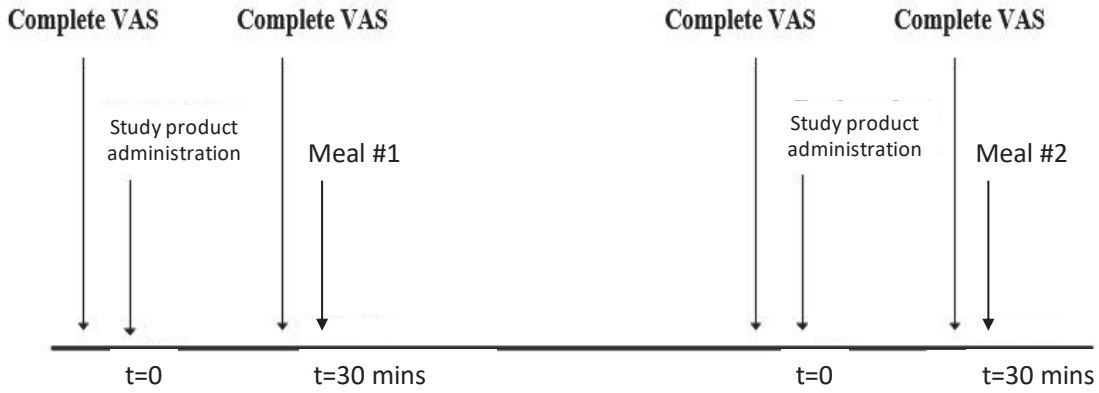
Each week subjects will be provided with a standardized questionnaire asking “yes or no” questions if they are adhering to the study diet and exercise guidance over a seven-day period. The questionnaire has approximately 10 questions and may be filled out at home or at the study site. Subjects will be told to answer the questions honestly, and that they will not be punished or rewarded based on how they answer the questions. The questionnaire will serve as a tool for the site staff on areas of the study diet and exercise guidance to focus instruction on during the visit.

7.10 Hunger and Satiety Visual Analog Scale (VAS)

In the week prior to the Day 1 visit, subjects will be asked to complete the hunger and satiety VAS to get them familiarized with the VAS self-assessment. Subjects will be asked several questions about hunger and fullness (satiety) and activity by marking on a scale associated with each question. Each of the questions in the VAS includes a scale that is 100 mm long. The study staff will measure the distance (in mm) from the left to right side of the scale, to the line that the subject marked as their answer. This length (in mm) is the score for that question.

The VAS assessment will be conducted biweekly during the administration period of Part 1 and Part 2. During each 2 week period subjects will choose a day to complete the VAS. For consistency, subjects should be instructed to try to complete the VAS on the same day of the week throughout the study. On the days the VAS is completed subjects will fill-out the VAS worksheets in conjunction with each study food product administration for that day (e.g., morning and evening) at two timepoints: 1) immediately prior to the study food product administration; and 2) immediately prior to eating the meal where the study food product is administered (see Figure 3). Subjects must return completed VAS worksheets for review at each study visit.

Figure 2: Timing of Visual Analog Scale Completion



7.11 Study Food Product Administration Diary

Subjects will be provided a diary to record study food product administration. Subjects will be required to complete the diary daily and return the diary to the clinic during each study visit for the site staff to review.

8 ADVERSE EVENTS AND SAFETY REPORTING

The Investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE as provided in this protocol.

8.1 Definitions

8.1.1 Adverse Events

An AE is any untoward medical occurrence associated with the use of a study food product or with study participation, regardless of the relationship of the occurrence to study food product or protocol. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the product, whether or not considered related to the product. An AE can arise from any use of the product, and from any route of administration, formulation or dose, including an overdose.

From the time of informed consent up to first administration of study food product on Day 1, any untoward medical occurrence considered related to study procedures will be recorded as an AE. Adverse events that occur from the first administration of study food product through the Follow-up Visit will be considered treatment-emergent AEs.

For each AE, start date, stop date, causality (relationship to study food product), action taken, outcome, and severity will be recorded in the source document and on the case report form (CRF).

8.1.2 Adverse Reaction and Suspected Adverse Reaction

All noxious and unintended responses to the study food product related to any amount administered should be considered adverse reactions. Suspected adverse reactions are any AEs for which there is a reasonable possibility that the product caused the AE.

AEs associated with the use of study food product outside what is described in the protocol, including misuse and abuse of the product, are considered adverse reactions.

8.1.3 Serious Adverse Event Definition

An SAE is any untoward medical occurrence at any dose that results in one or more of the following:

- Results in death
- Is life-threatening (at risk of death at the time of the event)
- Requires subject hospitalization or prolongation of existing hospitalization
  NOTE: Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered to be an AE.

- Results in disability/incapacity
  NOTE: The term disability is defined as a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (i.e., sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All SAEs must be reported to the Sponsor or designee within 24 hours of the site's awareness of the event. All available information should be included in the initial report. Additional follow-up information should also be reported within 24 hours.

8.1.4 Causal Relationship Assessment

The Investigator is required to provide an assessment of relationship of AEs and SAEs to study food product or protocol procedures, if applicable. To promote consistency, the following guidelines should be taken into consideration, along with good clinical and scientific judgement, when determining the relationship of AEs to study food products:

Definitely related: A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the study food product administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study food product should be clinically plausible.

Possibly related: A clinical event, including laboratory test abnormality, with a reasonable time sequence to the study food product administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the withdrawal of the study food product may be lacking or unclear.

Unlikely related: A clinical event, including laboratory test abnormality, with little or no temporal relationship to the study food product administration, and which other drugs or chemicals or underlying disease provide plausible explanations.

8.2 Categorization of Adverse Events

The intensity of an AE will be categorized as follows:

Mild: Mild events are those which are easily tolerated with no disruption of normal daily activity.

Moderate: Moderate events are those which cause sufficient discomfort to interfere with daily activity.

Severe: Severe events are those which incapacitate and prevent usual activity.

8.3 Recording and Reporting of All Non-Serious and Serious Adverse Events

Subjects will be required to report any AE that occur after informed consent is signed.

All AEs will be recorded from the time of informed consent until the last follow-up visit/call. Subjects will be instructed to report all AEs and will be asked a general health status question at each study visit.

An AE should be followed until it is either resolved, has returned to baseline, or is determined to be a stable or chronic condition.

If a non-serious AE is ongoing at the follow-up visit/call, the AE will be recorded as ongoing. AEs that are ongoing at the follow-up visit will be followed to resolution at the discretion of the Investigator.

At each study visit, all AEs that have occurred since the previous visit must be recorded. The Investigator or appropriate designee must determine the intensity of the AE.

Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually, with severity (mild, moderate, or severe).

All study food product-related AEs/SAEs will be followed to resolution (the subject's health has returned to his/her baseline status or all variables have returned to normal), or until an outcome is reached, stabilization occurs (the Investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained, regardless of whether the subject is still participating in the study. Where appropriate, medical tests and examinations will be performed to document resolution of the event(s).

8.4 Serious Adverse Event Reporting Procedures

All SAEs, from the signing of informed consent through the last follow-up visit, will be reported to the Sponsor or its representative, within 24 hours of the Investigator's first knowledge of the event, even if the event does not appear to be related to the study food product or the protocol.

The initial SAE report must be as complete as possible, including details of the current illness and (serious) AE, and an assessment of the causal relationship between the event and the food product. Information not available at the time of the initial report (e.g., an end date for the event, laboratory values received after the initial report, or hospital discharge summary) must be documented on a follow-up report. All follow-up information must be reported in the same timelines as the initial report.

At any time after completion of the AE reporting period, if an Investigator becomes aware of an SAE that is suspected by the Investigator to be related to the study food product, the event must be reported to the Sponsor or its representative.

The following information is a minimum set of information required for all initial SAE reports:

- Investigator name
- Subject identifiers
- AE term(s)
- Suspect study food product
- Study product event relationship
- Reason why the event is serious.

SAEs will be entered in the electronic data capture (EDC) system; the project contact for SAE receipt will be notified via the EDC system upon entry of an SAE in the system. In circumstances where the EDC system is not available, notification by telephone is acceptable. For telephone reports, the SAE must be entered into the EDC system as soon as the system is available for data entry.

The site will notify the Sponsor or its representative of additional information or follow-up to an initial SAE Report as soon as relevant information is available and within 24 hours of awareness. Follow-up information is reported in the EDC system.

8.4.1 Safety/SAE Reporting Contact Information

The preferred means of SAE reporting is via the EDC system, reference the Case Report Completion Guidelines for instructions. Instructions for initial SAE reporting and urgent safety issues/questions will be provided in the Safety Manual provided to the sites.

8.5 Data and Safety Monitoring Committee

There will not be an independent Data and Safety Monitoring Committee established for this study. The Investigators, Medical monitor and Sponsor will monitor all data for safety and protection of subjects.

8.6 Reporting to IRBs and Ethics Committees

The Sponsor or its designee is responsible for notifying the investigational sites of all expedited SAEs.

The Investigator will notify institutional review boards (IRBs) or Ethics Committees (ECs) of serious, related AE(s) or significant risks to subjects, per local country requirements. The Investigator must keep copies of all AE information, including correspondence with the Sponsor, IRBs and ECs on file. It is the responsibility of the Principal Investigator to notify the IRB/EC of all SAEs that occur at his or her site.

8.7 Contraception and Pregnancy

No human studies of the effects of AXA1957 on conception, pregnancy, or lactation have been performed to date. Therefore, female participants should not be exposed to the product if pregnant, breastfeeding, or attempting to conceive. Although there are no known or anticipated risks of potential harm to a foetus, there are currently no data to definitively prove whether a foetus would be at risk with this specific dietary supplement. Therefore, all female participants in this study will be asked to abstain from sexual intercourse during their participation in the study (including those that are not sexually active). During screening, the study site should allow for a private conversation with all potential female subjects to inquire if they are currently sexually active, or think it is likely/plan to be sexually active during the next 5- to 8-months (i.e. from first day of screening to last day of follow up, which is a mandatory 19 weeks in Part 1, and up to 31 weeks in the optional Part 2), and advise them of the study expectations as described below.

The following guidelines for contraception must be followed during the study:

- Heterosexually active female participants of childbearing potential (i.e. post-pubertal and /or post-menarchal, not surgically sterile [defined as tubal ligation, hysterectomy, or bilateral oophorectomy]) must agree to abstain from sexual intercourse for the entire duration of the study (i.e., from time ICF is signed to end-of-study follow up).

- Abstinence for the purpose of the study is defined as abstaining from sexual intercourse for from the time the ICF is signed until discharge from the study. Periodic abstinence (e.g., calendar, ovulation, symptothermal, etc. methods) are NOT acceptable.

- Hormonal contraception methods are considered prohibited medications and not allowed in the study as oestrogen can impact liver fat. As this study measures liver fat, this could potentially confound the study data and results.

- Sexual activity must be ascertained at each study visit, and every female participant in the study must complete a required pregnancy test at each study visit.

Male subjects with female partners of child-bearing potential must use at least 1 of the following contraceptive methods: complete abstinence, or barrier method of contraception. The contraception requirements must be followed from Screening (i.e. day the informed consent is signed) to last day of follow-up.

Pregnancy of a female partner of a male study participant must be reported to the Investigator, and, in turn, the pregnancy must be reported to the Sponsor or its representative within 24 hours of the Investigator's awareness of the pregnancy.

In the case of pregnancy of a female study participant during the study, study food product should be discontinued, and the Sponsor informed immediately. Follow-up of the pregnancy will occur until the outcome is available, including premature termination. The follow-up period will be deemed to have ended when the health status of the child has been determined upon birth.

9 STATISTICAL ANALYSIS

A detailed statistical analysis plan describing the specific analyses to be performed for this study will be completed prior to database lock. All analyses will be performed, and all tables, figures, and data listings will be prepared using SAS version 9.4 or higher. Summary statistics for continuous variables will include the mean, standard deviation, median, minimum and maximum value; categorical variables will be presented as counts and percentages. Some subgroup analyses (such as liver fat or ALT assessments) may be within each of the gender groups given the known and well-established gender differences in these parameters.

9.1 Sample Size

As this is food product study, no formal sample size calculations will be conducted. A sufficient number of subjects will be screened to have approximately thirty (30) subjects in total complete the study. This study is exploratory in nature, and the sample size is based on clinical judgement that this number of subjects will be sufficient to provide a characterization of the product safety.

Randomization will be stratified by gender with a block size of three (3). Randomization will occur via an interactive web response system (IWRS).

9.2 Analysis Sets

The Safety analysis set will include all subjects who receive at least 1 administration of either placebo or AXA1957. Any subject who is screened but who does not receive study food product will not be included in study reporting.

The Per protocol (PP) analysis set will include all subjects who receive at least 1 administration of AXA1957 or placebo with no major protocol deviations.

9.2.1 Safety Analyses

Safety analyses will be performed using the Safety analysis set. Safety and tolerability will be evaluated using descriptive statistics and listings of AEs, physical examination, including body

composition (lean and visceral fat mass) changes, clinical laboratory test values, including plasma amino acid levels, vital signs, weight, body temperature, ECGs and other safety parameters.

Analyses of AEs will be performed for those events that are considered product-emergent, where product emergent is defined as any AE with onset (or any changes in a pre-existing condition) after the first administration of either placebo or AXA1957. All efforts will be made to record start and stop dates for AEs. Adverse events with partial dates will be assessed using the available date information to determine product-emergent status. Adverse events with completely missing dates will be assumed to be product-emergent.

Adverse event verbatim terms will be coded to preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. AEs will be summarized by administration group using subject incidence rates. Therefore, in any tabulation, a subject contributes only once to the count for a given AE (preferred term). Separate tabulations will be produced for all product-emergent AEs, product-related AEs (those considered by the Investigator as possibly study food product related), SAEs, and discontinuations due to AEs. By subject listings will be provided for any deaths, SAEs, and AEs leading to discontinuation of study food product administration. If the number of AEs seen on the study is very small, listings only will be produced.

Clinical safety laboratory test values will be presented using conventional units. Listings of these data will be presented with out-or-normal range values flagged. Mean, standard deviation, median, min, and max will be calculated at each time point. Vital signs values will be presented in listings. Mean, standard deviation, median, min, and max will be calculated at each time point.

ECG values will be presented in listings. A shift table to indicate change in result (normal, abnormal – not clinically significant, or abnormal, clinically significant).

9.3 Interim Analysis

Interim analyses in addition to the final analysis are planned for this study. Up to two interim analyses are planned in Part 1. One after approximately 50% of subjects complete Visit 4 (Week 7) and another after approximately 50% subjects complete Visit 6 (Week 13).

9.4 Pharmacokinetics

Based on sparse plasma amino acid concentration sampling at two time points on Day 1, Week 13, and Week 25 (for those in Part 2), sparse population PK analysis may be performed to estimate PK parameters (Cmax, AUC, Tmax, etc.) as appropriate.

Details of PK computation (e.g., data-handling process) will be outlined in PK Analytical Plan (PKAP).

10 ETHICS AND ADMINISTRATIVE DETAILS

10.1 Ethics

This study is to be performed in accordance with the protocol, the Declaration of Helsinki, the International Council for Harmonization (ICH) Harmonized Tripartite Guideline for Good Clinical Practice (GCP), and all applicable local regulations.

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by health authorities. The Investigator must also comply with all applicable privacy regulations.

The protocol, consent form(s), diaries and any advertising materials along with any amendments will be reviewed and approved by an IRB/EC prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/EC in accordance with the standard operating procedures and policies of the IRB/EC, and the Investigator will keep the IRB/EC informed as to the progress of the study. The Investigator will obtain assurance of IRB/EC compliance with regulations.

Any documents that the IRB/EC may need to fulfill its responsibilities (such as protocol, protocol amendments, consent forms, information concerning subject recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/EC. The IRB/EC's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/EC's unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/EC approval except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/EC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/EC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB/EC; new information that may affect adversely the safety of the subjects of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

All study findings and documents will be regarded as confidential. Subject confidentiality will be strictly maintained to the extent possible under law. Subjects will be identified in the research records, case report forms, and other documents submitted to the Sponsor or its designated representative, by their assigned subject number. Documents that identify the subject (e.g., the signed informed consent form) should not be submitted to the Sponsor or its designated representative and must be maintained in confidence by the Investigator.

10.2 Informed Consent/Patient Information Sheet

Informed consent and assent, when required, will be obtained in accordance with the Declaration of Helsinki, ICH GCP, the EU Data Protection Directive 95/46/EC and local regulations.

The Investigator will prepare the site-specific informed consent/assent form and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/EC. The consent form(s) generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/EC. The written consent document(s) will embody the elements of informed consent and/or assent as described in the current ICH GCP E6 guideline and will also comply with local regulations. The Investigator will send an IRB/EC-approved copy of the informed consent form(s) to the Sponsor or designee for the study file.

Properly executed, written, informed consent(s) will be obtained from each subject, parent or legal guardian as deemed necessary, prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their parent (s)/legal representatives) must be given ample opportunity to inquire about details of the study. If a subject is unable to sign the informed consent form, a legal representative may sign for the subject. A copy of the signed consent form(s) will be given to the subject, parent and/or legal representative of the subject and the original will be maintained with the subject's records.

10.3 Administrative Details

10.3.1 Monitoring

The Sponsor or its representative (e.g., clinical research associate) may conduct a clinic visit to verify the qualifications of each Investigator, inspect the clinic's facilities, and inform the Investigator of responsibilities and procedures for ensuring adequate and correct study conduct and documentation.

Throughout the study, the monitor will make scheduled and possibly unscheduled clinic visits to discuss the progress of the study with the Investigator or his/her representatives, review the informed consent forms, review the CRFs for completeness and accuracy, review protocol compliance, compare CRFs and individual subject's medical records and source documentation, assess product accountability, and ensure that the study is being conducted according to pertinent regulations and GCP. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

Monitoring visits will be conducted by the Sponsor or its representatives according to ICH GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor or designee, and appropriate authorities, to conduct on-site monitoring and/or auditing of all appropriate study documentation.

10.3.2 Recording, Access and Retention of Source Data

The Investigator must make study data and documentation accessible to the monitor, and other authorized representatives of the Sponsor (or designee) and IRB/ECs upon request.

A file for each subject must be maintained that includes the signed informed consent form/assent form and copies of all source documentation related to that subject. The Investigator must ensure the reliability, integrity and availability of source documents from which the information on the CRF was derived.

All study documents (subject files, signed informed consent/assent forms, copies of CRFs, Investigator Site File, etc.) must be kept secured for a period of 7 years following the completion of the study unless there is prior written agreement with the Sponsor. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

10.3.3 Study Termination

If the Sponsor or Investigator discovers conditions arising during the study that suggest the study should be halted, then this can happen only after appropriate consultation between the Sponsor and Investigator. Conditions that may warrant study termination include, but are not limited to:

- The discovery of any unexpected, significant, or unacceptable risk to the subjects enrolled in the study.
- Failure of the Investigator to enter subjects at an acceptable rate.
- Unsatisfactory subject enrollment with respect to quality and/or quantity or data recording is inaccurate and/or incomplete on a chronic basis.
- Insufficient adherence to the protocol requirements.
- A decision on the part of the Sponsor to suspend or discontinue development of study food product.

10.3.4 Protocol Violations

A protocol violation occurs when the subject, Investigator or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria

- Use of a prohibited concomitant medication
- Failure to comply with GCP guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be recorded and reviewed with the Investigator.

10.3.5 Data Quality Assurance

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study food product.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific CRFs when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee) but will be identified by subject number and initials.

If a correction is required for a CRF, the time and date will be recorded by the person updating CRF data to create an audit trail. The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

The data will be entered into a validated database. A Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

10.3.6 Case Report Form

An electronic data capture (EDC) system will be used in the study.

10.3.7 Publication

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws.

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Appendix 1 Guidance and Lifestyle Recommendations for Adolescents with NAFLD

I. GUIDANCE (NICE NG49 AND CG189)

Per National Institute for Health and Care Excellence (NICE) Quality standard [QS152], adopting a healthy lifestyle can help to reduce the rate of progression of non-alcoholic fatty liver disease (NAFLD). Providing lifestyle advice to people with NAFLD can encourage them to consider changes they can make that might help them avoid more serious liver disease.

NICE guideline (NG49) for NAFLD assessment and management states that people with NAFLD should:

- Be advised that there is some evidence that exercise reduces liver fat content
- Be advised that, if they drink alcohol, it is important to stay within the national recommended limits for alcohol consumption.
- Not be offered omega-3 fatty acids because there is not enough evidence to recommend their use for people with NAFLD.
- Be offered advice on physical activity and diet in line with NICE's guidelines on obesity and preventing excess weight gain regardless of their BMI.

General lifestyle guidance per NICE Clinical guideline [CG189]

- Behavior change strategies (stimulus control, self-monitoring, goal setting, rewards for reaching goals, problem solving) should be part of general lifestyle changes
- Increase people's physical activity levels or decrease inactivity
- Improve eating behavior (but children should not go on exclusion diets)
- Improve the quality of the person's diet
- Have realistic targets for outcomes other than weight loss, such as increased physical activity and healthier eating

Physical activity guidance per NICE Clinical guideline [CG189]

- Encourage children and young people to increase their level of physical activity, even if they do not lose weight as a result, because of the other health benefits exercise can bring (for example, reduced risk of type 2 diabetes and cardiovascular disease). Encourage children to do at least 60 minutes of moderate or greater intensity physical activity each day. The activity can be in 1 session or several sessions lasting 10 minutes or more.
- Be aware that children and young people who are already overweight may need to do more than 60 minutes' activity.
- Encourage children and young people to reduce inactive behaviors, such as sitting and watching television, using a computer or playing video games.
- Give children and young people the opportunity and support to do more exercise in their daily lives (for example, walking, cycling, using the stairs and active play). Make the choice of activity with the child/adolescent, and ensure it is appropriate to their ability and confidence.
- Give children and young people the opportunity and support to do more regular, structured physical activity (for example football, swimming, walking, or dancing, etc.).

Make the choice of activity with the child/adolescent, and ensure it is appropriate to their ability and confidence.

Dietary guidance per NICE Clinical guideline [CG189]

- Tailor dietary changes to food preferences and allow for a flexible and individual approach to reducing calorie intake.
- Do not use unduly restrictive and nutritionally unbalanced diets, because they are ineffective in the long term and can be harmful.
- Encourage people to improve their diet even if they do not lose weight, because there can be other health benefits.
- Any dietary changes should be age appropriate and consistent with healthy eating advice.

II. LIFESTYLE RECOMMENDATIONS / ADVICE IN THIS STUDY

In this study which includes young people aged 12 to 17 years with fatty liver, lifestyle recommendations primarily centers on advice for making healthy food choices and encourages regular physical activity based on the circumstances of the individual. This advice is based on the principles of the NICE guidelines NG49 and CG189 outlined above. The parents/caregivers of study participants will be included in the consultation and advice for healthy lifestyle given to the family.

The dietary advice will be consistent with healthy eating and are as follows:

Limit/reduce:*

- Sugar sweetened beverages (coca cola, lemonade, sports or energy drinks) plus excessive fruit juice intake.
- Added sugar – high sugar cereals, yoghurts, and other milk-based drinks.
- High-sugar, high-fat snack intake (crisps, doughnuts, chocolate biscuits)
- Portion sizes of refined carbohydrates (white bread, white rice, cracker etc.)
- Fast-food

**Per the study protocol requirements, study participants while in the study are prohibited from consuming protein shakes, bars, gels, etc., or any other amino acid products, or any ketogenic products, or any other dietary supplements as noted in the study eligibility criteria and prohibited substances*

Encourage:

- More vegetables
- High-fiber versions of foods
- A switch from fruit juice to whole fruit.
- Healthy snacks: nuts and seeds, plain yoghurts, vegetables and dips.

Develop important skills with cooperation and support from the family members

- Reading nutrition labels
- Meal planning and purchasing
- Basic cooking skills, as age appropriate

Potential actions that the dietician or other qualified staff may take based on the review of subject's weekly compliance diaries may include:

- If subjects are following lifestyle guidance, then they should be encouraged to continue following them.
- If subjects are not following lifestyle guidance, then these recommendations and advice should be repeated and reinforced.