NASH CRN

Nonalcoholic Steatohepatitis

Clinical Research Network

Cysteamine Bitartrate Delayed-Release for the Treatment of Nonalcoholic Fatty Liver Disease (NAFLD) in Children (CyNCh) Trial

Standard Operating Procedures

Part I: Clinical Center Operations

21 March 2013

CyNCh SOP Part I: Clinical Center Operations

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CyNCh SOP Part I: Clinical Center Operations

1. Design overview

1.1. Design synopsis

Title	Cysteamine Bitartrate Delayed-Release for the Treatment of Nonalcoholic Fatty Liver Disease (NAFLD) in Children (CyNCh) Trial
Sponsor	NIDDK
Type of trial	Phase IIb randomized placebo controlled clinical trial
Objective	To evaluate whether 52 weeks of treatment with delayed release (DR) cysteamine improves NAFLD compared to treatment with placebo
Study design	Multicenter, double-masked, placebo-controlled study with 2 parallel treatment groups
Treatment groups	Group 1: Cysteamine bitartrate delayed-release (DR) capsules (300 mg, 375 mg or 450 mg orally twice a day) Group 2: Placebo

Study duration (per patient)

- Screening within 90 days of liver biopsy and randomization within 120 days of liver biopsy
- 52 week treatment period
- 24 week post treatment follow-up

Study duration (calendar time)

- Recruitment phase: 9 months
- Follow-up phase: 27 months
- Expected rate of recruitment is 16 patients per clinical center; approximately 2 patients per month

Sample size

- 160 patients (80 per group)

Number of clinics

- 10

Inclusion criteria

- Age 8-17 at screening
- Liver biopsy within 90 days of screening visit and no more than 120 days before randomization
- Clinical history consistent with NAFLD

- Definite NAFLD based on a liver Histology
- No evidence of other liver disease by clinical history or histological evaluation
- Histological severity of NAFLD Activity Score (NAS ≥4)
- Sexually active female participants of childbearing potential (i.e., not surgically sterile [defined as tubal ligation, hysterectomy, or bilateral oophorectomy]) must agree to use of two acceptable forms of contraception at screening and continuing through completion of the study, and to complete a pregnancy test at each study visit. Acceptable forms of contraception include hormonal contraceptives (oral, implant, transdermal patch, or injection) at a stable dose for at least 3 months prior to screening, and barrier (condom with spermicide, diaphragm with spermicide). Sexual activity will be ascertained at each study visit for post-menarcheal females and if sexually active, subject must verify use of the same 2 acceptable forms of contraception. For pre-pubescent children, a documented attestation from their parent or guardian will be acceptable
- Must be able to swallow DR Cysteamine capsules
- Signed informed consent from parent or guardian
- Signed informed assent from the child

Exclusion criteria: There will be no exclusion criteria based on race, ethnicity, or gender. Patients who satisfy any of the following exclusion criteria will be ineligible for enrollment in the trial:

1. Participants with a current history of the following conditions or any other health issues that make it unsafe for them to participate in the opinion of the Investigators:
    - Inflammatory bowel disease (if currently active) or prior resection of small intestine
    - Heart disease (e.g., myocardial infarction, heart failure, unstable arrhythmias)
    - Seizure disorder
    - Active coagulopathy
    - Gastrointestinal ulcers/bleeding
    - Renal dysfunction with a creatinine clearance < 90 mL/min/m^2
    - History of active malignant disease requiring chemotherapy within the past 12 months prior to randomization
    - History of significant alcohol intake (AUDIT questionnaire) or inability to quantify alcohol consumption
    - Chronic use (defined as more than 2 consecutive weeks) of medications known to cause hepatic steatosis or steatohepatitis in the past year:
        - systemic glucocorticoids
        - tetracycline
        - anabolic steroids
        - valproic acid
        - salicylates
        - tamoxifen

- The use of other known hepatotoxins within 90 days of liver biopsy or within 120 days of randomization
- Initiation of medications with the intent to treat NAFLD/NASH in the time period following liver biopsy and prior to randomization
- History of total parenteral nutrition (TPN) use in year prior to screening
- History of bariatric surgery or planning to undergo bariatric surgery during study duration
- Clinically significant depression (patients hospitalized for suicidal ideations or suicide attempts within past 12 months)
- Any female nursing, planning a pregnancy, known or suspected to be pregnant, or who has a positive pregnancy screen.

2. Non-compensated liver disease with any one of the following hematologic, biochemical, and serological criteria on entry into protocol:
   - Hemoglobin < 10 g/dL;
   - White blood cell (WBC) < 3,500 cells/mm^3 of blood;
   - Neutrophil count < 1,500 cells/mm^3 of blood;
   - Platelets < 130,000 cells/mm^3 of blood;
   - Direct bilirubin > 1.0 mg/dL
   - Total bilirubin >3 mg/dL
   - Albumin < 3.2 g/dL
   - International normalized ratio (INR) > 1.4
3. Poorly controlled diabetes mellitus (hemoglobin A1c (HbA1c) > 9%)
4. Evidence of other chronic liver disease:
   - Biopsy consistent with histological evidence of autoimmune hepatitis
   - Serum hepatitis B surface antigen (HBsAg) positive.
   - Serum hepatitis C antibody (anti-HCV) positive.
   - Iron/total iron binding capacity (TIBC) ratio (transferrin saturation) > 45% with histological evidence of iron overload
   - Alpha-1-antitrypsin (A1AT) genotype ZZ or SZ
   - Wilson's disease
5. Children who are currently enrolled in a clinical trial or who received an investigational study drug within 180 days of screening or liver biopsy.
6. Subjects who are not able or willing to comply with the protocol or have any other condition that would impede compliance or hinder completion of the study, in the opinion of the investigator.
7. Failure to give informed consent

1.1. Design synopsis

Outcome measures

- **Primary:**

  Centrally scored assessment of histologic improvement in NAFLD between the baseline liver biopsy to end of 52 weeks of treatment, where improvement is defined as: (1) decrease in NAS of 2 or more and (2) no worsening of fibrosis.

- **Secondary:**
  - Reduction in serum aminotransferase and gamma-glutamyl transpeptidase.
  - Reduction in MRI-determined hepatic fat fraction.
  - Changes to markers of oxidation and anti-oxidant status: maldondialdehyde, F2 alpha-isoprostane, total antioxidant capacity, oxidized LDL
  - Changes in fasting insulin and glucose
  - Changes in weight, height, BMI, and waist circumference
  - Changes in the PedsQL score
  - Changes to any symptoms the patient may have experienced
  - Proportion with a change from a histological diagnosis of definite NASH or indeterminate for NASH to not NASH at end of treatment
  - Individual histological characteristics at end of treatment compared to baseline such as steatosis, lobular inflammation, portal chronic inflammation, ballooning, fibrosis score and stage 1a vs 1b fibrosis
  - Change in mean NAS

Randomization:

- Centrally administered randomization stratified by clinical center and baseline body weight (3 groups: ≤65 kg, > 65-80 kg, > 80 kg)

Visit schedule

- Screening visit must occur within 90 days of liver biopsy and randomization within 120 days of liver biopsy.
- Randomization: Final pre-treatment patient interview followed by web-based randomization into one of 2 groups to receive either DR-cysteamine bitartrate or placebo. The randomization design will be stratified by clinical center and baseline body weight into one of three categories (less than or equal to 65 kg, greater than 65 kg up to 80 kg, or greater than 80 kg) with assignments in permuted blocks of random length within each stratum to achieve a target dose of 9 to 12 mg/kg per day up to a maximum total dose of 600-900 mg per day and dispensing of study drug
- Follow up visits (N=6):
  - 4, 12, 24, 36, and 52 treatment weeks after randomization
  - 76 weeks after randomization (24 weeks after treatment ends)

- Both groups will be administered current standard of care nutrition and exercise recommendations, as a series of one page hand-outs given to participants at randomization and each follow-up study visit (one hand-out per visit). These one-page handouts will include serial strategies to limit screen time, reduce saturated fat, simple carbohydrate and fructose intake, and increase physical activity, as well as fruit and vegetable intake.

Liver biopsy schedule

- Standard of care biopsy prior to screening for the trial
- 52 treatment weeks after randomization

MRI schedule

- Prior to treatment initiation
- 52 treatment weeks after randomization (to coincide with liver biopsy when possible)

Statistical Analysis

- The primary analysis is an intention-to-treat analysis in which the proportions of subjects in the active treatment group (Cysteamine DR orally twice daily) with histological improvement in NAFLD (primary outcome, defined above) is compared with the proportion of subjects in the placebo group in whom there is improvement. The comparison is made using a stratified (by clinical site) Mantel-Haenszel chi-square test; a P-value of 0.05 will be considered statistically significant. Subjects who do not undergo an end-of-treatment biopsy will be counted as not improved.

Sample size and assumptions

- Total of **160** participants in 2 groups of equal size (**80** per group)
- Primary comparison: Cysteamine DR vs. placebo
- Primary outcome measure: histological improvement in NAFLD
- Error protection
  - Type I = 0.05
  - Type II = 0.10 (90% power)
- Uncorrected chi-square test using Dupont and Plummer, Power and Sample size software (1998)
- Missing data: 10% will not have 52 week biopsy and will be considered not improved
- Minimum clinically important difference = 33% relative reduction in percent without clinically important improvement in NAFLD in the active treatment group compared to placebo group.
- Allocation ratio of active treatment to placebo groups = 1:1
- Assumed response rates: Expected percent with no clinically important improvement in NAFLD in the placebo group: 75% (based on TONIC data and assumed background use of medications that may influence histology = 33%)

- Expected percent with lack of clinically important improvement in NAFLD in the cysteamine DR group: 50%

Safety Monitoring

- NIDDK-appointed DSMB will monitor the data for safety and efficacy for outcomes such as hepatotoxicity, pregnancy, and any other outcomes or events identified as safety related.

1.2. Data collection schedule

Assessment/Procedure	Screening Visits	RZ	Follow-up visits Weeks from randomization f04	f12	f24	f36	f52	f76
Consent and HIPAA authorization	X	.	.	.	.	.	.	.
Baseline medical history	X	.	.	.	.	.	.	.
Follow-up medical history	.	.	X	X	X	X	X	X
Review for adverse effects	.	.	X	X	X	X	X	X
Review for concomitant medications	X	X	X	X	X	X	X	X
Alcohol questionnaire AUDIT (A) if interim (I)	A	.	I	I	I	I	I	I
Detailed (D) or focused (F) physical exam	D	F	F	F	D	F	D	D
Liver biopsy*	X*	.	.	.	.	.	X	.
MRI for hepatic fat (optional)	X	.	.	.	.	.	X	.
Nutritional assessment	X	.	.	.	.	.	X	.
Pediatric quality of life	X	.	.	.	.	.	X	X
Liver symptoms questionnaire	X	.	.	X	X	X	X	X
Standard of care materials provided	.	X	.	.	.	.	.	.
Eligibility confirmation	.	X	.	.	.	.	.	.
Study drug dispensing	.	X	X	X	X	X	.	.
Review of study drug adherence	.	.	X	X	X	X	X	.
Labs:								
Complete blood count	X	.	X	X	X	X	X	X
Comprehensive metabolic panel with uric acid	X	.	.	.	X	.	X	X
Hepatic panel with GGT, PT, INR	X	.	X	X	X	X	X	X
Fasting lipid profile	X	.	.	.	X	.	X	X
Fasting serum glucose, HbA1c, and insulin	X	.	.	.	X	.	X	X
Etiologic tests	X	.	.	.	.	.	.	.
Pregnancy tests	X	X	X	X	X	X	X	.
Banking:								
Fasting serum and plasma	X	.	.	X	X	X	X	X
DNA	X	.	.	.	.	.	.	.
Liver tissue	X	.	.	.	.	.	X	.
Closeout form	X	.	.	.	.	.	.	X

Complete blood count: Hemoglobin, hematocrit, mean corpuscular volume (MCV), white blood cell count (WBC), white blood cell differential, platelet count

Comprehensive metabolic panel: calcium, sodium, carbon dioxide, chloride, glucose, potassium, total protein, creatinine, blood urea nitrogen (BUN), uric acid

Hepatic panel: Total bilirubin, direct bilirubin, aspartate aminotransferease (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma glutamyltransferease (GGT), albumin, prothrombin time (PT), international normalized ratio (INR)

Lipid profile: triglycerides, total cholesterol, LDL and HDL

Etiologic tests: Hepatitis B surface antigen, hepatitis C antibody, alpha-1-antitrypsin level, ceruloplasmin. Autoantibodies: (ANA, AMA ASMA), serum iron, ferritin and total iron binding capacity (TIBC)

*The liver biopsy during screening is for the patient's evaluation of NAFLD

1.3. Blood draw schedule

		Study visit (wk)						
Procedure/amount in mL	**Screening**	**f04**	**f12**	**f24**	**f36**	**f52**	**f76**	**Total**
Fasting glucose, HbA1c and insulin	5	.	.	5	.	5	5	20
Fasting lipid profile	5	.	.	5	.	5	5	20
Complete blood count	5	5	5	5	5	5	5	35
Comprehensive metabolic panel	5	.	.	5	.	5	5	20
Hepatic panel with GGT, INR, PT	5	5	5	5	5	5	5	35
Etiologic tests	20	.	.	.	.	.	.	20
Plasma	10	.	10	10	10	10	10	60
Serum	20	.	20	20	20	20	20	120
DNA	20	.	.	.	.	.	.	20
Total	95	10	40	55	40	55	55	**350**

Complete blood count: Hemoglobin, hematocrit, mean corpuscular volume (MCV), white blood cell count (WBC), white blood cell differential, platelet count

Comprehensive metabolic panel: calcium, carbon dioxide, chloride, glucose, potassium, sodium, total protein, creatinine, blood urea nitrogen (BUN), uric acid

Hepatic panel: Total bilirubin, direct bilirubin, aspartate aminotransferease (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma glutamyltransferease (GGT), albumin, prothrombin time (PT), international normalized ratio (INR)

Lipid profile: triglycerides, total cholesterol, LDL and HDL

Etiologic tests: Hepatitis B surface antigen (HBsAg), hepatitis C antibody (anti-HCV), alpha-1-antitrypsin level, ceruloplasmin. Autoantibodies: (ANA, AMA, ASMA), serum iron, ferritin, and total iron binding capacity (TIBC)

1.4. Treatment groups and 'starting' dose at randomization

A patient who has signed or had a parent/guardian sign an informed consent statement and who meets eligibility criteria will be randomly assigned to one of two groups for 52 weeks of treatment:

Group 1: Cysteamine Bitartrate Delayed-release (75 mg capsules)
Group 2: Placebo (75 mg capsules)

Dose amounts of study drug or placebo will be assigned to patients by body weight stratum. Additionally, study drug or placebo will be increased gradually from Week 1 to the end of Week 4. If tolerated, the patient will continue with the dose amount dispensed at Week 4 according to his/her baseline body weight and the dose amount will remain fixed thereafter for the entire 52 week treatment period regardless of change in body weight.

Once the Randomization Checks (RZ) form is keyed and the randomization task is run, the randomization plan that was prepared by the Data Coordinating Center (DCC) will generate the study drug bottle numbers to be given to the patient; note that this will not require real time interaction with the DCC staff member. An assignment will be issued only if the database shows that the patient is eligible, the child/young adult/parent/guardian has signed the assent and/or consent statements, and the patient has had all required baseline data keyed to the database.

Dose increases based on body weight of child at baseline

Study drug will be dispensed in 75 mg capsules (either cysteamine bitartrate delayed- release or placebo). Daily dosage will be based on body weight at baseline and increased gradually beginning at Week 1 through the end of Week 4. The dose amounts dispensed at Week 4 will be the prescribed dose for the patient's weight stratum and, if tolerated, will remain fixed at that dose regardless of subsequent weight changes.

For children with baseline weight ≤65 kg:
Week 1: One 75 mg capsule in the morning and 1 in the evening (150 mg/day)
Week 2: Two 75 mg capsules in the morning and 2 in the evening (300 mg/day)
Week 3: Three 75 mg capsules in the morning and 3 in the evening (450 mg/day)
Week 4: Four 75 mg capsules in the morning and 4 in the evening (600 mg/day)

For children with baseline weight >65 kg - 80 kg:
Week 1: Two 75 mg capsules in the morning and 2 in the evening (300 mg/day)
Week 2: Three 75 mg capsules in the morning and 3 in the evening (450 mg/day)
Week 3: Four 75 mg capsules in the morning and 4 in the evening (600 mg/day)
Week 4: Five 75 mg capsules in the morning and 5 in the evening (750 mg/day)

1.4. Treatment groups

For children with baseline weight > 80 kg:
Week 1: Three 75 mg capsules in the morning and 3 in the evening (450 mg/day)
Week 2: Four 75 mg capsules in the morning and 4 in the evening (600 mg/day)
Week 3: Five 75 capsules in the morning and 5 in the evening (750 mg/day)
Week 4: Six 75 mg capsules in the morning and 6 in the evening (900 mg/day)

Distribution of study drug bottles by visit

The study drug assignment algorithm determining both the treatment assignment and the number of study drug bottles to be dispensed is determined by an automated program based on data recorded and keyed from the RZ form at Randomization.

The purpose of the Study Drug Dispensing and Return (RD) form is to capture data regarding dispensing of study drug bottles to patients. Study drug will be dispensed in 150-count bottles containing 75 mg strength capsules as specified below. A surplus of study drug is available depending on when the patient is scheduled within a time window.

For children with a baseline weight of ≤65 kg:
- rz visit: Two bottles (4 week supply + 2.8 weeks)
- f04 visit: Four bottles (8 week supply + 2.7 weeks)
- f12 visit: Six bottles (12 week supply + 4.1 weeks)
- f24 visit: Six bottles (12 week supply + 4.1 weeks)
- f36 visit: Seven bottles (16 week supply + 2.8 weeks)

For children with a baseline weight of >65 kg - ≤80 kg:
- rz visit: Three bottles (4 week supply + 3.6 weeks)
- f04 visit: Five bottles (8 week supply + 2.7 weeks)
- f12 visit: Seven bottles (12 week supply + 3 weeks)
- f24 visit: Seven bottles (12 week supply + 3 weeks)
- f36 visit: Nine bottles (16 week supply + 3.3 weeks)

For children with a baseline weight of > 80 kg:
- rz visit: Three bottles (4 week supply + 2.4 weeks)
- f04 visit: Six bottles (8 week supply + 2.7 weeks)
- f12 visit: Eight bottles (12 week supply + 2.3 weeks)
- f24 visit: Eight bottles (12 week supply + 2.3 weeks)
- f36 visit: Eleven bottles (16 week supply + 3.6 weeks)

If a patient cannot be scheduled for a followup visit before exhausting his supply of study drug, an additional bottle can be dispensed through the Study Drug task.

1.5. Study drug dispensing

At the randomization (rz) visit, two or three of study drug bottles are dispensed based on the child's weight. The data system will print a confirmation of CyNCh randomization and instructions for patient prescription which include the bottle numbers of the study drug to be given to the patient and the CyNCh visit window schedule. Keep the confirmation information for your study files.

At Followup visits, you will need to complete and key the DD (Drug Dispensing Documentation form) prior to followup visits f04, f12, f24, and f36 to receive the patient and visit specific drug bottle numbers. The data system will print a confirmation to CyNCh drug dispensing which includes the bottle numbers of the study drugs to be given to the patient.

If a patient needs additional study drug bottles before the next scheduled visit, you can dispense one bottle by using the visit code 'n' on the DD form and indicate in item 14 that one bottle should be dispensed. The Study Drug Dispensing and Return (RD) form should be keyed for this drug dispensing using a visit code of 'n'.

2. Eligibility and enrollment

2.1. Inclusion and exclusion criteria

Inclusion criteria

Patients must satisfy all of the following criteria to be eligible for enrollment:

- Children age 8-17 years inclusive.
- Liver biopsy within 90 days of screening visit and not more than 120 days before randomization.
- Clinical history consistent with NAFLD.
- Definite NAFLD based upon liver histology.
- No evidence of any other liver disease by clinical history or histological evaluation
- A histological severity of NAFLD Activity Score (NAS) ≥ 4.
- Sexually active female participants of childbearing potential (i.e., not surgically sterile [defined as tubal ligation, hysterectomy, or bilateral oophorectomy) must agree to utilize the same two acceptable forms of contraception from screening through completion of the study and to complete a pregnancy test at each study visit. The acceptable forms of contraception for this study include hormonal contraceptives (oral, implant, transdermal patch, or injection) at a stable dose for at least 1 month prior to screening, and barrier (condom with spermicide, diaphragm with spermicide). Sexual activity will be ascertained at each study visit for post-menarchal females and if sexually active, subject must verify use of the same 2 acceptable forms of contraception.
- Participants must be able to swallow DR cysteamine capsules.
- Written informed consent from parent or legal guardian.
- Written informed assent from the child.

Exclusion criteria

Exclusions will not be based upon gender, race, or ethnicity. Participants with a current history of the following conditions or any other health issues that make it unsafe for them to participate in the opinion of the Investigators:

- Inflammatory bowel disease (if currently active) or prior resection of small intestine
- Heart disease (e.g., myocardial infarction, heart failure, unstable arrhythmias)
- Seizure disorder
- Active coagulopathy (defined as <13,000 cells/mm^3 of blood)
- Gastrointestinal ulcers/bleeding
- Renal dysfunction with a creatinine clearance < 90 mL/min/m2
- History of active malignant disease requiring chemotherapy within the past 12 months prior to randomization
- History of significant alcohol intake (AUDIT questionnaire) or inability to quantify alcohol consumption
- Chronic use (defined as more than 2 consecutive weeks in the past year) of medications known to cause hepatic steatosis or steatohepatitis including:
    - systemic glucocorticoids
    - tetracycline

  - anabolic steroids
  - valproic acid
  - salicylates
  - tamoxifen,
- The use of other known hepatotoxins within 90 days of liver biopsy or within 120 days of randomization
- Initiation of medications with the intent to treat NAFLD/NASH in the time period following liver biopsy and prior to randomization
- History of total parenteral nutrition (TPN) use in the year prior to screening
- History of bariatric surgery or planning to undergo bariatric surgery during study duration
- Clinically significant depression (patients hospitalized for suicidal ideations or suicide attempts within past 12 months)
- Any female who is nursing, planning a pregnancy, known or suspected to be pregnant, or who has a positive pregnancy screen
- Non-compensated liver disease with any one of the following hematologic, biochemical, and serological criteria on entry into protocol:
  - Hemoglobin < 10 g/dL
  - White blood cell (WBC) < 3,500 cells/mm^3 of blood
  - NeutropFHl count < 1,500 cells/mm^3 of blood
  - Platelets < 130,000 cells/mm^3 of blood
  - Direct bilirubin > 1.0 mg/dL
  - Total bilirubin >3 mg/dL
  - Albumin < 3.2 g/dL
  - International normalized ratio (INR) > 1.4
- Poorly controlled diabetes mellitus (hemoglobin A1c (HbA1c) > 9%)
- Evidence of other chronic liver disease:
  - Biopsy consistent with histological evidence of autoimmune hepatitis
  - Serum hepatitis B surface antigen (HBsAg) positive
  - Serum hepatitis C antibody (anti-HCV) positive
  - Iron/total iron binding capacity (TIBC) ratio (transferrin saturation) > 45% with Histological evidence of iron overload
  - Alpha-1-antitrypsin (A1AT) genotype ZZ or SZ
  - Wilson's disease
- Children who are currently enrolled in a clinical trial or who have received an investigational study drug within 180 days of screening or liver biopsy
- Subjects who are not able or willing to comply with the protocol or have any other condition that would impede compliance or hinder completion of the study; in the opinion of the investigator
- Failure to give informed consent

2.2. Guidelines for repeat determinations of eligibility

While certain inclusion and exclusion criteria are more objective and are unlikely to change, others may change over time. Thus, children who are deemed ineligible at the time of initial screening may be re-screened at a later time as follows:

- An ineligible liver biopsy – the children may be re-screened after 12 months at the discretion of the investigator
- Ineligibility determined on measurements of albumin, INR, direct bilirubin, hemoglobin, WBC, neutrophils, total bilirubin, HbA1c and platelet count – the children may be re-screened at the discretion of the investigator
- Unwilling to participate – the participant may be re-screened after 3 months at the discretion of the investigator
- Unable to swallow study pills – if a child learns how to swallow pills, he could be rescreened

2.3. Randomization and eligibility checking

Randomization steps

- Complete collection of baseline data and key baseline data forms.
- Run electronic check on eligibility (i.e., run the Randomization Task, but opt out of randomization and resolve any ineligibility conditions).
- Run the Randomization Task and confirm that you want to randomize the patient "now." This task will officially randomize the patient in CyNCh and the randomization bottle numbers and materials needed in follow-up will be generated (i.e., visit time window schedule)

Randomization

- The date the clinical center runs the Randomization Task and confirms that the patient is to be randomized "now" and the treatment group is assigned is the date of randomization.
- The "time zero" for reckoning the time windows specified on the patient's CyNCh visit time window guide is the date of randomization.

2.4. Guidelines for transferring from NAFLD Pediatric Database 2 to CyNCh

Patients enrolled in NAFLD Pediatric Database 2 who want to enroll in CyNCh

- Transferring NAFLD Pediatric Database 2 patients into CyNCh necessitates that the screening procedures are conducted within the designated eligibility time window (e.g., liver biopsy obtained within 90 days of screening visit and not more than 120 days prior to randomization in CyNCh).
- NAFLD Pediatric Database 2 patients **without** a liver biopsy or a liver biopsy obtained more than one year prior to CyNCh registration are good candidates for screening in CyNCh.
- Physician discretion is recommended for NAFLD Pediatric Database 2 patients **with** a recent liver biopsy as to whether the patient should register for CyNCh; in this scenario, it may be reasonable to wait until the patient has completed their NAFLD Pediatric Database 2 annual t048 visit.
- Recent liver biopsies obtained in the NAFLD Pediatric Database 2 study and within the CyNCh eligibility window (within 90 days of screening and not more than 120 days prior to randomization) may be used for CyNCh screening to determine eligibility.
- Have the patient sign the CyNCh consent or assent form.
- Complete and key the CyNCh Registration (RG) form but do NOT issue a new patient ID number and code.
- Blood for serum and plasma may be used if collected for NAFLD Pediatric Database 2 within 90 days of biopsy used by CyNCh
- Regarding blood for genetics repository:
    - If not already collected, have patient sign the CyNCh genetic consent, collect a sample, and complete the CyNCh Genetic Consent and Blood Collection Documentation (CG) form
    - If blood for genetics testing was already collected, do not send another sample unless the yield was unsatisfactory.
        - If the yield on the sample drawn when the patient screened for the NAFLD Pediatric Database 2 was satisfactory, leave the NAFLD Pediatric Database 2 CG form in the data system and complete the CyNCh CG answering ‘yes’ to question about prior blood draw for the Pediatric Database 2; the patient does not need to sign the CyNCh genetic consent
        - If the yield on the sample drawn when the patient screened for the Pediatric Database 2 was unsatisfactory, have the patient sign the CyNCh genetic consent form and complete the CyNCh CG form; the Pediatric Database 2 CG form should remain in the data system.
- Lab results reported on the Pediatric Database 2 Laboratory Results - Screening and Followup (LR) and Laboratory Results - Test Done Only During Screening (LS) forms may be used on the CyNCh LR and LS forms if they were obtained within the time windows specified on the forms.

2.4. Guidelines for transferring from NAFLD Pediatric Database 2 to CyNCh

- All interviews and patient questionnaires (AUDIT, baseline history, liver symptoms, quality of life) must be completed anew for CyNCh.
- The physical exam (PE) form must be completed anew for CyNCh.
- If the biopsy used for CyNCh is the same one that was used for the NAFLD Pediatric Database 2, the biopsy for CyNCh must meet study medication requirements (e.g., no use of hepatotoxins 90 days prior to liver biopsy or within 120 days of randomization) not imposed in the NAFLD Pediatric Database 2. The Clinical Coordinator should transcribe the Histology data from the Liver Biopsy Histology Worksheet (HW) onto the CyNCh Liver Biopsy Histology Findings (HF) form. The CyNCh Liver Biopsy Materials Documentation (SD) form must be completed; transcribe information from the Pediatric Database 2 SD form. For items relating to slide sequence numbers, transcribe the slide numbers for the slides that were previously sent for the NAFLD Pediatric Database 2 (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue was obtained, the CyNCh Liver Tissue Banking (LT) form must be completed; transcribe information from the NAFLD Pediatric Database 2 LT form. Where the CyNCh LT form asks for the duplicate LT label to be affixed to the LT form, write in the label information from the NAFLD Pediatric Database 2 form and write in the margin "see NAFLD Pediatric Database 2 LT form". There will be more than one form in the data system pointing to the same numbered slides and liver tissue vials (Pediatric Database 2 SD/LT and CyNCh SD/LT forms), but this is okay since the patient enrolled in the Pediatric Database 2.
- If the patient is eventually randomized in CyNCh, have the patient complete CyNCh follow-up visits and forms; you do not need to complete the MV form for the missed NAFLD Pediatric Database 2 visits, but you do need to complete the NAFLD Pediatric Database 2 Closeout (CO) form to suspend the patient's participation in the NAFLD Pediatric Database 2. The CO form can be completed prior to or after randomization in CyNCh, but our advice is to complete it upon randomization in CyNCh. The patient remains enrolled in NAFLD Pediatric Database 2 while participating in CyNCh, but the patient is not subject to completion of NAFLD Pediatric Database 2 visits.
- Retain all NAFLD Pediatric Database 2 forms completed for the patient in the patient's NASH CRN file.
- Retain the patient's NAFLD Pediatric Database 2 visit windows schedule since it will be needed once CyNCh is completed.

Patients registered in NAFLD Pediatric Database 2 but never enrolled, now wants to register in CyNCh

- The patient should be closed out of the NAFLD Pediatric Database 2 by completing and keying the NAFLD Pediatric Database 2 Enrollment Form (EN) form to document the reason(s) why the patient did not enroll in the Pediatric Database 2. Answer as many of the questions in sections B, C, D, E, and F of the form EN as you can, coding an item as 'm' if you do not know the answer; if the patient is eligible for the NAFLD Pediatric Database 2 but is opting to go directly into CyNCh, answer 'no' to item 22 (no longer consents) and check 'Other reason' in item 23c and write in 'opted to go directly into CyNCh.'
- Have the patient sign the CyNCh consent form.

2.4. Guidelines for transferring from NAFLD Pediatric Database 2 to CyNCh

- Complete and key the CyNCh RG form but do NOT issue a new patient ID number and code.
- Blood for serum and plasma may be used if collected for NAFLD Pediatric Database 2 within 90 days of the biopsy used for CyNCh.
- Blood for genetics repository:
  - If not already collected, have the patient sign the CyNCh genetic consent, and collect a sample, and complete the CyNCh CG form.
  - If blood for genetic testing was already collected, do not send another sample unless the yield was unsatisfactory.
    - If the yield on the sample drawn when the patient screened for the NAFLD Pediatric Database 2 was satisfactory, key the Pediatric Database 2 CG form (if not already keyed) and complete the CyNCh CG form answering 'yes' to the question about prior blood draw for the Pediatric Database 2; the patient does not need to sign the CyNCh genetic consent.
    - If the yield on the sample drawn when the patient screened for the NAFLD Pediatric Database 2 was unsatisfactory, then have the patient sign the CyNCh genetic consent form, draw the replacement sample, and complete the CyNCh CG form. The Pediatric Database 2 CG form can remain in the data system.
- Interviews and questionnaires must be completed on the CyNCh forms:
  - If available, data from the Pediatric Database 2 AUDIT (AD) form may be transcribed to the corresponding CyNCh form, but the patient should be queried regarding any change since the previous interviews; the date in item 4 on the CyNCh AD form should be the date you review the information with the patient.
  - The CyNCh Baseline History (BH) form should be completed anew– it is different from the Pediatric Database 2 BG form, and the CyNCh BH form data will help establish that the biopsy is a medication free biopsy (medication use is not an issue with Pediatric Database 2 biopsies).
- The Physical Exam (PE) form must be completed anew.
- If the same biopsy is used for CyNCh that was used for the Pediatric Database 2, the biopsy for CyNCh must meet study medication requirements (e.g., no use of hepatotoxins 90 days prior to liver biopsy) not imposed in the NAFLD Pediatric Database 2. The Clinical Coordinator should transcribe the Histology data from the Histology Worksheet (HW) onto the CyNCh Liver Biopsy Histology Findings (HF) form. The CyNCh Liver Biopsy Materials Documentation (SD) form needs to be completed; transcribe information from the Pediatric Database 2 SD form. For items relating to slide sequence numbers, transcribe the slide numbers that were previously sent for the Pediatric Database 2 (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue was obtained, the CyNCh Liver Tissue Banking (LT) form must be completed; transcribe information from the Pediatric Database 2 LT form. Where the CyNCh form asks for the duplicate LT label to be pasted onto the LT form, write in the label information from the Pediatric Database 2 LT form and write in the margin 'see Pediatric Database 2 LT form'. The Pediatric Database 2 SD and LT forms can remain in the data system.
- Retain all Pediatric Database 2 forms completed for the patient in the patient's NASH CRN file.

2.4. Guidelines for transferring from NAFLD Pediatric Database 2 to CyNCh

Patient registered in CyNCh, but found to be ineligible, now wants to register in the NAFLD Pediatric Database 2

- The patient should be closed out of CyNCh by completing and keying the CyNCh RZ form to document the reason(s) the patient was found to be ineligible.
- Have the patient sign the NAFLD Pediatric Database 2 consent form.
- Complete and key the Pediatric Database 2 RG form but do NOT issue a new patient ID and code.
- Blood for genetics repository:
  - If blood for genetic testing was not already collected, have the patient sign the Pediatric Database 2 genetic consent, collect a sample, and complete the Pediatric Database 2 CG form.
  - If blood for genetic testing was already collected, do not send another sample unless the yield was unsatisfactory, but the CG form must still be completed.
- Interviews and questionnaires must be completed on the Pediatric Database 2 forms.
- If the same biopsy is used for the Pediatric Database 2 that was used for CyNCh, the Clinical Coordinator should transcribe the Histology data from the Histology Worksheet (HW) form onto the Pediatric Database 2 HF form. If slides were previously sent for CyNCh, the Pediatric Database 2 SD form must be completed referencing the slide numbers for the slides that were sent (i.e., only 10 unstained slides need to be sent from a single biopsy). If liver tissue was obtained, the Pediatric Database 2 LT form must be completed. Where the Pediatric Database 2 LT form asks for the duplicate LT label to be affixed to the LT form, write in the label information from the CyNCh LT form and write in the margin ‘see CyNCh LT form’. The CyNCh SD and LT forms can remain in the data system.
- Retain the CyNCh forms in the patient’s NASH CRN file.

These procedures are complicated. Please contact the DCC if you have questions or if you run into problems when trying to key forms or enroll/randomize a patient.

3. Certification

3.1. Certification overview

What is certification?

- It is a study-related requirement designed to identify the staff responsible for specific data items, data collection procedures, or decisions about eligibility.
- It is a managerial and quality assurance tool for the study.

Who and what does it apply to?

- It applies to:
  - CyNCh staff
  - Each clinical center
- Certification for CyNCh is required before any patient visits or data collection may occur; patients may not begin any screening examinations, sign any consent statements, or complete any study forms until the clinical site has been certified for the study.
- More than one staff member may be certified for a function and it is recommended that more than one staff member be certified for a function.

Why do we require it?

- Primary purpose is to help assure consistent conduct of the study over time, within and across clinics. The conduct of procedures should be similar across patients and in serial testing of the same patient over the duration of follow-up.
- Study procedures may vary from the usual practice of a participating clinical center, but it is important that methods be carried out in the same manner within and across clinical centers.
- It identifies the staff and sites that carry out study procedures and identifies to staff that they and their site are a part of the CyNCh trial.
- It provides a mechanism for tracking who collected key data items or made key decisions.
- The certification process may help a clinic prepare for study activities by presenting the training, facility, and equipment needs in an organized fashion and requiring acquisition or completion of these items before study specific activities may begin.

3.2. Clinical center certification

General comments

- Each clinical center participating in CyNCh must be certified.
- Completion of the Clinical Center Certification (CC) form will be required.
- IRB approval for the CyNCh protocol and consents will be required.

Purpose of clinical center certification

- Provide information regarding how the clinical center will conduct different aspects of the protocol and who will staff the study.
- Guide a clinical center through the steps of getting ready for CyNCh – provide a checklist of what needs to be in place before patient activities begin.

Requirements for certification of a site

- Complete the Clinical Center Certification (CC) form.
- Certify at least one person for each function that requires certification (a person may be certified for more than one function).
- Obtain IRB approval of the most current CyNCh protocol and consent documents.
- Obtain NIDDK Repository approval of your center's IRB approved consent document
- Receive written notice of approval (email) from the Data Coordinating Center that the site is certified.
- Provide assurances that the study participants' protected health information will be kept confidential.
- Provide assurances that the linkable information will not be transmitted to the DCC.

3.3. Personnel certification

Staff functions requiring certification

- Clinical Coordinator
- Pediatrician
- Pathologist
- Data Entry Technician
- Radiologist
- Imaging personnel (optional)

Requirements

- Everyone:
  - Read the CyNCh protocol
  - Complete the Knowledge Assessment (KA) form; this is a written general knowledge assessment about the CyNCh trial (open book)
  - Complete the Personnel Certification (PC) form; this form identifies the functions applied for and provides an assurance of data confidentiality and integrity
- Additional requirements for Pathologist:
  - Be approved by David Kleiner and Elizabeth Brunt
- Additional requirements for Data Entry Technician:
  - Complete the Data Entry Certification/Decertification Request (DC) form
  - Complete the data system tutorial (personnel previously certified as Data Entry Technician do not need to complete the data system tutorial a second time)
- Additional requirements for Radiologist and Imaging personnel:
  - Be approved by Radiology Reading Center
  - Follow instructions indicated in the CyNCh SOP Part VI: MRI Procedure Manual

Process

- Send required materials to the DCC
- The DCC will send written notice of approval for certification or pending certification
- Each staff member will be issued a Personnel Identification Number (PIN)

Staff PINs

- Each staff member certified for at least one function will be issued a PIN which will consist of 3 digits – the first digit will identify the clinical center and the next two digits will be a sequential number assigned by the Data Coordinating Center
- The PIN is used when completing forms
- The Data Entry Technician uses his/her PIN when signing on to the CyNCh data system
- Staff can be certified for more than one function, but will have only one PIN

4. Human subjects

4.1. Background

Consent to participate in the CyNCh Trial must be completed before screening for CyNCh may begin. The patient must consent to procedures offered to and performed on him/her for screening, as well as those for the follow-up visits which the patient will face in the future.

The consent process is a dynamic process involving explanations, time to think, questions, clarifications, and advice that a patient may seek from relatives, friends, or anybody else considered relevant. We wish to inform the prospective participant, and the parent, as much as possible and as accurately as possible about what will be offered to him/her, how it will be done, what are the reasonable risks and benefits, what are the alternatives, and what is expected of the patient. We wish to answer patients' questions in a consistent and complete way. A consent form signed by the parent and a patient assent form signed by the child or adolescent will be required.

The CyNCh consent process has four major stages:

- The patient (and parent) is asked to consent to screening and randomization into CyNCh.
- The patient (and parent) is asked to sign the HIPAA authorization to disclose protected health information.
- The patient (and parent) is asked to consent to the collection, storage, and use of blood samples for genetic research, which is optional.
- The patient is asked to consent to an MRI assessment, which is optional.

4.2. Institutional review board process

Three prototype consent statements have been prepared for the CyNCh trial:

- Consent for screening and enrollment in CyNCh
- Consent for the collection, storage, and use of blood samples for current and future genetic research (optional)
- Consent for MRI assessment (optional)

Clinics are expected to use these materials in their submissions to their institutional review boards (IRBs) for approval to participate in CyNCh. Each clinical center must send copies of the consent statements to be used at the clinical center, stamped with their IRB's seal, to the Data Coordinating Center prior to initiating patient activities in CyNCh. Data Coordinating Center staff will review and compare the approved local consents to the prototype consents. Specific local additions to and editing of the prototypes may be required at individual institutions, but deletion of material and major rewording of text may need to be explained and justified. Once a consent form has been approved by an institution's IRB, it cannot be changed without the IRB's approval.

The study protocol and consent forms will be submitted to each clinical center's IRB and to the DCC's IRB. Additionally, each clinical center will submit to their IRB any data collection forms or recruitment materials to be used at their site. A clinical center may not initiate any patient contact about CyNCh until the site has IRB approval for CyNCh and the DCC has certified the site for initiation of CyNCh patient activities. All study personnel will have completed training in the Protection of Human Subjects per NIH guidelines.

HIPAA authorization forms will be prepared by each clinical center according to local clinical center institutional requirements and guidelines.

4.3. Consent administration

CyNCh

Patients referred to a clinical center for screening may have heard about CyNCh, but their level of knowledge and expectations may differ. We wish to standardize the consent administration across clinical centers as much as possible. Administration of the CyNCh consents involves two tasks:

(1) A CyNCh staff member (pediatrician or clinical coordinator) must sit down with the patient and parent and review the contents of the statement; explain the risks, benefits, and responsibilities of participation; review the alternatives to participation; and answer questions.

(2) A CyNCh certified pediatrician or clinical coordinator must sign the consent statement, and in addition to the principal investigator, take overall responsibility for the patient's informed and voluntary consent.

Staff at each clinical center should be designated to carry out these tasks. The rationale for requiring that the consent statement be signed by a study physician is to help assure that the physician signing the consent is one who has a broad role in the study.

Generally, the consent statements should be offered to the patient and their parent to read through at least a day before signature is requested. The consent will then be reviewed with the patient by the staff member designated to obtain consent; the consenter may opt to read the statement to the patient, pausing to explain issues as needed. This activity should take place in a quiet, private, and relaxed setting in the clinical center.

The patient and parent should sign the consent statement in the presence of the CyNCh staff member after all questions have been answered and when the patient has asserted orally that he/she is ready to sign the consent. After the patient and parent have signed and dated the consent, the patient should meet with a CyNCh study physician for the physician to sign the consent statement. Ordinarily, this meeting should take place on the same day that the patient signed the consent statement. The physician should ask the patient to confirm his/her voluntary (assent) consent and query the patient about any questions or concerns the patient may have about participation. Both signatures on the consent form must be in a non-erasable ink pen. If the physician cannot meet with the patient on the same day that the patient signs the consent statement, the physician may sign on another day.

Consent for genetic research

The consent for collection and banking of blood for genetic research should be administered in the same way that the CyNCh consent is administered, except that it should not be signed until the patient has been determined to be eligible for the CyNCh trial. Patients who have already consented to collection and banking of blood for genetic research as part of the NAFLD Pediatric Database 2 do not need to sign this consent again as part of the CyNCh trial.

Consent for MRI research

The consent for participation of Magnetic Resonance Imaging (MRI) assessment should be administered in the same way that the CyNCh consent is administered, except that it should not be signed until the patient has been determined to be eligible for the CyNCh trial.

4.4. Time considerations for obtaining consent

- The **CyNCh Consent and HIPAA authorization** must be obtained at the start of the initial screening visit (CyNCh visit s); documents from the referring physician (if any) or from the NAFLD Pediatric Database 2 study should have been reviewed prior to the visit and the patient should be judged eligible for screening prior to the visit. Signature of this consent is required prior to sending the patient for any CyNCh diagnostic tests. A check for signature of this consent statement occurs on the Registration (RG) form.

- A patient may be given the consent statements to review prior to the initiation of the screening visit to meet patient needs with respect to review time. Whenever a consent is first given to a patient for review, it should be made clear to the patient that the consent should not be signed until requested by a CyNCh staff member. Whatever timing is used by a clinic, the patient should be allowed enough time to reflect about the proposed CyNCh procedures, pose questions, and consult with other individuals that he/she considers relevant to their participation in CyNCh. Patients may request and should be given time to "think it over" at home and come back at a later time.

- The **CyNCh Consent for Collection, Storage, and Use of Blood Samples for Current and Future Genetic Research** must be obtained after eligibility for CyNCh has been established during the screening visit or at the randomization visit. If the patient has already consented to genetic banking as part of the NAFLD Pediatric Database 2, the patient does not need to be presented this option as part of the CyNCh trial and does not need to sign this consent. Signature of this consent is required prior to drawing blood for genetic research; a check for signature of this consent statement occurs on the Genetic Consent and Blood Collection Documentation (CG) form. Signature of this consent statement is not required for CyNCh eligibility (i.e., the patient may choose not to participate in the genetic research component of CyNCh).

- The **Patient Consent for Magnetic Resonance Imaging Research, which is optional in CyNCh,** must be obtained after eligibility for CyNCh has been established during the screening visit and prior to randomization. If the patient verbally agrees to an MRI, the patient will be officially consented for the MRI procedure, per the institutional guidelines. A signed consent prepared by the institution where the CyNCh clinic is located is required prior to undergoing an MRI exam; a check for signature of this consent statement occurs on the MRI Consent and Report Form (MR). Signature of this consent statement is not required for CyNCh eligibility (i.e., the patient may choose not to participate in the MRI research component of CyNCh).

4.5. Consent and Assent handling

- Signed consent statements are important legal documents. These signed statements should be kept in the patient's CyNCh clinical center file together with his/her other CyNCh forms and documents. These forms may or may not be a part of the individual's institutional medical record, but are part of his/her study record in the CyNCh trial. Consent statements will be examined during site visits.

- Consents should be annotated with the patient's study identifiers (ID number and code).

- The CyNCh consent statement is an "all or none" form. The patient either accepts it in its entirety and signs it, or does not. The patient must consent to the evaluation procedures, the follow-up evaluations, and the banking of his/her serum, plasma, and liver tissue specimens. If the patient refuses any part, the patient may not enroll in CyNCh. If the patient was previously enrolled in the NAFLD Pediatric Database 2, the patient must also consent to the CyNCh evaluation, follow-up, and banking procedures to enroll in the CyNCh trial.

- The CyNCh Consent for Genetic Research has been made a separate consent statement so that the patient can opt out of genetic research and still participate in the CyNCh trial. If the patients previously consented to DNA banking as part of the NAFLD Pediatric Database 2 study, the patient will not need to sign a new consent unless the amount of blood was considered to be unsatisfactory.

- The CyNCh Consent for Magnetic Resonance Imaging (MRI) Research has been made a separate consent statement so that the patient can opt out of MRI assessment and still participate in the CyNCh trial.

4.6. Informing participants of changes to consent/assent statement after randomization

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As new data become available during the conduct of CyNCh, the consent statements may need to be changed to reflect the current assessment of risks and benefits to participants in the study.

Procedures for dissemination of revisions of consent statements from the DCC

- Changes deemed necessary will be made to the prototype consent statements.
- Revisions of the prototype consent statements will be distributed to sites via a numbered Policy and Procedure Memorandum (PPM) with instructions to submit the revised consent to their IRB

Procedures for reviewing changes to consent statements with participants

- Clinical center personnel will develop a chronology of IRB approved changes to the consent statements used at their site
- At each follow-up visit, staff will use the chronology of consent changes to review with the participant any changes to the consent since the last visit. This review does not require obtaining the participant's signature on a new consent statement, unless the local IRB requires obtaining a signature.
- Review changes to the consent statements with participants at follow-up visits.
- This review process is not intended to be a reaffirmation of consent. The clinical center, if required by their local IRB, may develop procedures for reaffirmation of consent.

4.7. HIPAA considerations

CyNCh study staff have access to patient health information and to patient identifiers, such as name, address, and telephone number. Study records are to be kept in a secure place. Only people working on CyNCh should have access to these records. However, these records could be reviewed to make sure that the study is being done as it should. People who may see medical records supporting study records are:

- Officials of your institution
- Your institution's research ethics committee
- Monitors from the NASH CRN Data Coordinating Center at the Johns Hopkins University, or other individuals selected by the NASH CRN Steering Committee to monitor the study
- Government officials from the Office of Human Research Protections, the National Institutes of Health, or the Food and Drug Administration

Each clinical center should take steps to protect patient privacy. The assigned patient ID number and code should be used to identify patients on forms and in the data files. Personal information such as name, address, and telephone number should be kept only at the clinical center where a patient completes visits.

People outside the clinical center who will receive CyNCh study data include:

- The NASH CRN Data Coordinating Center at the Johns Hopkins University in Baltimore, Maryland (or its successor) to maintain the central study database.
- The NASH CRN Radiology Reading Center, located at the University of California, San Diego, to receive MRI image transfers for analysis as well as imaging data recorded onto CD/DVD and sent via Federal Express. The data sent to the MRI Center are anonymized – identified only by a NASH CRN number and code
- The NASH CRN Data and Safety Monitoring Board to review the CyNCh data for performance and safety.
- The NIDDK Data Repository located at the Research Triangle International (RTI) in North Carolina
- The NIDDK Genetics Repository at Rutgers, the State University of New Jersey in New Brunswick, New Jersey (or its successor) to receive patients' blood to obtain DNA. The blood samples for a particular patient will be identified by the patient's study ID number and code, not by name.
- The NIDDK Biosample Repository at Fisher Bioservices in Germantown, Maryland (or its successor) to receive patients' plasma, serum, and liver tissue. The samples for a particular patient will be identified by the patient's study ID number and code, not by name.

- The NASH CRN investigators, as well as outside researchers, to analyze and report CyNCh trial data. Patient identity will not be disclosed in any reports or publications resulting from the study. While CyNCh is ongoing, the use of CyNCh data must be approved by the NASH CRN Steering Committee and by the research ethics committee at your institution.

Patient agreement to join the CyNCh trial indicates that the patient also agrees to the use of study data as described above. If a patient does not agree to the described uses of study data, the patient may not participate in CyNCh. The only exceptions are refusal to provide blood for genetic research or refusal of MRI procedure; patients may refuse to provide blood for genetic research or refuse the MRI procedure and still enroll in CyNCh.

5. Study visits

5.1. Overview of visit schedule

The patient-related activities of the CyNCh trial can be divided into 4 phases:

- **Screening for eligibility for enrollment** (1-2 visits over a maximum of 120 days)
  s: consent, registration, liver symptoms questionnaire, baseline history, review of concomitant drugs, physical exam, AUDIT questionnaire on alcohol use, quality of life, three 24-hour food recall, liver biopsy, fasting liver profile, complete blood count, metabolic panel, hepatic panel and GGT, fasting glucose and/insulin/PT and INR, HbA1c, pregnancy test, etiologic tests, nutritional interview, serum and plasma for banking, and MRI (optional)

- **Randomization to treatment** (1 visit)
  RZ: Consent and re-affirmation, review of concomitant drugs, pregnancy test, height, weight assignment to treatment group and dispense study drug

- **Follow-up treatment phase** (6 visits over 76 weeks)

  f04: Follow-up medical history, focused physical exam, review of concomitant drugs, pregnancy test, blood draw for CBC, hepatic panel, review of adverse events, study drug adherence, study drug dispensing, exercise and nutrition counseling

  f12: Follow-up medical history, review of concomitant drugs, review of adverse events, drug dispensing, study drug adherence, focused physical exam, interim drinking history, blood draw for CBC, hepatic panel and GGT, pregnancy test, serum and plasma for banking, liver symptoms questionnaire, exercise and nutritional counseling

  f24: Follow-up medical history, review of concomitant drugs, review of adverse events, drug dispensing, study drug adherence, detailed physical exam, interim drinking history, fasting lipid profile, complete blood count, metabolic panel, liver symptom questionnaire, hepatic panel and GGT, fasting glucose and insulin, HbA1c, pregnancy test, serum and plasma for banking, nutritional counseling, exercise prescription

  f36: Follow-up medical history, review of concomitant drugs, review of adverse events, drug dispensing, study drug adherence, focused physical exam, hepatic panel and GGT, pregnancy test, blood draw for CBC, serum and plasma for banking. nutritional counseling, exercise prescription, liver symptoms questionnaire

  f52: Follow-up medical history, review of concomitant drugs, review of adverse events, study drug adherence, detailed physical exam, health-related quality of life questionnaire, three 24-hour food recalls, liver symptoms questionnaire,

blood draw for CBC, fasting lipid profile, complete blood count, comprehensive metabolic panel, MRI (optional), blood draw for CBC, hepatic panel and GGT, fasting glucose/insulin/fatty acids, HbA1c, pregnancy test, serum and plasma for banking, liver biopsy

- **Post-treatment 24-week washout**

  f76: Follow-up medical history, review of concomitant drugs, review of adverse events, interim drinking history, detailed physical exam, health-related quality of life questionnaire, liver symptoms questionnaire, detailed physical exam, fasting lipid profile, blood for CBC, comprehensive metabolic panel, hepatic panel and GGT, fasting glucose and insulin, HbA1c, serum and plasma for banking, closeout

5.2. Visits, data forms, and procedures

Phase/ Visit	Form abbr	Procedure
Screening (s) visit		
	ND	Nutrition Data Documentation
	RG	Registration (document consent, sociodemographics, assign IDs)
	BH	Baseline History
	PE	Physical examination (detailed exam)
	SD	Liver biopsy materials documentation
	HW	Liver biopsy Histology worksheet
	HF	Liver biopsy Histology findings (reading at clinical center)
	LT	Liver tissue banking
	AD	Alcohol Use Disorders Identification Test (AUDIT)
	LR	Laboratory results done during screening and follow-up visits
	LS	Laboratory tests done only during screening
	PQ, PR	Parent Quality of Life
	PW, PY	Child/Teen Quality of Life
	CG	Genetic consent and blood collection documentation
	BP	Blood processing for plasma and serum
	MR	MRI consent and report form
	PL	Patient location (patient contact information)
	LP	Symptoms of Liver disease
Randomization (RZ) visit		
	RZ	Randomization checks
	RD	Study Drug Dispensing and Return
4 week follow-up (f04) visit		
	FH	Follow-up Medical History
	DD	Drug Dispensing Documentation
	LR	Laboratory results during screening and follow-up
	PF	Focused Physical Examination
	RD	Study Drug Dispensing and Return
	AE	Adverse Event Report

Phase/ Visit	Form abbr	Procedure
12 week follow-up (f12) visit		
	FH	Follow-up Medical History
	LR	Laboratory results done during screening and follow-up visits
	PF	Focused physical examination
	DD	Drug Dispensing Documentation
	BP	Blood Processing for Plasma and Serum
	LP	Symptoms of Liver disease
	RD	Study Drug Dispensing and Return
	AE	Adverse Event Report
24 week follow-up (f24) visit		
	FH	Follow-up Medical History
	LR	Laboratory results done during screening and follow-up visits
	DD	Drug Dispensing Documentation
	PE	Physical examination (detailed exam)
	BP	Blood Processing for Plasma and Serum
	LP	Symptoms of Liver disease
	RD	Study Drug Dispensing and Return
	AE	Adverse Event Report
36 week follow-up (f36) visit		
	FH	Follow-up Medical History
	LR	Laboratory results done during screening and follow-up visits
	PF	Focused physical examination
	DD	Drug Dispensing Documentation
	BP	Blood Processing for Plasma and Serum
	LP	Symptoms of Liver disease
	RD	Study Drug Dispensing and return
	AE	Adverse Event Report
52 week follow-up (f52) visit		
	FH	Follow-up Medical History
	LR	Laboratory results done during screening and follow-up visits
	SD	Liver biopsy materials documentation
	RD	Study Drug Dispensing and return
	LT	Liver Tissue banking
	PE	Physical examination (detailed exam)

Phase/ Visit	Form abbr	Procedure
	BP	Blood Processing for Plasma and Serum
	MR	MRI Consent and Report form
	PQ, PR	Parent Quality of Life
	PW, PY	Child/Teen Quality of Life
	ND	Nutrition Data Documentation
	LP	Symptoms of Liver Disease
	AE	Adverse Event Report
76 week follow-up (f76) visit		
	BP	Blood Processing for Plasma and Serum
	FH	Follow-up medical History
	PE	Physical examination (detailed exam)
	LR	Laboratory results done during screening and follow-up visits
	PQ, PR	Parent Quality of Life
	PW, PY	Child/Teen Quality of Life
	LP	Symptoms of Liver disease
	CO	Closeout
	AE	Adverse Event Report

As needed: RC, PL, DR, MV, CG, LT, SD, SR, TN

5.3. Guide for screening visits

Before the patient arrives for the visit

- Abstract data from patient's medical records as needed
- Gather the most recent prior liver biopsy slides as available
- Apply labels to forms as appropriate
- Gather specimen collection and mailing materials (labels, tubes, shippers)
- If advisable, alert phlebotomy lab staff of need to obtain plasma and serum samples
- Confirm eligibility with respect to whatever data have been keyed

Procedures

- Obtain signed consent and signed assent for child or adolescent and parent consent
- Obtain permission to abstract data from patient's medical records
- Obtain patient location information
- Initiate data collection for screening and baseline values
  - Physical exam and anthropometry
  - Interview for baseline history (responses may be modified or expanded upon chart review)
  - Laboratory testing
  - Alcohol use questionnaire
- If patient appears eligible at the close of screening visit
  - Schedule patient for second screening visit
  - Schedule patient for any needed etiologic tests
  - Schedule patient for biopsy, if appropriate
  - Schedule patient for MRI exam, if applicable
- Liver biopsy (pathologist should grade slides from most recent eligible biopsy and obtain 10 unstained slides if possible or arrange for biopsy if needed; if arranging for biopsy, prepare for collection of flash frozen liver tissue)
- MRI exam – optional (radiologist should prepare images and complete forms as indicated in the CyNCh SOP VI: MRI Procedure Manual)
- Complete quality of life questionnaire, and additional testing
- Obtain consent for DNA banking (if available)
- Collect blood for genetic specimen banking (2 tubes) and biosample banking (3 tubes)
- Confirm eligibility (hand/eyeball review of unkeyed data)
- Explain to the patient that you will electronically confirm eligibility after keying the data collected at this visit but that since you believe the patient to be eligible, you would like to schedule the patient for randomization

5.3. Guide for screening visits

Data collection forms

- Forms completed for all patients
  - RG - Registration
  - PE - Physical Examination
  - BH - Baseline History
  - SD - Liver Biopsy Materials Documentation
  - HF - Liver Biopsy Histology Findings
  - HW - Liver Biopsy Histology Worksheet
  - LS - Laboratory Results – Tests Done Only During Screening
  - LR - Laboratory Results - Screening and Follow-up
  - AD - Alcohol Use Disorders Identification Test (AUDIT)
  - -- LP - Symptoms of Liver Disease
  - BP - Blood Processing for Serum and Plasma
  - CG - Genetic Consent Documentation (this form documents both consent and refusal)
  - MR- MRI Consent and Report
  - -- PQ, PR - Parent Quality of Life
  - -- PW, PY - Child/Teen Quality of Life
  - ND - Nutrition Data Documentation

- Additional forms required under specific conditions
  - LT - Liver Tissue Banking (if liver tissue was obtained for banking)
  - RC - Rescreen in CyNCH

- Forms completed for all patients

Forms for clinical center use only

- PL - Patient Location
- Medical records release (use local form)

After the patient leaves the clinical center

- Register patient on clinic data system
- Apply labels to forms as needed
- Set up CyNCh chart for patient and file the materials generated at registration that will be used during visits
- Key completed data forms
- Package biopsy slides for sending to the DCC
- Batch ship flash frozen liver tissue specimen (if available) to NIDDK Biosample Repository by overnight delivery service
- Key data collection forms
- Run Randomization Task and re-check eligibility
- Package whole blood tubes for DNA banking for mailing and ship to Genetics Repository
- Submit MRI data to the RRC
- Process blood to serum and plasma and aliquot for banking; store in local freezer until batch sufficient for mailing accumulates

5.4. Randomization visit

Procedures

- Randomization visit to be conducted as a visit separate from the screening visit
- Patient will be randomized to study drug assignment
- Requests for randomizations will be made by clinical centers using a web based application
- A randomization assignment will be issued only if the database shows that the patient is eligible, has signed the consent statement, and has had all required baseline data keyed to the database
- Patient is given the assigned study drugs with a number unique to the patient, instructed about starting the drugs and monitoring for adverse effects, and begins taking study drugs

Data Collection Forms

- RZ - Randomization Checks
- RD - Study Drug Dispensing and Return
- the RD form should be entered into the data system within 48 hours of dispensing study drug to the patient

Comment

- The date of randomization visit is the date for reckoning all follow-up visits

5.5. Visit windows: randomization and follow-up

Randomization must occur within 120 days of liver biopsy

Visit	Window opens: weeks (days) after randomization	Window closes: weeks (days) after randomization	Ideal date
f04	2 weeks+1 day (15 days)	8 weeks (56 days)	4 weeks (28 days)
f12	8 weeks+1day (57 days)	18 weeks (126 days)	12 weeks (84 days)
f24	18 weeks+1day (127 days)	30 weeks (210 days)	24 weeks (168 days)
f36	30 weeks+1 day (211 days)	42 weeks (308 days)	36 weeks (252 days)
f52	44 weeks+1 day (309 days)	54 weeks (448 days)	52 weeks (364 days)
f76	64 weeks+1 day (449 days)	88 weeks (616 days)	76 weeks (532 days)

5.6. Interim (unscheduled) visits or telephone contacts

- Unscheduled visits or telephone contacts may occur as needed. No time windows or minimum time separations are imposed for such visits or contacts.
- Data are not collected at interim visits (i.e., data forms are not completed) unless reporting a death, a serious adverse event, or liver biopsy, or unless study drugs are needed between visits.
- If a liver biopsy is performed for a CyNCh patient at a time other than the baseline and f52, complete the forms related to liver biopsy (SD, LT forms).

6. Study procedures

6.1. Assignment of study identifiers

What

- The NASH CRN uses 2 identifiers for patients
  - ID number (4 digits)
  - ID code (3 alphabetic characters)
- These identifiers help assure confidentiality of patient identity

Materials

- ID number and code labels received from the Data Coordinating Center
- Registration (RG) form

When

- Eligibility evaluation visit (visit s)

By whom

- Clinical Coordinator

Procedures

- Complete the CyNCh Registration (RG) form; if the patient remains eligible at the close of the form, assign the ID number and code by peeling a label of the label sheet and affixing it to the specified item on form RG or note ID assigned previously in NASH CRN
- The patient will be known by these IDs for the duration of the NASH CRN, including participation in any other NASH CRN studies
- Key the Registration (RG) form into CyNCh data system; this must be the first form keyed
- The Registration (RG) form should be keyed for each patient screened for CyNCh, including patients already enrolled in the NAFLD Pediatric Database 2

Comments

- Once an ID number and its associated ID code are assigned, these IDs must be used by the patient for the duration of the NASH CRN and cannot be changed
- Do NOT reassign or reuse IDs assigned to patients found to be ineligible or who refuse enrollment

6.2. Baseline History (BH) Form

Who

- Complete for all CyNCh patients
- Study Physician and Clinical Coordinator sign the form

What

- The form queries:
  - History of liver disease
  - Liver biopsy History
  - Menstrual History (female patients) and pregnancy status
  - Medical History (answer items based on information from all sources available to you)
  - Medication use currently and in the past 6 months to 1 year (12 months)
  - Willingness to use birth control methods

When

- Visit s (but given that you need to do chart review, this may take multiple visit days)

How

- Mix of interview data and data obtained by chart review
- Other questions on the BH form can be answered by interview with the patient, or use all sources to get the most accurate information that you can

Definitions of hepatic events queried on Forms BH and FH

- The CyNCh trial will be tracking the occurrence of clinically significant hepatic events. These include variceal bleeding, ascites, edema, hepatic encephalopathy, hepatopulmonary syndrome, hepatorenal syndrome or liver cancer. Any occurrence of these should be documented on the FH form. The following guidelines may be useful in defining these events:

  - **Ascites.** The results of abdominal paracentesis and testing of ascetic fluid is the gold standard for the diagnosis of ascites. However, the appearance of ascites on imaging such as ultrasound or CT scan in the setting of portal hypertension is highly suggestive.
  - **Hepatic encephalopathy.** There should be sufficient documentation of a reversible decrease in neurologic function, in the absence of hypoxia, acidosis, drug toxicity, and other metabolic and toxic insults.

- **Hepatocellular carcinoma.** Cirrhosis may predispose patients to develop hepatocellular carcinoma, the most common primary malignant hepatic neoplasm. Physical findings such as ascites, fever, portal hyptertension, and jaundice are associated with hepatocellular carcinoma. Rise in serum alkaline phosphatase and low alpha-fetoprotein (AFP) level and imaging studies for evaluation of liver lesions (tumor) may lead to a liver biopsy for confirmatory diagnosis.
- **Pedal Edema.** Accumulation of excessive watery fluid in lower extremities will be evaluated by the investigator during physical examination.
- **Variceal bleeding.** The results of diagnostic testing including EGD, radionuclide imaging, or angiography, in the setting of portal hypertension should be confirmed.

6.3. Follow-up Medical History (FH) form

Who

- Complete for CyNCh patients
- Study Physician and Clinical Coordinator sign the form

What

- The form queries/reviews
  - Alcohol consumption since the last visit (AUDIT)
  - Medical History diagnoses and procedures since the last visit
  - Medication use since the last visit

When

- Follow-up visits: f04, f12, f24, f36, f52, f76.

How

- Mix of interview data and data obtained by chart review
- Drinking items should be obtained by an interview with the patient
- Other questions on the FH form can be answered by interview with the patient, or use all sources to get the most accurate information that you can

---

6.4. Physical examination (PE and PF forms)

Who

- All CyNCh patients

What

- Anthropometry
  - Height
  - Weight
  - Waist circumference (form PE only)
  - Hip circumference (form PE only)
- Vital signs
  - Temperature
  - Blood pressure
  - Resting radial pulse
  - Respiratory rate
- System review (form PE only)
  - Chest and lungs
  - Heart
  - Abdomen abnormalities
  -- Abnormality of extremities
  - Focused liver signs

When

- Detailed physical (form PE) at visit s, f24, f52 and f76
- Focused physical (form PF) at visits f04, f12, f36
- Fasting is irrelevant for Forms PE & PF

How

- Ideally, use a stadiometer for height measurement.
- Ideally, use the Gulick II tape measure for waist and Hip measurement; this device may be obtained from www.fitnessmart.com (608-735-4718, model 67019, listed at $36); it is manufactured by Country Technology Inc: 608-735-4718.

6.5. Height and weight measurements

Height

- Height may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a wall-mounted stadiometer with a horizontal measuring block (or fixed angle) is used; other height measuring devices are acceptable
- Follow the manufacturer's recommendation regarding method and frequency of calibration of the stadiometer
- The patient stands erect on the platform with his/her back parallel to the vertical mounted measure scale (but not touching the wall), looking straight ahead with his/her head in the Frankfort horizontal plane (the horizontal plane defined by the lower margin of the bony orbit (the bony socket containing the eye) and the most forward point in the supratragal notch (the notch just above the anterior cartilaginous projections of the external ear)
- The horizontal measuring block is brought down snugly, but not tightly, on the top of the head
- Record the height to the nearest tenth of the unit of measurement (1 decimal place)
- Ask the patient to step away, raise the measuring block, ask the patient to return, and repeat the measurement

Weight

- Follow the manufacturer's recommendation regarding method and frequency of calibration of the scale
- Weight may be recorded in pounds or kilograms
- Two measurements are recorded
- Ideally, weight is measured in the morning after voiding and before breakfast; this should be possible in the CyNCh since most follow-up visits are to be fasting; if this is not possible, try to measure the patient's weight at the same time of day and under the same conditions as the baseline measurements are obtained
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Patient should stand still in the middle of the scale platform with head erect and eyes looking straight ahead
- Record the weight to the nearest tenth of the unit of measurement (1 decimal place)
- Ask the patient to step away, zero the scale, ask the patient to return, and repeat the measurement
- Patients who have limb amputations or who are wearing casts should have weight measured, but note this on the form on the margin (the notes may be keyed at data entry in the General Comments area of the keying)

6.6. Waist and Hip circumference measurements

Waist

- Waist circumference may be recorded in inches or centimeters
- Two measurements are recorded
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Ideally, waist circumference is measured in the morning after voiding and before breakfast; this should be possible in the CyNCh since most follow-up visits are to be fasting; if this is not possible, try to measure the patient's waist at the same time of day and under the same conditions as the baseline measurements are obtained
- Patient should stand with feet together
- Pull an appropriate amount of tape out of the housing
- Ask the patient to bare his/her waist
- Wrap the tape once around the waist: the measure should be taken around the abdomen horizontally at the midpoint between the highest point of the iliac crest and lowest part of the costal margin in the mid-axillary line
- Mark the midpoint on both sides of the patient using a washable marker
- Patient may be asked to assist in passing the tape around the abdomen by holding the end of the tape in position
- When the tape is positioned in the horizontal plane at the correct height, the patient should be asked to keep his/her arms at the sides and breathe naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape, retract the tape, and repeat the procedure
- If the tape cannot be made horizontal across the waist markings, default to the right hip and note this in the margin of the form

6.6. Waist circumference measurement

Hip

- Hip circumference may be recorded in inches or centimeters
- Two measurements are recorded (ideally the two measurements should be within 4 inches (10.2cm) of each other)
- Ideally, a Gulick II Tape Measure will be used; this tape measure is designed to eliminate the guesswork by applying a known amount of tension (4 ounces) to the measuring tape; when used properly, tape tension is always 4 ounces; the self-retracting tape is kept at the desired length until the retract button is pushed
- If an ordinary tape measure (without the special 4 ounce tension indicator device) is used, the measurement will be affected by how tightly the tape is pulled
- Patient should be wearing light clothing (e.g, short sleeve shirt or blouse or surgical gown), shorts, socks and without shoes; pockets should be empty
- Ideally, hip circumference is measured in the morning after voiding and before breakfast; this should be possible in the CyNCh since most follow-up visits are to be fasting; if this is not possible, try to measure the patient's hip at the same time of day and under the same conditions as the baseline measurements are obtained
- Patient should stand with feet together
- Pull an appropriate amount of tape out of the housing
- Ask the patient to adjust his/her clothing to allow measuring the hips over the patient's underwear
- Wrap the tape once around the hips over the underwear: the measure should be taken at fullest part of the hips (maximum extension of the bullocks)
- Patient may be asked to assist in passing the tape around the hip by holding the end of the tape in position
- When the tape is positioned correctly, the patient should be asked to keep his/her arms at the sides and breathe naturally; ask the patient to breathe in and out and hold at the end of a normal exhalation
- Align the tape's zero line along side of the tape graduations; pull on the end of the tensioning mechanism until you see just one colored bead
- Record the measurement to the nearest tenth (one decimal place)
- Remove the tape and repeat the procedure

6.7. Nutrition Data Documentation (ND)

The Nutrition Data System for Research (NDSR) is the most accurate and comprehensive nutrient and food group serving count calculation software available for research purposes. Developed at the Nutrition Coordinating Center of the University of Minnesota's School of Public Health, NDSR is designed for the collection of 24-hour dietary recalls and the analysis of food records, menus, and recipes. NDSR also features an optional dietary supplement data collection module that may be included with 24-hour dietary recall or record protocols. The NDSR Dietary Supplement Assessment Module (DSAM) utilizes the most currently available NHANES Supplement Database with enhancements from NCC and allows for the collection of 24-hour and/or 30-day intake of all dietary supplements and antacids.

NDSR offers standardized dietary assessment methods, interactive interview modules, immediate nutrient calculations, quality assurance features, many hard copy report options and extensive data output files. Designed by researchers for researchers, NDSR features a dietary intake multiple-pass approach that prompts for complete food descriptions, detailed food preparation methods, additions, and diverse amount descriptions. The optional dietary supplement module provides a tiered interview approach that includes a series of data collection screens that prompt for detailed dietary supplement use.

The CyNCh trial protocol calls for three separate 24 hour food recalls to be obtained during screening and again at the f52 follow-up visit.

The Nutrition Data Documentation (ND) form will document completion of the 24-hour food recall using NDS-R on three different days. Centers will save the output files of the three recalls on a CD or USB drive to forward to the DCC twice a year. A copy of the NDSR Record Properties Report for each 24 hour recall conducted should be attached to the ND form. Six key nutritional values will be recorded on the ND form from each 24-hour food recall interview: Energy (kilocalories), Total fat, Saturated Fatty Acids (SFA), Total carbohydrates, Total sugars, and Total protein.

6.7.1. NDSR data collection and processing procedures

Nutrition Data System for Research (NDSR)

- Patient's dietary intake will be assessed via three 24-hour food recall interviews conducted at two visit points (screening and week 52 follow-up)
- Recall interviews will be conducted by a dietitian or trained research interviewer using the NDSR software and database
- The interviewer will conduct the diet recall using the multiple pass method contained in the NDSR computer program preferably in real-time to ensure the highest data quality. However, if necessary, the recall data may be recorded on an intake sheet and entered into the NDSR program at a later date (preferably not more than 24 hours after the interview)
- At the time of the recall interview, the most current version of NDSR should be used (note: the study may span more than one version)

NDSR Project Set-up

- Assign a project folder, name = **CyNCh**, and abbreviation per local standards.
- Select 'Recall' as the Record Type, and '24hr intake' for the Dietary Supplement Assessment Module (DSAM)

Record set-up

- At start of interview, enter following information into the Header page:
  - Participant ID and code (9999-xyz)
  - Date of intake
  - Date of Birth
  - Gender
  - Life Stage Group
  - Interviewer ID
  - Visit number (key 's' for Screening or f52 for the 52-week follow-up visit
  - Site ID (e.g., BCM, CINC, CU, EU)
- In **Notes** section of the Header page, key the following information for each interview as it applies to each interview:
  - in English OR Translator Used (choose one)
  - in person OR on the phone (choose one)
  - by dietician/nutrition research staff OR coordinator (choose one)
  - with Patient, Mother or female guardian; Father or male guardian; Other, specify (Choose all that apply)
- To see how Header Page should look, refer to section, 6.7.2, **NDSR Intake Properties Report** template page

By whom

- Dietitian or trained research staff interviewer

6.7.1. NDSR data collection and processing procedures

Forms

- ***CyNCh Nutrition Data Documentation (ND)*** form (see section 6.7.1 of CyNCh SOP 1)
- **NDSR Record Properties Reports** (see section 6.7.2 of CyNCh SOP 1)

When

- **Screening visit time window (between Baseline and Rz visit) and f52 time window:**
  - Recall interviews may be conducted in-person or via telephone
  - Three recall interviews will take place during the 's' time window (preferably prior to randomization) and during the f52 time window
  - **Note:** If the third recall interview needs to take place on day of randomization, you may key the ND form with data from the first and second recall interviews obtained during the 's' visit and enter the date of the third recall interview and enter '?' for items 19 a-f on the ND form. Edit the third recall information recorded on the ND form after a final NDSR Records Properties Report is provided by the nutritionist (but the data must be entered prior to randomization can occur)
  - At least one of the three recall interviews for both the screening visit and the f52 visit should obtain food intake information from a weekend day
  - At end of first recall interview during both the 's' and f52 visits, request days/times participant and/or parents are available for telephone interviews and obtain best telephone contact information, including a secondary telephone number if available

Recall interview recommendations

- Recommend that the first recall interview during 's' visit (and prior to randomization) be in-person to enable patient and family to meet dietitian, if possible; the standard script (see section 6.7.4) and Food Portion Size Guide (section 6.7.3) should be used
- Recommend that the third recall interview during 's' time window be conducted via telephone if possible to enable timely completion of ND form prior to randomization
- Recommend that the third recall interview during f52 time window may be conducted via telephone to enable timely completion of ND form

Conducting a standardized recall interview

- Recall interview may be conducted with the patient alone (patient $\geq$14 years)
- Recall interview may be conducted with parent/guardian alone (patient $\leq$10 years)
- Recall interview may be conducted with patient and parent/guardian together at any age

Interview materials

- A visual 'Portion Size Guide' should be used to assist patient and/or parents with estimating amounts of food or beverages consumed. Refer to section 6.7.3, **Food Portion Size Guide**

- Give patient a copy of the **Food Portion Size Guide** to take home and to use for telephone interviews
- If possible, complete the remaining two recall interviews within two weeks of study visit date
- Clinical centers will save all of the output files of the three recall interviews onto a CD or USB drive to forward to the DCC two times a year

Reporting

- ***NDSR Record Properties Report*** to be generated from food recall information obtained during screening and at f52 follow-up visits.
- Data will be keyed into the NDSR during real-time in-person or telephone recall interviews
- Generate and print the ***NDSR Record Properties Report*** for each of the three interviews at both the screening visit and the f52 visit
- Upon completing all three recall interviews with a patient, generate the NDSR Intake Properties Report for each recall and send to the Study Coordinator in pdf format (see section 6.7.2).
- The Coordinator will record relevant data from each of the ***NDSR Record Properties Reports*** onto the ***CyNCh Nutrition Data Documentation (ND)*** form and attach each of the three ***NDSR Record Properties Reports*** to the ***ND form***.
- Note the dates recorded on the ND form in items 8, 12 and 16 should be the date that the interview was done, NOT the "intake" date (date meals were eaten).

Off-Site Dietary Recalls (using CINC CR services)

- When the recalls are being conducted by an off-site center, all three recalls will be completed via telephone. The following procedures apply. At the screening visit, the study coordinator will:
    - inform the family that someone will be calling them at home to conduct the diet recall interviews;
    - Use the standardized script to instruct the family on how to respond to the diet interview and use the **Portion Size Guide** to estimate amounts (if applicable); and
    - Finally, the study coordinator will collect contact information and available days and times in the following two weeks when they will be available to respond to the diet recall interview over the telephone.

NDSR 2012 Record Properties Report

Header Information

Participant ID: #9#9
Participant Name: xyz
Date of Intake: 09/23/2012
Day of Intake: Sunday
Date of Birth: 12/21/2002
Gender: Female
Life Stage Group: Females, age 9-13 y
Interviewer ID: SS
Visit Number: S
Site ID: BCM
Header Notes: Recall administered by dietitian; in English; with mother and patient together; by phone.

Trailer Information

Amount of Intake Was: Close to the amount usually eaten.
Information Was: Reliable
Trailer Notes:

Collection Information

Date of Entry: 09/27/2012
Data Collected in NCC Database Version: 2012
Data Collected in Software Version: 2012
Data Collected in DSAM Database Version: 2012

Project Information

Project Name: Summer Quarter 2012 CyNCh Study SSS
Project Abbreviation: SQ12CYSS
Record Type: Recall
DSAM: 24-hour intake
Project Notes:

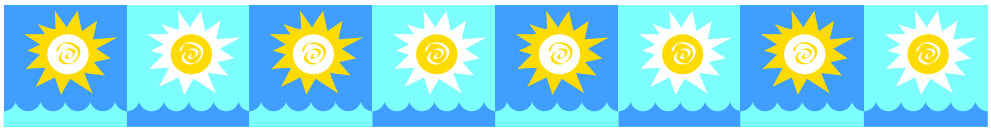
NDSR 2012 Record Properties Report

Selected Nutrients

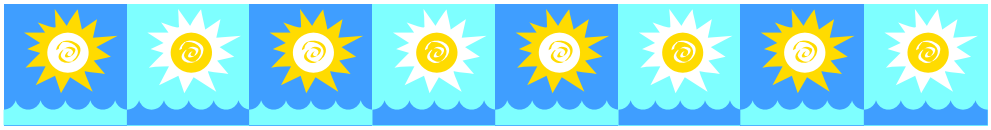
Nutrient	Amount Reported	Daily Value [1]	% Daily Value
Energy (kilocalories)	1285 kcal		
Calories from Fat	390 kcal		
Total Fat	43.889 g	65 g	68 %
Total Saturated Fatty Acids (SFA)	13.633 g	20 g	68 %
Total Trans-Fatty Acids (TRANS)	1.182 g		
Cholesterol	404 mg	300 mg	135 %
Sodium	3537 mg	2400 mg	147 %
Total Carbohydrate	157.916 g	300 g	53 %
Total Dietary Fiber	5.709 g	25 g	23 %
Total Sugars	54.247 g		
Total Protein	63.241 g	50 g	126 %
Total Vitamin A Activity (International Units)	1448 IU	5000 IU	29 %
Vitamin C (ascorbic acid)	9.363 mg	60 mg	16 %
Calcium	365 mg	1000 mg	36 %
Iron	9.004 mg	18 mg	50 %

[1] Daily Values are based on a caloric intake of 2000 kcal per day. Nutrient comparisons are not performed for all nutrients for which a Dietary Value has been established. From: *Code of Federal Regulations, Food and Drugs,* Title 21, Part 101.9, Nutrition labeling of food, 2007.

Note: DSAM nutrients are not included in these totals. Nutrient totals may not equal the sum of their parts. (Refer to the NDSR User Manual.)

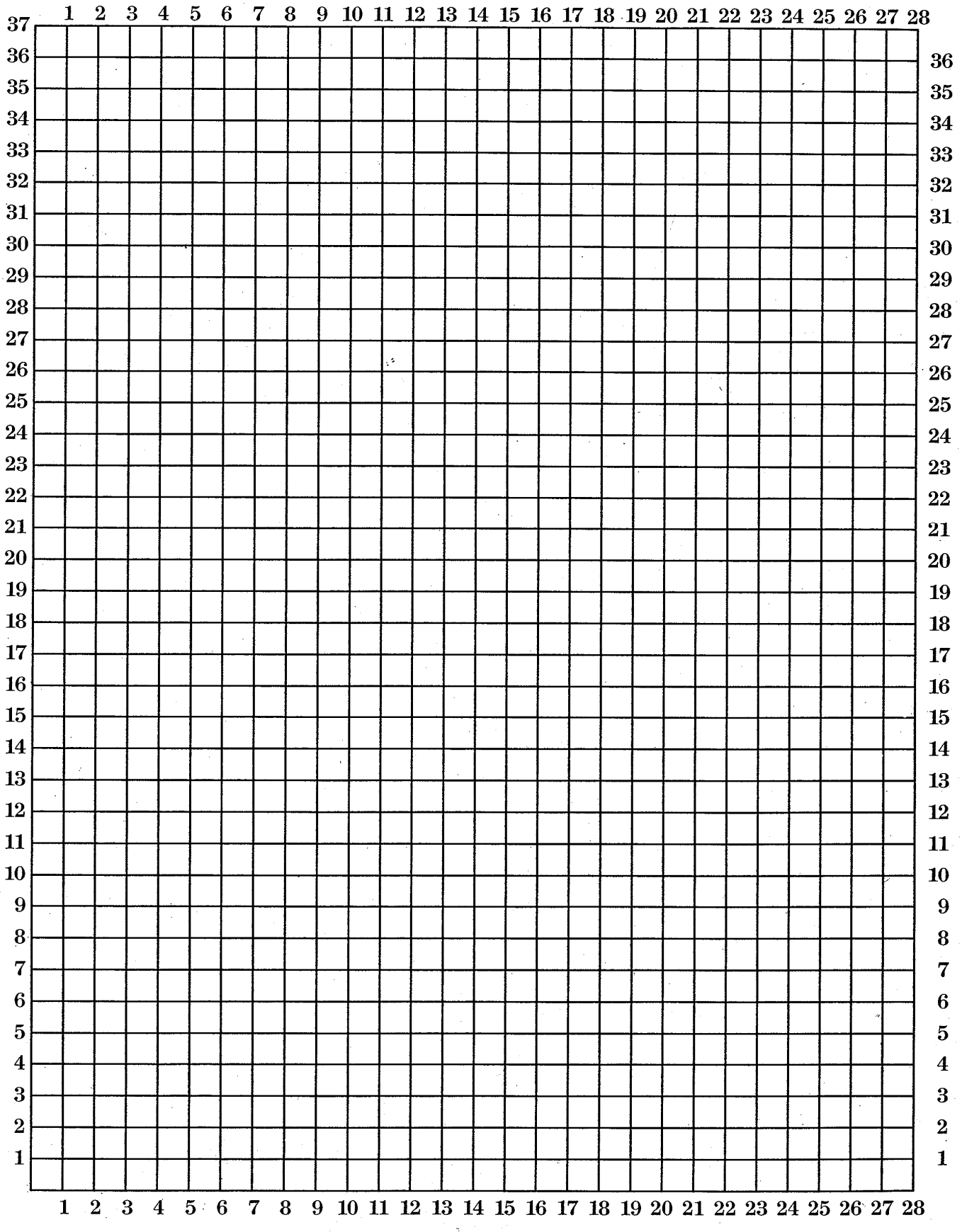


Food Portion Size Guide

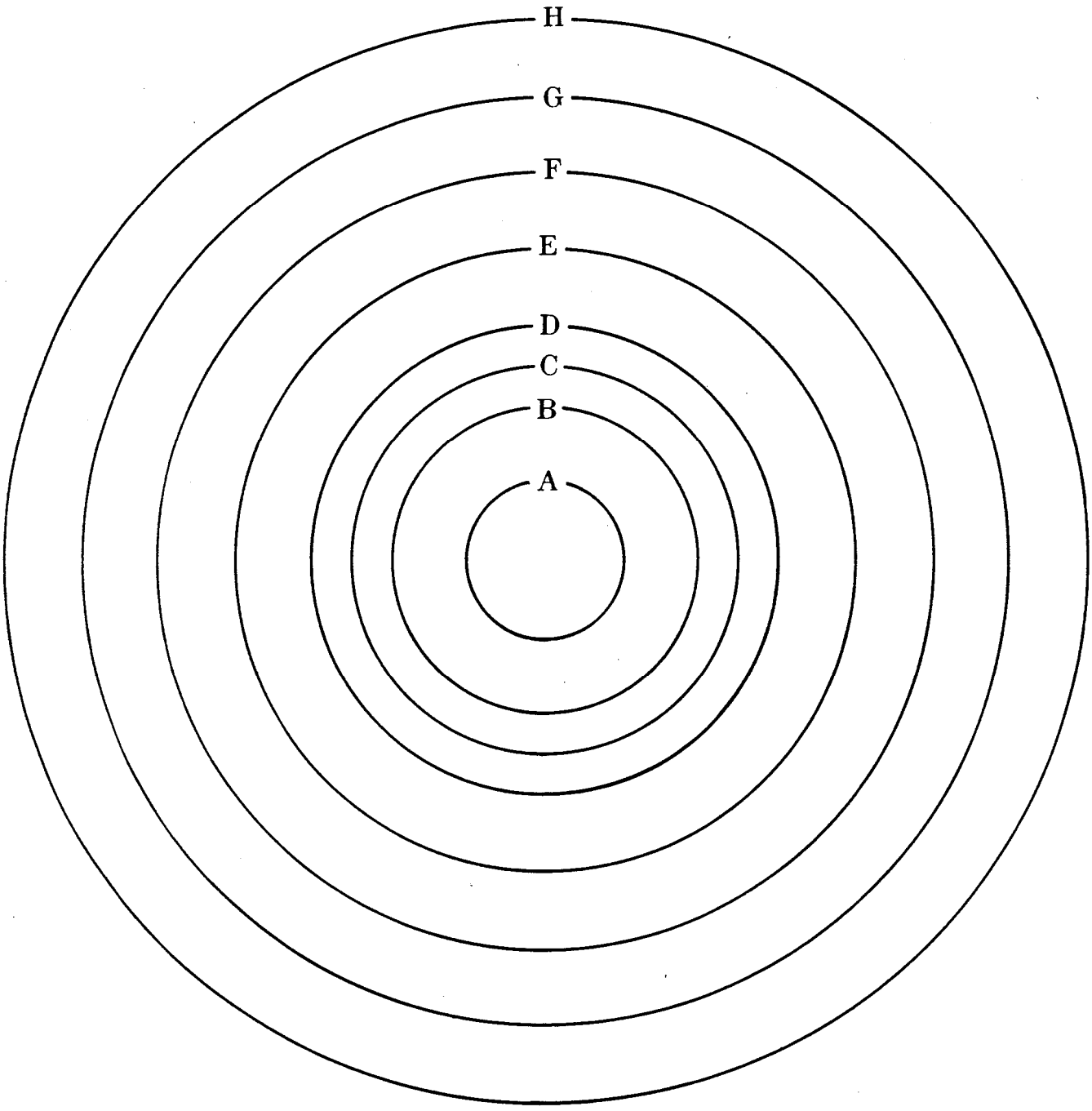


Please keep this packet near your phone

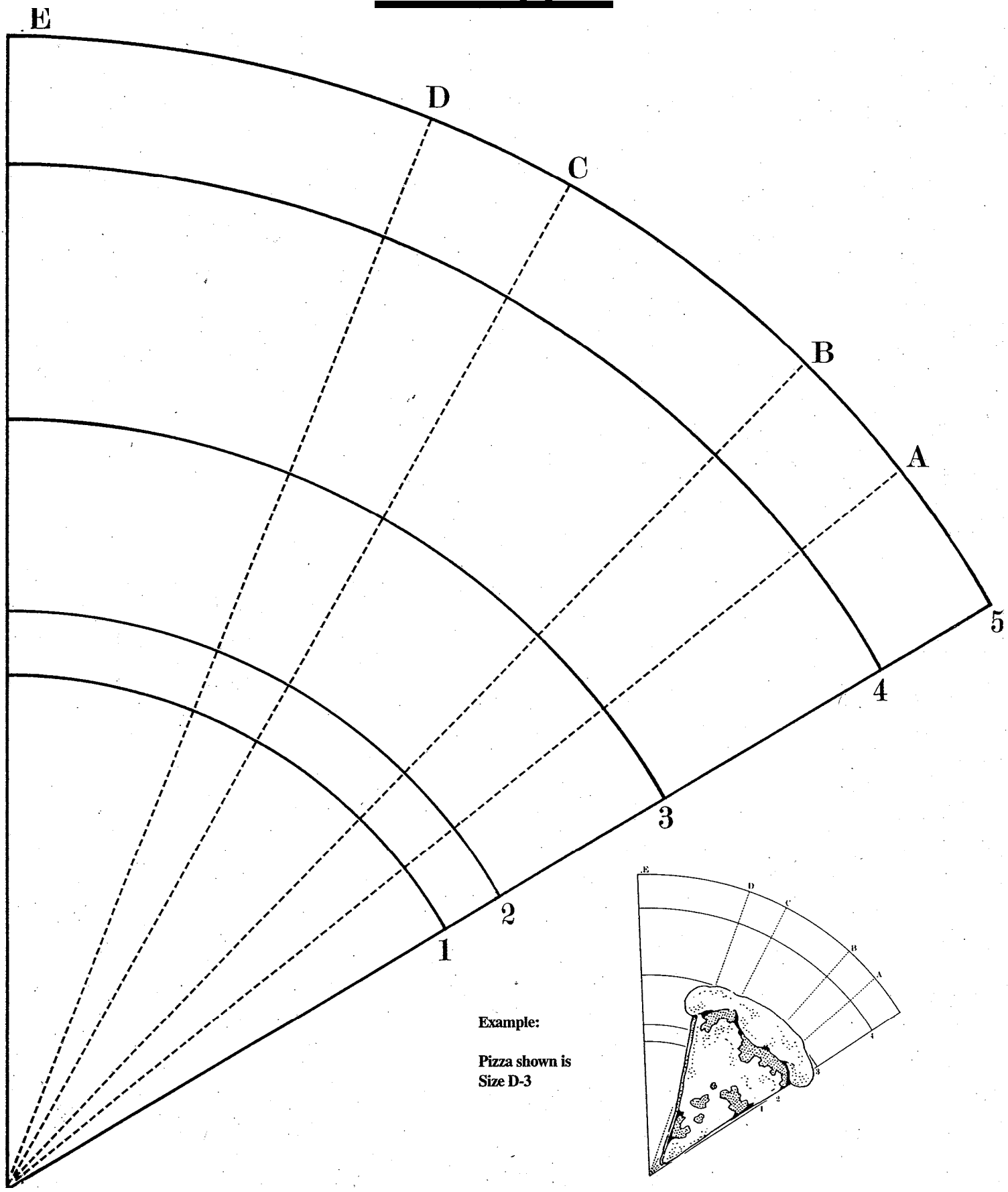
Squares and Rectangles



Circles



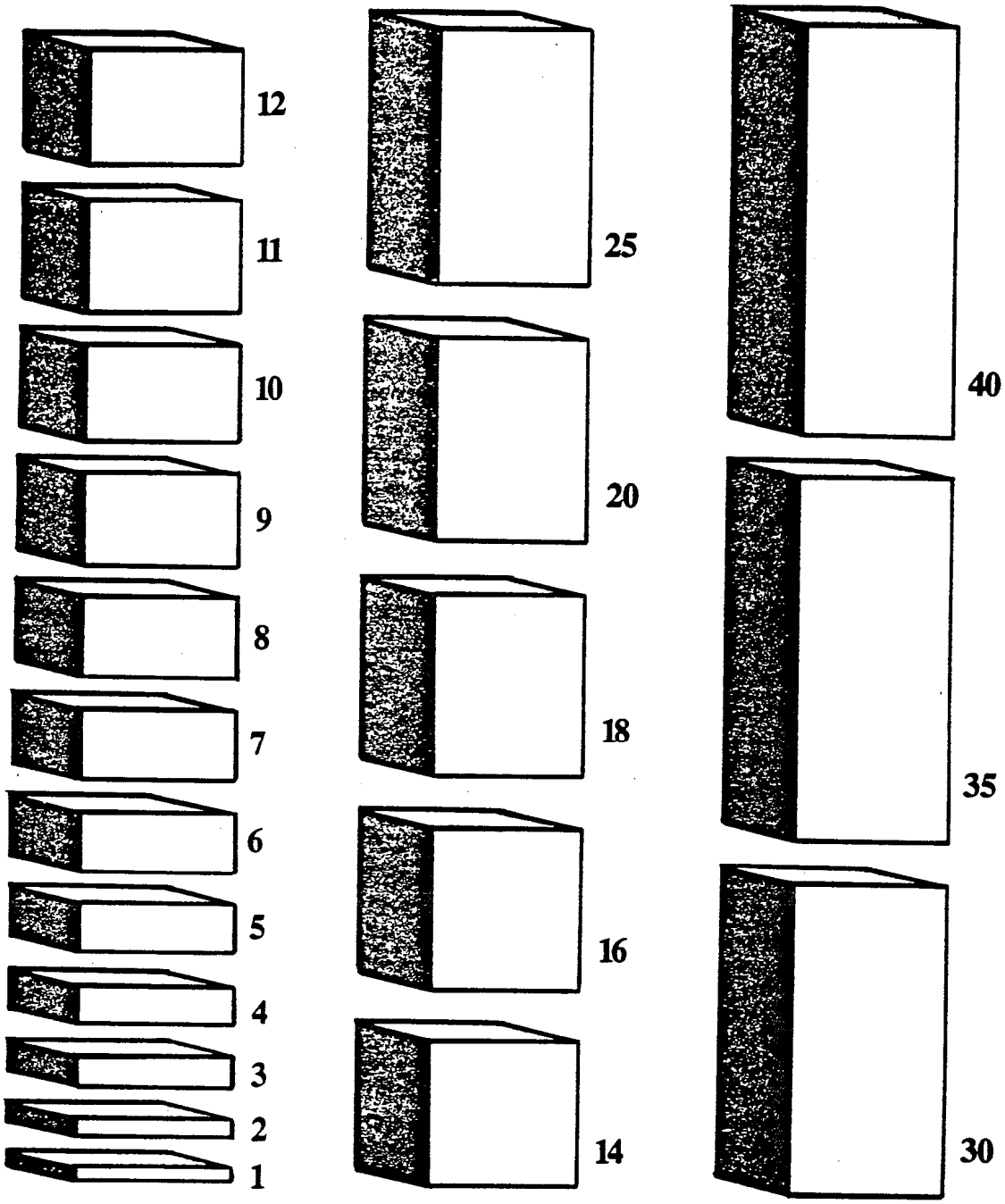
Wedges



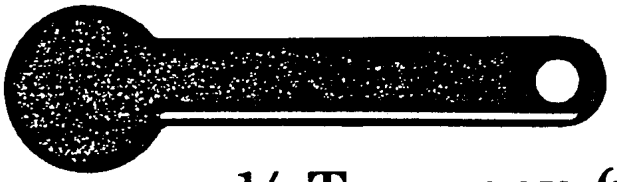
Example:

Pizza shown is Size D-3

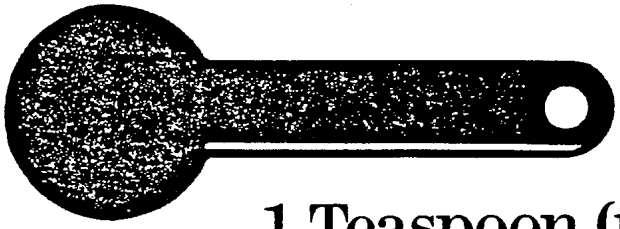
Thickness



Measuring Spoons



½ Teaspoon (tsp)

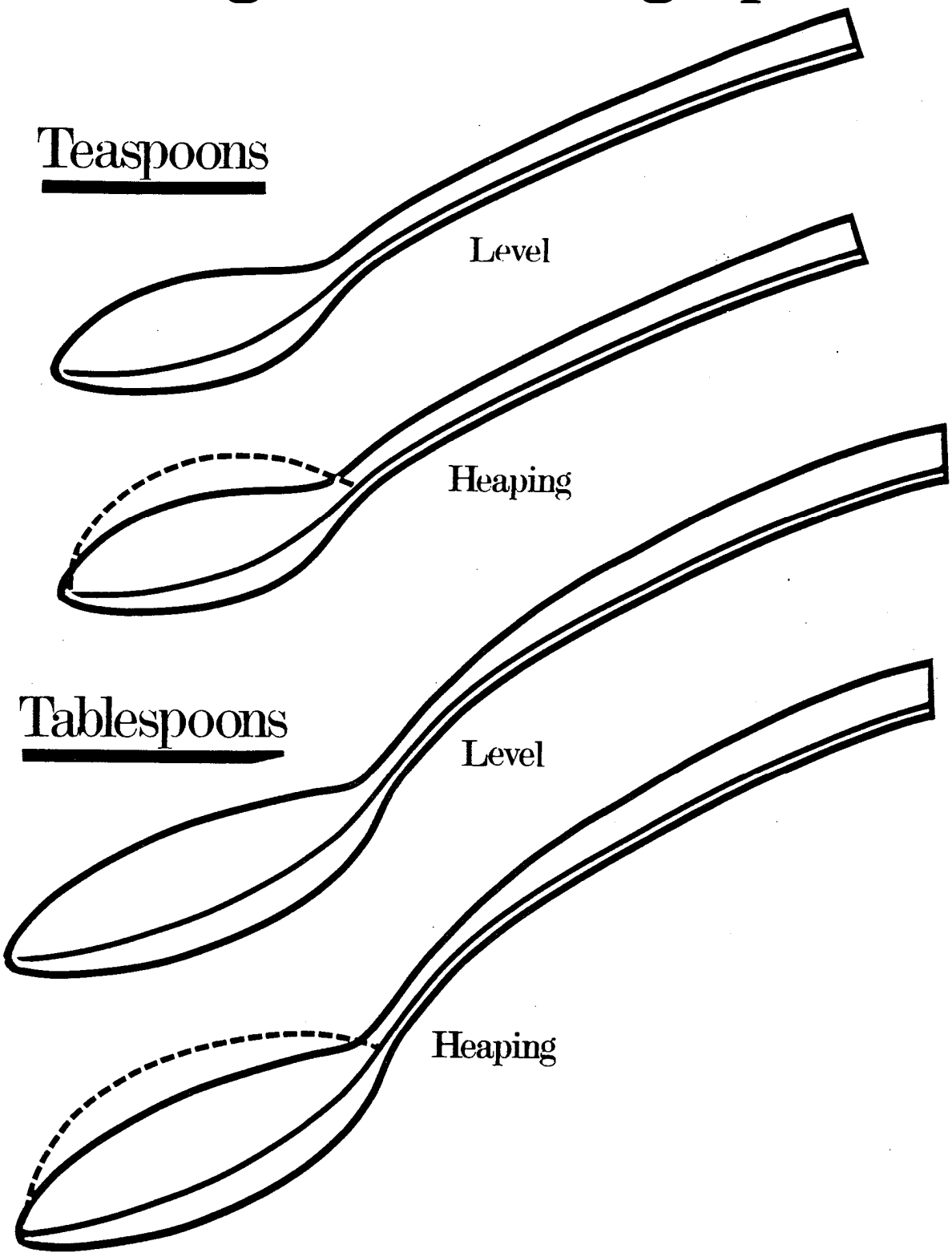


1 Teaspoon (tsp)

½ Tablespoon (Tbsp)

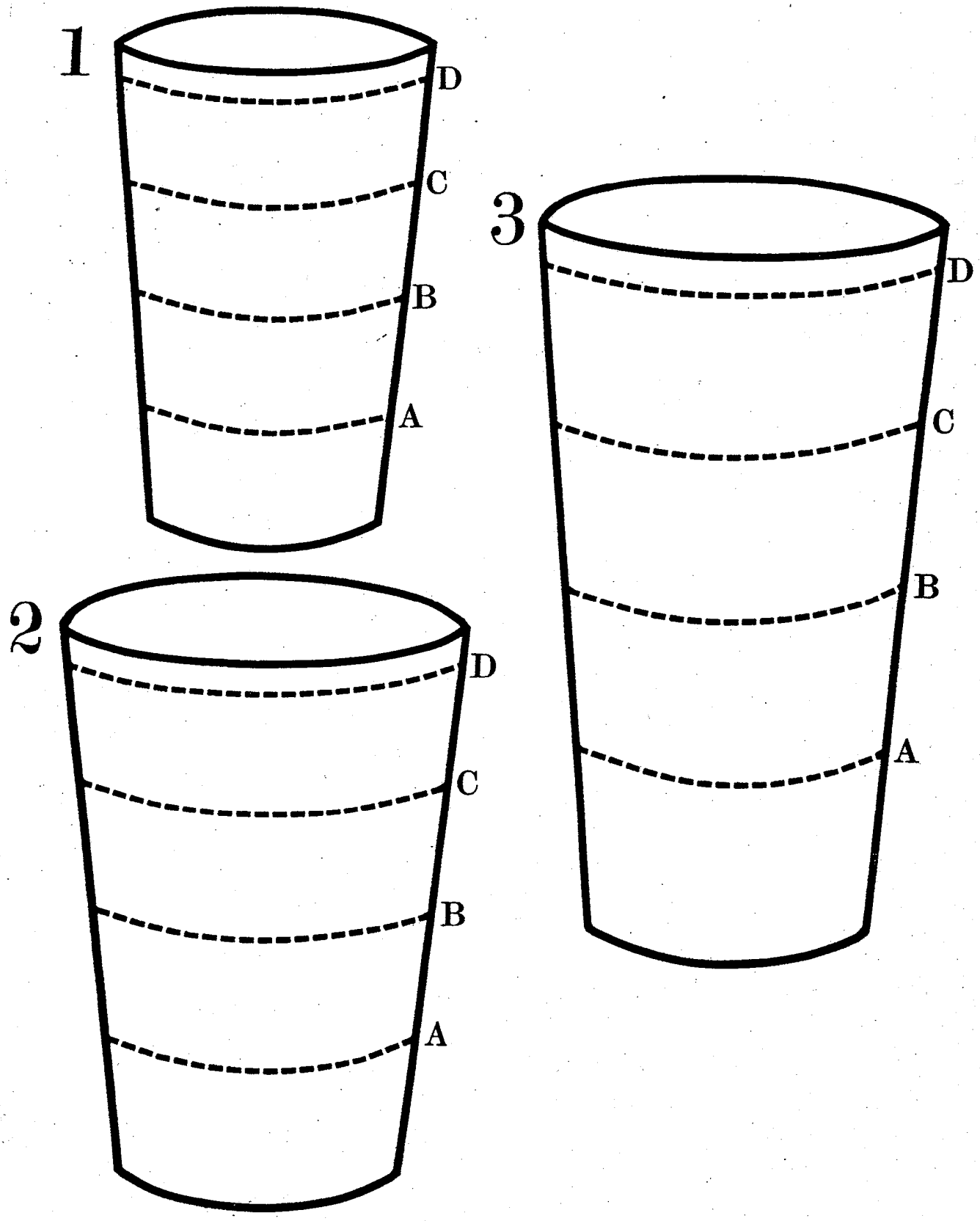
1 Tablespoon (Tbsp)

Eating and Serving Spoons

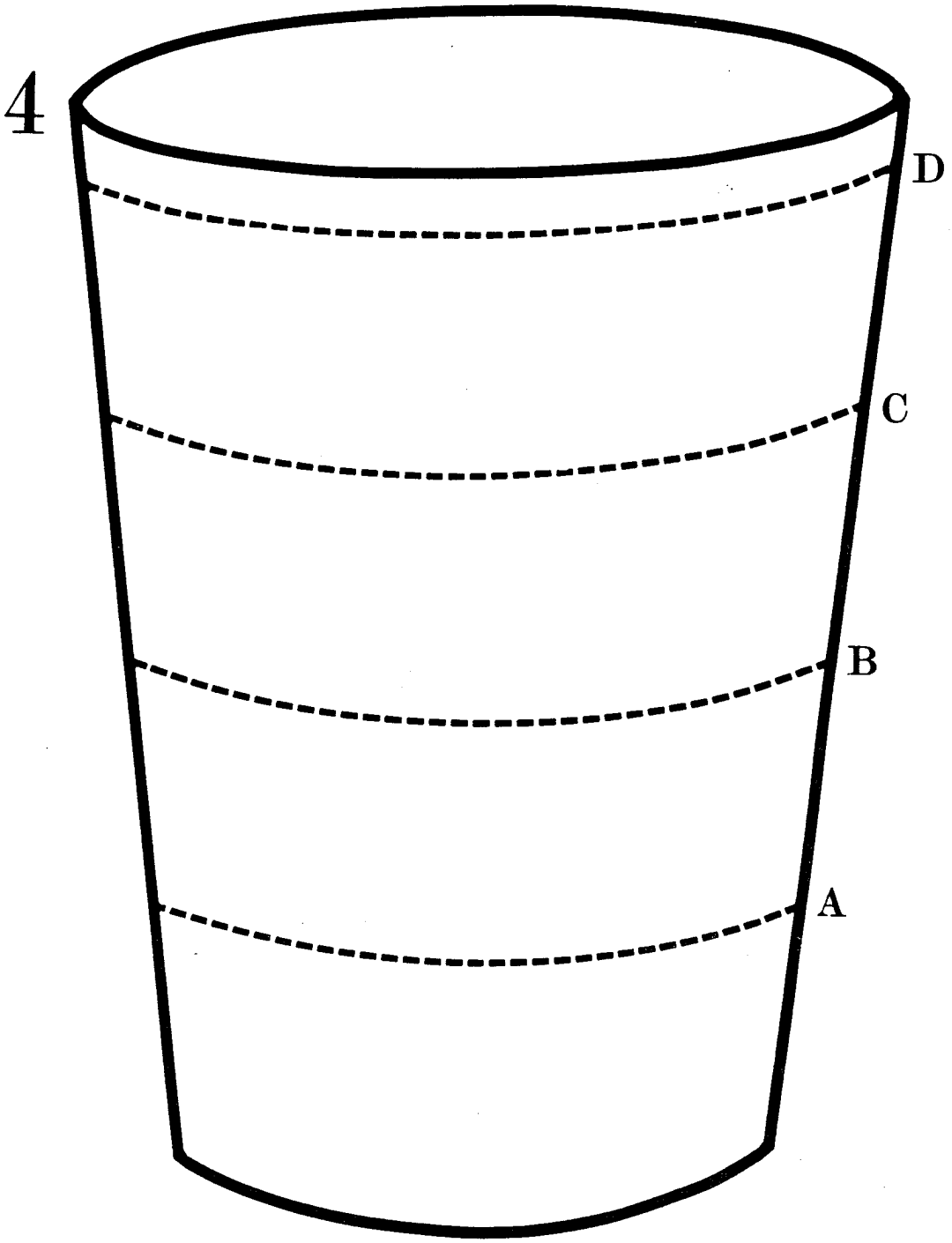


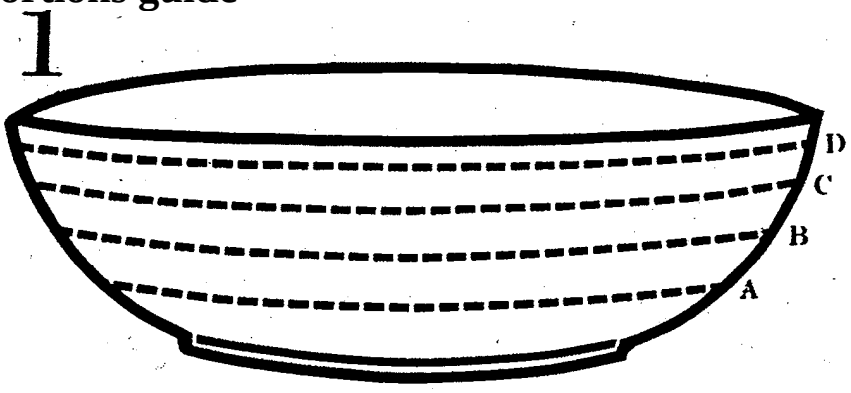
Measuring Cups

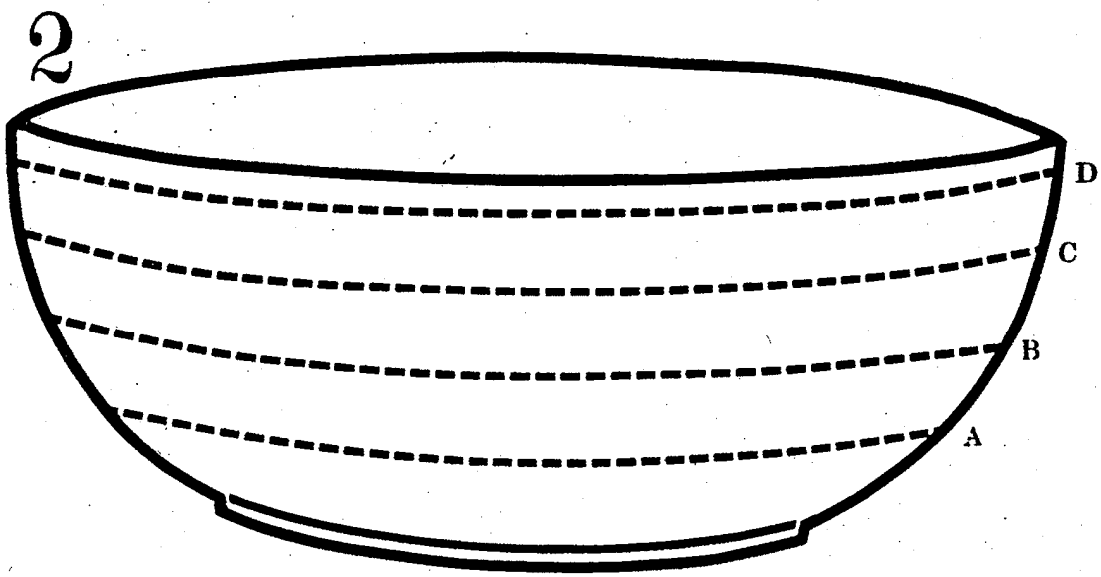
Glasses

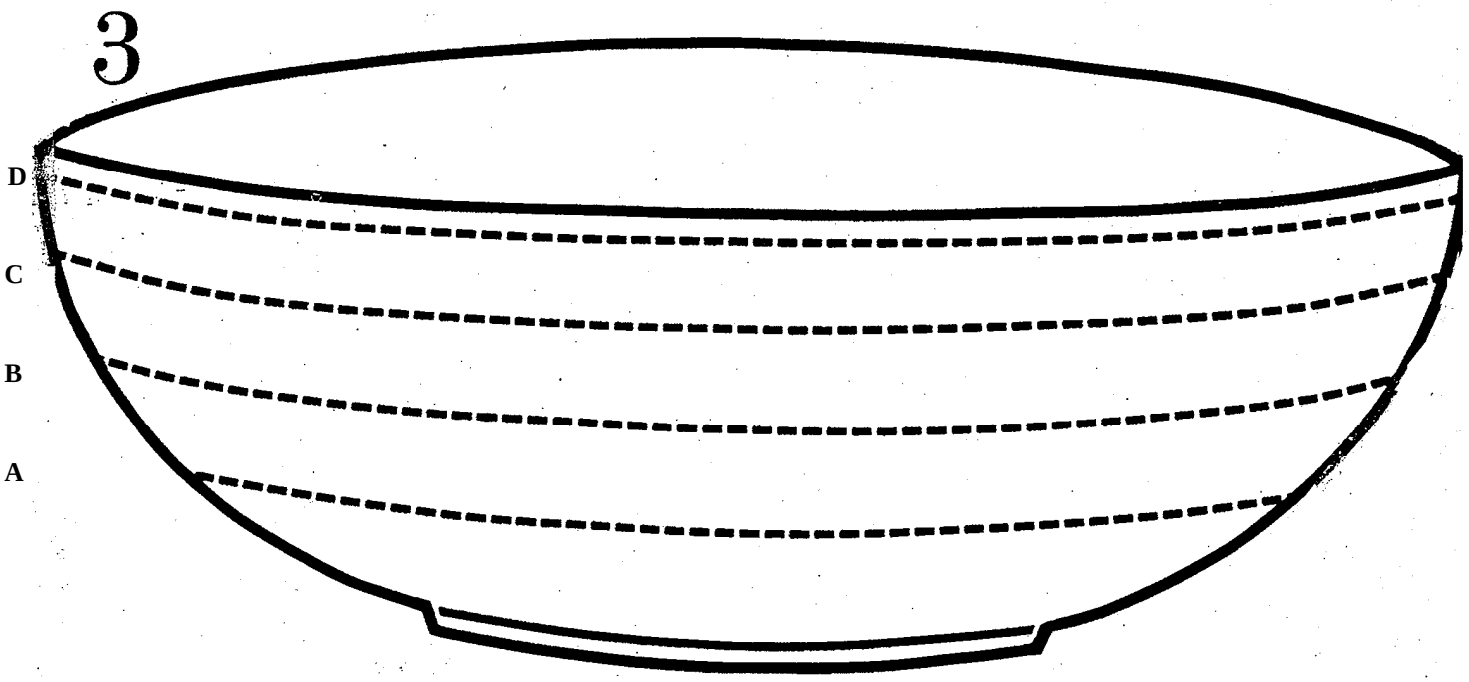


Glasses



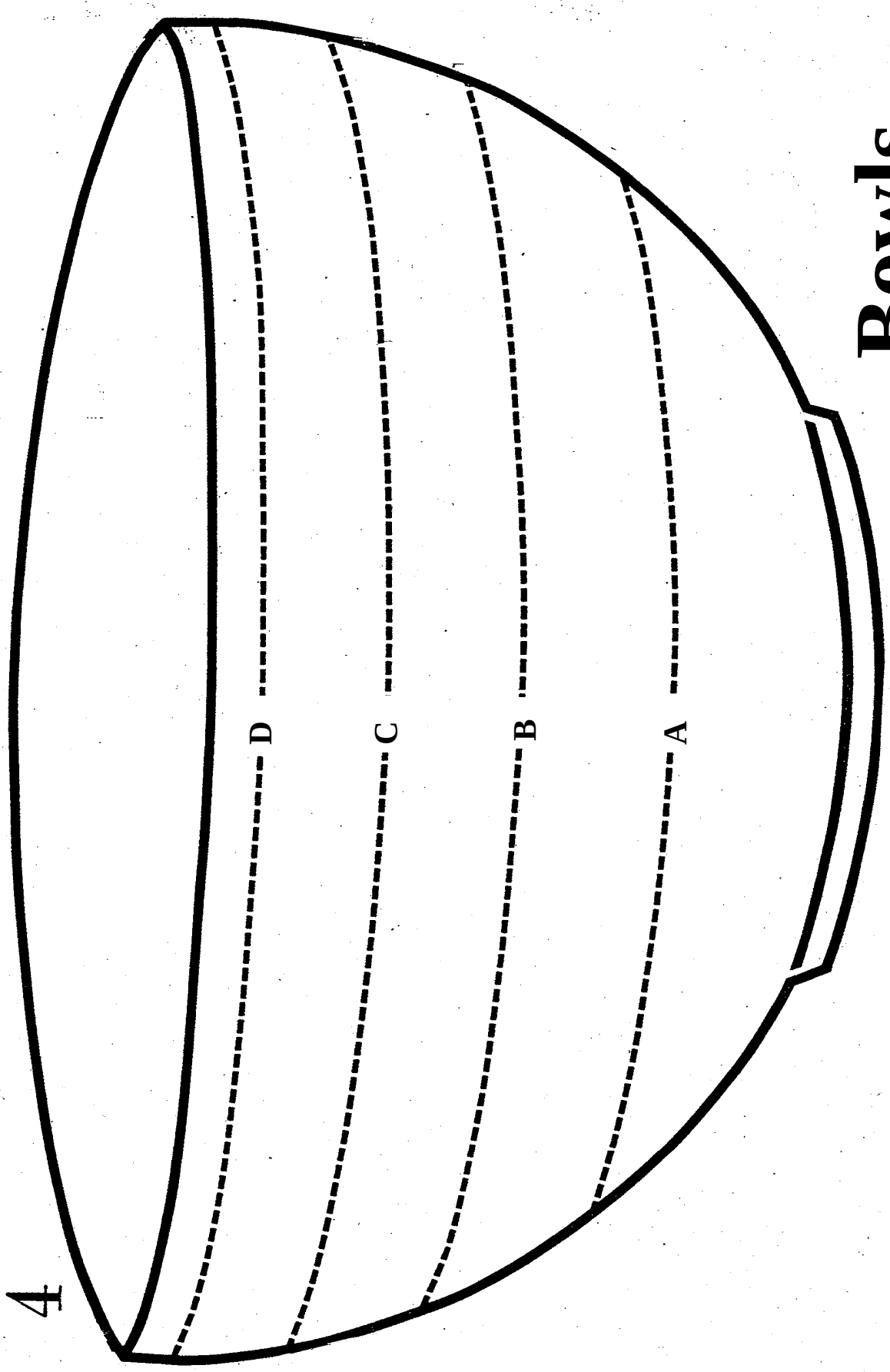






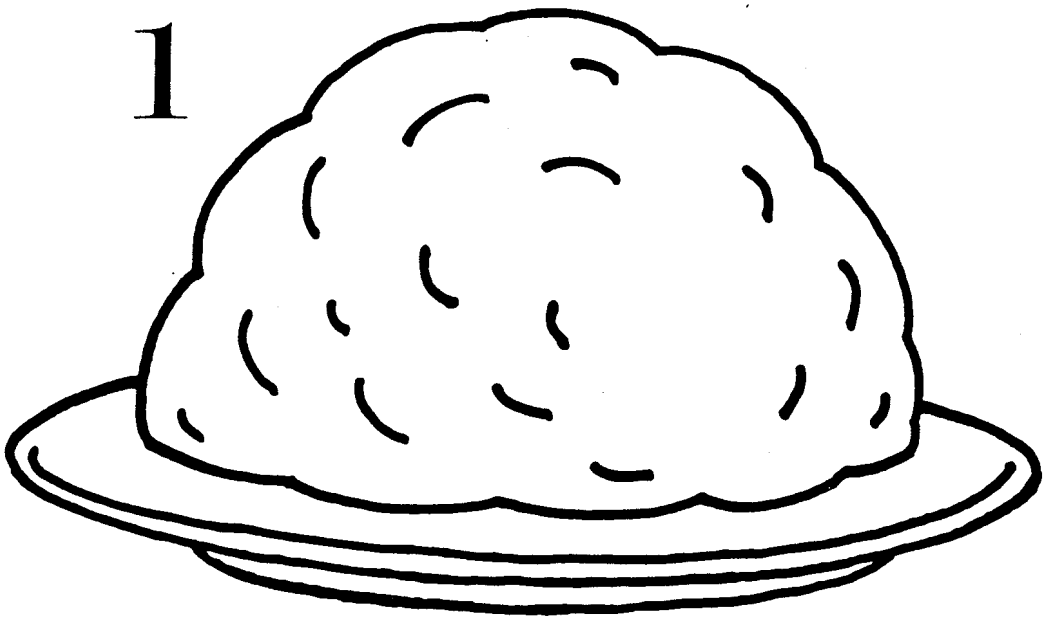
Bowls

Bowls



Mounds

1



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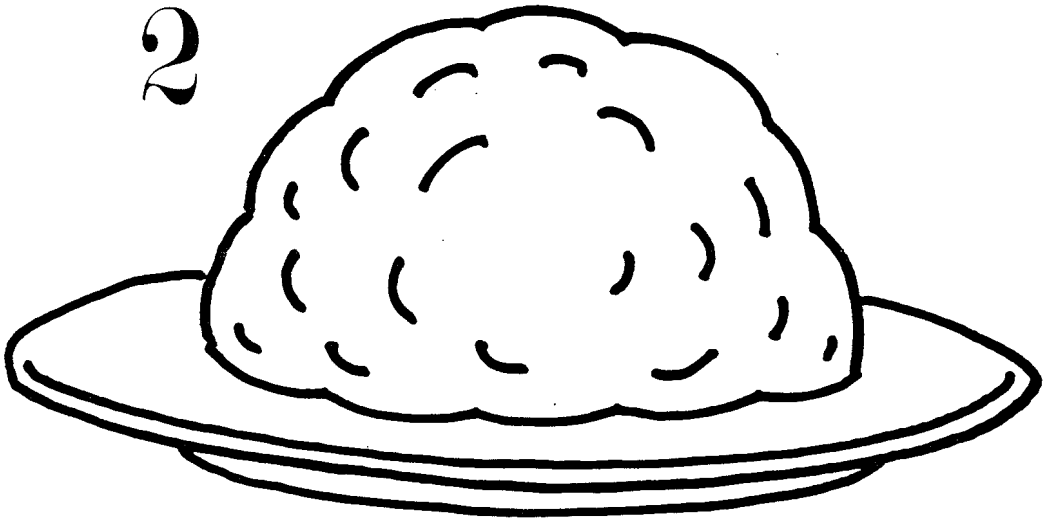
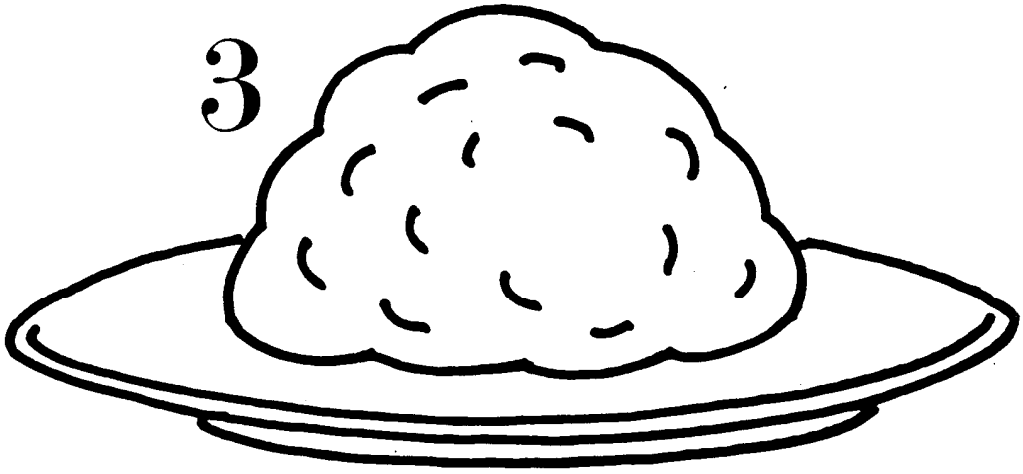
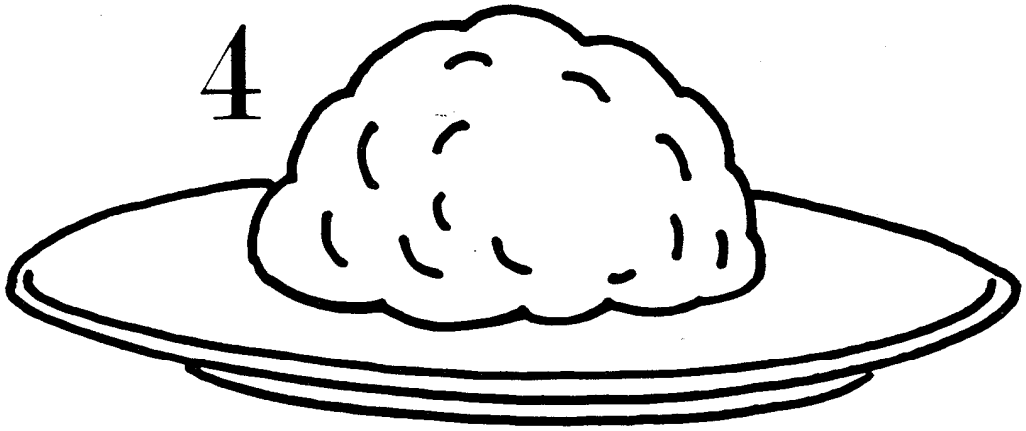
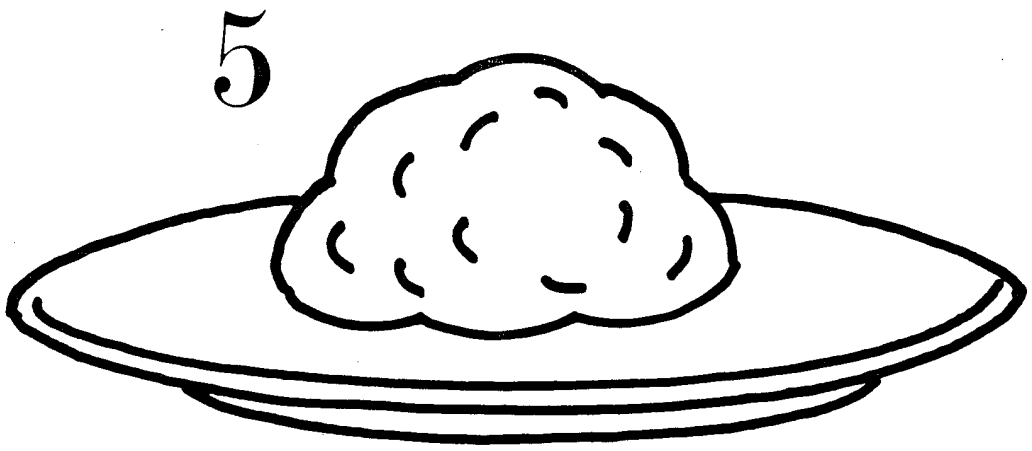


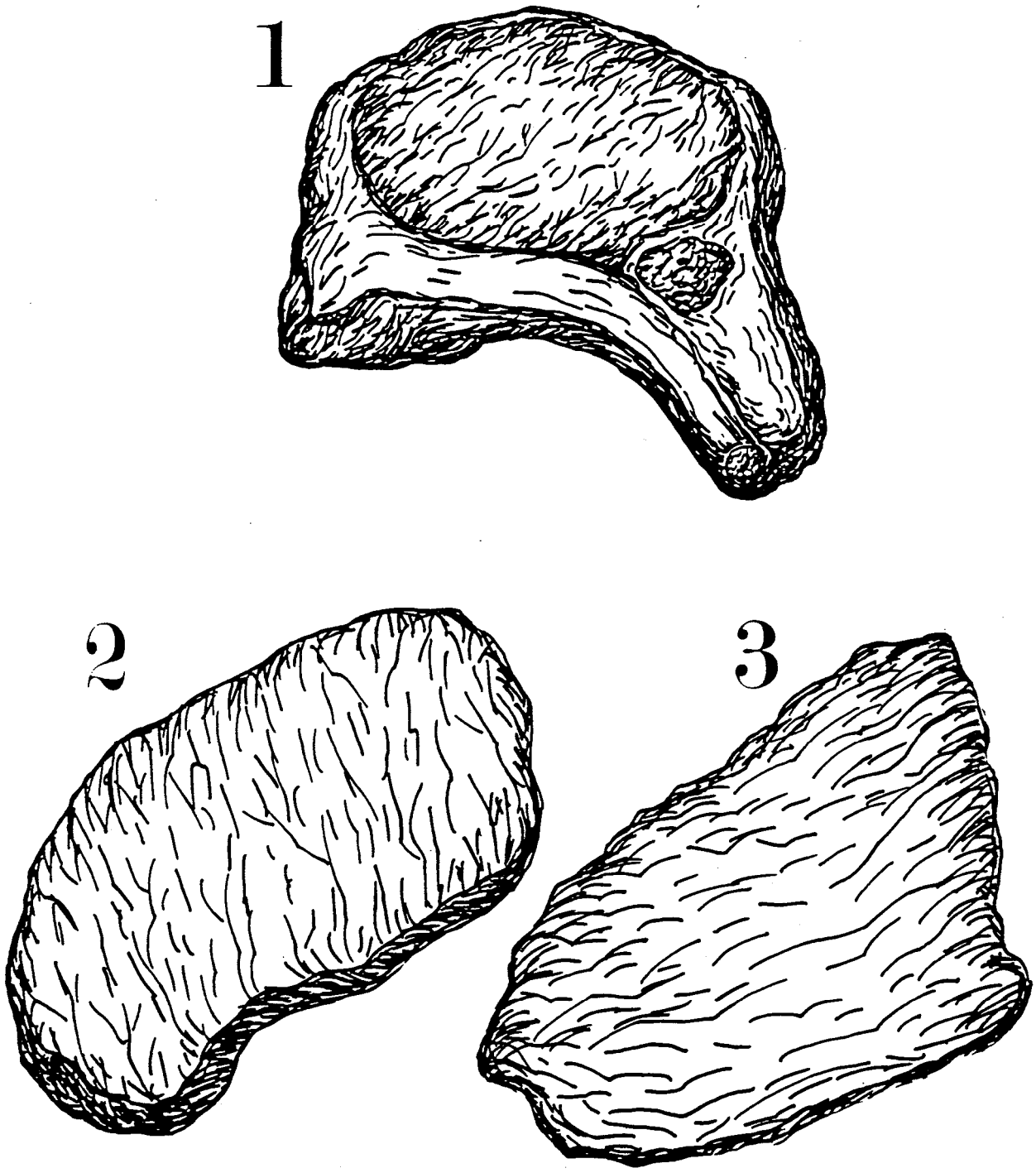
Mounds



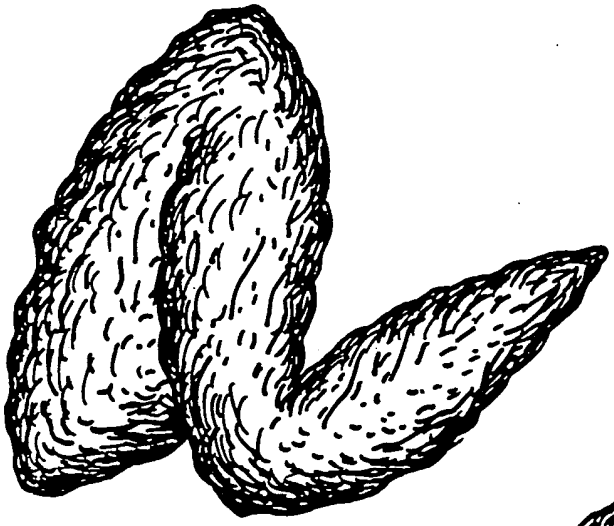




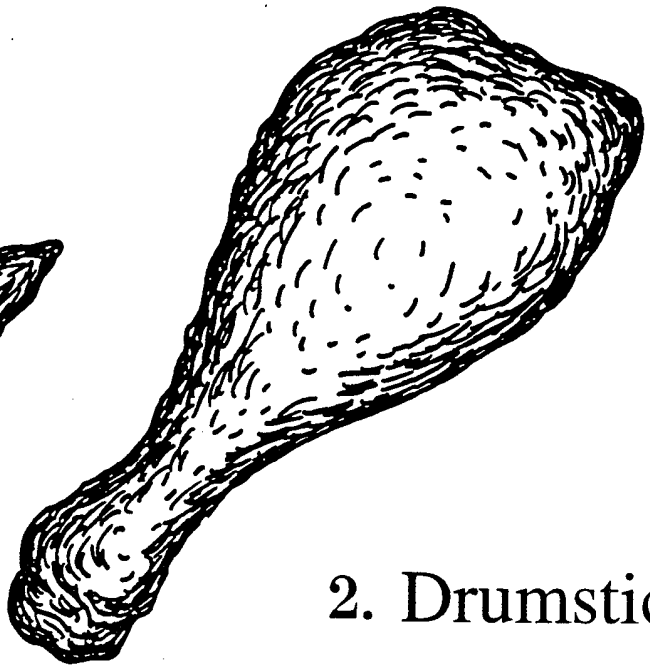
Meats



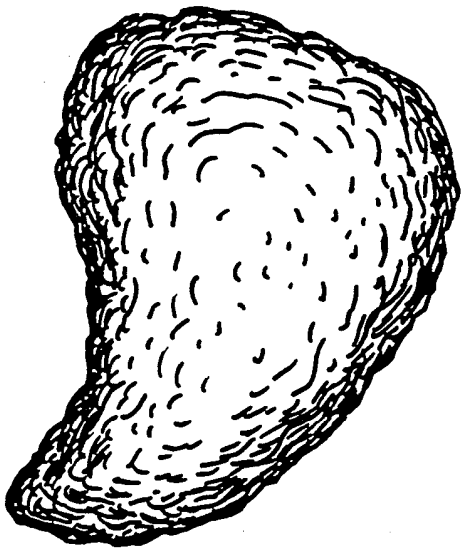
Chicken



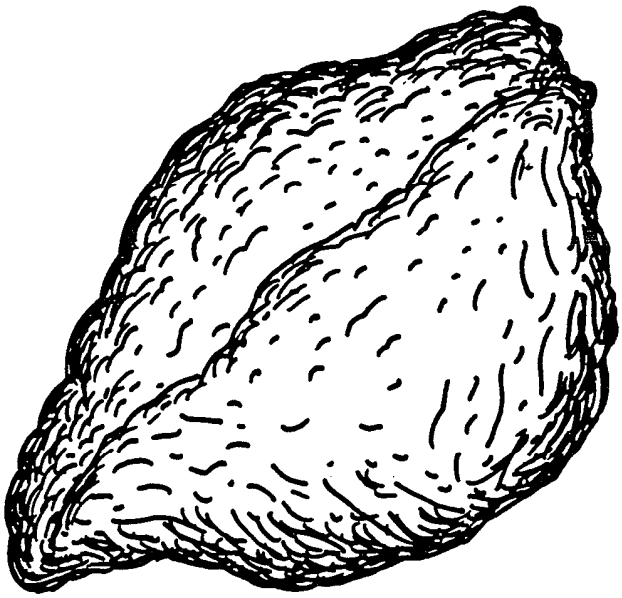
1. Wing



2. Drumstick



3. Thigh

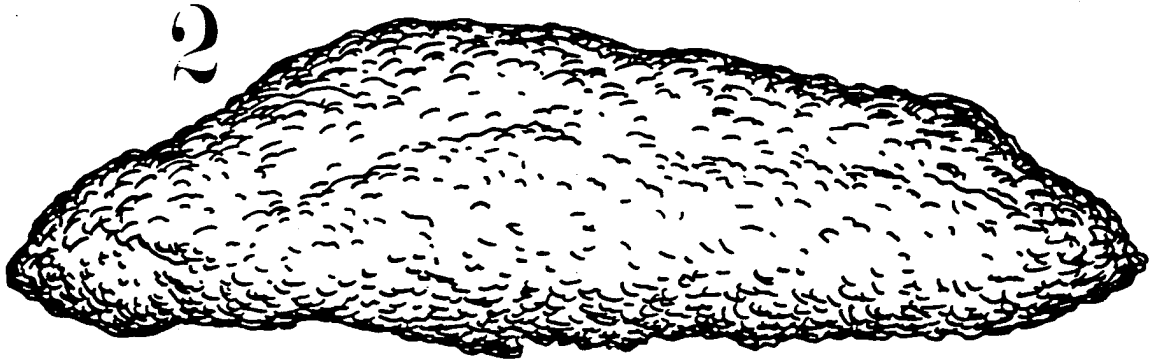


4. Breast

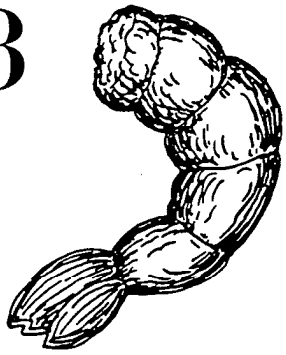
Fish

1

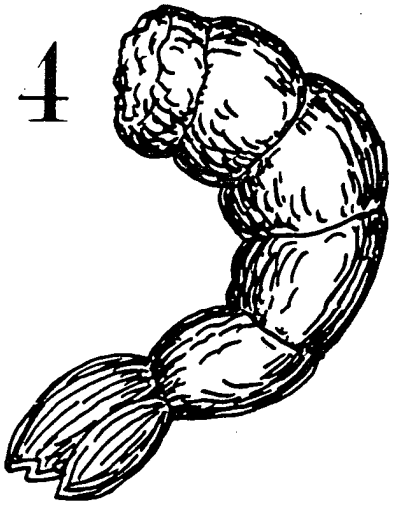
2



3



4



6.7.4. Guidelines for Instructing Subjects on the 24-hour Dietary Recall

(the following is a suggested "script" to use when talking to the patient/parent)

Trainer/Study coordinator: An important part of this research study is knowing what foods your child is consuming. In the next few weeks following this visit, you will be asked to participate in a series of three "dietary recall interviews" where our study dietitian will call and ask you questions in order to determine what your child had to eat and drink on the preceding day. You just need to answer the questions honestly and with as much detail as you are able to provide.

Trainer/Study coordinator: When reporting on what your child had to eat or drink, it is also important to know how much he or she consumed. Don't worry, we are not going to ask you to weigh and measure everything that he or she eats! Instead, we use common household measurement tools and units, as well as an illustrated portion guide, to help you estimate the amount of food or drink consumed.

Trainer/Study coordinator: Common household measurement tools include things like cups (or fractions of cups, for example ½ cup or 1/3 cup), teaspoons and tablespoons, fluid ounces for beverages, etc. You can use these terms to estimate a portion size. You are also encouraged to use food label information to provide portion sizes.

NEXT, SHOW THEM THE GUIDE

Trainer/Study coordinator: Here is our Food Portion Guide, which you may also use to estimate portions. But first, let's look through it together and I'll show you how we use it by going through some examples.

EXAMPLE 1: The dietitian asks you, "what was the first thing your child had to eat in the morning?" You reply "a bowl of oatmeal". The dietitian will ask you to turn to page 10 and look at the bowls there. Imagine they are actual size. Which bowl is closest to the bowl your child used for oatmeal? Let's say you reply "Bowl 3". Next, you will be asked how much of the bowl was filled with oatmeal. You reply "to line C". And that's it! You've estimated the amount consumed.

EXAMPLE 2: You report that your child had a brownie at 3pm for a snack. The dietitian will ask you to turn to page 1, "Squares and Rectangles", and ask you to picture the brownie placed on the page with the edges lined up to the lower left corner of the grid. Now, how big is the brownie using the numbers on the grid? You reply that the brownie fits into "square 12 by square 10".

6.7.4. Guidelines for Instructing Subjects on the 24-hour Dietary Recall

EXAMPLE 3: For dinner, you state that your child had a hamburger that was prepared at home. The dietitian will ask you to turn to page 2 and pick a circle that is closest to the size of the hamburger. You reply "circle E". We are halfway there. Next, the dietitian will ask you to turn to page 4 and look at the different thicknesses. How thick was the hamburger? You reply "thickness 14". We now have everything we need to estimate the size of the hamburger.

Trainer/Study coordinator: It may seem like this is a lot of detail to provide, but as you get used to the process it will become quite easy for you to estimate intake.

Trainer/Study coordinator: Do you have any questions?

Trainer/Study coordinator: Thanks for allowing me the time to review this process with you. Keep this guide in a handy location so that you can access it during the recall interviews.

6.8. Baseline and follow-up liver biopsy (SD and other forms)

Details of liver biopsy procedures, tissue banking, slide preparation, and shipment of slides to the DCC are discussed in the SOP Part IV, Liver Biopsy and NAFLD/NASH Histology Scoring System document.

- Baseline liver biopsy must be obtained within 120 days prior to randomization for all patients who have been found to be eligible for CyNCh with respect to all other criteria
- A follow-up liver biopsy should be obtained at the f52 visit for all patients enrolled in CyNCh
- Blood for serum and plasma banking may be drawn immediately prior to the liver biopsy, but cannot be obtained in the 72 hour period after a liver biopsy
- The MRI exam will ideally be scheduled as close to the time of the biopsy as feasible
  - Baseline MRI should be within 90 days of baseline biopsy and prior to randomization
  - Follow-up/post-treatment MRI should be no more than 6 weeks before and 12 weeks after the f52 visit biopsy
- A pregnancy test (for women of child-bearing potential) should be obtained prior to the liver biopsy and MRI exam

Forms

- Occurrence of liver biopsy(s) done before screening and occurrence of liver biopsy during screening are queried on the Baseline Medical History (BH) form
- Occurrence of a biopsy since the previous CyNCh visit is queried on the Follow-up Medical History (FH) form
- The Liver Biopsy Materials Documentation (SD) form must be completed to document the outcome of all biopsies obtained for CyNCh (screening and follow-up) with regard to availability of tissue for banking and stained and unstained slides for scoring and archiving
- If tissue was obtained for banking, the Liver Tissue Banking (LT) form must be completed.
- If the biopsy was done prior to or during screening, then the local CyNCh Study Pathologist must complete the Liver Biopsy Histology Worksheet (HW) and the Clinical Coordinator must complete the Liver Biopsy Histology Findings (HF) form
- Central scoring of biopsies, shipment of slides to the DCC, and shipment of frozen liver tissue in RNA*later*Solution® to the Biosample Repository must be documented on the Central Histology Review (CR) form
- The Histology Slide Transmittal Log (TS) form must be completed and accompany every shipment of slides sent to the DCC
- The Specimen Shipment Log (SS) form must be completed and accompany every shipment of frozen liver tissue to the NIDDK Biosample Repository

The pediatrician, the Study Pathologist, and the Clinical Coordinator must work together to accomplish these tasks and each complete their sections of the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

6.9. Baseline and follow-up MRI (MR and other forms)

Details of MRI procedures, case report forms, and submission of data to the Radiology Reading Center (RRC) are discussed in the SOP Part VI, MRI Procedure Manual.

- Baseline MRI must be obtained within 90 days of liver biopsy and prior to randomization for all patients who have been found to be eligible for CyNCh with respect to all other criteria
- A follow-up MRI should be obtained no more than 6 weeks prior to and no more than 12 weeks after the follow-up liver biopsy at the f52 visit for all patients enrolled in CyNCh.
  - It is preferred that the MRI exam is done while the patient is still using the study drug.
  - This MRI should be the same time of day as the baseline MRI.
- The MRI exam will ideally be scheduled as close to the time of the biopsy as feasible.
- A pregnancy test (for women of childbearing potential) should be obtained prior to the liver biopsy and MRI exam.

DCC Forms - entered into the NASH CRN data system

- The **MRI Consent and Report form (MR)** must be completed by the study pediatrician/radiologist and coordinator to document the occurrence of an MRI procedure obtained for CyNCh (screening and follow-up) and transmittal of images to the RRC.

RRC Forms - submitted to the RRC via fax (619-471-0503) or email (liclark@ucsd.edu)

- **MRI Radiologist Report CRF** (see SOP VI Appendix B) is completed by the Study Radiologist after the images are reviewed. If there are any significant findings, the Study Pediatrician must be notified.
- **MRI Data Transmittal CRF** (see SOP VI Appendix A) is completed by the Clinical Coordinator
- **MRI Adverse Event CRF** (see SOP VI Appendix C) is completed by the Clinical Coordinator for each MRI that is performed, whether or not there are adverse events.

MRI Related Adverse Events

If there are adverse events related to the MRI procedure, the Clinical Coordinator should notify a Study Pediatrician and complete an Adverse Event (AE) form for the DCC. More information regarding adverse events is discussed in Section 6.17 Adverse Event Reporting.

The Study Pediatrician, Clinical Coordinator, Study Radiologist, and other Imaging Personnel must work together to accomplish these tasks and each complete their sections of the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

6.10. Alcohol use questionnaires (AD)

What / Who

- AUDIT (AD) form
- Summary question on CyNCh Randomization Checklist (RZ) form
- Questions on interval alcohol consumption on Follow-up Medical History (FH) form
- Flash Card #9, Drink Equivalents, can be used with the alcohol questionnaires

Purpose

- At screening, obtain a detailed history of the patients alcohol consumption patterns from the onset of regular drinking
- Monitor alcohol use during follow-up

Who

- All CyNCh patients

How

- Form AD is self-administered for patients, without help from spouse or family

Comments

- At screening, the patient is never asked directly how much alcohol he/she drinks per week on average; clinic staff must judge this based on all information obtained from interview, chart review, discussions with those who know the patient etc; the clinic staff use their best judgement to answer the eligibility question on the CyNCh Randomization Checklist (RZ) form
- The Clinical Coordinator should complete section A on page 1 of Form AD and record patient ID on subsequent pages before asking the patient to complete the form

6.11. Quality of life questionnaires (PQ, PR, PW, PY)

Purpose

- To evaluate correlations between self-reported quality of life and disease severity

What / Who

- All CyNCh patients and parents

Forms

- PQ: Pediatric QOL: Parent Report for Teens, ages 13-17 (English or Spanish)
- PR: Pediatric QOL: Parent Report for Child, ages 8-12 (English or Spanish)
- PW: Pediatric QOL: Child Report, ages 8-12 (English or Spanish)
- PY: Pediatric QOL: Teen Report, ages 13-17, (English or Spanish)

When

- Visit s
- Follow-up visits f52 and f76

Procedure

- Clinical Coordinator should complete Part A and apply labels to subsequent pages as needed before giving the form to the patient to complete
- Self administered or by interview for patients and parent
- Clinical Coordinator should check returned forms for completeness before the family leaves the clinical center

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6.12. Laboratory measures (LS and LR)

Who

- All CyNCh patients

What

- Form Laboratory Results – Tests Done Only During Screening (LS) form covers assessments collected only at screening:
  - Screening etiologic tests
  - Iron assessments
  - Ceruloplasmin measurement
  - Alpha-1 antitrypsin assessment
  - Autoantibody studies

- Form Laboratory Results – Screening and Followup (LR) form covers assessments collected during screening and follow-up
  - Hematology
  - Chemistries
  - Prothrombin time, INR, and HbA1c
  - Liver panel
  - Fasting lipids
  - Fasting glucose
  - Pregnancy

When

- Form LS: Visit s
- Form LR: All visits
- Given the extent of information recorded on form LS, you may still be completing it at visit s2 or drawing blood for tests at visit s2; use visit code s anyway
- Requirements for fasting – nothing by mouth except water for at least 12 hours before blood draw

Instructions for Form LS

- Most of the tests on Form LS are intended to be obtained by chart review; time windows for the allowed age of tests are specified on the form
- Serological assessment to exclude viral causes of chronic liver disease is required for all patients
- Iron overload screening is required for all patients; hepatic iron index is recorded if available, but is not required
- Ceruloplasmin is required
- Alpha-1 antitrypsin assessment is required for all patients
- Autoantibody studies are required for all patients

Instructions for form LR

- The measures on form LR can also be obtained by chart review, both at screening and during follow-up; the time window for each type of assessment is specified on the form

6.12. Laboratory measures (LS and LR)

- During follow-up, the time window for the assessment is "in the time window for the follow-up visit (check the patient's Visit time window guide)" – e.g., f36 has an acceptable calendar time period within which it may be done; if you can find a hematology assessment in the patient's chart that was done under the same conditions as required by the study and which provides the required values and was done within the time window for visit f36, you do not need to order another hematology at f36
- If the chart review tests are out of the time window or the test conditions can't be ascertained or differ from what is required, the measures have to be done at the study visit and can be charged to the study
- For baseline, the required time window is within 90 days of the liver biopsy.
- All laboratory test results are required during screening.

6.13. Plasma and serum collection for Biosample Repository (BP)

Purpose

- Collection of whole blood from the CyNCh trial patients; when timed to coincide with a liver biopsy, blood should be collected ideally within ± 7 days and up to ± 90 days of the biopsy. Blood should not be collected in the 72 hour period after a biopsy
- Separation of plasma and serum at clinical center:
  - Screening visit, f12, f24, f36, f52, and f76: ten 0.5 mL aliquots of plasma and twenty 0.5 mL aliquots of serum are to be obtained in 2.0 mL cryogenic vials.
- Store plasma and serum aliquots at -70° C prior to batch shipping to the NIDDK Biosample Repository at Fisher BioServices

Fasting Instructions

- Patient instructed to fast 12 hours (recommended) prior to blood draw; an 8-hour fast prior to blood draw is allowable. *Note if the patient has not fasted a minimum of 8 hours at visits f04, f12 and f36, blood may still be collected for banking, please document this on the BP form item 8.

Forms / Materials

- BP - Blood Processing for Plasma and Serum
- Labels for heparin (green top) tube and serum separator SST tubes (red top)
- Labels for plasma and serum cryovials
- Barcode scanner
- SS - Specimen Shipment log and Excel Spreadsheet
- NIDDK Biosample Repository shipper

When

- Visit s
- Followup visits (f12, f24, f36, f52, f76)
- Batch shipments: Monthly or semi-monthly

By whom

- Phlebotomist
- Clinical Coordinator
- Person responsible for shipping to NIDDK Biosample Repository must have formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines

6.13. Plasma and serum collection for Biosample Repository

Equipment

Blood tubes/aliquot vials

- One 10 mL sodium heparin (green top) tube - *provided by clinical center*s
- Two 10 mL SST (red-gray top) tubes - *provided by clinical centers*
- Up to thirty 2.0 mL cryogenic vials - *provided by clinical centers*
  - vials should be able to withstand -196 degrees C
  - vials should be self standing (flat bottom, not curved), externally threaded, 13.5 mm wide x 48.3 mm tall, with silicone washers

Labels

- Preprinted labels for whole blood collection tubes (10 mL heparin tube and 10 mL SST tubes) and preprinted labels for Form BP – *labels are printed at the clinical center via web-based data management system; use MACO ML-5000 1" x 1 ½" labels, 50 labels/page*
- Preprinted polypropylene labels for 2.0 mL cryogenic vials – *provided by the DCC*

Equipment

- Centrifuge
- -70° C freezer
- Swing out rotor
- 5 mL pipettes

Blood processing procedures

- Patient instructed to fast 12 hours prior to blood draw (recommended); an 8-hour fast prior to blood draw is allowable. *Note if the patient has not fasted a minimum of 8 hours at visits f04, f12 and f36, blood may still be collected for banking, please document this on the BP form item 8.
- Collect whole blood into one 10 mL heparin (green top) tube for plasma
- During visit s and f76, collect whole blood into two 10 mL SST (red-gray top) tubes for serum.
- During other follow-up visits, collect whole blood into one 10 mL SST tubes for serum.
- If sample appears to have hemolyzed, do not aliquot. Re-draw blood

Plasma

- Collect blood into heparin (green top; Becton-Dickinson) tube. Ensure that heparin tubes have not expired. (*check that date shown above "Exp" in lower right corner of label is later than current month*)
- Completely fill vacutainer tube
- Mix gently by inversion 5 times
- Within 30 minutes, centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Immediately after centrifugation, insert a 5 mL pipette below surface of plasma

- Remove the clear plasma while avoiding blood cells
- Transfer plasma into aliquots of 0.5 mL each into 10 labeled 2.0 mL cryovials
- Freeze at -70° C immediately
- Processing of plasma should be completed within 30 minutes

Serum

- Collect blood into serum separator (red-gray top) tubes. Ensure that SST tubes have not expired. (*check that date shown above "Exp" in lower right corner of label is later than current month*)
- After filling, invert each SST tube gently at least 5 times
- Allow blood to clot for **at least 30 minutes** at room temperature
- Centrifuge at 1800 x g for 15 minutes at 4° C, preferably with a swing out rotor
- Transfer 0.5 mL of serum into 10-20 labeled 2.0 mL cryovials
- Freeze at -70° C immediately
- Processing of serum should be completed within two hours

Note: Separated serum and plasma may be stored at -20° C for up to one day before transfer to -70° C while ensuring samples remain frozen during the transfer.

Blood Processing for Plasma and Serum (BP) form

- Complete the Blood Processing for Plasma and Serum (BP) form
- Affix labels for the heparin plasma and the SST serum to the BP form
- Affix aliquot 00 cryovial labels to the BP form

Shipping samples to the NIDDK Biosample Repository

- Specimens are to be batch-shipped monthly to Fisher BioServices on Monday, Tuesday, or Wednesday
- Aliquots will be stored locally at the clinical center at -70° C prior to shipping
- Ship specimens in the STP 320 Saf T Pak shipper (provided by the NIDDK Biosample Repository)
- Open the template Excel file used for shipments and scan each cryovial using the carcode scanner provided to your clinical center. The file should have the filename: NASHCRNsiteXXX_shipdate.xls. Replace the 'xxx' with your clinical center three digit site ID and replace 'shipdate' with the date of shipment
- The Excel shipping file has column headings for barcode number: Site ID-Patient ID numbers; 3 letter patient code; date collected; specimen type: plasma, sera, liver tissue; volume; units of measure; study number; and visit code
- Record the Federal Express Airbill tracking number, at top of page 3, of the Specimen Shipping Log.
- Complete Section A. Center ID, shipment and study information and section B. Clinical Administrative information of the Specimen Shipment Log (SS). Enclose a printed copy of the Specimen Shipment Log and the Excel spreadsheet with each shipment of specimens.

- Keep a notebook of all original completed Specimen Shipment Logs (Form SS) and Excel spreadsheet so that you have a record of all shipments to the Biosample Repository
- Notify the Biosample Repository of the shipment via fax (301-515-4049) or email bio-niddkrespository@thermofisher.com) on the day the package is picked up by Federal Express. Include the tracking number in the subject line of the email.

Packaging Procedures

- Check that 1 absorbent pad is in the Saf T Pack Bio hazard plastic bag
- Insert frozen cryovial into small cardboard boxes with dividers. Place only one tube into each cardboard cell. Each cardboard box may hold 81 cryovials
- Insert each cardboard box with cryovials into its own plastic bag and seal
- Place each plastic bag with specimen box into its own STP-710 Tyvek envelope and seal.
- Place each Tyvek envelope into STP-111 inner brown cardboard box. No more than 3 Tyvek envelopes containing boxes with cryovials can be placed into the STP-111 inner brown cardboard box. If shipping only 1 or 2 specimen boxes, fill the rest of the space inside the cardboard inner box with bubble wrap to prevent movement
- Tape the inner cardboard box closed before placing the styrofoam cooler
- Place cardboard box in upright position in bottom of styrofoam cooler
- Surround the STP-111 inner brown cardboard box with abut 8 kg of 2" blocks or nuggets of Dry Ice
- Fill excessive room left in the insulated freezer box with bubble wrap to stabilize specimens in transit
- Place the polystyrene lid onto the freezer box
- Place the “Empty Packaging” cover and shipping form, with Excel spreadsheet on the top of the cooler lid
- Place a completed Specimen Shipment Log (Form SS) on top of the cooler lid
- Close and seal outer cardboard box with tape

Labeling Shipper:

- Place a checkmark in the block on the outer cardboard box next to “BIOLOGICAL SUBSTANCE, CATEGORY B”. Do not cover this marking with labels.
- Affix a label with your name and return address to the side of the box in the “Shipper:” block
- Affix the repository address label to the side of the box in the “Consignee:” block
- Affix the dry ice label below the repository address label. Enter the weight of dry ice on the label in kilograms
- Affix the “UN3373 BIOLOGICAL SUBSTANCE, CATEGORY B” label to the right of the dry ice label
- Use the preprinted Federal Express air bill to ship specimens to the NIDDK Biosample Repository. Complete return address (leave “Sender Federal Express account number” blank). Section 6, Special Handling: Check “Yes, Shippers Declaration not required,” check “Dry Ice” block and entry “1” x “8” kg. Section 7, Enter “1" under “Total Packages” and the total weight of 24 lbs. Place completed Federal Express Airbill on side of box adjacent to the labeled side. Call Federal Express at 1-800-463-3339; give them the account number in section 7 of the Airbill

  Do not write on exterior of box

  Do not ship frozen packages on Friday; the repository is closed on weekends

6.14. Symptoms of Liver Disease (LP)

The symptoms of Liver Disease (children) form is designed to document participant-reported symptoms associated with liver disease. See form: LP-Symptoms of Liver Disease, for additional information associated with how the LP form is administered and the symptoms queried.

6.15. Genetic consent and blood collection documentation (CG)

Purpose

- Collection of whole blood from CyNCh patients who consent for genetic research
- Shipment of whole blood to the NIDDK Genetics Repository at Rutgers University for DNA banking
- Do not repeat genetic consent or blood draw for patients who have had blood drawn for genetic research as part of other NASH CRN studies, unless original yield was low.

Forms

- CyNCh consent for genetic research
- Genetic Consent and Blood collection Documentation (CG) form
- NIDDK Genetics Blood Collection form

When

- Visit s (or as needed during follow-up due to a low yield [less than 50g] of DNA)
- Ship same day as whole blood collection

By whom

- Clinical Coordinator and Study Physician (to obtain consent)
- Phlebotomist (to obtain whole blood)
- Person responsible for shipping whole blood to NIDDK Genetics Repository must have formal, documented training to package and ship hazardous goods, per DOT/IATA guidelines

Equipment

- Two 10 mL NaEDTA vacutainer tubes (purple top) - *provided by NIDDK Genetics Repository*
- Preprinted whole blood tube labels and form CG labels - *provided by clinical centers (printed from web based data management system; clinical center provides MACO ML-5000 labels (1" x 1 ½ ", 50 labels per page, www.maco.com)*
- Shipper - *provided by NIDDK Genetics Repository*
  - One model 472 Thermosafe Safety Mailer (body and lid)
  - One 2 ½" x 9" pre-cut section of absorbent materials
  - Two 18" strips of red waterproof tape
  - One press-lock plastic bag
  - One corrugated shipping carton with locking tabs
  - One pre-printed Fed Ex airbill with third party billing
  - One NIDDK Genetics Blood Collection form
  - Instructions for Blood Sample Collection form

6.15. Consent and specimen collection for Genetics Repository (CG)

Blood collection procedures

- Affix MACO tube label onto the tube and avoid covering the barcode label
- Collect blood into two 10 mL NaEDTA (purple top) tubes
- Invert each tube gently 6 times to mix blood with additives
- Keep blood at room temperature
- Check that patient ID information on preprinted tube labels matches information recorded onto the NIDDK Genetics Blood Collection form

Packaging procedures

- Ship whole blood at ambient room temperature same day to the NIDDK Genetics Repository
- Package the whole blood tubes in the body of the Safety Mailer (Model 472 Thermosafe Safety Mailer)
- Place absorbent material so it exactly covers cavity of the Safety Mailer
- Place lid of Safety Mailer over body and absorbent material and press down firmly so that lid closes properly. Reposition absorbent material so that it does not get caught between the lid and body
- Peel backing from two 18" long pieces of red waterproof tape and seal the Safety Mailer lid to the body; peel backing from second piece of tape and continue sealing the mailer, overlapping the first piece of tape about two inches on both ends
- Place the sealed Safety Mailer into the press-lock plastic bag. **Do not seal the bag**.
- Place the NIDDK Genetics Blood Collection form in the mailer box outside the plastic bag
- Slide the Safety Mailer and open press-lock bag into the corrugated carton
- Seal the press-lock bag and close carton using the locking tabs
- Place sealing tape (not included) over them

Shipping procedures

- Use the preprinted Federal Express shipping label, marked for *Priority Overnight Delivery*, to ship whole blood to the NIDDK Genetics Repository
- Outside cardboard box must have stamped “Diagnostic Specimen Packed in Compliance with IATA Packing Instructions 650"
- Call Federal Express, 1-800-Go-FEDEX (1-800-463-3339) for courier*
- Notify Dana Witt-Garbolino or Dale Heyward at the NIDDK Genetics Repository that blood is being shipped and provide the Federal Express tracking numbers and the NIDDK ID numbers. Notification may be via:
  - Web portal: http://rucdr.rutgers.edu/shippingblood
  - email: witt@biology.rutgers.edu
    Heyward@dls.rutgers.edu
  - Fax: 1-732-445-1149
  - Telephone: 1-732-445-1498

- Ship whole blood to:
  Rutgers University/Cell Repository/NIDDK
  604 Allison Rd., Room C120A
  Nelson Laboratory
  Piscataway, New Jersey 08854-8000

- Do not schedule deliveries the day before or the day of a national holiday. Check with Federal Express and with the Genetics Repository if there are any questions about delivery availability or closure.

Genetics Repository Web Portal System
Rutgers University Cell and DNA Repository - (RUCDR) implemented Laboratory Information Management System (LIMS) software to track shipments. **Please note that the system is not compatible with Mac systems.**

- Please call 732-445-4429 with ANY questions about computer configuration for immediate support. The list of requirements are posted here: https://rucdrlims.rutgers.edu/starlims10.rucdrlims/support/default.htm

- Additionally, they have set up a support email account to specifically address LIMS questions. The address is starlimshelp@biology.rutgers.edu.

Establishing a Username and Password

http://rucdrlimsregister.rutgers.edu/

- Go to the URL listed above and then just follow the directions on the page. You will receive a confirmation email. You will then be contacted to establish your account.

Training videos

- There are training videos for several functions on their website: http://www.rucdr.com/training.htm
- **RUCDR STARLIMS Request for Supplies** (http://rucdrlimstraining.rutgers.edu/reqsupply_video.htm)

This video tutorial will teach you how to order supplies such as collection kits, phlebotomy forms and FedEx AirBills through the RUCDR STARLIMS system. The video will guide you through the appropriate steps of requesting supplies from the RUCDR. Should you need any additional help after watching the video, please contact our RUCDR STARLIMS helpdesk by phone at 732.445.4429 or email at starlimshelp@biology.rutgers.edu.

- **RUCDR STARLIMS Sample Submission** (http://rucdrlimstraining.rutgers.edu/presubmission_video.htm)

This video tutorial will teach you how to preregister your samples through the RUCDR STARLIMS system. By watching the video, you will learn the steps required to correctly preregister your samples before they are sent to the RUCDR.

Logging in to the System

- The URL for the RUCDR StarLIMS system is https://rucdrlims.rutgers.edu/starlims10.rucdrlims/start.lims Enter your username and password. If you ever forget your username or password there are options on this screen to retrieve a lost password or username. You will need to remember what email address you used to create your account to use this function!

Sample Submission

- Use the STARLIMS system to notify the NIDDK Genetics Repository that blood is being shipped and provide the Federal Express tracking numbers and the NIDDK ID numbers. Notification may be via:
  - STARLIMS system: https://rucdrlims.rutgers.edu/starlims10.rucdrlims/start.lims
  - Fax: 1-732-445-1149
  - Telephone: 1-732-445-1498

Self Help Resources

- This tab is a holding area for useful documents.

1. **FAQ** – If you have a question, hopefully it is already answered here.
2. **Download Center** – These instructions are here! Also, any paperwork enclosed with mailer kits is here in case you need to print off extras.
3. **View Announcements** – In case you missed the announcement page when you first logged in to the web portal you can read it again.
4. **Support Resources** – Links that may be of interest to visit.

Account Management

- From this tab you can "Modify Your Profile" or "Change Password".

Important Information Regarding Blood Shipments

- When a package is received, a mailer request is filled or a question is answered, you will receive an email from us and the status will be changed to "closed".

6.16. Study drug dispensing and return (DD and RD)

Forms

- DD - Drug Dispensing Documentation form
- RD - Study Drug Dispensing and Return form

Drug supply

- Cysteamine Bitartrate Delayed-Release: 75 mg/capsules, (150 count bottle) taken orally in the morning and the evening 30 minutes prior to meals
- Placebo: 75 mg capsules taken orally twice a day (qd)

Dispensing of study drug

- Study drug to be dispensed to participants at: Rz, f04, f12, f24 and f36 visits
- 3 month supply (3 bottles)
- Do not attempt to "re-use" bottles of study drug already dispensed to the patient, always collect all study drug bottles at each visit, and dispense new study drug bottles.

Checks on return of study drug

- Unused study drug to be returned by patient at: f04, f12, f24, f36, f52 and f76
- Children and parents should be reminded to return empty and partially used study drug bottles at all followup visits

Procedures at clinical center

- The DD form must be keyed at each followup visit to receive the bottle numbers to be dispensed to the patient; the DD form may be completed and keyed prior to the visit so the study drugs can be ordered from pharmacy
- The RD form **must be keyed within 48 hours of dispensing study drug to the participant** to document the capsules and bottle numbers returned as well as the bottle numbers dispensed at each visit

By whom

- CyNCh clinical coordinator or pharmacist

Ordering procedures at clinical center

- Inventory current drug supplies
- Study drug supplies are shipped to arrive within 2 working days of receipt of order
- Notify DCC if the supply falls below 30 bottles

Handling and disposal

- Unused portions of open bottles in the possession of patients should be considered contaminated and handled accordingly

6.16. Study drug dispensing and return (DD and RD)

- Returned capsules should be counted by the pharmacist and/or clinic coordinator and the number of capsules and the number bottles returned, should be recorded on the RD form and the CyNCh Trial Drug Accountability Record
- Expired study drug, partially used study drug, and bottles of study drug returned by patients may be destroyed following your institution's procedures for disposal of investigational study drug once the CyNCh Trial Drug Accountability Record has been audited and approved by the Data Coordinating Center
- Documentation should be recorded onto the CyNCh Trial study drug accountability records to account for all returned study drug as well as its destruction per your institutional guidelines

Storage and stability

- Store at room temperature (68-77 degrees F) in a cool dry place

6.17. Adverse event reporting

Definitions (21 CFR 312.32 IND Safety Reporting). To access CFR 312.32 IND Safety Reporting click on:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Life-threatening adverse event or life-threatening suspected adverse reaction. An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or serious suspected adverse reaction. An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Reportable CyNCh adverse events

- Adverse events should be recorded on the CyNCh Adverse Event Report (AE) data form whether or not thought to be associated with CyNCh or the study drug.
- Any event threatening the integrity of the CyNCh Trial or well-being of the participant (e.g., suspected fraud) is a reportable event. We recognize that this category is not well-defined; however, it is included as a reminder that reportable events can have a broader scope than events that happen to a patient. Some examples include:
  (1) events that impact the patient's treatment or participation in CyNCh;
  (2) adverse events that are recorded on the Follow-Up Medical History (FH) form;
  (3) adverse events that may or may not be related to study drug;
  (4) other events that clinical center staff feel should be reported;
  (5) when a follow-up report is needed for a previously completed Adverse Event (AE) form.
- Deciding whether an event is reportable to CyNCh (i.e., is in either of these categories) will be the responsibility of the Principal Investigator of the clinical center.

- The Data Coordinating Center will maintain a list of adverse events for reporting and review at Steering Committee meetings and DSMB meetings.

CTCAE v3.0

- The NASH CRN uses the Common Terminology Criteria for Adverse Events, (CTCAE v3.0) to specify and grade adverse events.
- This document is posted on the NASH CRN website (www.nashcrn.com – click on Studies and then click on CyNCh)
- Use the CTCAE v3.0 to specify the Short Name for the adverse event and the severity grade for the adverse event. All adverse events should be assigned a severity grade 1-5.
- Adverse events Grade 3 or higher must be faxed to the DCC within 1 week for immediate review by the Safety Officer, Dr. Jeanne Clark.

Local reporting requirements

- Your clinical centers' IRB has reporting requirements of its own regarding events occurring in the course of conduct of a study. These reporting requirements may be more stringent than those adopted by CyNCh. Regardless of what CyNCh requires, you must continue to meet your local IRB's requirements. If the local requirements are more stringent than CyNCh, you may report events locally that you do not report to CyNCh.
- It is possible that some CyNCh patients will develop significant liver-related morbidity or mortality during the course of followup. This information is important and should be documented on the Followup Medical History (FH) form and Adverse Event Report (AE) data form, but it may also be considered a reportable adverse event according to the local institutional guidelines.
- For more information please refer to FDA Guidance for Clinical Investigators, Sponsors, and IRB: Adverse Event Reporting to IRBs – Improving Human Subject Protection: https://jhuccs1.us/nash/closed/ctprot/CyNCh/GuidanceAEreporting.pdf

6.18. Serious adverse event reporting

Definitions (21 CFR 312.32 IND Safety Reporting)

Serious adverse event or serious suspected adverse reaction. An adverse event or suspected adverse reaction is considered **"SERIOUS"** if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the CyNCh study drug caused the adverse event. For the purposes of IND safety reporting**, "REASONABLE POSSIBILITY"** means there is evidence to suggest a causal relationship between the CyNCh study drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction. An adverse event or suspected adverse reaction is considered **"UNEXPECTED"** if it is not listed in the most current cysteamine bitartrate brochure or is not listed at the specificity or severity that has been observed. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Expedited review process

- Adverse events that are judged by the principal investigator to be **SERIOUS**, **UNEXPECTED** and have a **REASONABLE POSSIBILITY** of being caused by CyNCh study drug should be recorded on the Serious Adverse Event/IND Safety Report (SR) form. The SR form should be entered into the data system and faxed to the DCC within 2 business days along with a narrative of the event and a copy of the clinical center's IRB report.

- The narrative should describe the serious adverse event and include History with the CyNCh study drug, any comorbidities, concurrrent medications with doses, and any recent changes hospitalizations or ER visits and justification of how the serious adverse event is determined to be serious, unexpected, and have reasonable possibility of being caused by CyNCh study drug as defined above and in 21CFR312.32.
- The Data Coordinating Center along with the Safety Officer, Dr. Jeanne Clark, will review the materials to make sure that the required information is included. At any time, the DCC may ask for revisions of the supporting documentation and further clarification of the serious adverse event until the amount of information is sufficient.
- The DCC will notify NIDDK of the event within 3 business days of receiving the SR form and submit a preliminary report for further review of the material. At any time NIDDK may ask for additional information or further clarification of the serious adverse event.
- Not every SR form that is submitted will result in an expedited IND Safety Report to the FDA. The final decision will be the responsibility of the NIDDK (sponsor).
- If NIDDK determines that the serious adverse event requires an expedited IND Safety Report, they will notify the FDA within 7 business days of the SR form receipt at the DCC.
- Within 1 month of the initial SR form, the clinical center must submit to the DCC and the NIDDK a follow-up report when:
  (1) serious adverse event is resolved;
  (2) there has been a significant change in the patient's condition;
  (3) in the physician's judgment about the serious adverse event (and periodic updates if needed) to report the details of the disposition of the serious adverse event.
- For more information, please refer to the FDA Final Rule: IND Safety Reporting requirements for Human Drug and Biological Products: http://www.gpo.gov/fdsys/pkg/FR-2010-09-29/pdf/2010-24296.pdf

Data Coordinating Center responsibilities

- The Data Coordinating Center will catalog all serious adverse events for reporting and review at Steering Committee meetings and DSMB meetings.
- The DSMB will review each serious adverse event report and provide comments to the NIDDK project officer and Steering Committee. If requested by any member of the DSMB, a teleconference will be scheduled to discuss the serious adverse events and recommend any actions to the NIDDK.
- If the FDA determines that a change to the investigators brochure, IND or protocol is needed, the Data Coordinating Center will send a copy of the report to all clinical centers, with instructions to forward the report to their IRB. Copies of the report will also be sent to the NIDDK, Data and Safety Monitoring Board (DSMB).

6.18. Serious adverse event reporting

Local reporting requirements

- When you receive a report from the Data Coordinating Center regarding occurrence of an event reportable to the CyNCh trial at another NASH CRN clinical center, you must forward that report to your IRB. It may be that your IRB has no comment on events occurring elsewhere; nevertheless, the notification of your IRB is still a CyNCh requirement.
- Your clinical centers' IRB has reporting requirements of its own regarding serious adverse events occurring in the course of conduct of a study. These reporting requirements may be more stringent than those adopted by CyNCh. Regardless of what CyNCh requires, you must continue to meet your local IRB's requirements. If the local requirements are more stringent than CyNCh, you may report events locally per your IRB guidelines.
- For more information, please refer to the FDA Guidance for Clinical Investigators, Sponsors, and IRB: Adverse Event Reporting to IRBs – Improving Human Subject Protection: https://jhuccs1.us/nash/closed/ctprot/FLINT/GuidanceAEreporting.pdf

6.19. Anticipated adverse events

Essentially all children with NAFLD have metabolic syndrome or overt, previously diagnosed or undiagnosed Type 2 diabetes. Furthermore, advancement of metabolic syndrome and diabetes and emergence of the multitude diabetic complications are typically gradual, ongoing processes. Please consult the cysteamine bitartrate brochure for a complete and current listing of anticipated adverse events: https://jhuccs1.us/nash/closed/cped/CYNCH/InvestigatorBrochure_13Jan12.pdf.

Cardiovascular, Cerebrovascular, and/or Peripheral Vascular:Angina, atherosclerosis, acute coronary syndrome, cardiac ischemia, myocardial infarction, cerebrovascular accident (CVA) or stroke (ischemic or hemorrhagic), cerebral ischemia, transient ischemic attack, claudication, decreased peripheral pulses, and abnormal ankle-brachial index.

Dermatologic: Acanthosis nigricans, foot ulcers, and stasis ulcers.

Endocrine: Hypo- and hyperglycemia, elevated hemoglobin A1C, insulin resistance, dyslipidemia, ketoacidosis, and hyperosmolar hyperglycemia.

Gastrointestinal: Gastric and intestinal dysmotility (related to autonomic neuropathy) and mesenteric ischemia (intestinal atherosclerosis).

Immunologic/Infectious: Infections including foot infections, urinary tract infections (including fungal infections and emphysematous urinary tract infections), superficial fungal infections (oral and/or esophageal candidiasis, onychomycosis, candidal intertrigo, and vulvovaginal candidiasis), zygomycosis (mucomycosis), malignant (necrotizing) external otitis, pyomyositis (primary bacterial infection of skeletal muscle characterized by the formation of one or more intramuscular abscesses), and necrotizing infections of the skin and fascia (which may necessitate surgery and amputations).

Neurologic: CVAs. Diabetic neuropathy and related signs and symptoms including numbness, tingling, decreased sensation, neuropathic pain, and sensory or autonomic neuropathy.

Ophthalmic: Diabetic retinopathy and related signs and symptoms including nerve-fiber layer infarcts (cotton-wool spots), intraretinal hemorrhages, hard exudates, retinal microvascular abnormalities (including microaneurysms, occluded vessels, and dilated or tortuous vessels), macular edema, macular ischemia, neovascularization, preretinal and vitreous hemorrhage (with or without subsequent fibrosis), and traction retinal detachment. Acute and/or chronic vision loss related to diabetic retinopathy may also occur.

Orthopedic: Charcot foot, bony infarcts, and amputations secondary to these complications, ulcers, or peripheral vascular disease.

6.19. Anticipated adverse events

Renal: Diabetic nephropathy and related signs and symptoms including micro- and macroalbuminuria, proteinuria, glucosuria, ketonuria, glomerular hyperfiltration, mesangial expansion, glomerular basement membrane thickening, and nodular glomerulosclerosis (Kimmelstiel-Wilson lesions). Acute and/or chronic renal failure (characterized by rising serum creatinine and BUN levels and/or electrolyte disturbances) and acute and/or chronic worsening of hypertension related to diabetic nephropathy may also occur. These complications may necessitate renal dialysis and renal transplantation

If such an event occurs, appropriate medical care should be provided immediately in the clinic. If a suspected anticipated event is reported by telephone at the time of the event or later, the participant should be evaluated in the clinic by medical staff or referred to an appropriate facility for evaluation and management.

6.20. Procedures for unmasking treatment assignment

- Treatment assignments are unmasked after all data collection for the CyNCh trial has been completed (i.e., after completion of the 24 week post trial followup for all patients)
- Unmasking of study drugs will occur under the following conditions:
  - **Severe allergic reaction (Stevens-Johnson Syndrome)**: Study drugs will be stopped indefinitely. The patient, primary care provider (PCP), local principal investigator and pharmaceutical manufacturer may be unmasked.
  - **Pregnancy during the study**: Study drug will be stopped indefinitely, and the coded medication will be unmasked. The patient, PCP, and investigator will be notified of the assigned treatment and the associated risks of teratogenicity.
- In unforeseen situations where the clinical center principal investigator considers unmasking is in the best interest of the participant's health and well being, unmasking may be done after notifying the Executive Committee.
- The Data and Safety Monitoring Board will review all instances of unmasking that occur.

6.21. Procedures for missed or incomplete visits (MV)

Purpose

- Record data about missed or incomplete visits

Form

- Missed or Incomplete Visit (MV) form

When

- At close of a visit window for any missed follow-up visit or for any follow-up visit with specific forms not completed

By whom

- Clinical Coordinator

Procedures for missed or incomplete in-person visits

- For a missed visit:
  - Date of missed visit is the last date of the visit window
  - Indicate reason(s) for missed visit
- For an incomplete visit:
  - Date of incomplete visit is the date on which a partial set of procedures was performed
  - Indicate reason(s) for missed procedures

6.22. Procedures for patients lost to follow-up

Purpose

- Ascertain vital status of patient
- Document reason(s) patient did not attend visit
- Ascertain if patient is lost to followup

When

- Whenever patient misses a study visit and is difficult to contact

By whom

- Clinical coordinator

Search strategies

- Contact all persons identified on the Patient Location (PL) form
  - Telephone different times during the day/evening
  - Send letter via regular or certified registered mail to determine if patient is still at listed address
- Check current telephone directory for listings both for the patient and the patient's contacts specified on the PL form, e.g., next of kin, health care professionals
- Check post office for forwarding address; ask patient's contacts for forwarding address
- Check obituaries
- Check state vital records

6.23. Procedures for mortality closeout (DR)

Purpose

- Record participant death

Forms

- Complete the Death Report (DR) form

By whom

- Study Physician and Clinical Coordinator

6.24. Medical management of patients and side effects

To keep recommendations and care for participants in the study as uniform as possible, investigators should generally discuss with participants what is laid out in the CyNCh SOP V: Standards of Care Documents for Pediatric patients with fatty liver disease.

Diabetes

Since patients with diabetes may be entered into the CyNCh trial, is is likely that some will develop diabetic-related events. The criteria for diabetes is as follows (Diabetes Care, January 2010, 33: S11-S61):

- Fasting blood glucose test: ≥126 mg/dL (7.0 mmol/L)
- Blood glucose level ≥ 200 mg/dL (11.1 mmol/L) after two hour OGTT (75 g load)
- Hemoglobin A1c measurement ≥ 6.5%

Few adverse events related to study drugs are expected. The most common side effects are abdominal pain, nausea, vomiting, anorexia (loss of appetite), fever, diarrhea, drowsiness, and rash. These side effects will often get better within a couple of weeks of starting cysteamine. Other potential adverse events are those related to blood draws, liver biopsy and MRI procedures. If such an event occurs, appropriate medical care should be provided immediately in the clinic and documented in the study chart.

6.25. Closeout and transferring into NAFLD Pediatric Database 2 Study (CO)

Purpose

- To close out a patient's participation in CyNCh and document the patient's consent to join or re-enter the the NAFLD Pediatric Database 2

Form

- Closeout (CO) form

When

- The Closeout form should be completed at the f76 visit or at the close of the f76 window for all patients randomized in CyNCh.

By whom:

- Clinical coordinator

Instructions

- Ask the patient if he/she consents to re-entering or enrolling in the NAFLD Pediatric Database 2

- Patients willing to re-enter or join the NAFLD Pediatric Database 2 should sign the most recent version of the NAFLD Pediatric Database 2 informed consent approved by your IRB (follow your institutional IRB guidelines for re-consenting participants previously enrolled in the NAFLD Pediatric Database 2.

- Each consenting patient should be scheduled for a NAFLD Pediatric Database 2 follow-up visit approximately 12 months after the date of their CyNCh f76 visit. For patients previously enrolled in the NAFLD Database 2, consult the patient's CyNCh visit schedule (time windows guide) generated at their enrollment and schedule the NAFLD Pediatric Database 2 visit that is open 12 months from the date of their CyNCh f76 visit.

- For patients who were not previously enrolled in the NAFLD Pediatric Database 2, a new NAFLD Pediatric Database 2 visit schedule (time windows guide) will be automatically generated when the CyNCh Closeout form (CO) is keyed into the online database. The new visit schedule will use the CyNCh randomization date as the effective date of enrollment into the NAFLD Pediatric Database 2. Schedule the participant approximately 12 months from their CyNCh f76 visit for their t144 NAFLD Pediatric Database 2 follow-up visit.

- For CyNCh participants who decline to participate in the NAFLD Pediatric Database 2; inform them that the study results and their treatment assignment will be available to them sometime after the close of the CyNCh trial.

7. Forms management

7.1. Clinical center ID codes

Alphabetic IDs

- Alphabetic clinic IDs are used on forms, lists, and tables.
- Alphabetic clinical center IDs are based on the name of the institution with which the clinical center is affiliated.
- Assigned IDs:

Cincinnati Children's Hospital	CINC
Columbia University	CU
Northwestern Univ: Children's Memorial Hospital	NWU
Indiana University	IU
Saint Louis University	SLU
Baylor University: Texas Children's Hospital	BCM
University of California, San Diego	UCSD
University of California, San Francisco	UCSF
Univ of Washington: Seattle Children's Hospital	UW
Emory University	EU

Numeric site IDs

- The NIDDK Genetics and Biosample Repositories use numeric IDs to identify the NASH CRN clinical centers.
- These will be used on the specimens (whole blood and plasma, serum, and liver tissue samples sent to the Genetic and Biosample Repositories, respectively).
- Assigned IDs (satellite centers should use site ID of parent clinic):

Cincinnati	220
Columbia University	828
NWU	222
Indiana University	221
Saint Louis University	223
University of California, San Diego	224
University of California, San Francisco	225
Emory University	227

7.2. Patient identifiers

What

- Patient ID number
- Patient code

Patient ID number

- 4 characters, all numeric
- ID number labels will be distributed to clinics by the Data Coordinating Center
- The ID number for a patient will remain the same for the duration of the NASH CRN, even if the patient enters another NASH CRN study or if the patient fails screening and is subsequently re-evaluated – the ID never changes

Ranges of patient IDs assigned to clinics

Case Western Reserve University	CWRU/CCF/CINC	1001	-	1999
Columbia University	CU	9001	-	9999
Duke University	DUKE/NWU/JHU	2001	-	2999
Indiana University	IU	3001	-	3999
Saint Louis University	SLU/BCM	4001	-	4999
University of California, San Diego	UCSD	5001	-	5999
University of California, San Francisco	UCSF	6001	-	6999
Virginia Mason Medical Center	VMMC/UW	7001	-	7999
Virginia Commonwealth University	VCU/MSCH/EU	8001	-	8999

Patient code

- 3 character alpha code assigned by the Data Coordinating Center and printed on the ID number label
- Each patient code is unique across the NASH CRN

7.3. Visit ID code

- 1 to 3 character alpha-numeric code
- Determined by purpose of visit and timing with respect to visit windows
- Visit ID codes

s	Screening visits
Rz	Randomization
f04	4 weeks follow-up visit (approximately 1 month)
f12	12 weeks follow-up visit (approximately 3 months)
f24	24 weeks follow-up visit (approximately 6 months)
f36	36 weeks follow-up visit (approximately 9 months)
f52	52 weeks follow-up visit (approximately 12 months)
f76	76 weeks follow-up visit (approximately 24 months)
n	Unscheduled visit

7.4. General guidelines for forms completion

Ink

- Forms should be completed in blue or black ink that is dark enough to photocopy legibly.

Changing responses on forms

- If an error is made on the form, correct the response by marking through the response with one or two lines and writing the correct response next to or above the original response. The staff member making the correction should put their initials and the date in the margin by the correction. A brief explanation for the change should also be written in the margin; e.g., 'error', 'pt changed mind', 'wrong response checked'.
- Do not obliterate, erase, or white-out incorrect responses.
- The idea is to preserve an audit trail of the original response and subsequent changes to it.

Multipage forms

- The patient ID number should be written on the top right of every page of every form in the space provided -- protect yourself against ineffective staples and photocopying mishaps.

Miscellaneous

- All written responses should be printed legibly so the responses can be keyed to the database.
- Do not use abbreviations or short-hand that may not be easily understood or keyed in the written responses.
- Numeric data should be recorded in the units prescribed on the form and to the level of precision (number of digits) indicated on the form.
- All numbers should be right justified and leading and trailing zeroes should be recorded on the form where applicable (e.g., an age of 8 would be written and keyed as "08").
- All letter codes should be left justified with the remaining spaces left blank (e.g., a visit ID for the s visit code would be completed and keyed as "s").
- The clinical coordinator should review all responses for completeness and accuracy before signing off on the form.
- Wherever possible, forms should be completed in real time. Interviews and questionnaires should be completed on the actual data form.
- The data on some forms, such as the Laboratory Results form, will be transcribed from worksheets or lab reports, but the visit date on the form should correspond to the date the testing took place.
- Staple relevant lab reports and worksheets to the data form; if your lab reports are transferred to you electronically, print a paper copy of the report and staple the copy to the form.

Calculations

- All calculations should be performed using a calculator.
- Values should be rounded according to the NASH CRN data rounding rule (see section on data rounding rule, later in this chapter of the SOP).

7.5. Instruction box

- Each form includes an instruction box at the top of the first page. This instruction box gives the purpose of the form, when it should be completed, who administers the form, the respondent, and specific instructions for the form.

7.6. Form skips, stops, ineligibility symbols

Skip pattern

- Form navigation (skip pattern) instructions are indicated in **boldface**. Skips are designated by an arrow from that response to a box with the number of the next item to be completed.

Caution sign

- Cautions are designated by a triangle with enclosing a C

Stop sign

- Stops are indicated with an arrow from the response to a stop sign – instructions are given that must be fulfilled in order to continue with the form. For example, Form RG (Registration) asks if the patient has signed the consent form; if the response is "no", the form is stopped with the instructions that *'the consent form must be signed prior to continuing with screening'*.

Ineligibility sign

- Ineligible conditions are designated by an arrow from the response to the symbol:

Other

- Other special instructions are indicated on the form in *italics*. Some examples are:
  – *check only one*: only one of the listed responses should be checked
  – *check all that apply*: one or more of the listed responses may be checked
  – *specify*: a response should be printed on the line(s) provided

7.7. Headers and footers

- Data Collection Forms include headers and footers at the top and bottom of each page, respectively, which identify the form and the patient. The top right of the first page of each form has a space to check when the form is keyed [( )keyed]. The top right of subsequent pages is reserved for the patient ID number. The footers include the form abbreviation, form revision number and date, the form name, and the page number. For example:

CyNCh Patient ID: __ __ __ __

Form RG CyNCh
Revision 1 (07Jan11) RG - Registration Page 2 of 3

- The keyed box should be √ed when the form is keyed; the person keying the form should also date and initial the form by the keyed box.
- The patient ID number should be written on each page of the form.

7.8. Key fields

- The first 7 items of each form include the key fields which identify the clinical center, patient, visit and study

A. Clinical center, patient and visit identification
   1. Center ID: __ __ __ __
   2. Patient ID: __ __ __ __
   3. Patient code: __ __ __
   4. Date form completed: __ __ - __ __ __ - __ __
      day mon year
   5. Visit code: __ __ __
   6. Form & revision: __ __ __
   7. Study: CyNCh 8

- The form and revision number will be printed on the forms in item 6; if a form is only used for one specific visit, the visit code will also be printed on the forms.
- When a form revision affects the data that are collected, the form revision number and date will change; if this occurs, older revisions of that form should no longer be used for data collection.
- If the form is revised without affecting the data collection - i.e., the wording of an item is revised - only the revision date of the form will be changed.

7.9. Missing data

- If a data item is missing and cannot be obtained when the form is completed or reviewed, write the appropriate code in the first left hand space of the empty data field:

  ? = data temporarily missing or inconsistent; to be collected or resolved in the near future; items keyed with a ? will need to be followed up on and resolved
  d = patient does not know the answer
  n = not applicable in this situation
  m = data missing
  r = patient refused

- When using any of the above codes, the entire data field does not need to be filled with the code (e.g., a missing height would be completed as m _ _ _._).

- If data are missing on the form, an explanation for the missing values should be written on the form and keyed to the database in the General Comments section of the keying.

- It is important to keep the number of missing data items at a minimum since resulting conclusions and paper publications depend on complete data, especially at the Baseline Visits.

- If an item is missing at the time the form is filled out, but is expected to be collected in the near future, record "?" rather than "M" code for the item on the form. The "m" code should only be recorded for data that are actually missing. The screening visit windows are broad enough to allow you to collect all data within the allotted time windows.

- If the data system will not accept a value, because it is out of range, please contact the DCC. In the meantime, record "?" rather than "m" on the form.

- If there is a valid reason that a required baseline laboratory value is missing, fax the LR or LS form to the DCC with the reason for the missing value.

- Participants cannot be enrolled until all "?"s keyed on the forms have been resolved.

7.10. Administrative sign off

- Each form contains a section for administrative sign off.
- These items include the Clinical Coordinator PIN and signature and the date the form was reviewed.
- Depending on the form, they may also include the PIN and signature of other staff.

It is the standard of practice with NIH funded studies to certify study physicians who assume responsibility for the accuracy and integrity of the sponsored studies. Coordinators are certified separately based on their professional qualifications and privileges even though the functions fulfilled may overlap functions of the study physician.

On the CyNCh data collection forms that require the Physician's signature, the signature is the assurance that as the clinical center's principal investigator, they are assuming responsibility for the accuracy of the data recorded on the study form. This does not require that the study physician completes the forms or performs the procedures, but does require assumption of responsibility signified by signing the CyNCh forms. This is also the standard of practice required by the FDA for case-report forms completion.

7.11. Handling forms

Form duplication

- The forms will be available on the NASH CRN website.
- You can print master copies from the website and then photocopy as needed or print as needed from the website – if you print copies ahead of time, do not print huge quantities as forms may be revised, especially in the early days of a study.
- The forms will also be available for printing from the data system.
- If a master copy gets frayed or faded, print a new master — always use clear copies for reproduction masters.

Form storage

- Forms for patients registered but not enrolled in CyNCh should be kept in a single folder in a locked room or locked filing cabinet.
- Each patient who is enrolled in CyNCh will have a patient file – either a notebook or file folder which is kept in a locked room or locked filing cabinet. The patient file should contain all CyNCh documents for the patient – consents, forms, appointment schedule, labels, and randomization materials. The forms should be arranged in the notebook or folder chronologically by visit. Tabs can be used to separate the visits.

7.12. Data rounding rules

To round data, examine the digits following the last position required on the form:

- If the first digit following the last data position required for the response is less than 5, leave the digit in the last data position required for the response unchanged, e.g., if you need to round to _._, then 4.73 rounds to 4.7 and 1.44 rounds to 1.4
- If the first digit following the last data position required for the response is 5 or more, round up the digit in the last data position required for the response, e.g., if you need to round to _._, then 4.78 rounds to 4.8 and 4.75 rounds to 4.8

When completing a calculation for CyNCh, apply the rounding rule only at the last step, when required to record a quantity on the CyNCh form.

7.13. Data audits and edits

Data audits

- The Data Coordinating Center will serve as the site monitor.
- The Data Coordinating Center will conduct periodic data audits as a quality control measure.
- Audits may be done by mail or on-site.
- During an audit, the forms will be reviewed to see if they were completed and keyed correctly; the forms will also be checked against the source documents to be sure that values were transcribed correctly.

Source documents include but are not limited to:

- Liver biopsy pathology reports
- MRI reports
- Laboratory test result reports
- Medical records for archival information
- Institutional drug accountability logs
- There are no source documents for questionnaires (the questionnaires are the original documents for the data collection)

Data edits

- Computerized data edits will be sent to the clinics periodically.
- The data edits check for consistency and questionable values in the database.

Changes resulting from audits or edits

- Changes made to the forms as a result of an audit or an edit should be marked "per audit" or "per edit" and should be dated and initialed.

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8. Quality assurance

8.1. Site visits

Purpose

- Conduct an audit of selected patient data
- Review documentation and procedures for CyNCh
- Tour facilities
- Discuss with clinical center personnel any problems that have occurred or that are expected to occur in conducting the study

The following regulatory and study documents should be available or accessible:

- IRB communications organized with original approval letters, revision approvals, annual renewals, serious adverse event forms, and any communications regarding concerns or special requests from clinical center review board
- Signed and dated consent and assent forms for all participants including the date and signature of a witness
- Documents including CyNCh Protocol, PPMs, and SOPs
- Study forms for participants should be available for data audit

Participants

- At least two DCC personnel will attend the site visit. At least one person from another CyNCh clinical center will also attend. Representatives from other resource centers associated with the NASH CRN may also attend.
- NASH CRN certified staff from the clinical center

Reviewed during site visit

- IRB documentation
  - Original approval
  - Annual renewals (if applicable)
  - IRB submissions
  - Approval for updated consent and assent forms and protocol

- Documents
  - Directory
  - SOPs
  - Forms Book
  - PPMs
  - Protocol

- Enrollment and retention
  - Status
  - Recruitment and retention strategies
  - Problems
  - Losses to follow-up

- Personnel
  - Certification status
  - Personnel changes
  - Backup plans for personnel in event of absence

- Clinical management
  - Adverse event reporting procedures
  - Study procedures
  - Clinical center coordination
  - Study drug storage and dispensing
  - Scheduling
  - Clinical center concerns or problems

- Participant files
  - Security
  - Organization
  - Consent statements

- Specimen shipment
  - Comparison of specimens expected and received
  - Shipping procedures and problems
  - Shipping supplies

- Protocol performance
  - Protocol deviations

- Forms and data management
  - Monthly form status reports
  - Source documentation
  - Data audit (selected patients)
  - Eligibility criteria
  - Adverse events
  - Death reports

- Previous site visit report
  - Action items follow-up
  - Data audit follow-up

Site visit followup

- A list of action items is compiled at the end of the site visit to identify items which require further action. The procedure for site visit action item follow-up is:
  - Action items will be listed at the end of the site visit report.
  - Clinical centers will be required to respond to action items within 30 days of receipt of the site visit report. Responses should be in writing and sent to the DCC.
  - The DCC will be required to respond to the action items within 30 days of the completion of the site visit report. The DCC will send a written report to the clinical center.

8.2. Performance monitoring

- The DCC will generate enrollment reports that will provide a count of participants enrolled at each clinical center.

- On approximately a quarterly basis, the DCC will generate reports summarizing the performance of all clinical centers. These reports will include information on enrollment and the percentage of expected visits for which documentation has been entered into the CyNCh data system. Also, for those visits for which data have been entered, the report will show the percentage of missed visits, the completeness of data collection, the timeliness of data entry, and protocol deviations. Performance reports will be reviewed by the Steering Committee, and the Steering Committee will make decisions regarding actions to be taken in the event that a clinical center is performing poorly.

8.3. Data quality surveillance

General procedures

- Quality assurance of data accuracy will occur routinely through three main procedures: data entry checks, monthly checks for completeness and edits, and form audits.

- In addition, detection of problems may occur during data analysis. For example, in preparing reports for Steering Committee meetings, problems may be discovered. Outliers and unusual variations or patterns in the data are examined and may reveal problems.

- Quality assurance of data analysis is achieved by independent replication of key analyses within the DCC and review of reports by multiple individuals before distribution.

Data entry checks

- The data system will contain checks during the data entry process of range, logic, and consistency of items within forms.

- The data system will perform checks between forms to ensure that the same fields entered on different forms match.

- A double data entry system will be used for all forms.

Monthly check for completeness and edits

- On a monthly basis, after receipt of data transmittals from the clinical centers, the DCC will generate a database report of:
  - number of participants enrolled
  - missed visits
  - incomplete visits (missing or pending forms)
  - missed specimen collection or shipment
  - edits (see below)

- Edits are run on the database of the keyed forms monthly. Checks for missing, out-of-range, unusual and inconsistent values, cross-form checks and arithmetic errors are some of the types of checks performed. A listing of edits is sent to each clinical center for resolution. The clinical center must respond to each edit on the listing, make appropriate changes to the forms and database, compute documentation of each change, and file the documentation with the edited data collection form. Items that cannot be corrected (e.g., missing values, unusual measures) are entered into a database at the DCC. These items are excluded from future edits. A hard copy of the edits with each resolution should be kept in a notebook located at the clinical center.

Forms audits

On a periodic (approximately monthly) basis the DCC selects and requests copies of forms for specific participants be sent by each clinical center to the DCC for auditing.

- Audited forms are compared with the database; discrepancies are noted and queried.
- Audited paper forms are also inspected for other problems, which are noted and queried.
- Each clinical center will be required to resolve discrepancies from the audit report and fax the resolutions to the DCC within 5 days.
- The DCC will generate a summary report of the audit discrepancies by clinical center to be distributed to all CyNCh centers.
- Discrepancy rates over time by clinical center are reported to the Steering Committee.

NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

CyNCh
Standard Operating Procedures
Part IV:

Liver Biopsy
and
Histology Scoring System

April 2012

Contents

CyNCh SOP IV: Biopsy and Histology Scoring

1. Overview

1.1. Philosophy

Nonalcoholic fatty liver disease (NAFLD) is defined by hepatic steatosis in the absence of known causes of liver disease and significant alcohol consumption. Nonalcoholic steatohepatitis (NASH) is a subset of NAFLD defined histologically by steatosis, spotty lobular inflammation, and distinctive pattern of zone 3 injury characterized by hepatocellular ballooning; Mallory's hyaline and perisinusoidal fibrosis (psf) may also be present. In some as yet poorly defined fraction of patients, there is progression of disease to cirrhosis and hepatocellular carcinoma. The NASH Clinical Research Network (NASH CRN) is a clinical research network sponsored by the NIDDK to study the natural history of NAFLD in both adult and pediatric patients and to carry out (1) treatment trials of NASH in adult patients and (2) treatment trials of NAFLD in pediatric patients. This document specifies the procedures for liver biopsy and histology scoring for the CyNCh trial which will evaluate whether 52 weeks of treatment with delayed release (DR) cysteamine in children improves NAFLD as measured by changes in histology, compared to treatment with placebo. Procedures for other NASH CRN studies, including the NAFLD Database 2 and FLINT trial, will be specified in separate manuals specific to the particular study.

The procedures specified by the NASH CRN for their studies are based on two concepts: one is the principle that a primary goal of the NASH CRN is to collect a database of specimens along with clinical and laboratory data, so that this information remains available for research after the NASH CRN has disbanded. The other concept behind these procedures is the necessity of a system of evaluation of NASH that can be utilized in evaluating efficacy in treatment trials. Because there are no serum markers or other diagnostic modalities that can accurately stage or grade the liver disease in these patients, a liver biopsy remains the standard against which all other methods will be compared. Every effort should be made to build a liver tissue archive and slide archive that are as complete as possible, so that what is accomplished will have value for the future as well as the present. With this principle in mind, study consent statements should include language that permits such approved uses of liver biopsy materials without the need for re-consent at a later date; patients who refuse access to their liver biopsies or who refuse to provide specimens for banking should not be enrolled in the NASH CRN.

1.1. Philosophy

Histologically confirmed NAFLD is an inclusion criterion for the CyNCh trial. The baseline biopsy may have been done prior to screening (within specified time limits) or it may be done as standard of care as part of the screening procedure. CyNCh patients will also have a followup biopsy after 52 weeks of treatment in the trial. Unscheduled biopsies also may occur after screening. Ideally, the CyNCh trial will obtain a piece of liver tissue for banking and 10 unstained slides for archiving from each of these biopsies. However, because some of the biopsies evaluated for CyNCh may not provide these materials (eg, not enough tissue is obtained or the paraffin block is exhausted), contingency plans have been developed, including requesting to borrow the institutional slides and returning them after central scoring has been completed.

It should be emphasized that a surgical pathology report alone is not sufficient for satisfying inclusion/exclusion criteria related to liver histology nor for comparison with other biopsy data. Tissue slides must be available for review and must be judged by the CyNCh pathologist to be adequate for scoring according to the NASH CRN scoring protocol. However, a copy of the surgical pathology report must be obtained for all slides. The surgical pathology report serves two purposes: (1) to verify and document the identity of the patient whose tissue is on the slides that are being evaluated and (2) to provide the date of the biopsy.

1.2. Tasks and forms related to liver biopsy

The CyNCh eligibility criteria include histologic evidence of NAFLD according to the NASH CRN protocol for histology scoring. The biopsy that is used to satisfy eligibility may be a historical biopsy (done in the 120 days prior to enrollment and the patient must not have used specified medications in the 90 days prior to the biopsy) or it may be done prospectively under the care of the CyNCh investigator as a screening procedure. Each randomized patient will have another biopsy after 52 weeks of treatment. In addition, a patient may have an interim biopsy as needed for standard of care. As a check to be sure that all biopsies that occur are caught, occurrence of a biopsy since the previous CyNCh visit is queried on the Followup Medical History (HI) form.

Information about the biopsy procedure and materials is captured on the Liver Biopsy Materials Documentation (SD) form. Cautions about the use of proscribed medications in the 90 days prior to the biopsy used for eligibility screening are noted on the Baseline History (BH) form; lack of use of proscribed medications is confirmed on the Liver Biopsy Materials Documentation (SD) form. The SD form also documents the outcome of the biopsy with regard to availability of tissue for banking and availability of stained and unstained slides for scoring and archiving. If tissue was obtained for banking, the Liver Tissue Banking (LT) form must be completed. If the biopsy was a screening biopsy (ie, done/evaluated to determine eligibility for CyNCh), then the local CyNCh Study Pathologist must complete the Liver Biopsy Histology Worksheet (HW) form. If the NAFLD activity score (NAS) for the screening biopsy is less than 4, then the patient is not eligible for CyNCh but may be an appropriate candidate for the Pediatric Database 2 Study. Other forms that the CyNCh trial uses to document activities and materials related to liver biopsy are the Liver Biopsy Histology

1.2. Tasks and forms related to liver biopsy

Findings (HF) form and Central Histology Review (CR) form and logs for shipping tissue and slides (forms SS and TS). In summary, these seven forms (SD, LT, HW, HF, CR, TS, SS) are used to:

- Document what slides, if any, are available for scoring and sending to the DCC and whether liver tissue was obtained for banking (form SD)
- If the biopsy is the screening biopsy, document lack of use of proscribed medications during the 90 days prior to the biopsy (form SD) and remind the clinical center that the screening biopsy cannot be older than 120 days at the time of randomization
- If liver tissue was obtained for banking, document collection of extra liver tissue and procedures for banking (form LT)
- Document local scoring of baseline biopsies (forms HW and HF)
- Document shipment of slides to the DCC (form TS)
- Document shipment of liver tissue in RNA*later*® Solution to the Biosample Repository (Form SS)
- Document scoring of baseline and followup biopsies by the NASH CRN Pathology Committee (form CR)

The CyNCh hepatologist, the Study Pathologist, and the Clinical Coordinator must work together to accomplish these tasks and complete the required forms. Considerable cooperation and close communication will be required to complete these tasks successfully.

2. Obtaining liver biopsy materials for scoring for CyNCh

2.1. Overview

Baseline (screening) liver biopsy specimens can be divided into two categories: (1) those performed prior to consent for screening, and (2) those performed as part of standard of care during the screening process, but after consent for screening for CyNCh has been obtained. In the case of (1), we will try to obtain 10 unstained slides for the exclusive use by CyNCh, but limited biopsy materials may require that the institution's biopsy slides be borrowed. In the case of (2), sufficient tissue should be collected to provide for the institutional pathology slides AND 10 unstained slides for CyNCh exclusive use AND for banking a sample at the Biosample Repository. Followup liver biopsies should all be in category (2).

Baseline biopsies will be scored by the local CyNCh Study Pathologist (to determine eligibility) and also centrally (after randomization) by the Pathology Committee. Biopsies obtained 52 weeks after randomization will be scored centrally only by the Pathology Committee. Unscheduled biopsies will be read locally for standard of care and will also be scored centrally by the Pathology Committee.

2.2. Baseline biopsies performed prior to consent for screening

Because these biopsies were obtained prior to consent for CyNCh screening, investigators may have little control over variables such as fixation or biopsy size, but these considerations should not prevent investigators from trying to obtain unstained slides for CyNCh purposes. However, it is an unfortunate fact that the amount of tissue available in archived paraffin blocks may be very limited.

Upon learning that a patient had a recent biopsy and after checking that no proscribed medications were used in the 90 days prior to the biopsy, the Clinical Coordinator should obtain the original pathology materials: the surgical pathology report, the institution's H&E and Masson's trichrome slides, and the paraffin block so that 10 unstained slides may be obtained (see below for how to prepare and label those slides).

Upon receipt of the original pathology materials, the Clinical Coordinator should verify that all materials pertain to the CyNCh patient they are said to and should annotate the surgical pathology report with the patient's NASH CRN ID number and code and black out the patient's name. The annotated report should be attached to the Liver Biopsy Materials Documentation (SD) form and a copy of the annotated report must be sent to the DCC when the biopsy slides are sent to the DCC. The Clinical Coordinator and/or Study Pathologist must determine the following so that form SD may be completed:

2.2. Baseline biopsies performed prior to consent for screening

- Confirm that no proscribed medications were used in the 90 days prior to the biopsy (baseline biopsy only)
- Determine the number and type of the institution's stained slides available for the local review of the biopsy and determine if any stained slides need to be sent to the DCC; if fewer than 2 unstained slides are available for sending to the DCC, the DCC must be sent the institution's stained slides
- If the DCC will be sent stained slides, label those stained slides with NASH CRN slide labels for stained slides (which are not the same as the labels for unstained slides); transcribe the slide sequence numbers (printed on the slide label) to form SD
- If the DCC will be sent stained slides, determine if CyNCh is borrowing the stained slides from the institution or if CyNCh is taking possession of the stained slides; if borrowing the stained slides, determine the date by which the slides need to be returned to the institution and note the source of the slides; record this information on form SD
- If unstained slides are obtained, label them with the NASH CRN slide labels for unstained slides (which are not the same as the labels for stained slides); transcribe the slide sequence numbers from the slide labels to form SD

The Study Pathologist should complete the Liver Biopsy Histology Worksheet (HW) form using the institution's H&E stained slide and Masson's trichrome stained slide, as described later in this document. The Clinical Coordinator will transcribe the data from the Histology Worksheet to the appropriate HF form. If the NAFLD activity score (NAS) is 3 or less the patient is ineligible for CyNCh, but may be eligible for the Pediatric Database 2 Study.

If there is no H&E stained slide or if there is no Masson's trichrome stained slide, the biopsy is insufficient for evaluation for entry into CyNCh.

If only the H&E and Masson's trichrome slides are available (i.e., no unstained slides available), these should be reviewed locally. If the patient is found to be eligible, these slides will need to be sent to the DCC for central review by the Pathology Committee. Both of these slides must be available for central review for the patient to be found eligible for CyNCh.

The CyNCh trial should request that the slides be provided outright, with no arrangements to return the slides at the end of the trial. In the event that any of the slides from a prior biopsy need to be returned to the original laboratory, the first plan should be for those slides to be returned at the end of the trial. If that is not acceptable to the original pathology laboratory, the slides will be returned to the original pathology laboratory after the central review is completed.

Slides for patients who are not randomized in CyNCh should be returned to the original pathology laboratory upon determination that the patient will not be randomized.

2.3. Baseline and followup biopsies performed after consent for screening - biopsy procedure

Each clinical center investigator will notify their CyNCh Study Pathologist (e.g., via local institutional requisition sheet) when a biopsy is performed on a CyNCh patient so that when the block is initially cut for the local institution's requirements, the CyNCh trial's additional 10 unstained slides can be cut at the same time. This will minimize the chances of loss of tissue with refacing the block.

In order to insure adequate material for histologic review, investigators should obtain needle core biopsies of at least 2.0 cm length using a 16 gauge or greater needle. If there is adequate tissue beyond 2.0 cm, the extra tissue may be stored in RNA*later*® Solution and banked at the Biosample Repository.

2.4. Preparation of slides

Biopsy tissue for slides should be placed in buffered formalin as soon as possible; investigators should use whatever formaldehyde solution is used by the site's pathology laboratory.

If this is a baseline biopsy, each clinical center will obtain locally stained H&E and Masson's trichrome slides as part of their standard institutional pathology materials; these will be read by the local CyNCh Study Pathologist for the local evaluation (i.e., for completion of form HW).

For both baseline and followup biopsies, the local laboratory should cut 10 additional slides (additional to what is cut for the institutional pathology needs) at the same time as the institutional slides are obtained in order to decrease the chances of loss of tissue with refacing the block. The local NASH CRN Study Pathologist should advise on the selection of a maximum of 10 unstained slides to be submitted to the Histology Review Center at the DCC for banking if there are (1) more than 10 unstained slides available or (2) if the unstained slides were cut from different tissue blocks obtained from the same liver biopsy core(s). The NASH CRN Pathology Committee's recommendation is that all unstained slides submitted come from the same, single liver biopsy core when possible. Centers should be careful not to submit unstained slides with insufficient or fragmented biopsy tissue since it is not possible for DCC staff to sort those out before sending the slides for central staining.

Tissue should be placed on pre-cleaned, "charged" slides to allow for possible future use with immunohistochemistry. The specifications for the recommended brand of slides are:

SuperFrost Plus slides, Precleaned
Distributor: Fisher Scientific
Catalog No.: #12-550-15
Size: 25/75/1.0 mm
Estimated cost: $133.07 per gross (144 slides/gross); $1,118.24 per case of 10 gross
Tele: 1-800-766-7000

Coverslips should not be placed on the slides. Slides should be labeled with the patient specific preprinted NASH CRN labels for unstained slides. The 10 (or as many that can be obtained up to 10) uncoverslipped, unstained slides will be forwarded to the DCC for the purposes of central staining, review, and archiving.

2.5. Labeling stained and unstained slides at the clinical center

Each of the slides (stained and unstained) sent to the DCC must be labeled with a NASH CRN slide label. Two types of slide labels are used in the NASH CRN:

(1) permanent labels are used for unstained slides; these labels can withstand the chemicals used during staining
(2) removable labels (overlabels) for stained slides that are borrowed from an institution.

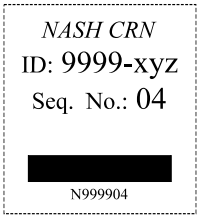
The requirements for the labeling scheme for slides are:

- Each slide must be uniquely identifiable
- The unique identifier for the slide has to be built from information that can be specified when the label is printed
- The slide label must be able to be read by human eyes
- Labels for unstained slides must be resistant to solvents used in the staining process and to over-labeling by interim processes (e.g., when sent for central staining)
- Labels for borrowed stained slides must be removable since those slides must be returned to the original pathology laboratory

The solution to these requirements is to use Brady labels in the format shown below: (1) labels for unstained slides and (2) labels for stained slides which are borrowed.

2.5. Labeling stained and unstained slides at the clinical center

(1)



(2)

Slide labels are provided to each clinical center in preprinted form, 1 set of labels per patient ID, by the DCC. Each set includes labels for unstained slides and labels for stained slides.

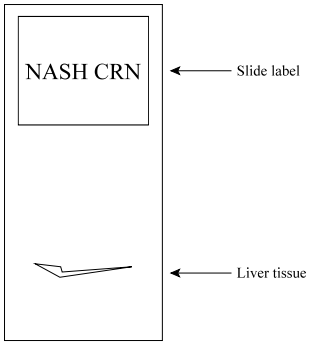
The unique identifier for a slide will consist of the patient ID number and a sequential number. The sequence numbers on the labels for unstained slides run from 01 to 60 (60 is the maximum number of unstained slides that we think we will ever get for any one patient over the life time of all NASH CRN studies). The sequence number on the labels for stained slides run from 81 to 90 (10 is the maximum number of stained slides that we think we will ever borrow for any one patient over the lifetime of all NASH CRN studies).

The slide labels include the following information:

- NASH CRN (printed on label)
- Patient ID number (4 digit number printed on label) and patient code (3 character alphabetic code printed on label)
- Slide sequence number (printed on label)
- Bar code constructed from N (for NASH CRN), 4 digit ID number, and 2 digit slide sequence number

Permanent labels should be positioned squarely at the top end of the slide, on the same side that the liver tissue is on and over the white part of the glass, with no edges hanging off the slide. Overlabels should be placed on the back of the slide, directly on the glass and opposite the existing

label. Both permanent labels and overlabels should be positioned so that the print is oriented vertically with the narrow end of the slide (see diagram below).



2.6. Liver tissue for banking at Biosample Repository

The extra piece of liver tissue (minimum 1-2 mm or greater) will be stored in RNA*later*® Solution as follows:

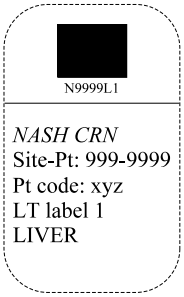
Labeling Procedures

- Apply pre-printed label provided by DCC to the cryogenic vial according to the following steps:

  - Attach the label to the vial when the vial is at room temperature
  - Leave the cap on the vial when labeling to ensure the inside of the vial remains sterile
  - Position the label on the vial so that the long edge of the label is parallel to the floor when the vial is held in an upright position; the label should not trail off the bottom of the vial or over the cap
  - While holding the vial in an upright position, affix the colored (white) portion of the label to the vial first
  - Wrap the clear tail around the perimeter of the vial; the end of the clear tail should overlap the colored portion of the label
  - Press firmly on the entire label; verify that all edges of the label adhere to the vial
  - When possible, allow newly labeled vials to set at room temperature for several hours prior to subjecting them to colder temperatures (24 - 48 hours is optimal)

2.6. Liver tissue for banking at Biosample Repository

- The liver vial labels have the following format:



- The vial used for banking extra liver tissue should be a 2.0 mL polypropylene cryogenic vial (13.5 mm wide x 48.3 mm tall) that is self-standing and externally threaded vials and silicone washers. This vial is designed for ultra low temperatures (-196 ° C, liquid nitrogen vapor) (supplier: CIC, catalog number 5012-0020; phone 877-738-8247)

- Preferably within one minute and no more than five minutes after biopsy, place the liver tissue into the vial, pre-filled with approximately 1 mL of RNA*later*® Solution. If the sample is not placed in RNA*later*® Solution within 5 minutes discard the sample. Refrigerate the cryovial at 4° C overnight to allow thorough penetration of the liver tissue and then transfer to -70° C freezer for storage.

- RNA*later*® Solution may be ordered online at http://www.ambion.com/catalog/catnum.php?AM7020. The catalog number for 100 mL of Ambion RNA*later*® Solution cat#AM7020 and sells for $96.

- Complete the Liver Tissue Banking (LT) form; the duplicate liver vial label should be attached to the LT form

- Make sure you use the "Cryovial" and "LT form" labels from the same set (i.e.., with the same sequence number)

- Complete the Specimen Shipment Log (SS) form. In the NASH CRN Excel shipment file under column J, enter "R"

- Batch ship cryovials to the NIDDK Central Biosample Repository on Monday, Tuesday or Wednesday; after refrigeration overnight at 4° C, store temporarily in -70° C freezer at the clinical center until the next batch shipment to Fisher Bioservices.

3. Development of the histology scoring system

3.1. Background

The NASH CRN has designed and validated a feature scoring system that can be used for the spectrum of NAFLD, both to correlate histologic features with clinical and physiologic measurements and to document changes in liver tissue observed over time in patients in natural history studies and clinical trials.

3.2. Methods and validation

Pathologists from the NIH and from the 8 clinical centers participating in the NASH CRN met in March 2002 to discuss the features to monitor and the criteria for scoring these features. Each study pathologist was asked to contribute cases of adult and pediatric liver biopsies. The cases were chosen from patients referred for consideration of NASH who had been histologically diagnosed as either diagnostic, borderline/suspicious, or not diagnostic of NASH. The cases were to have no confounding liver diseases. Subsequent to the March 2002 meeting and after criteria for the scoring system were established based on the discussions at the meeting, additional cases were requested from each center's pathologist. The original and newly received cases were anonymized and were assembled into what is called the anonymized study set. This set consists of 50 cases (32 adult, 18 pediatric) with H&E and Masson stains for each case.

During the summer and fall of 2002, the anonymized study set was circulated to each study pathologist for review to establish agreement for features and for diagnosis according to the three categories of definite, borderline, and not NASH. The adult cases were scored by each pathologist twice and the pediatric cases once.

Weighted group and pair-wise Kappa statistics were used to evaluate inter- and intra-rater agreement. Inter-observer agreement was assessed for both adult and pediatric cases and intra-observer agreement was determined using the adult cases.

Cochran's Chi-square test for trend and GEE logistic regression were used to analyze univariate and multivariate relationships between feature scores and the diagnosis of NASH. Adult cases were analyzed separately from pediatric cases.

3.2. Methods and validation

Fourteen features of NAFLD were evaluated in the scoring system. Four of the features were evaluated semi-quantitatively:

- Steatosis percent (0-3)
- Modified Brunt criteria for fibrosis (0-4); the modification, specifically, was subdivision of stage 1 into 1a, 1b, and 1c:
  - 1a: zone 3 perisinusoidal fibrosis that does not require a trichrome stain
  - 1b: zone 3 perisinusoidal fibrosis that does require a trichrome stain
  - 1c: portal fibrosis only

  The remainder of the fibrosis scoring used Brunt criteria.
- Lobular inflammatory foci/20x (0-3)
- Hepatocellular ballooning (0-2).

Location of steatosis was recorded but not scaled. An additional 9 features were recorded as either present or absent.

Weighted group kappa statistics for the inter-rater agreement on adult cases were 0.84 for fibrosis, 0.79 for steatosis, 0.56 for hepatocellular ballooning and 0.45 for lobular inflammation. Agreement on the diagnosis of NASH had a kappa of 0.61. Kappa values for intra-rater agreement were higher for all features, while inter-rater agreement on pediatric cases was generally lower. By multivariate analysis 5 features independently correlated with the diagnosis of NASH in adults: hepatocellular ballooning ($P<0.0001$), perisinusoidal fibrosis ($P=0.0009$), lobular inflammation ($P=0.002$), steatosis ($P=0.004$) and acidophil bodies ($P=0.02$).

In summary, we have devised a feature scoring system for NAFLD and NASH that has inter- and intra-rater agreement similar to other semiquantitative scoring systems for chronic liver disease. The pediatric NAFLD features were not as reproducible as those of adults, possibly due to the presence of varied patterns of injury. In accordance with the idea that NASH is a pattern of injury comprised of several features, this scoring system demonstrates that a firm diagnosis of NASH correlates with the degree of steatosis, characteristic pattern of fibrosis, lobular inflammation, and hepatocellular ballooning. Based on this evaluation, the NASH CRN will use this system to evaluate liver biopsies for features of NAFLD.

4. Evaluation at the clinical center (for forms HW and HF)

4.1. Introduction

The local site CyNCh Study Pathologist will evaluate baseline biopsies only, for eligibility determination only. CyNCh patients must have histologically confirmed NAFLD with a NAS ≥4.

The local site CyNCh Study Pathologist should make his/her evaluation using the locally stained H&E slide (all features except fibrosis) and Masson's trichrome slide (fibrosis only) and should complete the Liver Biopsy Histology Worksheet (HW) form. The information on the HW form will be transcribed to the study-specific HF form by the clinical coordinator. A copy of the HF and HW forms are included at the end of this document for your information; please obtain blank forms for completion for a patient from the study website (https://jhuccs1.us/nash) or from the Clinical Coordinator.

4.2. Guidelines for features scored in the local evaluation

The following guidelines are provided for uniformity of reading among the CyNCh pathologists. The system is based entirely on H&E stain for all lesions except fibrosis, and on Masson's trichrome stain for fibrosis.

4.2.1. Length of biopsy

The tissue is measured (mm) on the glass slide prior to reading the slide.

4.2.2. Steatosis grade (0-3; 4x or 10x)

0: <5%
1: 5 -33%
2: 34 - 66%
3: > 66%

In the recognition that (1) deciding on less than 5% can be challenging and (2) that this really is a significant cut-off for allowing patients into long-term follow-up studies such as the NASH CRN is conducting, we think it is best to err on the side of grade 1 rather than grade 0 when faced with a borderline case. Evaluation is best at 4x or 10x at most.

4.2.3. Steatosis location

Steatosis location has 4 choices for characterization:

Zone 3: distribution means not panacinar and not strictly zone 1
Zone 1
Azonal: this pattern is the random scattered macrosteatosis
Panacinar: implies severe steatosis that appears to involve the whole acinus equally, rather than the more random azonal pattern

4.2.4. Fibrosis stage (0-4; requires Masson's trichrome slide)

0: None
1a: Zone 3, perisinusoidal fibrosis (requires trichrome)
1b: Zone 3, perisinusoidal (easily seen on H&E; verified on trichrome)
1c: Portal/periportal only
2: Zone 3 and periportal, any combination
3: Bridging
4: Cirrhosis

4.2.5. Portal chronic inflammation (0-1)

0: None to minimal
1: Mild
2: More than mild

4.2.6. Lobular inflammation (0-3; 20x)

Amount of lobular inflammation combines mononuclear, fat granulomas, and polymorphonuclear (pmn) foci.

0: None
1: < 2 / 20x mag
2: 2-4 / 20x mag
3: >4 / 20x mag

4.2.7. Hepatocellular ballooning (0-2)

0: None
1: Few
2: Many

4.2.8. Steatohepatitis diagnosis

At some point in your evaluation of the case, decide how you would sign this case at your institution, using whatever criteria you currently use to make a diagnosis of steatohepatitis. The question asked is "Is steatohepatitis present?" and the choices for response are:

0: Not NAFLD
1: NAFLD, but not NASH
2: Suspicious, Zone 3 pattern (1A)
3: Suspicious, Zone 1 pattern (1B)
4: Yes, definite steatohepatitis

This choice has to be made based on the H&E and Masson's trichrome slides, even if you have access to other stains (such as ubiquitin immunohistochemistry).

4.2.9. Exclusion of other liver disease and other features

- Primary biliary cirrhosis
- Wilson's disease
- Chronic cholestatic liver disease
- Inflammation suggestive of AIH, HCV
- Pigment suggestive of HH
- Globules suggestive of A1AT
- Hepatocellular changes suggestive of HBV
- Granulomas suggestive of sarcoid, PBC, infection

Diagnosis of primary biliary cirrhosis, Wilson's disease, bile duct obstructions, primary sclerosing cholangitis, autoimmune liver disease, HCV, hemochromatosis, A1AT deficiency, and HBV by the study physician are exclusionary; these diagnoses are marked with Caution symbols on the HF form.

4.2.10. NAFLD Activity Score (NAS)

The NAFLD Activity Score (NAS) is a composite score that is calculated from the steatosis grade (0-3), the lobular inflammation grade (0-3), and the hepatocellular ballooning score (0-2); the scores for these three components are summed. The NAS may range from 0 through 8. Patients with

a NAS of 0-3 on screening are ineligible for CyNCh, but may be considered for the Pediatric Database 2 Study.

4.2.11. Comments

This item is for any comments you want to share with the rest of the group on the case such as comments on unusual features or on a difficult scoring choice.

4.3. Unscheduled liver biopsy

Unscheduled biopsies (i.e., biopsies done after screening and at a followup time other than 52 weeks after randomization) will be evaluated locally for standard of care and also centrally by the Pathology Committee. Forms HW and HF will not be completed for unscheduled liver biopsies, but the biopsy slides should be obtained and the SD form should be completed. Form CR will be completed upon central review by the Pathology Committee. The CR form will use visit code n or the visit code for the visit that was open when the biopsy was obtained.

5. Central pathology evaluation (for Form CR)

5.1. Procedures

Each pathology review session will include attendance by at least three Pathology Committee members and will include at least one of the co-chairs. Central reviews are planned for every 3-4 months. Review sessions will last 1 - 2 days. One or two DCC members will attend each central review to complete the Central Pathology Review (CR) form for each biopsy reviewed. These completed data forms will be keyed at the DCC.

Following central review, slides will be returned to storage at the DCC. Slides that are only loaned to the CyNCh trial for a specified time will be returned to the clinical center at the required time. Slides will be packed and shipped for return to the clinical center as specified later in this manual.

5.2. Documentation of which slides were used for evaluation

The sequence numbers of the slides viewed will be recorded on the CR form for each case.

5.3. Guidelines for features scored in the central evaluation

The following features are evaluated both centrally and locally, and the guidelines to be used for the central evaluation of these features are the same as the guidelines used for the local evaluation:

- Steatosis grade
- Steatosis location
- Fibrosis stage
- Lobular inflammation
- Portal chronic inflammation
- Hepatocellular ballooning
- Cirrhosis diagnosis
- Steatohepatitis

Steatohepatitis diagnosis and amount of portal inflammation are scored both centrally and locally, but the scoring of these features for the central evaluation differs from the scoring for the local evaluation.

Guidelines for features scored only in the central evaluation or scored differently in the central evaluation from the local evaluation are described below.

5.3 Guidelines for features scored in the central evaluation

5.3.1. Steatosis

5.3.2a Types of Macrovesicular steatosis

0: Predominantly large droplet macrovesicular steatosis
1: Mixed large and small droplet macrovesicular steatosis
2: Predominantly small droplet macrovesicular steatosis

5.3.2b Microvesicular steatosis, continguous patches

0: Absent
1: Present

The definition agreed on was the pathologists' definition of microsteatosis: foamy appearance to the cytoplasm, nucleus centrally located. Remember, this is not the small droplet type of "macro" steatosis that does not completely fill the cell. Microsteatosis typically needs ORO for confirmation in routine practice.

5.3.2. Microgranulomas seen (yes/no)

5.3.3. Large lipogranulomas seen (yes/no)

5.3.4. Ballooning

5.3.4a: Severe ballooning present

0: No
1: Yes

A score of "severe ballooning present" should be made if large, classical balloon cells are seen from low magnification in multiple areas throughout the biopsy. The biopsy should already have a score of "2" for ballooning to qualify for severe ballooning.

5.3.4b: Classical balloon cells present

0: No
1: Yes

Classical balloon cells are ones that are easily recognized at low to medium magnification, stand out from the surrounding parenchyma and have cytoplasm that is clumped. They may have Mallory-Denk bodies. A positive score requires only one classical balloon cell.

5.3.5. Pigmented macrophages (Kupffer cells)

0: Rare/absent
1: Many

5.3.6. Megamitochondria

0: Rare/absent
1: Many

5.3.7. Mallory - Denk bodies

0: Rare/absent
1: Many

5.3.8. Glyogen nuclei

0: Rare/absent
1: Present in patches

5.3.9. Glycogenosis of hepatocytes

0: Not present
1: Focal, involving less than 50% of the hepatocytes
2: Diffuse, involving more than 50% of the hepatocytes

5.3.10. Fibrosis

5.3.10a: Perisinusoidal fibrosis grade

0: No perisinusoidal fibrosis present
1: Perisinusoidal fibrosis present that requires a Masson stain to identify
2: Perisinusoidal fibrosis present that is visible on the H&E stain

Note that stage 1A fibrosis would automatically get a score of 1 and stage 1B fibrosis would automatically get a score of 2 on this scale.

5.3.10b: Predominant location of fibrosis (scored only if fibrosis stage is 1A, 1B, 1C, 2 or 3)

0: Fibrosis is more prominent around or between portal areas
1: No portal or central predominance to the fibrosis
2: Fibrosis is more prominent around or between central veins

Note that stage 1A and 1B fibrosis would automatically get a score of 2 and stage 1C fibrosis would automatically get a score of 0 on this scale.

5.3.11. Iron: hepatocellular grade (0-4)

0: Absent or barely discernible, 40x (skip hepatocellular iron distribution)
1: Barely discernible granules, 20x
2: Discrete granules resolved, 10x
3: Discrete granules resolved, 4x
4: Masses visible by naked eye

5.3.12. Iron: hepatocellular distribution

0: Periportal
1: Periportal and midzonal
2: Panacinar
3: Zone 3 or nonzonal

5.3.13. Nonhepatocellular iron grade (0-2)

0: None (skip nonhepatocellular iron distribution)
1: Mild
2: More than mild

5.3 Guidelines for features scored in the central evaluation

5.3.14. Nonhepatocellular iron distribution

0: Large vessel endothelium only
1: Portal/fibrosis bands only, but more than just in large vessel endothelium
2: Intraparenchymal only
3: Both portal and intraparenchymal

5.3.15. Comments

General comments on the biopsy can be recorded by the Pathology Committee.

6. Shipping slides to the Data Coordinating Center

6.1. Packing and shipping slides

The steps in shipping slides to the Data Coordinating Center are:

- Complete the Histology Slide Transmittal Log (Form TS), specifying the number of stained and unstained slides for each patient included in the shipment; specify the date and visit code for the slides for each patient included in the shipment; make a copy of the completed TS form for your clinic records and include the original in the shipment
- Attach a copy of the surgical pathology report for each slide set included in the shipment; make sure that each report is annotated with the patient's NASH CRN ID number and ID code and that the patient's name has been blacked out
- Packing
  - Pack slides in standard slide shipping boxes, which hold 25 slides (VWR International catalog number 82003-418, HS15989A (blue), 82003-420 (green); phone 800-932-5000)
  - Use tissue paper or something similar, inside the box, to prevent the slides from moving during shipment
  - Seal the box shut with tape
  - Wrap the box in bubble wrap
  - Place the bubble wrapped box inside a padded jiffy bag. Place the jiffy box and pathology reports and Form TS in a Federal Express box; stuff the Federal Express box as needed with newspaper or other packing material so that the bubble wrapped box does not move around
  - Ship second day arrival to:

    Pat Belt
    NASH CRN Data Coordinating Center
    615 North Wolfe Street, Room W5010
    Baltimore, MD 21205
    410-614-6021

    You may bill the shipment to the DCC's slide shipment Federal Express account (#2991-6250-8)
  - Notify Pat Belt to expect the shipment (email pbelt@jhsph.edu or fax 410-955-0932)

6.2. Receipt of slides at the Data Coordinating Center

When slides are received, staff at the Data Coordinating Center will:

- Notify the clinic that the shipment was received
- Review the shipment for consistency with the accompanying shipping log, for damage, for appropriate labeling and other quality control items
- Log the slides into the DCC slide inventory system and designate a storage location
- Send slides for central staining as appropriate (currently, 3 stains are planned)
- If the received slides are not adequate for evaluation, the institution's slides may be requested and used to evaluate a biopsy

6.3. Returning slides to the clinical center

- Log the slides out of the DCC slide inventory system
- Complete a shipping log for return of slides to the clinical center
- Pack up slides for return to the clinical center
- Notify the clinical center of the impending return of the slides
- Obtain confirmation of receipt of the returned slides from the clinical center

7. Appendices

NASH CRN

Nonalcoholic Steatohepatitis Clinical Research Network

CyNCh

Standard Operating Procedures

Part V

Standard of Care for Children with Fatty Liver Disorders

November 2012

1. Introduction

The purpose of this document is to articulate a uniform set of practices to be applied by investigators in the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) to the care of pediatric patients with fatty liver disorders. As directed by the NASH CRN Steering Committee, these standards were developed so that pediatric patients with fatty liver disorders will receive uniform treatment across study sites, thereby reducing the extent to which care at a particular site will independently influence outcomes. These standards of care were derived by expert opinion, specifically the opinions of the NASH CRN investigators, and as such cannot and should not be construed to mean that they should be applied outside of the NASH CRN clinical centers or that the failure to apply these standards is a breach of community standards of care.

The NASH CRN Standards of Care Committee developed these standards in their initial draft form after the first meeting of the Committee on July 29, 2002 in Baltimore, MD. After review and revision, it was submitted to the NASH CRN Steering Committee and approved in principal at its meeting on September 22, 2002 in Atlanta, GA. The document was revised for the continuation of the NASH CRN in 2009 and approved by the Steering Committee in 2009. Committee members are aware that application of the standards described here could be viewed as an intervention in itself. However, the committee also felt that these standards are essential to fulfill our obligation to provide the best care to the participating pediatric patients.

2. Specific recommendations

2.1. Evaluation of patients with suspected NASH

a. Obtain the following**:**
   i. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), fasting lipid profile, insulin and glucose
   ii. Alpha-1-antitrypsin (A1AT) level, ceruloplasmin, and hepatitis B and C panels will be obtained. Auto-antibody studies for autoimmune hepatitis (AIH), antinuclear antibody (ANA), antismooth muscle antibody (ASMA), and antibody to liver-kidney microsomal antigen (anti-LKM1) may be drawn at the discretion of the study physician.
   iii. Imaging study (ultrasound, CT scan, or magnetic resonance image) to evaluate fat in the liver

b. Liver biopsy can be considered in patients with suspected NAFLD to confirm the diagnosis and stage the degree of injury. Imaging and degree of abnormality in serum aminotransferases may suggest the diagnosis but may not reflect the degree of injury or establish diagnosis

2.2. Dietary intake

a. A “heart healthy”, Choose My Plate (www.choosemyplate.gov, United States Department of Agriculture) diet will be recommended to patients. Health and Nutrition Information for children over five is available at www.choosemyplate.gov/children-over-five.html. Specific recommendations may include:
   i. Less than 30% of calories from fat
   ii. Less than 10% from saturated fats by selecting non-fat dairy products such as milk, yogurt, cheese, fish or poultry without skin, and preparing food with small amounts of unsaturated oils such as olive and canola oils.

iii. Eat foods free of commercial trans fatty acids
iv. Fill half the plate with fruits and vegetables of different colors to consume at least five servings of fruits and vegetables per day.
v. If subject is overweight, then modest total calorie restriction, calculated from expected needs based on height and age, with weight loss goal of 1-4 pounds per month.
vi. Patient's diet must meet recommended dietary allowances (RDAs) for micronutrients including calcium and Vitamin D.

b. Patients with known or newly discovered type 2 diabetes will receive specific recommendations as promulgated by the American Diabetes Association (ADA).
c. Recommendations regarding the use of specific nutritional supplements are addressed below.
d. Dietary recommendations may not apply to all persons or situations.

2.3. Weight loss

a. Weight loss recommendations will be individualized. Very overweight subjects (BMI ≥ 95%) may be given a target goal of losing and sustaining the loss of 10% of body weight or down to BMI< 85%. This weight loss target may be achieved at a rate of 2 - 4 pounds per month per expert committee recommendations.
b. Food guide pyramid and other age-appropriate materials will be used as instructional material.
c. Patients will be instructed not to fast as a means of achieving weight loss.
d. Alternative diet plans will not be recommended.
e. Family education regarding healthy eating, fast food, etc. will take place. Educational materials on parenting skills and obesity will be developed.

2.4. Alcohol consumption

a. Pediatric patients will be reminded that alcohol consumption is prohibited.

2.5. Exercise

a. Patients will be instructed to engage in moderate to vigorous exercise for a minimum of 60 minutes or more daily. This will be defined as continuous physical exertion sufficient to raise the heart rate to 130 and "break a sweat." Patients/families will be advised to limit TV-watching and video/ computer game time to less than 2 hours per day. Further recommendations can be found at: http://www.cdc.gov/physicalactivity/everyone/guidelines/children.html

2.6. Preventive medicine

a. **Vaccination for viral hepatitis**. Hepatitis B vaccine is standard of care for children. While hepatitis A vaccine is now routine, many teenagers may not have undergone vaccination yet; these teens with underlying liver disease should receive the hepatitis A vaccine. Compliance will not be monitored for the study since disparities in clinical center practices would have no impact on the studies of the NASH CRN.
b. **Hepatocellular carcinoma screening**. Although recent data suggest that cirrhosis caused by NASH is associated with a similar risk of developing hepatocellular carcinoma as other major causes of cirrhosis, there is lack of consensus in the field regarding an optimal cost-effective screening strategy, particularly in pediatrics. Screening methods will not be standardized across sites, but will be in accordance with local standards.

2.7. Management of coexisting morbidities

a. **Type 2 diabetes**

i. Some patients will not have a pre-existing diagnosis of type 2 diabetes but meet diagnostic criteria as a result of testing performed for NASH CRN studies. These patients will be referred to a pediatric endocrinologist for appropriate management.

ii. Patients with controlled diabetes (hemoglobin A1C < 7%) will be continued on their current treatment regimens.

iii. Patients with suboptimally controlled diabetes (hemoglobin A1C > 7%) will receive a recommendation for follow-up with their pediatric endocrinologist for improved glycemic control.

b. **Hypertriglyceridemia**

For patients with fasting triglycerides > 150, referral to a dietician should be considered For patients with triglycerides > 250 further evaluation should be considered.

c. **Hypercholesterolemia**

Patients with fasting total cholesterol levels > 200 mg/dL will be referred to a dietitian for step 1 diet (NCEP) and those with a total cholesterol > 220 or low-density lipoprotein (LDL) cholesterol of ≥ 130 should be further evaluated. Treatment should be considered for LDL cholesterol levels ≥ 160 if risk factors such as a family history of early heart disease or diabetes is present, or LDL cholesterol levels ≥ 190 if no risk factors are present. Study physicians may refer patients to a lipid specialist at their discretion. Further recommendations can be found at: http://aappolicy.aappublications.org/cgi/content/full/pediatrics;122/1/198#SEC5

d. **Hypertension**

For patients with repeated systolic blood pressure or diastolic blood pressure > 95% for age and height (NHLBI), referral for further evaluation should be considered.

e. **Sleep apnea**

Symptoms suggestive of sleep apnea (snoring, observed periods of apnea, disruptive sleep disturbances) will be specifically sought as part of the review of symptoms. If these symptoms are present, patients will be referred for a possible sleep study. The rationale for this assessment is that disrupted sleep will contribute to fatigue and the role of periods of hypoxia in the pathogenesis of liver injury is unknown.

f. **Hyperandrogenism and polycystic ovary syndrome (PCOS)**

Girls with hirsutism (facial and/or chest hair) and menstrual irregularities not associated with prepubescence will be referred to the appropriate specialist.

g. **Occupational exposure to hepatotoxins, recreational drugs, etc.**

A history of ongoing exposure to volatile hydrocarbons or recreational drugs will be sought. All subjects with positive histories will be cautioned regarding dangers of use and instructed to avoid/stop usage.

h. Evaluation of pediatric NASH patients with age < 5 years or developmental delay will include urine organic acids (and perhaps other metabolic testing) to evaluate for underlying metabolic disease.

2.8. Possibly helpful concomitant medication use

a. Vitamin E
   i. Patients receiving a stable dose of Vitamin e for at least 6 months may continue use of Vitamin E.
   ii. For therapeutic trials, Vitamin E may not be initiated in the time period between liver biopsy and randomization.
   iii. For therapeutic trials, if a patient is taking Vitamin E but adherence is poor, Vitamin E may be stopped.

b. Ursodeoxycholic acid (UDCA, Actigall, Urso, Ursodiol)
   i. UDCA will generally be stopped unless new data is published to indicate a significant benefit for patients with NASH.
   ii. UDCA will be continued in patients who have shown a significant improvement associated with treatment in liver histology or surrogate markers for NAFL such as ALT or imaging studies. 90 day UCDA washout may be required before therapy studies.

c. Metformin
   i. Patients receiving metformin as a treatment for diabetes will remain on the drug.
   ii. Patients receiving metformin as a treatment of other disorders of insulin resistance (e.g., PCOS) will remain on the drug.
   iii. For therapeutic trials, if a patient is taking Vitamin E but adherence is poor, Vitamin E may be stopped.

d. Fibrates: There is little experience in children. Use will be decided on an individual case basis in children with hypertriglyceridemia.

e. Statins: There is little experience in children. Use will be decided on an individual case basis in children with hypercholesterolemia.

f. Thiazolidinediones (TZDs; pioglitazone, rosiglitazone)
   i. Patients receiving a TZD as a treatment for diabetes will remain on the drug.

2.9. Possibly harmful concomitant medication use

a. Acetaminophen
   i. Acetaminophen should be restricted to < 45 mg/kg/d in any given day.
   ii. Repeated use of > 20 mg/kg daily for more than 3 consecutive days should be discouraged.
   iii. Families should be warned that many over-the-counter (OTC) medications contain acetaminophen. Labels should be read carefully.

b. Anticonvulsants: Children with seizure disorder will continue on previous anticonvulsants. Neurologists treating children with NASH with Valproate will be asked to change to a different anticonvulsant if possible.
   i. Valproate washout may be required before therapy studies.

c. Estrogens (oral contraception, hormone replacement therapy)
   i. Estrogen use as oral contraception will be permitted.

d. Iron supplements
   i. Patients with iron deficiency will be allowed to continue the use of iron supplements until iron stores are sufficient. In the case of ongoing blood loss (e.g., meno/metrorrhagia, refractory GERD, portal hypertensive gastropathy), iron supplements will be continued as long as the transferrin saturation remains < 50%.
   ii. Patients having normal iron status (serum ferritin > 15 ng/mL or transferrin saturation > 20%) and taking iron supplements will be requested to discontinue the supplements.

e. Accutane
    i. Accutane may cause elevations in liver enzymes as well as lipids. In the context of NASH, this drug should be discontinued.
f. Prednisone
    i. Use should be minimized.

2.10. Possibly helpful concomitant dietary supplement use

a. Multivitamins. A daily multivitamin with iron content < 20 mg daily will be allowed.
b. Betaine. Betaine should not be used outside of a trial. 90 day betaine washout may be required before therapy studies.
c. S-adenosylmethionine. SAM should not be used outside of a trial. 90 day SAM washout may be required before therapy studies.
d. Creatine and other bodybuilding supplements will be forbidden.

2.11. Possibly harmful concomitant dietary supplement use

a. Vitamin A supplements in excess of that contained in a daily multiple vitamin (5000 IU) should not be used.
b. Herbal supplements.
    i. For therapeutic trials, herbals may not be initiated in the time period between liver biopsy and randomization.
    ii. If a herbal supplement is believed to be hepatotoxic, then use of that supplement should be discontinued.

3. Implementation

The intention of the NASH CRN is to implement these standards of care immediately in the patients followed at all clinical centers. There will be no standardized lead-in period during which these standards will be applied before patients are enrolled in NASH CRN studies. Implementing the standards in patients with only steatosis on a liver biopsy will be at the discretion of the hepatologist. The committee believes that implementing these standards of care constitutes good clinical practice and does not constitute an intervention; moreover, data will not be collected regarding outcomes after implementation of these standards. As such, their implementation is not under the purview of local Institutional Review Boards.

Responsibility for implementation. The NASH CRN will develop uniform teaching materials to provide patients with the information detailed above. Clinical centers will be responsible for printing costs. The materials will be distributed to patients by their hepatologists or other health care worker such as nurses and nutritionists. Distribution of materials to patients will be documented in their medical records.

Compliance and reinforcement. Patients' adherence to the above guidelines will be established by the study personnel. The practices will be reviewed by the hepatologist or associated health care worker with the patient during the clinic visit. Patients will be given the opportunity to take the teaching materials outlining the above recommendations at each hepatology clinic visit.

Frequency of follow-up. Patients will be seen at least once yearly by the hepatologist. The visit should include an interim history, review of symptoms, updated medication list, physical exam, lab work, and discussion of adherence to the standard of care recommendations.

4. Preparation and dissemination of materials needed to implement the standard of care

4.1. Physician summary of guidelines

a. Physicians at the study sites will need ongoing reminders of the specifics of the standards of care.
b. Perhaps a pocket card and a small poster for patient care areas are needed.

4.2. Patient brochures

a. What brochures are needed:
    i. Healthy eating
    ii. Healthy weight loss
        (1) BMI formula and curves
        (2) Goals
    iii. Handout on parenting skills (See Section 5.1)
    iv. General NASH brochure to cover most other recommendations
        (1) Acetaminophen use
            a. Allowable amounts
            b. List of medications
        (2) Supplemental iron use
        (3) Vitamins
            a. Allowable vitamin A
            b. MVI daily
        (4) Warning about herbal remedies
        (5) Symptoms to report
            a. Sleep apnea
            b. Irregular menstruation, facial hair
b. Brochure development
        i. Content: Standard of Care Committee (See Section 5.2)
        ii. Design: Need a professional aesthetically pleasing design
        iii. Printing: Local center to arrange for printing, distribution, cost recovery
c. Updates to the brochures
        i. Content to be reviewed annually and discussed at Steering Committee meetings
        ii. Revised content and design to be prepared within 4 weeks of review at Steering Committee. Revisions to be distributed to the Steering Committee members for final approval.

4.3. Referring physician information

a. Implementation of the standard of care will occasionally require communication of findings such as newly diagnosed diabetes, hypertension or hyperlipidemia. This communication should be made according to local protocol and physician judgment. Documentation of referrals should be kept in the patient's NASH CRN file.

4.4. Website

A public website will be considered in the future, but will not be implemented during the first year of the trial.

5. Parent guidelines and patient brochure

Printable versions of the Parent Guidelines and Patient Brochure are available on the NASH CRN website: http://www.jhuccs1.us/nash/closed/cped/CYNCH/sop.htm. If you print the guidelines or brochure from the SOP, the headers and footers will cause the documents to not fit the page correctly; therefore, you must go to the website to print the documents.

5.1. Lifestyle intervention approach in the CyNCh trial

Attaining a healthy weight is the cornerstone of our current treatment of pediatric nonalcoholic fatty liver disease (NAFLD), given the lack of proven pharmaceutical therapy in this age group and the strong association of NAFLD with excess adiposity, in particular central adiposity.(1) Weight loss has been associated with improvements in liver enzymes and histology in adults with NAFLD, while weight loss in children with NAFLD has been shown to improve serum aminotransferase levels in small pilot studies.(2-4) Therefore, lifestyle intervention through changes in diet and exercise will be encouraged for participants in both the placebo and intervention arm of the CyNCh trial, as this represent the current standard of care for this disease in children.

The standard of care lifestyle intervention designed for this trial will incorporate components of the American Academy of Pediatrics' 2007 Expert Committee Recommendations Regarding the Prevention, Assessment, and Treatment of Child and Adolescent Overweight and Obesity that can be reproduced across the study sites.(5) CyNCh study participants will be given a study-specific set of written materials that will include discrete evidence-based strategies to achieve a healthier diet and increase physical activity, as endorsed by the American Academy of Pediatrics as well as the CDC and NIH.(5-7) A family-based, patient-centered and stepped approach to making lifestyle changes will be employed as recommended by the AAP.(5) Accordingly, the lifestyle materials will be reviewed with study participants and their family members at each study visit (RZ, F4, F12, F24, F36).

Topics to be covered in the materials will include:

RZ VISIT: 1) Reduce fat intake and sugar intake to 0 servings per day of sweets.
F4 VISIT: 2) Reduce screen time to 2 hours or less per day
F12 VISIT: 3) Increase physical activity to 1 hour or more per day
F24 VISIT: 4) Increase fruits and vegetable intake to 5 or more servings per day
F36 VISIT: 5) Make healthier choices when eating out

These topics and the specific strategies to be included in the lifestyle intervention materials are in accordance with healthy weight strategies currently recommended by the Centers for Disease Control and the National Institutes of Health National Heart Lung and Blood Institute's "We Can" program.(6-8) Therefore, we will also include references in the study's lifestyle intervention materials to these freely accessible, federal government sponsored websites so that participants and their families can easily access additional information on these lifestyle changes and strategies to achieve them.

References

1) Loomba R, Sirlin CB, Schwimmer JB, Lavine JE. Advances in pediatric nonalcoholic fatty liver disease. Hepatology 2009;50;1282-93.
2) Neuschwander-Tetri BA. Lifestyle modification as the primary treatment of NASH. Clin Liver Dis 2009;13:649-65.
3) Peterson KF, Dufour S, Befroy D et al. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance and hyperglycemia by moderwate weight reduction in patients with type 2 diabetes. Diabetse 2005;54:603-8.
4) Nobili V, Manco M, Raponi M, Marcellini M. Case management in children affected by non-alcoholic fatty liver disease. J Paediatr Chid Health 2007;43:414.
5) Barlow SE. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics 2007;120 Suppl 4:S164-92.
6) http://www.cdc.gov/healthyweight/children/, accessed September 22, 2010
7) http://www.nhlbi.nih.gov/health/public/heart/obesity/wecan/index.htm, accessed September 22, 2010
8) http://www.fruitsandveggiesmatter.gov/ accessed September 22, 2010

NASH CRN CYNCH Trial

Achieving a healthy weight can improve fatty liver disease

TIP # 1: REDUCE SWEETENED FOODS AND BEVERAGES– AIM FOR 0 SERVINGS PER DAY ON MOST OR ALL DAYS OF THE WEEK.

Limiting fat and sugar intake can help you achieve a healthy weight. Many prepared foods and drinks have added sugars, both table sugar and high fructose corn syrup. Both types of sweeteners can contribute to unwanted weight gain and make fatty liver disease worse. Sugar is found naturally in some foods, like fructose in fresh or dried fruits or lactose in milk. The grams of sugar in these foods are too small to worry about unless you eat or drink these foods in very large amounts.

Saturated fats (fats that tend to be solid at room temperature, like butter and whole fat milk) can promote weight gain. Foods with saturated fats or made with saturated fats are typically higher in calories per serving.

Beware of foods that are labeled fat-free or reduced fat. Many of these prepared foods have added sugars and the same number of calories as regular food. The easiest way to reduce fat and sugar intake is to eat foods that are not processed. Choose foods that you prepare yourself from raw ingredients.

Here are some ideas on how you can avoid added sugars and saturated or trans fats:

1) Avoid beverages or sodas sweetened with sugar. Choose water rather than fruit juice or soda. Choose diet drinks if you must drink a soda.
2) Choose low fat dairy products (skim milk, low fat milk or cheese products) for cooking or snacks. For example, use part skim mozzarella cheese in lasagna.
3) Read labels and avoid foods that contain saturated or trans-fats.
4) If you love sweets, limit your intake of cookies or sweets to 1 or 2 days per week and stick to 1 serving.
5) Choose fresh or frozen fruit with low fat unsweetened yogurt for dessert.
6) Limit your fast food intake to no more than one time per week or once every other week. Most fast food choices are loaded with added sugar, salt and fat.

For more information visit:
http://www.choosemyplate.gov/food-groups/downloads/TenTips/DGtipsheet13CutBackonSweetTreats.pdf
In Spanish: http://www.choosemyplate.gov/downloads/DGTipSheet13CutBackonSweetTreats-sp.pdf
http://www.nhlbi.nih.gov/health/public/heart/obesity/wecan/index.htm
http://www.choosemyplate.gov/physical-activity.html

TIP #2: CUT DOWN ON SCREEN TIME TO 2 HOURS OR LESS PER DAY

Reduce your "screen time" – this is the total time you spend watching TV, DVDs, playing video games, texting or using a phone or the computer for *anything* other than school or work.

- It's important for the whole family, including parents and kids, to sit less and move more to have more energy, learn new skills and be at a healthy weight.
- Be a good role model. Parents *and* kids should limit screen time to no more than 2 hours per day.
- Don't use TV or video game time as reward or punishment.

Here are some ideas on how to cut down your family's screen time:

1. Keep track of how much time you and your family spend in front of a screen (TV, computer or phone) and how many hours you spend being active (walking, active playing, gardening, swimming, running) on a weekly or monthly calendar. Have a family competition to see who can increase their active time and decrease their screen time the most in one month!
2. When you do spend time in front of a TV or computer, take breaks to be active. During commercial breaks, get up stretch, walk around the house, do yoga, leg lifts, or lift weights. Compete with your family members to see who can do the most jumping jacks or sit ups during breaks.
3. Move TVs out of your bedrooms.
4. Make meal times about family time and turn off the TV.
5. Set a house rule on the amount of TV and screen time per day at home and stick with it! Remember your goal of no more than 2 hours of screen time per day.
6. Go for a half hour walk or any other physical activity instead of watching TV.

For more information, visit:
http://www.nhlbi.nih.gov/health/public/heart/obesity/wecan/reduce-screen-time/tips-to-reduce-screen-time.htm

TIP # 3: INCREASE YOUR PHYSICAL ACTIVITY TO 1 HOUR OR MORE PER DAY

Getting more active can boost your energy, cardiovascular fitness, help you achieve a healthy weight and reduce the fat around your middle and in your liver.
Start with small steps to get more active every day: Choose a different tip each week to gradually increase your daily activity.

Here are some ideas on how you can get more active:

1. Walk to nearby destinations or friends' houses instead of driving whenever possible.
2. Walk to school if you can.
3. Take the stairs instead of the elevator or escalator at school, work or when out.
4. Take a daily family walk together after dinner or make a weekend walk a family habit.
5. Get off the bus one stop early and walk to your destination.
6. Park further away from the store or mall to get in a little extra walking while shopping.

7. Take your dog on longer walks.
8. Play outside for at least 30 minutes 2 times per day to achieve your goal of 1 hour.
9. Dance to music or a music DVD at home instead of watching a TV or cable show.
10. Help around the house: wash the car, work in the yard, clean your room, and help with laundry.

For more information visit:
http://www.nhlbi.nih.gov/health/public/heart/obesity/wecan/get-active/getting-active.htm
http://www.choosemyplate.gov/physical-activity.html

TIP #4: FRUITS AND VEGETABLES MATTER – AIM FOR 5 A DAY

- Use MyPlate (www.choosemyplate.gov/food-groups) to determine how many servings of fruits and vegetables you should eat each day.
- Choose fresh, frozen or canned fruits and vegetables
- Rinse canned products because of the added salt and sugar

Here are some ideas on how to add more fruits and vegetables to your meals and snacks:

1. Blend a fruit smoothie using your favorite fruits and low fat milk or yogurt.
2. Add extra fresh, frozen or canned chopped vegetables to your family's favorite recipes.
3. Add extra vegetables to salads – grated carrots, sliced cucumbers, cherry tomatoes.
4. Choose sliced fruit for breakfast on your cereal, whole grain waffle or pancake.
5. Pre –plan by packing a school lunch the night before – add a serving of fruit or vegetable.
6. Keep ready-to-grab healthy snacks in your kitchen – veggies rinsed and cut up in the refrigerator, and apples, banana, and grapes ready to wash and eat.
7. Put fresh fruit such as melon chunks in the freezer for a frozen treat.

For more ideas and information visit:
http://www.choosemyplate.gov/food-groups/downloads/TenTips/DGTipsheet11kidfriendlyveggiesandfruits.pdf
In Spanish: http://www.choosemyplate.gov/downloads/DGTipSheet11kidfriendlyveggiesandfruits-sp.pdf
http://www.nhlbi.nih.gov/health/public/heart/obesity/wecan/index.htm
http://www.cdc.gov/healthyweight/children/
http://www.fruitsandveggiesmatter.gov/

TIP# 5: MAKE HEALTHY CHOICES WHEN EATING OUT OR ORDERING TAKE OUT FOOD.

Foods cooked in restaurants or fast food chains are often loaded with sugar, fat and salt.
It is more challenging to eat well and stay healthy if you eat out more than one time per week.

Your best strategy is to reduce the number of times you eat out to no more than one time per week. Here are some ideas on how to make healthier choices and reduce your intake of sugar and fat when you choose to eat out or order carry out food.

Here are some ideas on how you can make healthier choices when eating out or taking out food:
1) Choose items from the menu that are steamed, broiled, baked, roasted, or poached.
2) Ask for low fat salad dressing on the side and use no more than 1 tablespoon.
3) Limit high calorie toppings (dried fruit, croutons, bacon bits) on your salad to 1 tablespoon.
4) Ask the waiter to leave out the gravy or sauce on your entrée or sandwich
5) Choose fruits or steamed vegetables instead of fried foods as a side dish.
6) Split restaurant entrees and share with a family member (restaurant portions are often several servings)
7) Cut back on eating out by one or more meals per week, and prepare more meals at home.

For more information visit:
http://www.choosemyplate.gov/food-groups/downloads/TenTips/DGTipSheet12BeaHealthyRoleModel.pdf
In Spanish: http://www.choosemyplate.gov/downloads/DGTipSheet12BeAHealthyRoleModel-sp.pdf
http://www.nhlbi.nih.gov/health/public/heart/obesity/wecan/eat-right/eating-out.htm
http://www.nhlbi.nih.gov/health/public/heart/obesity/wecan/index.htm