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Treatment of Nonalcoholic Fatty Liver Disease in Children: TONIC Trial Design

The NASH CRN Research Group^{1,*}

Abstract

Background—Nonalcoholic fatty liver disease (NAFLD) in children can lead to steatohepatitis, cirrhosis, and end-stage liver disease. The cause of NAFLD is unknown, but it is commonly associated with obesity, insulin resistance, and dyslipidemia.

Objectives—TONIC is conducted to test whether treatment with metformin, an insulin sensitizer, or vitamin E, a naturally available antioxidant, will lead to improvements in biochemical and histological features of nondiabetic children with biopsy-proven NAFLD.

Design—TONIC is a randomized, multicenter, double-masked, placebo-controlled trial of 96 weeks of treatment with metformin or vitamin E. The primary outcome measure chosen for the trial is improvement in serum alanine aminotransferase (ALT) levels with treatment as compared to placebo. An improvement in ALT is defined as reduction in serum ALT levels to below 50% of the baseline values or into the normal range (40 U/L or less) during the last 48 weeks of treatment. Histological improvement is defined by changes in liver histology between a baseline and end-of-treatment liver biopsy in regards to (1) steatohepatitis, (2) NAFLD Activity Score, consisting of scores for steatosis, lobular inflammation, and hepatocellular injury (ballooning), and (3) fibrosis score.

Methods—Between September 2005 and September 2007, 173 children were enrolled into TONIC at 10 clinical centers in the United States. Participants were randomized to receive either metformin

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Corresponding author: Aynur Ünalp (Telephone: 410 614 4851; Fax: 410 995-0932; aunalp@jhsph.edu).

¹A list of the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) investigators appears in the Appendix.

***Writing Committee:** Joel E. Lavine^a, Jeffrey B. Schwimmer^a, Jean P. Molleston^b, Ann O. Scheimann^c, Karen F. Murray^d, Stephanie H. Abrams^e, Philip Rosenthal^f, Arun J. Sanyal^g, Patricia R. Robuck^h, Elizabeth M. Bruntⁱ, Aynur Ünalp^{j,†}, James Tonascia^j

a Joel E. Lavine, Jeffrey B. Schwimmer, University of California San Diego, La Jolla, CA

b Jean P. Molleston, Indiana University School of Medicine, Indianapolis, IN

c Ann O. Scheimann, Johns Hopkins Hospital, Baltimore, MD

d Karen F. Murray, Seattle Children's, Seattle, WA

e Stephanie H. Abrams, Baylor College of Medicine, Houston, TX

f Philip Rosenthal, University of California San Francisco, CA

g Arun Sanyal, Virginia Commonwealth University, Richmond, VA

h Patricia R. Robuck, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

i Elizabeth Brunt, Washington University School of Medicine, St. Louis, MO

j, † Aynur Ünalp, James Tonascia, The Johns Hopkins University, Bloomberg School of Public Health, 615 North Wolfe Street, Room W5010, Baltimore Maryland 21205

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(500 mg b.i.d.), vitamin E (400 IU b.i.d.), or placebo for 96 weeks. This protocol was approved by all participating center Institutional Review Boards (IRBs) and an independent Data and Safety Monitoring Board (DSMB). (ClinicalTrials.gov number, NCT00063635.)

Keywords

nonalcoholic fatty liver; nonalcoholic steatohepatitis; metformin; vitamin E; RRR- α -tocopherol; randomized controlled trial; children

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common and increasingly recognized disorder in children characterized by the presence of fat in at least 5% of hepatocytes, usually found in association with obesity, insulin resistance, or dyslipidemia [1–5]. NAFLD is probably the most common cause of liver disease in children and adolescence [4,5]. First described in adults, NAFLD was subsequently reported in children [6] who were obese and without another identifiable cause of chronic liver disease. Liver biopsies from these children showed changes similar to adults with nonalcoholic steatohepatitis (NASH) [6]. Subsequent reports of NASH in children have appeared from Japan [7], the United States [8,9], Canada [10], Australia [11], and Italy [12]. Reports have also shown that NASH can progress to cirrhosis in children [10,13].

Obesity and insulin resistance are the most common risk factors for NAFLD in children and adolescents as well as adults [4,9]. As part of the growing epidemic of childhood obesity in the United States, it is estimated that the prevalence of NAFLD has risen up to 10% in children [4,14,15]. In a community representative autopsy study which was based on liver histology and was conducted from 1993 to 2003 in the county of San Diego, California, the standardized prevalence of fatty liver disease in children age 2 to 19 years was estimated as 9.6% (7.4 to 11.7% with a 95% confidence interval) [4]. However, since NAFLD is diagnosed by liver biopsy, it is hard to estimate the prevalence in children in a population based study [16]. In addition, several investigators performed studies of fatty liver prevalence using serum alanine aminotransferase (ALT) as a screening tool [7,12]. In the National Health and Nutrition Examination Survey 1999 to 2004 (NHANES III), the prevalence of elevated ALT (> 30 U/L) levels was 8% among adolescent participants age 12 to 19 years [5]. However, ALT appears to be both an insensitive and nonspecific screening surrogate, as recent evidence in adults provides ample evidence that “normal ALT NASH” occurs [17].

NAFLD encompasses a histological spectrum from isolated hepatic steatosis to steatosis with inflammation, cell injury and possible fibrosis. Some cases may progress to cirrhosis, portal hypertension, and liver-related death in early adulthood [13,18,19]. Longitudinal studies in adults have shown that isolated steatosis and NASH have distinct outcomes: steatosis is largely benign with regard to liver outcomes and has a minimal risk of cirrhosis, whereas NASH is a progressive liver disorder which can lead to cirrhosis and liver failure in a substantial proportion of patients [20–22]. Several agents have been found to be promising as therapy of NAFLD in small clinical trials, but none have been proven to alter its natural history or outcome, and none are approved for general use in this disorder.

The pathogenesis of NAFLD is not fully known. It is however generally agreed that insulin resistance plays a central role in the development of the disease [23–29]. The insulin resistant state is associated with increased delivery of fatty acids to the liver for re-esterification to triglycerides [26] and a simultaneous increase in de novo lipogenesis and decrease in very low density lipoprotein secretion. These factors combine to cause hepatic steatosis. The metabolic derangements due to the insulin resistant state as well as the altered cytokine profile associated

with insulin resistance activate intracellular pathways that can lead to adaptation to the altered metabolic state or, alternatively, can induce cell injury and apoptosis [30,31]. These pathways are most activated in patients who have NASH, the progressive form of NAFLD. Intracellular oxidative stress due to mitochondrial failure to convert energy from oxidized fatty acids to adenosine triphosphate and increased cytochrome p450 activity plays an important role in the genesis of cell injury and in perpetuating the insulin resistant state in the liver [32–34]. This understanding of the pathogenesis of NASH provides the rationale to consider insulin resistance and oxidative stress as therapeutic targets.

Treatment options for pediatric NAFLD remain unproven. An open-label pilot study demonstrated encouraging results using the insulin-sensitizer metformin to treat NAFLD in ten nondiabetic children [35] following the lead on reversal of fatty liver disease in genetically obese, leptin deficient mice [36]. Other pilot studies demonstrated that vitamin E, a naturally occurring antioxidant, improves serum biochemical tests in both children and adults with NAFLD [37–41]. Although vitamin E antioxidant therapy suppressed steatosis and inflammation in a recent murine dietary steatohepatitis study [42], definitive studies evaluating its effect on hepatic histology in humans are lacking. The Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) was established by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in 2002 to assess the natural history, pathogenesis and therapy of this disease in both adults and children [43,44].

To assess whether metformin or vitamin E are efficacious in children with NAFLD, the NASH CRN developed a multicenter, double-masked, double-dummy, placebo-controlled study entitled “Treatment of NAFLD in Children (TONIC).” TONIC is being conducted by the 10 Pediatric Clinical Centers and a central Data Coordinating Center of the NASH CRN. Metformin is being used as the insulin sensitizer, and RRR- α -tocopherol is the vitamin E formulation used. The NIDDK appointed Data and Safety Monitoring Board (DSMB) reviewed and approved the study protocol and an Investigational New Drug application (IND) was obtained by the NIDDK on behalf of the NASH CRN from the United States Food and Drug Administration. This manuscript describes the design of the TONIC trial.

2. Methods

Design overview

TONIC is a multi-center, randomized, placebo-controlled, double-masked, double-dummy clinical trial of treatment with metformin, or vitamin E, or placebo for nondiabetic children with histologically confirmed NAFLD. The screening period for evaluating eligibility and collecting baseline data lasted for up to 16 weeks before randomization. Eligible participants are randomized to receive either metformin (oral 500 mg b.i.d.), or vitamin E (oral 400 IU b.i.d.), or placebo for 96 weeks followed by a 24 week washout period. The primary objective of the TONIC trial is to determine whether either metformin or vitamin E will lead to improvements in biochemical and histological features of NAFLD compared to placebo. The comparisons will be made using an intention-to-treat analysis of the primary outcome: reduction in ALT at every follow-up visit during the last half of treatment to either 50% or less of the baseline value *or* to 40 U/L or less. The estimate of reduction in ALT will be based upon all values from week 48 to week 96 visits on therapy. Histological improvement will be determined from standardized histological scoring of liver biopsies taken at baseline before treatment and again at week 96 that will be read centrally in a masked fashion for an assessment of steatohepatitis, fibrosis stage, and a NAFLD Activity Score (NAS) consisting of steatosis, lobular inflammation, and hepatocyte ballooning. A schematic of the trial design is presented in Figure 1.

The scoring system utilized by the Pathology Committee was developed and validated by the same committee for the express purpose of semi-quantitative analysis for treatment trials [45]. The scoring system includes assessment of activity (steatosis, lobular inflammation and ballooning) as well as fibrosis and architectural remodeling. Both features are reported using numeric values; the former, considered to be potentially reversible, are indicated in the NAS and the latter, less reversible, by a fibrosis “stage”.

Participant selection

Eligible children (8 through 17 years of age at entry) were identified and recruited at the 10 participating clinical centers (the list of centers is included in the acknowledgments) starting in September 2005, and by September 2007, 173 participants were enrolled. Determination of eligibility was based on standard of care tests and procedures that were completed during screening. Each child and his/her parent or guardian signed the consent at the screening visit to obtain any tests and procedures needed to finalize eligibility and had a history and physical examination to identify other illness and contraindications for participation.

Inclusion criteria

The study entry criteria (Table 1a) were based on serum ALT elevation (> 60 U/L) on two occasions more than 30 days but less than one year apart, and a liver biopsy indicating NAFLD obtained within 6 months before randomization (participant must not have used medications suspected of having an effect on NAFLD in the 3 months before the biopsy). The histological evidence of NAFLD is defined as a minimum of 5% of hepatocytes with macrovesicular fat, without other etiologies for the presence of fat being identified.

Exclusion criteria

Reasons for exclusion of participants included significant alcohol consumption, history of diabetes mellitus, and evidence of cirrhosis or other forms of chronic liver disease, normal ALT or lack of liver biopsy within the designated time (Table 1b). Alcohol consumption was ascertained by the self-administered “alcohol use disorders identification test” (AUDIT) with flash cards to remind study participants of drink equivalents. Use of drugs historically associated with causing NAFLD such as systemic glucocorticoids, tetracyclines, anabolic steroids, valproic acid, or other known hepatotoxins was also assessed during screening and their use constituted an exclusion.

Run-in period

Participants were not allowed to use any prescription or over-the-counter medication or herbal remedy taken with an intent to improve or treat NAFLD, liver disease, obesity, or diabetes for the 3 months before randomization. Such agents included: metformin, vitamin E, thiazolidinediones, ursodiol, S-adenosyl methionine (SAM-e), betaine, milk thistle (silymarin), and probiotics. Prohibited antidiabetic agents included: insulin, biguanides, sulfonylureas, metformin and thiazolidinediones. These agents were not to be used during screening nor for the duration of the trial (except in the form of assigned study treatment or treatment for new onset diabetes). Participants were allowed to continue prescription anti-hyperlipidemic agents for the duration of the trial. However, any over-the-counter medication or herbal remedy that was being taken with an intent to improve hyperlipidemia was not allowed for at least 3 months before randomization and were discouraged after randomization. Participants were interviewed in a detailed fashion at screening, randomization, and at every clinic visit to document the absence of such use.

Treatment groups

Participants who gave their assent, whose parents or guardians signed an informed consent statement, and who met the eligibility criteria were randomly assigned to one of three groups for 96 weeks of treatment:

Group 1: Metformin (500 mg twice daily) and vitamin E-placebo (twice daily)

Group 2: Vitamin E (400 IU twice daily) and metformin-placebo (twice daily)

Group 3: Metformin-placebo (twice daily) and vitamin E-placebo (twice daily)

Metformin is administered orally as a single tablet of 500 mg with the morning meal and a second 500 mg tablet with the evening meal per day. A similar appearing placebo tablet is taken twice a day by participants assigned to either the placebo group or the vitamin E group due to the double-dummy design of the TONIC trial. The rationale for choosing this dosage was based upon an earlier pilot study that examined the safety and efficacy of metformin in children with NAFLD [35].

The formulation of vitamin E (Nature Made®, Pharmavite, LLC, Mission Hills, CA) used in this study is the natural form of vitamin E (RRR- α -tocopherol, formerly known as *d*- α -tocopherol) and is given in a dose of 400 IU as a single softgel orally twice a day. A similar appearing placebo softgel is taken twice a day by participants assigned to either the placebo group or the metformin group due to the double-dummy design of the TONIC trial. Double-masked trials and large population studies have shown that oral vitamin E at 800 IU daily dose is safe with minimal or no side effects [46,47]. The vitamin E dose chosen for this trial (400 IU twice daily) is within the range of vitamin E dosage that has been tested for the treatment of NAFLD in previous pilot studies [37–41]. As there is no pharmacologic therapy of proven benefit for NAFLD in children, using a placebo for comparative purposes is justified.

In addition to study medications, all participants receive standardized recommendations concerning life-style modification (dietary modification, weight loss, exercise), use of prescription or non-prescription medicines or herbal remedies or dietary supplements, avoidance of alcohol, and management of various co-morbid illnesses. The placebo group in TONIC trial was deemed essential to account for changes that may result from adherence to these recommendations, to determine the natural history of pediatric NAFLD with histology, and to establish a baseline for comparing changes which might result from treatment with vitamin E or metformin. Per TONIC protocol in addition to screening phase, participants are asked to avoid prohibited agents such as thiazolidinediones, UDCA, SAM-e, betaine, silymarin, and probiotics during the treatment phase of the trial. The life-style recommendations were prepared by the NASH CRN Standards of Care Committee and were approved by the Steering Committee to help ensure that the study participants receive consistent standard of care treatment at each of the 10 pediatric clinical centers.

Outcome measures

The primary outcome measure used in the trial is a reduction in serum ALT levels during the last half of the 96 weeks of treatment to either 50% or less of the baseline value *or* to normal (40 U/L or less). The reduction is operationally defined as all ALT values from the 48 week through the 96 week visits fitting the criteria of being either less than or equal to 50% of the baseline ALT value or less than or equal to 40 U/L. Other laboratory results evaluated as secondary outcome measures include a reduction in serum aspartate aminotransferase (AST) and serum γ -glutamyl transferase (GGT) levels during the last half of the 96 weeks of treatment to either 50% or less of the baseline value *or* 40 U/L or lower. Although histology is usually considered the outcome of greatest importance in evaluating NAFLD, a sustained reduction in ALT was chosen as the primary outcome measure in TONIC trial, since there was insufficient

information on the histology of NAFLD in children at the time of study design for calculation of an appropriate sample size.

The histological outcome measures require improvement in NAFLD Activity Score (NAS), after 96 weeks of treatment as determined by comparison of liver biopsy scores on samples taken before and at the end of treatment [45]. Histological outcome measures include (1) change in total NAS (i.e., the sum of scores for steatosis, portal and lobular inflammation, and ballooning degeneration) as well as the change in each of the three components of the NAS; (2) change in fibrosis (“stage” and architectural alterations); (3) improvement in other histological features associated with NASH such as presence of Mallory bodies and megamitochondria.

Other secondary outcomes to be assessed in the trial include changes in (a) anthropometric measures including body weight, height, waist-to-hip ratio, waist circumference, triceps skin-fold thickness and total body fat, (b) Tanner staging, (c) insulin resistance, (d) serum vitamin E levels, (e) cytokines, fibrosis markers, and lipid profile; and (f) health-related quality of life (PedsQL®).

Sample size justification

The planned sample size for the TONIC trial is 180 participants with equal allocation to each of the three treatment groups (60 per group). The sample size estimates were based upon a two-group, binomial comparison of the proportions of participants satisfying the primary outcome, reduction in serum ALT to either 50% or less of the baseline value *or* to 40 U/L or lower during the last half of the 96 weeks of treatment either with metformin or vitamin E. Since TONIC had three treatment groups and two primary hypotheses, the assumption was made that the two primary comparisons, metformin versus placebo and vitamin E versus placebo, required the same sample size with type I error estimate reduced from 0.05 to 0.025 (Bonferroni correction). Expected proportions of improvement were approximated using pilot data from a 24-week metformin study and from a consensus among investigators as to the response in the placebo group [35]. There were no available data at the time of study design to estimate histological response with vitamin E, which was assumed, for purposes of planning the trial, to be the same as for metformin.

The sample size calculations were performed using the nQuery Advisor 5.0 software [48] with an expected proportion improved in placebo group (assumed=0.20), with an expected proportion improved in metformin or vitamin E group (0.50), with an α level of two-sided type I error (0.025, Bonferroni corrected for two comparisons), and with a β level of type II error (0.10; i.e., 90% power). The number per group, using the above assumptions was 60 or a total of 180 participants for the trial. Participants with missing primary outcome data (serum ALT) will be considered unimproved and will be included in the intention-to-treat analyses.

Interim analysis

An independent Data and Safety Monitoring Board (DSMB), membership appointed by the NIDDK and advisory to the NIDDK, approved the protocol for the TONIC trial and is responsible for monitoring the accumulated interim data as the trial progresses to ensure participant safety and to review efficacy. In addition, the DSMB is charged with reviewing the quality and timeliness of data collection. All of the summary recommendations by the DSMB and communication from the NIDDK regarding the DSMB are forwarded to all of the Institutional Review Boards (IRBs) overseeing the study.

Interim data on safety measures requested by the DSMB are reviewed at each of the scheduled semi-annual meetings. The DSMB reviews all serious adverse events as they occur and receives

quarterly reports of hepatic adverse events by masked treatment groups as well as counts of participants who require more frequent liver function testing due to rises in ALT levels of more than 1.5 times baseline ALT or beyond 250 U/L. Hepatotoxicity is defined as the development of jaundice with a serum direct bilirubin of > 1.0 mg/dL or a doubling of the serum baseline ALT **and** to a value > 400 U/L during treatment. The DSMB also examines the trends in ALT or AST levels for each participant who experiences a rise in ALT. A single interim efficacy analysis of the primary outcome measure is planned when approximately 50% of the data are complete or when approximately 87 of the 173 participants have completed the 96 week course of treatment. O'Brien-Fleming statistical stopping guidelines for efficacy with an overall two-sided type I error of 0.025 and a P-value of 0.0008 are used at the time of the interim efficacy analysis [49].

Statistical analysis

Statistical analyses for the two primary hypotheses will follow the intention-to-treat paradigm, which means that all randomized participants with baseline and follow-up serum ALT values will be included in the treatment group to which they were assigned. Any randomized participant who does not have the requisite follow-up ALT values will be considered unimproved on the primary outcome measure and compared by assigned treatment group.

Since the primary outcome measure is a binary indicator of sustained reduction in ALT after 96 weeks of treatment compared to baseline and since the randomization is stratified by clinic, P-values will be derived from the Mantel-Haenszel χ^2 test for stratified 2x2 tables [50]. Two P-values will be derived: one comparing proportions improved in the group assigned to metformin compared to the group assigned to placebo and another comparing the group assigned to vitamin E to the group assigned to placebo. Since two primary comparisons are planned, a P-value of 0.025 will be considered significant, applying a Bonferroni correction for multiple comparisons.

2. Conduct of the trial

Study visit overview

The participant-related activities of the TONIC trial is divided into 4 phases: (1) screening for eligibility for enrollment (2 visits over a maximum of 16 weeks), (2) randomization (one visit), (3) treatment (9 visits over 96 weeks), and (4) post-treatment observation (one visit 24 weeks after stopping study drugs).

The visit and data collection schedule is summarized in Table 2. Anthropomorphic assessments include body weight and height, body mass index, waist and hip circumference, waist-to-hip ratio, triceps skin fold thickness, mid-upper arm circumference.

Randomization

The NASH CRN web-based data management system includes software to check participant eligibility based on keyed case report forms. The web-based eligibility system provides immediate information on which eligibility features that the participant had failed and provides a summary finding of whether the participant is eligible or ineligible. The randomization visit cannot take place until the eligibility check indicates that the participant is eligible in all items except those that could be completed at the randomization visit only.

The randomization plan is prepared and administered centrally by the Data Coordinating Center (DCC). Requests for randomizations are made by the clinical staff using a web-based application. A study drug assignment is issued only if the TONIC database shows that the participant is eligible, has signed the consent statement, and has all required baseline data keyed

to the database. The random assignment is in permuted blocks of treatments stratified by center to minimize local effects of differences in patient populations and management.

Follow-up visits

Participants return for follow-up visits on treatment at 4, 12, 24, 36, 48, 60, 72, 84, and 96 weeks and once after stopping treatment at 120 weeks after randomization. The follow-up visit 24 weeks after stopping therapy will to assess whether changes in biochemical laboratory tests are sustained. The details of various data to be obtained at each of these follow-up visits are shown in Table 2.

A second liver biopsy is scheduled to be obtained at or around the week 96 visit while the participant is still receiving study drugs. Predefined general guidelines for obtaining the biopsy specimen are provided to each site. Wherever possible, a 16 gauge biopsy needle and a specimen length of at least 1.5 cm are preferred. The slides have to be of adequate size (1.5 cm or more) and adequate quality for interpretation. The liver tissue is prepared locally for light microscopy interpretation with stains including hematoxylin and eosin, Masson's trichrome and iron stain and slides are forwarded to the DCC for central reading by the study pathologists.

Standardized questionnaires

Several standardized questionnaires are administered to participants enrolled in the TONIC trial. Questionnaires are administered at baseline (before randomization) and during follow-up at specified intervals (Table 2). The focus of the questionnaires is to obtain standardized information regarding alcohol intake, nutrition, functional activity, health-related quality of life, and liver-related symptoms. The questionnaires were chosen by the NASH CRN Measures and Assessments Committee during development of the TONIC trial and included the AUDIT questionnaire for capturing alcohol consumption, the Block Brief Nutrition Questionnaire for estimating food frequency and quantity over the preceding 12-month period, the Modifiable Activity Questionnaire (MAQ) for Adolescents as a measure of functional activity, the Pediatric Quality of Life (PedsQL[®]) and a liver symptom questionnaire (see Appendix) developed by the NASH CRN to capture liver-related symptoms during the trial.

Case report forms include baseline and follow-up physical exam and medical history to capture co-morbidities and co-medications in the trial database. Other case report forms constituting the TONIC trial database include laboratory tests results for eligibility checks at baseline and safety monitoring during follow-up, local and central histology reviews of liver biopsy slides, dual energy X-ray absorptiometry (DEXA) scan for body fat, and study drug dispensing form for study drug adherence and accountability.

Specimen banking

Specimens are collected and stored in a central repository for use as approved by the Steering Committee of the NASH CRN. Specimens include serum, plasma, and DNA. The blood samples collected at screening visit 2, and at 24, 48, 72, and 96 week visits are divided into 0.5 mL aliquots and stored frozen at -70 degrees C. Additional blood is collected at the screening visit for extraction of DNA which is stored at -20 degrees C. The biosamples collected throughout the TONIC trial are available to outside investigators for specific research projects as part of an ancillary study proposal mechanism as outlined on the NASH CRN web page [51].

3. Results

Enrollment in TONIC started in September 2005 and ended in September 2007 with 173 participants randomized (96% of target enrollment). A total of 229 participants were registered and screened for the TONIC trial, 56 of whom (24%) were found ineligible. Serum ALT <60 U/L (n=41) and the failure to meet histological entry criteria (n=8) were the most frequent reasons for ineligibility.

Baseline liver biopsies were scored by the local study pathologist for histological eligibility for trial entry according to the NASH CRN histological scoring system [45]. In addition, all baseline biopsies are reviewed centrally and all follow-up biopsies are scheduled to be reviewed centrally at a multihead microscope using consensus scoring by the NASH CRN Pathology Committee for efficacy assessments. Although the reading of the local pathologist was used to determine histological eligibility for trial entry, the histological readings and the NAS and fibrosis stage assigned by the pathology committee at central reviews, will be used in efficacy analyses.

The follow-up of TONIC participants is currently ongoing and data collection including histological findings obtained after 96 weeks of treatment with study medications will be completed in early 2010.

4. Summary

TONIC is a multicenter, randomized, placebo-controlled therapeutic clinical trial of NAFLD in children. Three treatment groups include metformin 500 mg twice a day, vitamin E 400 IU twice a day and placebo administered for 96 weeks. The primary outcome measure is a reduction in ALT levels during the last half of 96 weeks of treatment. Histological changes are the major secondary outcome measures and include changes in NAS, fibrosis, as well as changes in other histological features commonly found in NASH.

Compliance with trial protocol and safety of therapeutic interventions are followed closely by an independent DSMB. The enrollment was completed by September 2007 and final results are expected by the end of 2010. The full TONIC protocol can be requested from the NASH CRN DCC via the Internet [52].

Writing Committee

Members consist of Joel E. Lavine, Jeffrey B. Schwimmer, Jean P. Molleston, Ann O. Scheimann, Karen F. Murray, Stephanie H. Abrams, Philip Rosenthal, Arun J. Sanyal, Patricia R. Robuck, Elizabeth M. Brunt, Aynur Ünalp, James Tonascia, and these authors take full responsibility for the contents of this manuscript.

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Appendix

The following members of the Nonalcoholic Steatohepatitis Clinical Research Network have been instrumental in the design and conduct of TONIC trial.

Case Western Reserve University, Cleveland, OH: Diane Bringman, RN, BSN (2003–2008); Srinivasan Dasarathy, MD; Ariel Feldstein, MD; Carol Hawkins, RN; Yao-Chang Liu, MD; Arthur McCullough, MD (Principal Investigator); Ruth Sargent, LPN; Margaret Stager, MD

Duke University Medical Center, Durham, NC: Manal Abdelmalek, MD; Anna Mae Diehl, MD (Principal Investigator); Marcia Gottfried, MD (2004–2008); Cynthia Guy, MD; Paul Killenberg, MD (2004–2008); Samantha Kwan, Yi-Ping Pan, Dawn Piercy, FNP; Melissa Smith, and *Johns Hopkins University School of Medicine, Baltimore, MD:* Kimberly Pfeifer, RN; Ann O. Scheimann, MD, MBA; Michael Torbenson, MD

Indiana University School of Medicine, Indianapolis, IN: Prajakta Bhimalli, Elizabeth Byam, Naga Chalasani, MD (Principal Investigator); Oscar W. Cummings, MD; Ann Klipsch, RN; Lydia Lee, Jean P. Molleston, MD; Linda Ragozzino, Girish Subbarao, MD; Raj Vuppalanchi, MD

Saint Louis University School of Medicine, St Louis, MO: Sarah Barlow, MD (2002–2007); Elizabeth M. Brunt, MD; Jose Derdoy, MD; Joyce Hoffmann, Debra King, RN; Joan Siegner, RN; Susan Stewart, RN; Brent A. Neuschwander-Tetri, MD (Principal Investigator); Judy Thompson, RN; and *Baylor College of Medicine, Houston, TX:* Stephanie H. Abrams, MD; Diana Arceo, MD, MS; Denise Espinosa, Leanel Angeli Fairly, RN

University of California San Diego, San Diego, CA: Cynthia Behling, MD, PhD; Lisa Clark, PhD, MPH; Janis Durelle, Tarek Hassanein, MD; Joel E. Lavine, MD, PhD (Principal Investigator); Susana Mendoza, Zana Parman (2003–2007), Heather Patton, MD; Jeffrey B. Schwimmer, MD; Claude Sirlin MD; Tanya Stein, MD; Zobeida Palomares

University of California San Francisco, San Francisco, CA: Kiran Bambha, MD, Nathan M. Bass, MD, PhD (Principal Investigator); Linda D. Ferrell, MD; Danuta Filipowski, MD; Raphael Merriman, MD (2002–2007); Mark Pabst, Monique Rosenthal, Philip Rosenthal, MD; Tessa Steel (2006–2008)

University of Washington Medical Center (2002–2007), Virginia Mason Medical Center, Seattle, WA: Melissa Coffey, Kris V. Kowdley, MD (Principal Investigator); Jody Mooney, MS; Karen F. Murray, MD; James Nelson, PhD; Cheryl Saunders, MPH; Matthew Yeh, MD, PhD; Melissa Young

Virginia Commonwealth University, Richmond, VA: Sherry Boyett, RN; Daphne Bryan, MD; Melissa J. Contos, MD; Michael Fuchs, MD; Amy Jones, Velimir AC Luketic, MD; Bimalijit Sandhu, MD; Arun J. Sanyal, MD (Principal Investigator); Carol Sargeant, RN, MPH; Melanie White, RN; and *Children's National Medical Center, Washington DC:* Parvathi Mohan, MD; Kavita Nair

National Cancer Institute, Bethesda, MD: David E. Kleiner, MD, PhD

National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD: Edward Doo, MD; Jay Hoofnagle, MD; Patricia R. Robuck, PhD, MPH

Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD: Terry T-K Huang, PhD

Johns Hopkins University, Bloomberg School of Public Health (Data Coordinating Center), Baltimore, MD: Pat Belt, BS; Fred Brancati, MD, MHS; Jeanne Clark, MD, MPH; Ryan Colvin, MPH; Michele Donithan, MHS; Mika Green, MA; Rosemary Hollick (2004–2005); Milana Isaacson, Wana Kim, Alison Lydecker, MPH (2006–2008); Pamela Mann, MPH; Laura Miriel, Alice Sternberg, ScM; James Tonascia, PhD (Principal Investigator); Aynur Ünalp-Arida, MD, PhD; Mark Van Natta, MHS; Laura Wilson, ScM; Kathie Yates, ScM

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TONIC Trial Design

- Randomized, multicenter, masked, placebo-controlled study
- Metformin, 500 mg *bid*, or vitamin E, 400 IU *bid*, compared to placebo

Figure 1.
Design schematic

Table 1**TONIC Trial Design****Table 1a. TONIC inclusion criteria**

- 1 Liver biopsy indicating NAFLD obtained within 6 months before randomization
- 2 ALT greater than 60 U/L on two separate occasions more than 30 days but within one year
- 3 Histological evidence of NAFLD (minimum of 5% of hepatocytes with macrovesicular fat)
- 4 Informed consent and assent provided

Table 1b. TONIC exclusion criteria

- 1 Current or history of significant alcohol consumption
- 2 Current or history of diabetes mellitus
- 3 ALT greater than 400 U/L
- 4 Clinical or histological evidence of cirrhosis
- 5 Evidence of other Chronic liver disease
- 6 Serum creatinine of 1.5 mg/dL or greater for boys or 1.4 mg/dL for girls
- 7 Use of drugs historically associated with NAFLD for more than 2 consecutive weeks in the 2 years prior to randomization
- 8 Use of antidiabetic drugs in the 3 months prior to randomization
- 9 Use of anti-NAFLD drugs (metformin, vitamin E, UDCA, SAM-e, betaine, milk thistle, probiotics) in the 3 months prior to randomization
- 10 Use of any over-the-counter or herbal remedy for hyperlipidemia in the 3 months prior randomization
- 11 History of metabolic acidosis
- 12 History of renal dysfunction
- 13 History of coagulopathy
- 14 History of bariatric or hepatobiliary surgery
- 15 History of total parenteral nutrition during the past 3 years prior to screening
- 16 Inability to swallow study medication
- 17 Vitamin E supplementation of greater than 100 IU per day
- 18 Disease considered by study physician to be significant
- 19 Females of childbearing potential: positive pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
- 20 Females of childbearing potential: Breast feeding
- 21 Any other condition, which in the opinion of the investigator would impede compliance or hinder completion of the study

Table 2

Data collection schedule

Assessment/Procedure		Screening visits		Follow-up visits weeks from randomization											
		S1	S2	RZ	4	12	24	36	48	60	72	84	96	120	
Consent															
Baseline (B) or interim (I) medical history	B	.	.	X	.	.	.	.	.	.	.	.	.	.	.
Review for adverse effects	X	.	.	.	I	I	I	I	I	I	I	I	I	I	I
Review of concomitant medications	X	.	.	.	X	X	X	X	X	X	X	X	X	X	X
AUDIT (A) or interim (I) alcohol quest	A	.	.	.	X	X	X	X	X	X	X	X	X	X	X
Study drug dispensing	.	.	.	X	.	X	X	X	X	X	X	X	.	.	.
Review of study drug adherence	.	.	.	X	X	X	X	X	X	X	X	X	X	.	.
Detailed (D) or focused (F) physical exam	D	.	.	.	F	F	F	F	D	F	F	F	D	D	D
DEXA scan for body fat	.	X	.	.	.	.	.	.	.	.	.	.	X	.	.
MRI for body fat (optional)	.	X	.	.	.	.	.	.	.	.	.	.	X	.	.
Liver biopsy (H for ≤6 months)	H	.	.	.	.	.	.	.	.	.	.	.	X	.	.
Block Brief nutrition questionnaire	.	X	.	.	.	.	.	.	X	.	.	.	X	X	X
Modifiable activity questionnaire	.	X	.	.	.	.	.	.	X	.	.	.	X	X	X
Pediatric quality of life	.	X	.	.	.	.	.	.	X	.	.	.	X	X	X
Liver symptom questionnaire	.	X	.	.	.	.	.	.	X	.	.	.	X	X	X
Labs															
Metabolic panel	X	.	.	X	X	X	X	X	X	X	X	X	X	X	X
Hematology	X	.	.	.	.	X	.	X	.	X	.	X	X	X	X
Fasting lipid profile	X	.	.	.	.	.	X	.	X	.	X	.	X	X	X
Fasting serum glucose	X	.	.	.	.	.	X	.	.	.	X	.	.	.	.
Hepatic panel	.	X	.	X	X	X	X	X	X	X	X	X	X	X	X
Vitamin B12	.	X	.	.	.	X	.	X	.	X	.	X	.	X	X
GGT, prothrombin time, HbA1c	.	X	.	.	.	.	.	.	X	.	.	.	X	X	X
OGTT with insulin and C-peptide	.	X	.	.	.	.	.	.	X	.	.	.	X	X	X
Free fatty acid, leptin, C-reactive protein	.	X	.	.	.	.	.	.	X	.	.	.	X	X	X
Urine pregnancy test (females)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Banking															

Assessment/Procedure	Screening visits			Follow-up visits weeks from randomization									
	S1	S2	RZ	4	12	24	36	48	60	72	84	96	120
Fasting serum, plasma banking	.	X	.	.	.	X	.	X	.	X	.	X	.
Serum vitamin E (on banked specimen)	.	X	.	.	.	.	.	X	.	.	.	X	.
DNA for banking	.	X	.	.	.	.	.	.	.	.	.	.	.
Closeout form (CO)	.	.	.	.	.	.	.	.	.	.	.	.	X

Detailed (D) physical includes measurement of height, weight, waist, hips, vital signs (temperature, heart rate, blood pressure); triceps skin fold thickness; mid-upper arm circumference; examination for scleral icterus and pedal edema and auscultation of the heart and lungs; general physical findings (hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting, factor); Tanner staging.

Focused (F) physical includes measurement of height, weight, vital signs (temperature, heart rate, blood pressure); examination for scleral icterus and pedal edema and auscultation of heart lungs.

OGTT: Blood samples will be obtained only at baseline and at 2 hours after administration of glucose solution.

Lipid profile: total cholesterol, triglyceride, LDL, HDL.

Hematology: Hemoglobin, hematocrit, WBC, platelet count.

Metabolic panel: sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin total protein.

Hepatic panel: total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase.

Fasting visits: All visits except RZ.