NASH CRN

Nonalcoholic Steatohepatitis
Clinical Research Network

Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis: A Multicenter, Randomized, Double-Masked, Placebo-Controlled Trial (PIVENS)

IND #69, 751

Protocol

CONFIDENTIAL

18 April 2007

Contents

Design synopsis

Title	Piogliatazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis: A Multicenter, Randomized, Double-Masked, Placebo-Controlled Trial (PIVENS)
Sponsor	NIDDK
Type of study	Phase III randomized clinical trial
Objective	To evaluate whether 96 weeks of treatment with either pioglitazone or vitamin E lowers NASH activity as determined from hepatic histology in nondiabetic adults with NASH compared to treatment with placebo
Study design	Multicenter, double-masked, double-dummy, placebo-controlled study with 3 parallel groups
Treatment groups	Group 1: Pioglitazone (30 mg q.d.) and vitamin E-placebo (q.d.) Group 2: Vitamin E (800 IU, natural form, q.d.) and pioglitazone-placebo (q.d.) Group 3: Pioglitazone-placebo (q.d.) and vitamin E-placebo (q.d.)
Study duration	Up to 6 months screening prior to randomization, including at least 3 months of drug washout for those using antiNASH or antidiabetic medications prior to baseline liver biopsy or prior to randomization 96-week treatment period 24-week washout period
Sample size	240 (80 per group)
Number of clinics	8
Inclusion criteria	- Histological evidence of NASH activity based on standardized scoring of a liver biopsy obtained no more than 6 months prior to randomization - Age 18 years or older at initial screening interview
Exclusion criteria	- Significant alcohol consumption more than 20 g/day for females and more than 30 g/day for males on average, either currently or for a period of more than 3 consecutive months in the 5 years prior to screening - Inability to reliably quantify alcohol intake - Clinical or histologic evidence of cirrhosis

- Evidence of other forms of chronic liver disease
- Serum alanine aminotransferase (ALT) greater than 300 U/L
- Fasting plasma glucose of 126 mg/dL or greater at initial screening or at the oral glucose tolerance test done at the second screening visit
- Serum creatinine of 2.0 mg/dL or greater
- Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, other known hepatotoxins) for more than 2 weeks in the 2 years prior to randomization
- Use of antidiabetic drugs (insulin, biguanides, glucosidase inhibitors, sulfonylureas, meglitinides, metformin, thiazolidinediones) in the 3 months prior to liver biopsy or the 3 months prior to randomization
- Use of antiNASH drugs (thiazolidinediones, vitamin E, metformin, UDCA, SAM-e, betaine, milk thistle, gemfibrozil, anti-TNF therapies, probiotics) in the 3 months prior to liver biopsy or the 3 months prior to randomization
- Use of a non-stable dose of statins (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin) or fibrates (clofibrate, fenofibrate) in the 3 months prior to liver biopsy or the 3 months prior to randomization
- Known intolerance to thiazolidinediones or vitamin E
- Vitamin E supplementation of greater than 100 IU/day
- Inability to safely obtain a liver biopsy
- History of diabetes mellitus
- History of total parenteral nutrition in the year prior to screening
- History of bariatric surgery or currently undergoing evaluation for bariatric surgery
- History of biliary diversion
- Known positivity for antibody to Human Immunodeficiency Virus
- Known heart failure of New York Heart Association class 2, 3, or 4
- Active, serious medical disease with likely life-expectancy less than 5 years
- Active substance abuse, such as alcohol or inhaled or injection drugs, in the year prior to screening
- Women of childbearing potential: positive pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
- Women: breast feeding

		- Any other condition, which in the opinion of the investigator would impede compliance or hinder completion of the study - Failure to give informed consent
Outcome measures	***Primary:***	Improvement in NASH activity defined from change in standardized scoring of liver biopsies at baseline and after 96 weeks of treatment
	Secondary:	- Change in composite histology score - Change in fibrosis score - Change in serum aminotransferase levels - Change in anthropometric measurements (weight, BMI, waist to hip ratio, waist circumference, triceps skin fold thickness, upper arm circumference, body composition) - Change in insulin resistance (assessed by HOMA) - Change in serum vitamin E levels - Change in cytokines, leptin, fibrosis markers, and lipid profile - Change in HR-QOL scores
Randomization	Centrally administered randomization stratified by clinical center and blocked by calendar time	
Statistical analysis	All analyses will be on an “intention-to-treat” basis	
Safety monitoring	NIDDK appointed DSMB will monitor the data for safety and efficacy for outcomes such as hepatotoxicity, hypoglycemia, pregnancy, new onset diabetes, and any other outcomes or events identified as safety-related	

1.Primary hypothesis and principal objective

Insulin resistance and oxidative stress (resulting in lipid peroxidation) are considered to be the two most important mechanisms in the pathogenesis of NASH. We hypothesize that pioglitazone and vitamin E will lead to improvement in hepatic histology in nondiabetic adults with biopsy proven NASH through changes in insulin resistance or oxidative stress.

The principal objective of this randomized, multicenter, double-masked, double-dummy, placebo-controlled trial is to evaluate whether 96 weeks of treatment with either pioglitazone or vitamin E lowers NASH activity as determined from hepatic histology in nondiabetic adults with NASH compared to treatment with placebo.

2. Background and significance

2.1. Introduction

In 1980, Ludwig et al. coined the term "nonalcoholic steatohepatitis" (NASH) to describe a previously recognized clinicopathological entity that occurred in obese, diabetic women with no excessive alcohol consumption in whom the hepatic histology was similar to alcoholic hepatitis (1). In the original description, hepatic histology was characterized by striking macrovesicular steatosis and evidence of focal necrosis with mixed inflammatory infiltrate, and Mallory bodies. Fibrosis was present in most specimens, with 15% of the patients having established cirrhosis. Since that report, it has become increasingly clear that NASH is a common disease and can have serious clinical sequelae. Hepatic steatosis is the precursor for NASH, but the pathogenesis of liver cell injury, inflammation, and hepatic fibrosis that characterize NASH are not well understood.

2.2. Definition, prevalence, and risk of progression of NASH

Recently several groups of investigators have proposed the term "nonalcoholic fatty liver disease" (NAFLD) to describe a spectrum of histological findings ranging from (a) steatosis alone to (b) steatosis with lobular inflammation to (c) steatosis with ballooning degeneration of hepatocytes to (d) steatosis with "alcoholic hepatitis" like lesions (sinusoidal fibrosis and polymorphonuclear infiltrates, with or without Mallory bodies) (2;3). These investigators have suggested that NASH represents the most advanced form of NAFLD, and only conditions described by (c) or (d) should be referred to as NASH.

NAFLD is probably the most common cause of abnormal liver enzymes in the U.S. population (4). Falck-Ytter et al. recently summarized studies that reported the prevalence of NAFLD in cohorts of patients undergoing liver biopsy, abdominal imaging (ultrasound, CT scan), and postmortem examination; the authors estimated the prevalence of NAFLD in the general population at approximately 20% (5). These rates are similar to those reported from the analyses of NHANES III, a U.S. population based survey from which the prevalence of NAFLD was estimated to be 7.9% (4).

While the natural history of NASH has not been fully defined, several lines of evidence strongly suggest that it is a progressive disorder with significant risk of developing cirrhosis and liver failure:

- In combined analyses of major series with follow-up liver histology data, 43% of patients with NASH had histological progression over a follow-up period of 1 to 7 years (with 14% developing cirrhosis) (6-8)

- In a recent single center study from Cleveland based upon 136 patients with NASH (72% with at least 10 years of follow-up), the cumulative risk of cirrhosis was 25% and liver related mortality was 31% (5)

- In several recent studies, a significant proportion of patients with cryptogenic cirrhosis may indeed represent a burn-out form of NASH (9-12).

Cryptogenic cirrhosis, the term applied when the etiology of liver disease is unexplained, is a common condition and accounts for up to 14% of liver transplants performed in the United States (13). Evolving data also suggest that a proportion of subjects with cirrhosis due to NASH develop hepatocellular carcinoma (14).

2.3. Factors associated with NASH

Factors associated with increased risk of NASH are listed below. Older age, female gender, diabetes, obesity, and hypertriglyceridemia are the most frequently reported risk factors for NASH. NASH is also associated with congenital and acquired forms of lipodystrophy, Alstrom's syndrome (a syndrome of retinal degeneration, deafness, obesity, and type II diabetes), and the metabolic syndrome (syndrome X: visceral fat accumulation, insulin resistance, type II diabetes, hypertriglyceridemia, and hypertension). NASH may also be more common in patients with hypothyroidism and sleep disorders.

Factors associated with NASH

Older age	Lipodystrophy
Female gender	Alstrom's syndrome
Diabetes mellitus	Obstructive sleep apnea (?)
Obesity	Hypothyroidism (?)
Hypertriglyceridemia	Polycystic ovary syndrome (?)
Metabolic syndrome	Prader-Willi Syndrome
Peroxisome biogenesis disorders	

2.4. Pathogenesis of NASH

The pathogenesis of NASH is not well understood, and understanding of this disorder is in evolution (15-17). Most evidence suggests that NASH is caused by two "hits", one that causes hepatic steatosis and the second that causes hepatocellular necrosis and inflammation which can lead to fibrosis. Steatosis induced by obesity or dysmetabolic syndrome is believed to represent the "first hit". Three overlapping mechanisms have been proposed as the "second hits":

1) Insulin resistance and disordered fatty-acid metabolism
2) Oxidative stress and subsequent lipid peroxidation
3) Abnormal cytokine production.

Of these, insulin resistance has been most investigated and is one of the most plausible mechanisms in the pathogenesis of NASH.

2.4.1. Insulin resistance

The two metabolic abnormalities most strongly associated with NASH are insulin resistance and an increased delivery of free fatty acids to the liver. NASH associated with diabetes and obesity appears to be primarily due to peripheral insulin resistance, and accordingly, is associated with high concentrations of serum insulin. Insulin blocks mitochondrial fatty-acid oxidation and, in common with other factors that precipitate NASH in obesity-related steatosis (rapid weight loss, surgery, alcohol intake), results in increased intracellular fatty acids, which may be directly toxic or lead to oxidative stress.

It is now evident that insulin resistance is very common even in nondiabetic subjects with nonalcoholic fatty liver and NASH. Marchesini et al. initially reported that subjects with nonalcoholic fatty liver (biopsies not performed) had substantially higher fasting insulin and glucose levels and marked insulin resistance as determined by the homeostasis model assessment method (HOMA) relative to a control population without fatty liver (18). As liver biopsies were not performed, this study could not address if NASH was associated with a higher degree of insulin resistance compared to simple fatty liver. Some of these questions were addressed in a recent study by Sanyal et al., who performed clamp studies in subjects with NASH or fatty liver, and normal controls (19). They found that the glucose infusion rate required to maintain euglycemia significantly decreased in a step-wise fashion from normal controls to subjects with fatty liver to those with NASH. Additionally, the suppression of lipolysis induced by insulin infusion was lower in subjects with fatty liver and NASH than in normal volunteers. Thus, NAFLD and, to a greater extent, NASH are associated with peripheral insulin resistance at the level of skeletal muscle (glucose disposal) and adipose tissue (lipolysis). At least 3 other studies confirmed that insulin resistance is a metabolic hallmark of nondiabetic patients with nonalcoholic steatohepatitis (20-22).

While the high prevalence of insulin resistance in nondiabetic patients with NASH no longer is in dispute, it continues to be unclear whether insulin resistance is the cause or the consequence of NASH. Preliminary data from Indiana University shed some light in this aspect. A prospective study was conducted in 18 nondiabetic patients with histologically proven NASH and in 18 age-, gender-, ethnicity-, and BMI-matched controls to characterize the metabolic response to a mixed meal and to identify anthropometric determinants of insulin resistance (23). Compared to controls, subjects with NASH had significantly higher fasting insulin and C-peptide levels at baseline and following the mixed meal. However, glucose levels were not different either at baseline or following the meal. The

NASH group had higher fasting levels of free fatty acids (FFA) than the controls (459 ± 190 mmol/L vs 339 ± 144 mmol/L, respectively, P=0.03); however, meal induced suppression in lipolysis was similar between the two groups (39 ± 113% vs 46 ± 60%, P=0.8). As expected, insulin resistance had a significant correlation with BMI (r=0.39, P=0.02) and visceral fat (r=0.50, P=0.004). While BMI, % total body fat, and subcutaneous abdominal fat were similar between the groups, the NASH group had significantly higher percent visceral fat compared to controls (28 ± 10% vs 22 ± 14 %, P=0.02). As visceral fat is considered to be an important determinant of insulin resistance, this finding lends support to the hypothesis that insulin resistance is the cause rather than the consequence of NASH. This study also showed that fasting FFA levels were significantly higher in nondiabetic subjects with NASH and correlated with BMI and visceral fat mass. Previous studies have shown that elevated levels of FFA can lead to insulin resistance, and enlarged visceral fat mass can contribute sufficient amounts of FFA to the systemic circulation to cause peripheral insulin resistance (24-26).

Several lines of evidence support the concept that insulin resistance and resulting hyperinsulinemia play a fundamental role in the pathogenesis of NAFLD and NASH:

(1) The degree of insulin resistance correlates with the degree of severity of NAFLD. Insulin resistance is rather mild in patients with simple fatty liver, whereas it is pronounced in patients with advanced forms of NAFLD, i.e. NASH (19).

(2) In mice that are genetically altered to overexpress lipoprotein lipase in the liver, hepatic insulin signaling is selectively inhibited and NAFLD develops (27;28).

(3) Insulin resistance and hyperinsulinemia have been shown to increase hepatic CYP2E1 activity which can contribute to oxidative stress and lipid peroxidation (29;30).

(4) It was recently demonstrated that IKK-beta is chronically activated in ob/ob mice and other murine models of chronic insulin resistance (IKK-beta is the kinase that activates NF-κB)(31;32). When IKK-beta was inhibited in these experiments using genetic and pharmacologic methods, insulin resistance was abolished, indicating IKK-beta activation is one of the important molecular mechanisms for insulin resistance. TNF-α is a well-recognized IKK-beta activator, and the resultant induction of NF-κB promotes the transcription of TNF-α, suggesting a positive-feedback mechanism that could lead to a state of chronic insulin resistance.

(5) Hyperinsulinemia may play a direct role in the pathogenesis of NAFLD. Hyperinsulinemia decreases mitochondrial β-oxidation of fatty acids by increasing levels of malonyl CoA, an allosteric inhibitor of carnitine palmitoyltransferase that transports long chain fatty acids from the cytosol into the mitochondria (33). Furthermore, subjects with renal failure undergoing peritoneal dialysis regularly with dialysate containing insulin developed severe sub-capsular hepatic steatonecrosis indicating a direct effect of insulin on the liver

parenchyma (34).

2.4.2. Oxidative stress and lipid peroxidation

Sources of hepatic oxidative stress can include microsomes, mitochondria, or peroxisomes. Although peroxisomal and mitochondrial oxidative stress has been shown in experimental models of NASH, no human studies published to date have demonstrated their role in the pathogenesis of NASH. This is in contrast to microsomal oxidative stress where the evidence is mounting that microsomal oxidative stress plays a role in the human and animal models of NASH. Weltman et al. published the first human study that explored the role of CYP2E1 in the pathogenesis of NASH (35). In this study, the authors measured the levels of CYP2E1 in paraffin-embedded liver biopsy material (31 with NASH and 10 histologically normal livers) by immunohistochemistry using specific anti-human CYP2E1 antibodies. While the normal livers showed a CYP2E1 immunostaining confined to a rim two or three cells thick around the central vein, NASH livers had a significantly increased intensity of CYP2E1 immunostaining that closely followed the distribution of steatosis (most prominent in acinar zone 3, however extending into zones 2 and 1 in some cases).

In order to examine CYP2E1 activity and its determinants in humans with NASH, the investigators from Indiana University have recently measured hepatic CYP2E1 activity using chlorzoxazone (CHZ) elimination as an in vivo CYP2E1 probe in a well-characterized cohort of nondiabetic subjects with NASH and age-, gender-, and BMI-matched controls with no liver disease (36). This study found that nondiabetic subjects with NASH had significantly increased hepatic CYP2E1 activity, as reflected by oral clearance of CHZ, compared to well-matched controls (39 ± 12.5 vs 28 ± 10, $P=0.029$). Lipid peroxidation has been demonstrated both in animal and human models of NASH, and the proinflammatory, profibrogenic properties of its aldehyde end-products (malondealdehyde and 4-hydroxynonelol) potentially can account for all of the typical histological features observed in this disorder (16). The correlation between the degree of lipid peroxidation and magnitude of hepatic steatosis also provides an explanation for the association between severity of steatosis and the risk of NASH (34).

The first human study to demonstrate increased lipid peroxidation in patients with steatosis and NASH was conducted by Sanyal et al (19). In this study, the authors assessed the lipid peroxidation in the liver biopsy specimen by immunohistochemical staining for 3-nitrotyrosine (3-NT), a marker for lipid peroxidation. While minor amounts of staining for 3-NT were noted in normal livers, a considerable amount of staining for 3-NT was noted in patients with fatty liver and NASH; the degree of staining for 3-NT was significantly higher in patients with NASH than in patients with fatty liver alone.

More recently, investigators from Indiana University conducted in-vivo assessment of peripheral lipid peroxidation in 20 nondiabetic patients with biopsy proven NASH and 17 age-, gender-, and BMI-matched controls (37). The lipid peroxidation was assessed through photometric measurement

of total plasma thiobarbituric acid reacting substances (TBARS) and expressed as malondialdehyde equivalents. The plasma levels of TBARS were significantly higher in patients with NASH relative to their controls (3.2 ± 1.3 vs 2.0 ± 1.1 nmol/ml, P=0.004). These studies together indicate that subjects with NASH have increased levels of hepatic and peripheral lipid peroxides when compared to appropriate controls.

2.4.3. Abnormal cytokine production

A role for abnormal cytokines in the pathogenesis of NASH was initially suspected from the development of NASH in patients with jejunoileal bypass (21). This liver disease often responded to metronidazole and was therefore thought to be due to bacterial overgrowth and portal endotoxemia (15). Other studies in obese mice and, more recently, in humans with NAFLD have further suggested that abnormal cytokine production may have a role in the pathogenesis of NASH (38). A recent report found that humans with NASH had significantly higher prevalence of small intestinal bacterial overgrowth and elevated levels of TNFα relative to age- and gender-matched controls (39).

2.5. Review of published therapeutic studies of NASH

A number of reports have appeared in the literature evaluating the role of different therapies in the management of patients with NASH. These studies have shown promising but inconclusive data because of their small sample size, lack of adequate follow-up, and lack of use of liver histology as an endpoint. Consequently, none of the current treatments for NASH listed below has been shown to be both effective and safe.

Current treatments for NASH

Lifestyle modification	Insulin sensitizers
UDCA	- Metformin
Antioxidants	- Troglitazone
- Vitamin E	- Pioglitazone
- Betaine	- Rosiglitazone
- SAM-e	
Lipid lowering agents	
- Clofibrate	
- Gemfibrozil	
- Atorvastatin	

2.5.1. Lifestyle modification

Preliminary data suggest that gradual weight reduction may be beneficial for NASH. In a recent

report of 15 obese patients with NAFLD who had a 10% reduction in their BMI over a 3-month period using a calorie restricted diet (25 cal/kg/day) and exercise, there were significant improvements in liver biochemistries and the degree of steatosis. Improvement in the degree of inflammation and fibrosis also occurred in some patients (40). In contrast, more rapid and drastic weight reduction may worsen hepatic inflammation and fibrosis in morbidly obese patients with NAFLD (41). Other published reports in the literature also suggest gradual weight loss may lead to an improvement in liver histology (42-44).

However, the rate and degree of weight loss required for improving liver histology have not been established. Furthermore, techniques used to induce a weight loss may play an important role in liver histology, and little is known about the relative importance of changing diet composition as opposed to general calorie restriction (27). In animal studies, diets high in sucrose and fat are more likely to cause insulin resistance and hepatic steatosis than are equi-caloric diets enriched with glucose or protein (27).

Therefore, although subjects with a diagnosis of NASH are routinely advised by their physicians to modify their lifestyle (diet and exercise), there exist no convincing data to show it is of benefit to patients with NASH. The difficulty in achieving long-term weight control with calorie restriction and exercise has made it difficult to obtain prospective, controlled data on the role of weight loss and life-style modification on NASH.

2.5.2. Thiazolidinediones

Mouse models of insulin resistance and fatty liver disease have shown that thiazolidinediones improve hepatic histology (27). In a small series of patients, Caldwell et al., reported that troglitazone treatment led to improvement in liver enzyme abnormalities to a significant degree and mild improvement in the hepatic histology (45). Troglitazone was withdrawn from the market due to its potential to cause life-threatening hepatotoxicity.

Second generation thiazolidinediones are less hepatotoxic. To our knowledge, there are three ongoing single center, uncontrolled studies of thiazolidinediones (2 with pioglitazone and 1 with rosiglitazone) (46-48). All three studies have shown that a 24-48 week course of thiazolidinediones leads to an improvement in serum aminotransferase levels and hepatic histology. The degree of improvement was marked in those studies using a full 48-week course of treatment. In some cases, hepatic inflammation as well as fibrosis improved significantly.

2.5.3. Vitamin E

Lavine et al. conducted an open-label, pilot study to determine whether supplemental oral vitamin (α-tocopherol) was effective in lowering serum aminotransferases and alkaline phosphatase levels in children with NASH associated with obesity (49). Eleven children younger than 16 years of

age with elevated ALT levels and steatosis by imaging were given oral vitamin E (400-1000 IU/day) for 4-10 months. Daily oral vitamin E administration normalized serum aminotransferase and alkaline phosphatase levels in children with NASH despite no change in the BMI. Mean ALT levels decreased from 175 ± 106 IU/L to 40 ± 26 IU/L ($P<0.01$) and mean AST levels decreased from 104 ± 61 IU/L to 33 ± 11 IU/L ($P<0.002$). Interestingly, the levels of transaminases returned to abnormal levels in those individuals who elected to stop therapy with vitamin E. Serum α-tocopherol levels were within normal range at the beginning of the trial and increased significantly with supplementation.

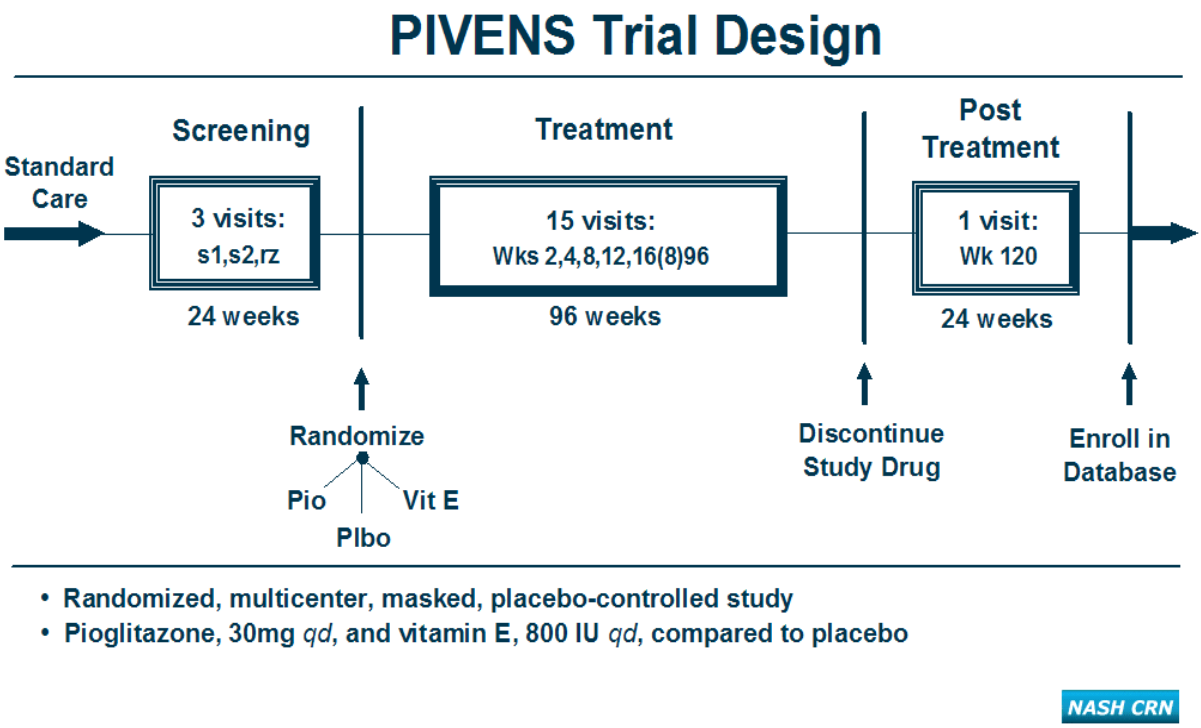
Hasegawa et al. conducted a pilot study evaluating plasma TGF β1 levels and the efficacy of α-tocopherol in adults with NASH (50). TGF β1 levels were normal in healthy controls, but were elevated in patients with NASH. After one year of therapy with α-tocopherol, serum aminotransferases and TGF β1 were significantly reduced. The authors concluded that the long-term therapy with vitamin E was safe and effective in reducing the inflammatory and fibrosis markers in patients with NASH.

In a study of Zucker rats, Soltys et al. demonstrated significantly higher levels of oxidative stress in these animals and that tocopherol therapy significantly improved the levels of oxidative stress and their survival following exposure to lethal levels of ischemia (51).

3. Study design

3.1. Design overview

PIVENS is a multi-center, randomized, placebo-controlled, double-masked, double-dummy clinical trial of treatment with pioglitazone, vitamin E, or placebo for patients with NASH. Screening for eligibility and collection of baseline data will span up to 24 weeks. Eligible patients will be randomized to receive either pioglitazone (30 mg q.d.), vitamin E (800 IU q.d.), or placebo for 96 weeks. There will be a 24 week washout period at the end of the treatment phase to assess durability of effects, if any, and to ensure patient safety following the end of treatment. Patients will be asked to participate in the NAFLD Database with annual follow-up visits after the trial has ended. The primary comparisons will be made using an intention-to-treat analysis of the change in NASH activity, as determined from standardized histologic scoring of liver biopsies taken at baseline and at week 96. Secondary outcome measures include change in serum aminotransferase levels, change in anthropometric measurements, change in insulin resistance, change in serum vitamin E levels, and change in health related quality of life scores. A schematic of the trial design is presented below:



3.2. Treatment groups

Patients who have signed an informed consent statement and who meet the eligibility criteria will be randomly assigned to one of three groups for 96 weeks of treatment:

Group 1: Pioglitazone (30 mg q.d.) and vitamin E-placebo (q.d)
Group 2: Vitamin E (800 IU, natural form, q.d.) and pioglitazone-placebo (q.d.)
Group 3: Pioglitazone-placebo (q.d.) and vitamin E-placebo (q.d.)

The randomization scheme will assign patients in randomly permuted blocks of assignments stratified by clinical center; block size will be determined randomly. This scheme will ensure that the three groups will be balanced by calendar time of enrollment (to minimize secular effects) and by clinic (to minimize clinic-specific effects of differences in patient populations and management).

The randomization plan will be prepared and administered centrally by the Data Coordinating Center (DCC) but will not require real time interaction with a DCC staff member. Requests for randomizations will be made by the clinics using a web-based application. An 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.

3.3. Pioglitazone

Pioglitazone will be administered as a single tablet of 30 mg per day orally with the morning meal. A similar appearing placebo tablet will be taken daily by patients assigned to either the placebo group or the vitamin E group.

The rationale for choosing this dosage hinges on the earlier studies that examined the safety and efficacy of pioglitazone in patients with NASH (46;47). In an open-label, pilot study, Promrat et al. reported that pioglitazone administered in a dose of 30 mg per day is safe and leads to biochemical and histological improvement (47). Similarly, in a prospective study of 21 patients with NASH, Sanyal et al. reported that pioglitazone administered in 30 mg per day in combination with vitamin E led to a significant improvement in hepatic steatosis, ballooning, and Mallory's hyaline compared to vitamin E alone (46).

Pioglitazone, a thiazolidinedione derivative, is an oral antidiabetic agent that primarily works by increasing insulin sensitivity. Studies have shown that pioglitazone improves insulin sensitivity in muscle and adipose tissue and inhibits hepatic gluconeogenesis. It is a highly selective and potent agonist for the peroxisome proliferator-activated receptor-gamma (PPARγ).

In humans, PPARγ receptors are found in key target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPARγ nuclear receptors regulates the transcription of insulin responsive genes involved in the control of glucose production, transport, and utilization. In addition, PPARγ responsive genes participate in the regulation of fatty acid metabolism.

3.3.1. Rationale for using thiazolidinediones

Points establishing a rationale for the use of thiazolidinediones (TZDs) for treatment of NASH are:

- Insulin resistance is a metabolic hallmark of patients with NASH, and TZDs are effective in improving insulin sensitivity as described above.

- TZDs are effective in the management of several insulin resistance syndromes such as polycystic ovary syndrome (PCOS) (52;53), lipodystrophy associated with HIV (54), and vascular insulin resistance associated with hypertension (55).

- Mouse models of insulin resistance and fatty liver disease have shown that TZDs improve hepatic histology (56).

- Caldwell et al. treated 10 NASH patients with the first clinically available TZD for varying duration up to 6 months and noted improved liver enzymes and decreased necroinflammatory changes in some patients (45).

- In an open label study, Tetri et al. administered 4 mg rosiglitazone twice daily for 48 weeks to 30 patients with biopsy proven NASH. Rosiglitazone was well tolerated and led to improvement in insulin sensitivity, serum ALT levels, hepatic steatosis as demonstrated by CT imaging, and hepatic histology (48).

- In a prospective study of 21 patients with NASH, Sanyal et al. showed that pioglitazone administered for 6 months in combination with vitamin E resulted in a significant improvement in hepatic steatosis, ballooning degeneration, and Mallory's hyaline in comparison to vitamin E alone (46).

- In a recent study by Promrat et al., a 48-week course of pioglitazone (30 mg daily) led to significant improvements in insulin resistance, serum ALT levels, and hepatic histology in 18 patients with NASH (47).

- A recent study by Galli et al. examined the effects of rosiglitazone and pioglitazone on liver fibrosis in rats induced either by toxin administration or bile duct ligation (57). Oral administration of TZDs reduced extracellular matrix deposition and hepatic stellate cell activation in both toxic and cholestatic models of hepatic fibrosis. In vitro, TZD-induced

PPARγ activation inhibited collagen synthesis induced by TGF-β1 in human hepatic stellate cells. These data indicate that PPARγ activation retards fibrosis in vivo and suggests the use of TZDs for the treatment of liver fibrosis.

Collectively, these studies indicate that pioglitazone is likely to result in marked improvement in insulin resistance in patients with NASH and that the change may be associated with substantial improvements in hepatic pathology.

3.3.2. Adverse effects of thiazolidinediones

There are a number of adverse effects that have been attributed to thiazolidinediones (TZDs), which are summarized below:

- **Hepatotoxicity:** The first generation TZD, troglitazone, was associated with idiosyncratic hepatotoxicity and very rare cases of liver failure, liver transplantation, and death. Troglitazone has been withdrawn from clinical use. The second generation TZDs appear to be significantly less hepatotoxic. Second generation TZDs include rosiglitazone and pioglitazone:

  Rosiglitazone: In pre-approval clinical studies encompassing 3600 patient-years of exposure, there was evidence of drug-induced hepatotoxicity or associated elevations in ALT levels with treatment. In these controlled trials, 0.3% of subjects receiving rosiglitazone had elevations in ALT levels of more than 3 times the upper limit of normal compared to 0.2% on placebo and 0.5% on active comparators. In the postmarketing experience, reports of hepatitis and liver enzyme abnormalities have appeared. Rarely, these reports have included acute liver failure with or without fatal outcome. In the study of rosiglitazone in patients with NASH by Tetri et al. (48), one of 30 subjects was withdrawn from the study due to a sudden 5-fold increase in ALT. Incidentally, this subject had received a short course of prednisone for an unrelated indication.

  Pioglitazone: In clinical studies worldwide, over 4500 subjects have been treated with pioglitazone, and there was no evidence that it was associated with drug-induced hepatotoxicity or associated elevations in ALT levels. In these trials, the frequency of increase in ALT of more than 3 times the upper limit of normal was 0.26% with pioglitazone and 0.25% with placebo. However, post-marketing reports suggest that pioglitazone usage can lead to increases in the ALT levels, and case-reports have appeared in the literature suggesting that pioglitazone usage can lead to liver dysfunction and jaundice. Pinto et al. from Indiana University reported a case in which pioglitazone use was associated with severe but reversible cholestatic jaundice (peak bilirubin more than 35 mg/dl) (58). In the study of pioglitazone in patients with NASH by Sanyal et al., one of 11 subjects

was withdrawn from the study due to a sudden 6-fold increase in ALT levels (46).

In the study of pioglitazone in patients with NASH reported by Promrat et al., none of 18 patients were withdrawn because of liver test abnormalities (47).

- **Effects on lipids:** Rosiglitazone can lead to an increase in the levels of total cholesterol, LDL, and HDL, and decreases in the levels of free fatty acids. The changes in triglyceride levels during therapy with rosiglitazone are variable. Patients treated with pioglitazone had mean improvements in triglycerides and HDL-cholesterol, but no consistent increases in LDL or total cholesterol (59).

- **Dose dependent weight gain:** When administered at 8 mg per day, rosiglitazone led to a median weight gain of 2.6 kg in clinical studies. In U.S. placebo-controlled monotherapy trials, pioglitazone led to a weight gain ranging between 0.5 to 2.8 kg (59). In the study of Promrat et al., a one-year course of pioglitazone in patients with NASH led to an average weight gain of 3.2 kg, most of which was shown to be due to increase in fat mass (47).

- **Hypoglycemia:** Infrequently, hypoglycemia can occur when TZDs are used as monotherapy for diabetes. When rosiglitazone was used as monotherapy in a dose of 8 mg per day, the incidence of hypoglycemia was 1.6%. The frequency of hypoglycemia was 2% when pioglitazone was used in combination with sulfonylureas (59).

- **Edema:** TZDs are associated with the development of mild-moderate edema (59). Therefore, caution should be applied while using these agents in subjects with edematous conditions. The edema appears to be due to an increase in the plasma volume.

- **Ovulation:** TZDs may result in ovulation in some anovulatory premenopausal women (59). Thus, subjects taking TZD agents are at an increased risk of becoming pregnant. Therefore, adequate contraception is necessary in premenopausal women.

- **Hemoglobin/hematocrit:** The use of TZDs leads to a consistent but mild drop in hemoglobin and hematocrit (mean drop in hemoglobin less than 1 g/dL) (59). This is thought to be dilutional in nature as a result of increase in the plasma volume.

- **Risk of drug interactions:** Pioglitazone is metabolized by multiple cytochrome P450 isoforms such as CYP2C8 and CYP3A, and there are some data which suggest that pioglitazone may induce CYP3A. This leads to a theoretical possibility that co-administration of a CYP3A inhibitor (such as diltiazem, macrolides, imidazoles, etc) may lead to disturbed in vivo disposition of pioglitazone. However, this is not believed to be a significant concern as pioglitazone will be metabolized by CYP2C8 in situations when CYP3A is inactive.

Strategies for protecting patients against the possible adverse effects of pioglitazone are discussed in section 5.8.1.1.

3.4. Vitamin E

The formulation of vitamin E to be used in this study is the natural form of vitamin E (RRR-α-tocopherol, formerly known as *d*-α-tocopherol) at a daily dosage of 800 IU administered orally via a single softgel. A similar appearing placebo softgel will be taken daily by patients assigned to either the pioglitazone group or the placebo group.

The rationale for the use of vitamin E for treatment for NASH was developed in section 2.5.3. Double-masked trials and large population studies have shown that oral vitamin E at 800 IU daily dose is safe with no significant side effects (60;61). The vitamin E dose chosen for this trial (800 IU q.d.) is within the range of vitamin E dosage that has been tested for the treatment of human NASH in previous pilot studies (46;49;62;63). For example, in a pilot study, Kugelmas et al. tested the effect of step I American Heart Association diet plus exercise with or without 800 IU of vitamin E daily (63).

Although the use of antioxidants may improve insulin resistance and thereby may reduce the incidence of new-onset diabetes, it is reasonable to assume that the magnitude of risk of new-onset diabetes in patients randomized to the vitamin E group is the same as placebo (i.e., 18 patients during the 96 weeks of treatment). Measures to identify patients who develop diabetes during the trial and their management strategy are described in section 5.8.1.2.

3.5. Rationale for placebo treatment group

Currently, there is no proven pharmacotherapy available for the treatment of NASH. In the current study, we intend to test the hypothesis that therapeutic agents to modify insulin resistance (pioglitazone) or oxidative stress (vitamin E) improve liver histology in patients with NASH. The efficacy of each of these agents will be compared to placebo. As there is no proven pharmacologic therapy for NASH, using a placebo for comparative purposes is justified. In this study, subjects will take pioglitazone-placebo and vitamin-E placebo.

3.6. Standard treatment recommendations

In addition to the study medication, patients will receive a standardized set of recommendations about life-style modification (dietary modification, weight loss, exercise), use of prescription or non-prescription medicines or herbal remedies or dietary supplements, consumption of alcohol, and management of various co-morbid illnesses. These recommendations have been prepared by the NASH CRN Standards of Care Committee and are approved by the NASH CRN Steering Committee. This will help ensure that the patients in all groups receive standard of care treatment for NASH.

4. Patient selection

4.1. Recruitment

Approximately 240 patients will be recruited from the eight clinical centers of the NASH CRN (averaging 30 patients per center) over a two-year period:

- Case Western Reserve University, Cleveland, OH (PI: Arthur McCullough, MD),
- Indiana University, Indianapolis, IN (PI: Naga Chalasani, MD),
- Duke University, Durham, NC (PI: Anna Mae Diehl, MD),
- Saint Louis University, St Louis, MO (PI: Brent Tetri, MD),
- University of California, San Diego, CA (PI: Joel Lavine, MD),
- University of California, San Francisco, CA (PI: Nathan Bass, MD),
- University of Washington, Seattle, WA (PI: Kris Kowdley, MD),
- Virginia Commonwealth University, Richmond, VA (PI: Arun Sanyal, MD).

Eligible patients will be identified and recruited at the participating clinical centers subject to the inclusion and exclusion criteria listed later in this chapter. Clinics will be required to recruit sufficient overall numbers of minorities and women so that results can be generalized to these populations.

Each clinic will develop a recruitment plan. These plans will vary from clinic to clinic depending on the available pools of patients and local recruitment resources.

4.2. Inclusion criteria

In order to qualify for inclusion in the trial, patients must satisfy the following inclusion criteria:

1. Based on a liver biopsy obtained no more than 6 months prior to randomization (patient must not have used antiNASH medications in the 3 months prior to the biopsy), histological evidence of NASH as defined by a NASH activity score, which is **EITHER** (1) a composite score of 5 or greater (where each component score for steatosis, hepatocyte ballooning, and lobular inflammation is 1 or more) as judged by the local NASH CRN pathologist with a finding of possible (defined as suspicious/borderline/indeterminate) or definite steatohepatitis as judged by the local NASH CRN pathologist, **OR** (2) a composite score of 4 (where each component score for steatosis, hepatocyte ballooning, and lobular inflammation is 1 or more) as judged by the local NASH CRN pathologist and a finding of definite steatohepatitis as judged by the majority of the local NASH CRN pathologist and two additional NASH CRN pathologists (i.e., at least two of the three pathologists must find definite steatohepatitis).

2. Patient must be 18 years of age or older as of the initial screening interview.

4.3. Exclusion criteria

Patients who satisfy any of the following exclusion criteria will be ineligible for enrollment in the trial:

1. Current or history of significant alcohol consumption for a period of more than 3 consecutive months within 5 years prior to screening (significant alcohol consumption is defined as more than 20 g/day in females and more than 30 g/day in males, on average) or inability to reliably quantify alcohol consumption
2. Clinical or histological evidence of cirrhosis as judged by study physician (clinical cirrhosis is defined by presence of any of the following: albumin less than 3 g/dL, INR greater than 1.3, conjugated bilirubin greater than 2 mg/dL, varices, or ascites)
3. Evidence of other forms of chronic liver disease:
   - Hepatitis B as defined by presence of hepatitis B surface antigen (HBsAg)
   - Hepatitis C as defined by presence of hepatitis C virus (HCV) RNA or positive hepatitis C antibody (anti-HCV)
   - Evidence of ongoing autoimmune liver disease as defined by the presence of anti-nuclear antibody (ANA) of greater than 1:80 and consistent liver histology; or history of autoimmune hepatitis as judged by study physician
   - Primary biliary cirrhosis as defined by elevation of alkaline phosphatase greater than upper limit of normal and anti-mitochondrial antibody (AMA) of greater than 1:80 and consistent liver histology
   - Known primary sclerosing cholangitis and suggestive liver histology
   - Wilson's disease as defined by ceruloplasmin below the limits of normal and consistent liver histology
   - Alpha-1-antitrypsin deficiency as defined by a suggestive liver histology (confirmed by alpha-1 antitrypsin level less than normal at the discretion of the study physician)
   - History of hemochromatosis or iron overload as defined by presence of 3+ or 4+ stainable iron on liver biopsy
   - Drug-induced liver disease as defined on the basis of typical exposure and history
   - Known bile duct obstruction
   - Suspected or proven liver cancer
   - Any other type of liver disease other than NASH
4. Serum alanine aminotransferase (ALT) greater than 300 U/L
5. Fasting plasma glucose of 126 mg/dL or greater at initial screening or at the oral glucose tolerance test done at the second screening visit
6. Serum creatinine of 2.0 mg/dL or greater

7. Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, or other known hepatotoxins) for more than 2 consecutive weeks in the 2 years prior to screening
8. Use of antidiabetic drugs (insulin, biguanides, glucosidase inhibitors, sulfonylureas, meglitinides, metformin, thiazolidinediones) in the 3 months prior to liver biopsy or the 3 months prior to randomization
9. Use of antiNASH drugs (thiazolidinediones,vitamin E, metformin, UDCA, SAM-e, betaine, milk thistle, gemfibrozil, anti-TNF therapies, probiotics) in the 3 months prior to liver biopsy or the 3 months prior to randomization
10. Use of a non-stable dose of statins (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin) or fibrates (clofibrate, fenofibrate) in the 3 months prior to biopsy or the 3 months prior to randomization
11. Known intolerance to thiazolidinediones or vitamin E
12. Vitamin E supplementation of greater than 100 IU/day
13. Inability to safely obtain a liver biopsy
14. History of diabetes mellitus
15. History of total parenteral nutrition in the year prior to screening
16. History of bariatric surgery (jejunoileal bypass or gastric weight loss surgery) or currently undergoing evaluation for bariatric surgery
17. History of biliary diversion
18. Known positivity for antibody to Human Immunodeficiency Virus
19. Known heart failure of New York Heart Association class 2, 3, or 4
20. Active, serious medical disease with likely life-expectancy less than 5 years
21. Active substance abuse, such as alcohol or inhaled or injection drugs, in the year prior to screening
22. Women of childbearing potential: positive pregnancy test during screening or at randomization or unwillingness to use an effective form of birth control during the trial
23. Women: Breast feeding
24. Any other condition which, in the opinion of the investigator would impede compliance or hinder completion of the study
25. Failure to give informed consent

4.4. Run-in period

Patients must not have used any prescription or over-the-counter medication or herbal remedy taken with an intent to improve or treat NASH or liver disease or obesity or diabetes for the 3 months prior to liver biopsy and for the 3 months prior to randomization. AntiNASH agents include: thiazolidinediones, vitamin E, metformin, UDCA, SAM-e, betaine, milk thistle, gemfibrozil, anti-TNF therapies, and probiotics. Antidiabetic agents include but are not limited to: insulin, biguanides, sulfonylureas, metformin and thiazolidinediones. These agents are 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).

Any over-the-counter medication or herbal remedy that is being taken with an intent to improve hyperlipidemia will not be allowed for at least 3 months prior to randomization and will be discouraged after randomization. Patients will be allowed to continue on prescription anti-hyperlipidemic agents. Patients will be interviewed in a detailed fashion at screening, randomization, and at every clinic visit to document the absence of such use. If using a statin or fibrate medication, the patient must have been on a stable dose in the 3 months prior to liver biopsy and must have been on a stable dose in the 3 months prior to randomization.

5. Trial protocol

5.1. Visit schedule overview

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

- Screening for eligibility for enrollment (2 visits over a maximum of 24 weeks)
- Randomization to treatment (1 visit)
- Treatment phase (15 visits over 96 weeks)
- Post-treatment washout phase (1 visit at 120 weeks)

The visit and data collection schedule described below in detail is summarized in Appendix 9.3.

5.2. Screening and baseline data collection

Patients who appear to be eligible after chart review and completion of standard of care tests and procedures for NASH will be invited to undergo screening. Recording of screening data on NASH CRN forms may not start until the patient has signed the consent statement. Screening and baseline data collection procedures will include questionnaires, physical examination, measurement of fasting serum glucose, routine liver tests, and urine analysis. Prior therapy for NASH will be reviewed, and patients will be asked to stop any specific antiNASH treatment such as thiazolidinediones, vitamin E, metformin, UDCA, SAM-E, milk thistle, betaine, or gemfibrozil, anti-TNF therapies, or probiotics. Patient charts will be reviewed for historical information and previous liver biopsy findings. Patients who have not had a liver biopsy previously or whose previous liver biopsy does not meet the requirements for this trial will undergo a liver biopsy as part of screening for the trial.

All participants who sign the consent statement will be registered in the trial database. Each participant who starts screening will be accounted for at the end of screening, as either a screening success (enrolling in the trial) or a screening failure. A screening failure is defined as a participant who signed the consent form, but is found to be ineligible prior to randomization; screening failures include patients who meet medical eligibility criteria but who refuse enrollment in the trial. Reason for screening failure will be recorded in the trial database.

Screening and baseline data collection will be conducted over two clinic visits completed on separate calendar days. The goal of the first screening visit is to obtain consent and record data regarding the trial's inclusion and exclusion criteria; the goal of the second screening visit is to complete collection of baseline data on patients who appear eligible. If a liver biopsy is required, it will be done after the patient has been found to be eligible with respect to other inclusion and exclusion criteria. The procedures completed during screening are:

- **Screening visit 1**: Determination of eligibility will be based mostly on chart review of standard of care tests and procedures that were completed before screening visit 1. The patient will sign the consent at screening visit 1 to obtain any tests and procedures needed to finalize eligibility after chart review and will undergo a history and physical examination to identify other illness and contraindications for participation. Anthropomorphic assessments (body weight [kg], body height [m], body mass index [BMI], waist circumference [cm], hip circumference [cm] and waist-to-hip ratio, triceps skin fold thickness, mid-upper arm circumference); vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature); and general physical findings, including hepatosplenomegaly, peripheral manifestations of liver disease, ascites, wasting or fetor, will be collected and recorded. History of prior liver biopsies and use of antiNASH, antidiabetic, statin and fibrate medications in the 3 months prior to the biopsy will be obtained and recorded. Laboratory test results that need to be recorded from chart review or obtained as part of screening visit 1 include: tests for hepatitis B (HBsAg) and hepatitis C, ANA, anti-mitochondrial antibody, ceruloplasmin (if patient is less than 40 years old), A1AT concentration, iron overload and hepatic iron index, fasting serum glucose, CBC, metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, total protein), fasting lipid profile (total cholesterol, triglyceride, LDL, HDL). Frequency and amount of alcohol intake will be obtained. Women of childbearing potential must have a negative pregnancy test.

- **Baseline liver biopsy**: Patients who have not had a liver biopsy within 6 months of randomization or whose previous liver biopsy is not available for review or whose previous liver biopsy is of inadequate quality or whose previous biopsy was done within 3 months of using antiNASH medication (eg, thiazolidinediones, vitamin E, metformin, UDCA, SAM-e, betaine, milk thistle, gemfibrozil, anti-TNF therapies, or probiotics) must have a liver biopsy prior to randomization. The biopsy should be done once the patient has been found to be eligible with respect to all other criteria. Biopsy slides must be of adequate size (1.5 cm or more) and of adequate quality for interpretation. The patient must have not been using antiNASH drugs in the 3 months prior to biopsy, and the patient must have histologic evidence of definite or possible NASH as described in section 5.7 of this protocol.

  In the case of a biopsy done previously as standard of care, the NASH CRN study physician should check if tissue blocks and/or additional slides can be obtained from the original biopsy. If the liver biopsy is completed as part of screening for this trial, the liver tissue will be prepared for light microscopy and stains will include hematoxylin and eosin, Masson's trichrome and iron stain; additionally, a piece of liver tissue will be snap frozen and stored at -70 degrees C and set aside for banking.

Clinic staff should note that the date of the biopsy establishes a hard window for completion of screening for randomization – randomization must take place within 6 months (183 days) of the date of biopsy. If the biopsy is more than 3 months old when the patient enters screening and if the patient has medications that need to be washed out, the biopsy will be too old by the time randomization occurs. Clinic staff will have to monitor completion of screening procedures and speed things up if the closing date on the 6 months biopsy window is getting close.

- **Screening visit 2**: Patients will provide blood for banking (measurements to be done on banked serum will include vitamin E level). Patients will complete a nutritional questionnaire (Block 98), a health-related quality of life questionnaire (SF-36), life time drinking history questionnaire (Skinner), functional activity questionnaire, and liver symptoms questionnaire. Laboratory tests include: GGT, prothrombin time, HbA1c, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase), microalbuminuria. A standard 75 g OGTT with fasting serum glucose, insulin, and C-peptide will be administered. Women of childbearing potential must have a negative pregnancy test. Patients will undergo a DEXA scan to estimate total body fat. All patients will be given information on a healthy life style and diet appropriate for their weight and other factors.

The NASH CRN clinic data system will include software to check patient eligibility based on keyed data forms. The eligibility check task may be run at any time, and there is no limit on the number of times it may be run. The output from the task will list the eligibility checks that the patient has failed and a summary finding that the patient is eligible or ineligible for the trial. Thus staff can use this task to identify the items that still need to be completed, keyed, or verified after data from screening visit 1 are keyed and again after data from screening visit 2 are keyed. The randomization visit should not take place until the eligibility check indicates that the patient is eligible except for the items that can be completed only at the randomization visit.

5.3. Randomization visit

The randomization visit is the visit at which randomization takes place and the patient is issued the study medication randomly assigned to the patient. Randomization is the act of generating the random study medication assignment and is the procedure which defines a patient's enrollment into the trial. Randomization can only occur after eligibility has been fully checked and all data collected at screening visits 1 and 2 have been keyed to the trial database. Since these processes take time, randomization cannot be done at screening visit 2, and since study medication needs to be issued to the patient, the randomization visit must be completed in person with the patient. Therefore a visit separate from screening visit 2 is necessary. Since this will be a visit on a different calendar day and medication will be started at this visit, good clinical practice requires that a few basic checks of the patient's well-being be completed at the randomization visit.

The procedures completed at the randomization visit are: pregnancy test for women of child bearing potential, verification that the patient is feeling well, affirmation of consent, and generation of the random treatment assignment. The generation process includes the same electronic check on eligibility that the staff may run prior to the randomization visit. The medication assignment will not be generated unless the check finds that the patient is eligible, and the clinic staff indicate that they want to randomize the patient.

The random treatment assignment will consist of medication bottle numbers; these numbers will be unique and will be specific to the particular patient and visit it was generated for. They will correspond to numbered bottles of medications which have been sent to the clinical center's research pharmacy (or clinical coordinator if not using a pharmacy) by the NASH CRN Drug Distribution Center. The research pharmacy (or clinical coordinator) will issue the specific numbered bottles to the patient. Each patient's random treatment assignment will be generated for that specific patient and will not be transferable to another patient. Once the assignment has been generated, the patient should be issued the assigned study drugs (in person) and instructed about starting the drugs and monitoring for adverse effects.

The date of randomization is the 0 time for reckoning all Follow-up visits (ie, all Follow-up visits are scheduled at specific times measured from the date of randomization). The randomization computer program will generate a personalized appointment schedule for the patient; this schedule will indicate the ideal date for each Follow-up visit, as well as the time window around the ideal date during which the Follow-up visit may be done and the data collected at the visit may be used in the trial.

5.4. Follow-up visits

Patients will return for follow-up visits at 2, 4, 8, 12, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, and 120 weeks after randomization. Thus, starting at 16 weeks after randomization, patients will be seen at 8 week (2 month) intervals through 96 weeks. Each visit will have an interval of time surrounding the ideal date for the visit during which the visit may be done and the data included in the trial database. The ideal date for a visit is the exact anniversary from randomization. Visit windows will be constructed to be contiguous, so that at any point in time, some visit window is open, subject to a check on the minimum separation required between consecutive visits. The specific procedures to be completed at each of the follow-up visits are:

- **Week 2 visit**: Blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential).

- **Week 4 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination, including height, weight, waist, hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver signs; blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline

phosphatase); pregnancy test (for women of child-bearing potential). Dispense study drug and review study drug adherence with patient.

- **Week 8 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination, including height, weight, waist, hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver signs; blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential). Dispense study drug and review study drug adherence with patient.

- **Week 12 visit**: Blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential). Review study drug adherence with patient.

- **Week 16 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination, including height, weight, waist, hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms; blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential); blood draw for banking at central repository. Dispense study drug and review study drug adherence with patient.

- **Week 24 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; detailed physical examination, including height, weight, waist and hip measurements, triceps skin fold, and mid-upper arm circumference, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms; blood draw for CBC, metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, globulin, total protein), hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase), fasting glucose; pregnancy test (for women of child-bearing potential). Dispense study drug and review study drug adherence with patient.

- **Week 32 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination, including height, weight, waist, hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms; blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential); blood draw for banking at central repository. Dispense study drug and review study drug adherence with patient.

- **Week 40 visit**: Blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential). Review study drug adherence with patient.

- **Week 48 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; detailed physical examination, including height, weight, waist and hip measurement, triceps skin fold, and mid-upper arm circumference, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms; blood draw for CBC, metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, globulin, total protein), hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase), GGT, prothrombin time, OGTT with fasting serum glucose, insulin, and C-peptide, fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), HbA1c; pregnancy test (for women of child-bearing potential), microalbuminuria; nutritional, functional activity, HR-QOL and liver symptom questionnaires; blood draw for banking at central repository. Dispense study drug and review study drug adherence with patient.

- **Week 56 visit**: Blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential). Review study drug adherence with patient.

- **Week 64 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination including height, weight, waist, hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms; blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential); blood draw for banking at central repository. Dispense study drug and review study drug adherence.

- **Week 72 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination including height, weight, waist, hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms; blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase), fasting glucose; pregnancy test (for women of child-bearing potential). Dispense study drug and review study drug adherence with patient.

- **Week 80 visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination including height, weight, waist, hip measurement, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms; blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential); blood draw for banking at central repository. Dispense study drug and review study drug adherence with patient.

- **Week 88 visit**: Blood draw for hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase); pregnancy test (for women of child-bearing potential). Review study drug adherence with patient.

- **Week 96 (final treatment phase) visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; detailed physical examination including height, weight, waist and hip measurement, triceps skinfold and mid-upper arm circumference, vital signs (temperature, heart rate, respiratoryrate, blood pressure), and liver signs; blood draw for CBC, metabolic panel (sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, uric acid, albumin, globulin, total protein), hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase), GGT, prothrombin time, OGTT with fasting serum glucose, insulin, and C-peptide, serum vitamin E, fasting lipid profile (total cholesterol, triglyceride, LDL, HDL), HbA1c; pregnancy test (for women of child-bearing potential), microalbuminuria; blood draw for banking at central repository; nutritional, functional activity, HR-QOL and liver symptom questionnaires; DEXA scan for body fat composition; liver biopsy; review of study drug adherence; withdrawal of study medication.

- **Follow-up liver biopsy**: A follow-up liver biopsy will be obtained at the week 96 visit. Guidelines for obtaining the biopsy specimen are provided in the NASH CRN Liver Biopsy Procedure and NAFLD/NASH Histology Scoring System Manual; a 16 gauge needle is preferred and the specimen should be least 1.5 cm in length. The slides must be of adequate size (1.5 cm or more) and adequate quality for interpretation. The liver tissue will be prepared for light microscopy and stains will include hematoxylin and eosin, Masson's trichrome and iron stain; additionally, a piece of liver tissue will be snap frozen and stored at -70 degrees C and set aside for banking.

- **Week 120 (24 weeks after withdrawal of study medication) visit**: Follow-up medical history including review of medications, adverse effects, and interim drinking history; focused physical examination including height, weight, waist, hip measurements, vital signs (temperature, heart rate, respiratory rate, blood pressure), and liver symptoms; blood draw for CBC, hepatic panel (total and direct bilirubin, AST, ALT, alkaline phosphatase), OGTT with fasting serum glucose, insulin, and C-peptide, fasting lipid profile (total cholesterol, triglyceride, LDL, HDL); pregnancy test (for women of child-bearing potential), microalbuminuria, blood draw for banking at central repository, nutritional, functional activity, HR-QOL, liver symptom questionnaires; DEXA scan for body fat composition and closeout form.

5.5. Standardized questionnaires

Several standardized questionnaires will be administered to patients enrolled in the PIVENS trial. Questionnaires will be administered at baseline (prior to randomization) and during follow-up at specified intervals (see Appendix 9.3 for the data collection schedule). The purpose of the questionnaires is to obtain important information regarding alcohol intake, nutrition, functional activity, health-related quality of life, and liver-related symptoms.

Alcohol questionnaires: Patients enrolled in the treatment protocol will complete AUDIT and Skinner (lifetime drinking history) questionnaires during screening. Patients will complete interim alcohol questionnaires (AUDIT-C) at follow-up visits (included in the Follow-up Medical History). The purpose of these questionnaires is to ascertain that there is no significant alcohol consumption at enrollment or during the study period and to evaluate the effect of alcohol consumption on the response to various interventions.

Nutrition Questionnaire: The Block 98 nutrition questionnaire will be administered to each patient during screening and after 48 and 96 weeks of treatment, and at the 120 week visit (24 weeks after withdrawal of study medication). This instrument is detailed and likely to take 30-40 minutes to complete. It estimates food frequency and quantity over the preceding 12-month period. The objectives of administering nutrition questionnaire are (a) to assess the baseline nutritional comparability among randomized patients and (b) to assess the possible effect of nutrient intake on the response to various treatments.

Measure of functional activity: The NHANES III Activity Questionnaire will be administered during screening and after 48 and 96 weeks of treatment, and at the 120 week visit (24 weeks after withdrawal of study medication). The scores can be converted to METS; then the activity of NASH patients can be compared to U.S. population, and the activity of NASH patients can be compared to other liver disease patients.

Health-related quality of life: The SF-36 questionnaire will be administered during screening and after 48, and 96 weeks of treatment, and at the 120 week visit (24 weeks after withdrawal of study medication).

Liver-related symptoms: A questionnaire on liver symptoms has been developed by the NASH CRN Measures and Assessments Committee and will be administered during screening and after 48 and 96 weeks of treatment, and at the 120 week visit (24 weeks after withdrawal of study medication).

5.6. Specimen repository

Specimens will be collected and stored in a central repository for use as approved by the Steering Committee of the NASH CRN (see Appendix 9.4 for whole blood draw schedule). Specimens include serum, plasma, DNA, and liver tissue. The blood collected into a serum separation tube at screening visit 2, and at 16, 32, 48, 64, 80, 96 and 120 week visits will be divided into 0.5 mL aliquots. Aliquots will be kept in a storage facility at -70 degrees C. Blood will be collected at the screening visit 2 for extraction of DNA which will be stored at -20 degrees C. When possible, a portion of the liver biopsy specimen will be collected and stored.

5.7. Overview of scoring of liver biopsies

The tissue will be examined by light microscopy and scored based upon the NASH CRN NAFLD/NASH scoring system. The scoring system is detailed in the Liver Biopsy Procedure and NAFLD/NASH Histologic Scoring System Manual prepared by the NASH CRN Pathology Committee and approved by the NASH CRN Steering Committee. Briefly, biopsies will be assessed for the following:

(1) Adequacy of the biopsy sample for reading;

(2) Steatosis (including grade of 0-3, location, and whether microvesicular);

(3) Fibrosis based upon Masson's trichrome stain as 0-4 with:
0 = none
1a = mild zone 3 perisinusoidal
1b = moderate zone 3 perisinusoidal
1c = portal/periportal only
2 = zone 3 and periportal
3 = bridging
4 = cirrhosis

(4) Lobular inflammation as 0, 1 (<2 areas seen), 2 (2-4 areas seen), or 3 (>4 areas seen under 20-fold magnification) and as presence or absence of microgranulomas and lipogranulomas

(5) Portal inflammation as 0 (none), 1 (mild), or 2 (greater than mild)

(6) Liver cell injury: Ballooning as 0 (none), 1 (few) and 2 (many), with notation of presence or absence of acidophilic bodies (0 =rare or 1 = many), pigmented macrophages (0 = rare/absent, 1 = many), and megamitochrondria (0 = rare/absent, 1 = many)

(7) Mallory bodies as 0 (rare/absent) or 1 (many)

(8) Glycogen nuclei as 0 (rare/absent) or 1 (many)

(9) Hepatocellular iron grade based on iron stain as 0 (absent or barely discernible, 40x), 1 (barely discernable granules, 20x), 2 (discrete granules resolved, 10x), 3 (discrete granules resolves, 4x), and 4 (masses visible by naked eye)

(10) Steatohepatitis finding (No; Suspicious/borderline/indeterminate; or Yes, definite)

The component scores will be used to determine eligibility as well as primary histologic outcome measure defined elsewhere in this protocol. Baseline liver biopsies will be scored by the local NASH CRN pathologist for eligibility. Baseline and follow-up liver biopsies will be scored by central review of the slides by the Pathology Committee. The scores obtained centrally will be used for data analysis.

5.8. Safety issues

Safety issues can be divided into (a) safety concerns relating to the therapeutic interventions, (b) safety concerns related to liver biopsy, (c) concerns related to patient privacy, and (d) issues related to the central specimen repository.

5.8.1. Safety concerns related to the therapeutic agents

The following paragraphs discuss the important potential adverse effects and the proposed safeguards to minimize the risks involved.

5.8.1.1. Safety issues related to thiazolidinediones

• **Hepatotoxicity**: The first generation TZD, troglitazone, was associated with idiosyncratic hepatotoxicity, and very rare cases of liver failure, liver transplantation, and death. Troglitazone has been withdrawn from clinical use. The second-generation thiazolidinediones appear to be significantly less hepatotoxic. A review of risk of hepatotoxicity associated with second-generation TZD agents is provided in section 2.4. We intend to minimize the risk of hepatotoxicity (or its consequences) (a) by closely monitoring the aminotransferase levels during the study period (at 2, 4, 8, 12, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, and 120 weeks after randomization), (b) by discontinuing study medication in patients with severe hepatotoxicity (defined as ALT or AST greater than 10 times the ULN in the local lab OR absolute AST or ALT greater than 500 U/L OR serum total bilirubin at least 2.5 mg/dL and at least half of it is direct bilirubin), (c) by avoiding the other potential hepatotoxic agents, and (d) by instructing the patients about self-identification of symptoms associated with hepatotoxicity (i.e., yellow eyes, dark urine, unexplained anorexia, asthenia, or right upper quadrant abdominal pain).

Those who exhibit ALT or AST elevation that is not to the extent described above but who develop a 1.5-fold or greater elevation in ALT or AST from their baseline value and

absolute ALT or AST greater than 250 U/L OR total bilirubin at least 1.5 mg/dL and at least half of it is direct bilirubin OR develop symptoms suggestive of acute hepatotoxicity (i.e., jaundice, nausea, vomiting, abdominal pain, fatigue, anorexia, dark urine) will have their liver biochemistries checked on a weekly basis. If ALT, AST, and/or bilirubin levels decline or stabilize, subjects will have their liver biochemistries checked monthly for 3 months and then according to the protocol schedule. If ALT or bilirubin continues to rise on two consecutive measurements following the initial rise in ALT, AST or bilirubin or if the patient develops symptoms of hepatotoxicity, subjects will be considered to have developed significant hepatotoxicity and the study medication will be discontinued indefinitely.

Patients in whom the study medication has been discontinued due to significant hepatotoxicity will be followed according to the protocol and will be offered a liver biopsy at the conclusion of the study.

- **Hypoglycemia** is infrequently seen when thiazolidinediones are used as monotherapy. Subjects will be instructed about symptoms associated with hypoglycemia and the ways to correct it (eg, consuming fruit juice or a candy bar). Study medication will not be unmasked.

- **Ovulation**: Thiazolidinediones may result in ovulation in some anovulatory premenopausal women. Thus, subjects taking thiazolidinediones are at an increased risk of becoming pregnant. We intend to minimize this risk in absence of female sterilization or complete abstinence (a) by requiring that women of child-bearing potential practice two reliable birth control methods including systemic hormones, intrauterine device and barrier methods (diaphragm, male or female condom, cervical cap) with concomitant intravaginal spermicide, (b) by performing pregnancy tests in women of child-bearing potential at frequent intervals (at 2, 4, 8, 12, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, and 120 weeks after randomization), and (c) by discontinuing the study medication indefinitely in women who become pregnant or who wish to become pregnant during their participation in the trial. The study medication will be unmasked.

5.8.1.2. Safety issues related to placebo

In the Diabetes Prevention Program (DPP), the incidence of diabetes in the placebo group was 11.0 cases per 100 person-years (64). Based on this, it is anticipated that at least 18 subjects in the placebo group will develop new-onset diabetes during their participation in the trial. Participants will be seen frequently during the study period for assessment of symptoms consistent with uncontrolled hyperglycemia and will have fasting glucose levels measured at 24, 48, 72, 96, and 120 weeks after randomization (at 48, 96 and 120 weeks this measurement is part of the OGTT). Patients with a fasting glucose of at least 126 mg/dL will have the test repeated within 6 weeks to confirm the diagnosis of new-onset diabetes. Patients with a diagnosis of new-onset diabetes will be managed according to the following stepped care approach and the primary care physician will be notified.

(a) Lifestyle counseling to reduce excess body weight and increase exercise
(b) Education on diabetic diet

(c) If fasting glucose remains greater than 140 mg/dL after three months or if patient develops symptomatic diabetes, treatment will be initiated. The suggested treatment would begin with metformin or sulfonylurea as the preferred agent.
(d) If patient still is unable to maintain glycemic control, primary care physician may request unmasking of the study medication and should institute necessary measures for diabetes management. Study medication will not be unmasked to the investigator. These patients will be followed at scheduled intervals to collect other outcome data; however, they will not have the OGTT scheduled for the visits at 48, 96, and 120 weeks but will have only fasting glucose levels measured.

5.8.1.3. Safety issues related to Vitamin E

Vitamin E at 800 IU daily dose has no significant toxicity in adults (60;61). Although use of antioxidants may improve insulin resistance and thereby may reduce the incidence of new-onset diabetes, it is safe to assume that the magnitude of risk of new-onset diabetes mellitus in patients randomized to vitamin E is the same as placebo (i.e., 18 subjects during the 24 months treatment). Measures to identify patients who have developed new-onset diabetes mellitus and their management strategy are described in section 5.8.1.2.

5.8.1.4. Management of adverse effects attributed to study medication

During the trial, if a participant develops side effects thought to be due to the study medication and requires cessation of study medication, the medication will be stopped for 4 weeks. If the side effects disappear, an attempt will be made to reintroduce the study medication after 4 weeks. If the symptoms reappear, study medication will be once again stopped and the patient will no longer receive the study medication, but will be followed in the study according to the protocol, in keeping with the “intention-to-treat” paradigm.

5.8.2. Safety issues related to liver biopsy

Patients will have up to two liver biopsies for research purposes during their participation in this protocol. About 20% of people who have a liver biopsy have some degree of pain over the liver that may last a few minutes up to several hours. This occasionally requires pain medication and usually disappears completely within a day or two. A rare complication of liver biopsy is severe bleeding such that a blood transfusion or even radiological/surgical interventions are required to stop the bleeding (less than 1 in 1,000). Very rarely (less than 1 in 10,000 reported cases) death has occurred from bleeding after a biopsy. We intend to minimize the risks associated with liver biopsy (a) by requiring that each of the physicians who will obtain liver biopsies in the NASH CRN be very experienced in safely obtaining the liver biopsy specimens, (b) by not enrolling subjects with cirrhosis or subjects with coagulopathy, (c) by adhering to the good clinical practice in performing the liver biopsy, (d) by assuring that an attending hepatologist or radiologist directly supervises if a physician trainee is performing the procedure, and (e) by considering a transjugular liver biopsy in morbidly obese patients in whom a percutaneous, mid-axillary approach may not be feasible.

5.8.3. Safety issues related to patient privacy

It is the investigator's responsibility to conduct the protocol under the current version of Declaration of Helsinki, Good Clinical Practice, and rules of local IRBs. The investigator must ensure that the patient's anonymity be maintained in their data submission to the Data Coordinating Center. Patients will be identified only by an identification code but not by their name, SSN, or hospital medical record number. Investigators will maintain a separate confidential enrollment log which matches identifying codes with the patients' names and addresses (i.e., available only to local clinic staff). All study material will be maintained in strict confidence.

5.8.4. Safety issues related to specimen repository

It is anticipated that serum, plasma, DNA, and liver tissue from the participants will be stored for future studies related to NASH and possibly other liver/metabolic diseases. These samples will be stored in a central repository. The NASH CRN Steering Committee will develop specific guidelines addressing the issues such as (a) obtaining a separate informed consent, (b) storage, (c) transportation of the material, (d) who will have access to the material, and (e) what investigations to be conducted.

5.9. Adherence and retention

Two important goals of this protocol are to optimize adherence to the pharmacological regimen and to maximize the retention of participants in the study. Assessment of adherence to the assigned study medication will provide clinic staff a means to identify participants having problems with adherence. Adherence will be assessed by:

- Counts of pills in the patient's returned pill containers
- Conducting a brief, structured interview, in which the study coordinator will assist the patients to identify problems in taking the study medication and to estimate adherence to the prescribed medicine since their previous visit.

These assessments will guide the consideration of strategies to improve adherence. Resources will be provided to remove barriers to participation such as child or elder care, transportation, and parking expenses. These resources can be provided as cash, transportation vouchers, or parking passes.

An honorarium may be paid to patients in recognition of their time and effort twice during the trial when scheduled visits and procedures are completed successfully.

Certificates of appreciation may be given at enrollment and at conclusion as an incentive.

5.10. Management of concomitant conditions

Hypertension and hyperlipidemia will be managed in conjunction with the patient's primary care physician according to the protocols described in the Standards of Care document prepared by the Standards of Care Committee of NASH CRN.

Pregnancy and diabetes will be managed according to the guidelines shown in sections 5.8.1.1. and 5.8.1.2.

In the event of major dermatological reactions such as generalized urticaria, bullous rashes,

exfoliative dermatitis, or Stevens-Johnson Syndrome, study medication will be discontinued immediately and not restarted. For local skin reactions, study medication may be discontinued if the skin reactions are potentially drug related. If the rashes clear, the study medication may be restarted. If local skin reactions recur with restarting the study medication, study medication should be discontinued. In cases where the study medication has been discontinued, the study medication will be unmasked and the participant, investigator, and the primary care provider will be notified in order to prevent future exposures.

5.11. Food and Drug Administration

The PIVENS trial will be conducted under an Investigational New Drug (IND #69,751) held by the NIDDK Project Officer. The investigators will complete a Statement of Investigator (FDA Form 1572) per the Code of Federal Regulations before the initiation of the PIVENS trial. The safety data required to meet IND regulatory requirements will be collected through adverse event reporting by the clinic investigators and will be provided by the Data Coordinating Center to the NIDDK for transmission to the FDA.

5.12. Adverse event reporting

The PIVENS trial will monitor and report adverse events to ensure patient safety. There are two separate sets of government regulations that apply to unanticipated or adverse events in research studies: (1) 45 CFR Part 46, Subpart A; the "Common Rule", shared by 17 Departments and Agencies and (2) 21 CFR 312, the FDA regulation for adverse events. The Common Rule requires written procedures and policies for ensuring reporting of "unanticipated problems" involving risks to participants to IRBs, appropriate institutional officials, and the Department or Agency Head. The FDA regulation requires notification of the FDA and participating investigators of any adverse event associated with the use of a test article that is "both serious and unexpected." Since the definitions and reporting requirements for unanticipated events differ between the two sets of Federal regulations, the PIVENS trial definitions and procedures for adverse events are designed to satisfy both sets of requirements.

5.12.1. Definitions

Adverse event. An adverse event is any untoward medical occurrence that may present itself during treatment or administration with a pharmaceutical product or clinical procedure and which may or may not have a causal relationship with the treatment. Adverse events include any unanticipated problems involving risks to participants, or breaches of protocol which might entail risk to participants. The term "unanticipated problem" includes both new risks and increased rates of anticipated problems.

Serious adverse event. A serious adverse event (SAE) is an adverse event occurring at any time during the study that results in death, life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Other events may also be considered an SAE if, based on medical judgement, the event jeopardized the patient to the point of requiring medical or surgical intervention to prevent the occurrence of any of the conditions for an SAE listed above.

Unexpected adverse event. An unexpected adverse event is any adverse event with specificity or severity that is not consistent with the risk information in the study protocol, current investigator brochure, or current package insert.

Associated with the use of the drug means that there is a reasonable possibility that the adverse experience may have been caused by the drug.

5.12.2. Monitoring for adverse events

Adverse events will be recorded on study data forms whether or not they are thought to be associated with the study or with one of the study drugs. Adverse events may be discovered during regularly scheduled visits or through unscheduled patient contacts between visits.

Summary data on adverse events will be monitored by the DSMB quarterly and at its semi-annual meetings or more frequently, as needed. These summaries will include analyses comparing rates of adverse events by treatment group, by clinic, or in other subgroups requested by the DSMB. Where applicable, signs and symptoms associated with the adverse event will be graded as to severity by the clinical site staff as mild, moderate, or severe using the Common Terminology Criteria for Adverse Events (65).

After each meeting, the DSMB will issue a written summary of its review of the study data, including adverse events, for transmission to the IRBs at each of the study centers. Analyses or listings of adverse events will not be provided to the IRBs; however, adverse events involving unanticipated problems involving risks to participants, or breaches of protocol which might entail risk to participants must be reported to local IRBs as soon as possible after they are discovered. Each participating center is responsible for ensuring that all local IRB requirements for reporting adverse events are met.

A summary of adverse events will be reported to the FDA as part of the IND annual report.

5.12.3. Reporting serious adverse events

Serious adverse events (SAE) must be reported upon discovery at the clinical center. This will involve completing an SAE data form describing the severity and details of the event. The SAE form, together with a memo summarizing the circumstances of the event and the current status of the patient, must be faxed to the Data Coordinating Center and to the NIDDK project officer within one working day of the discovery of the SAE. Also within one day, the clinical center must notify the NIDDK and Data Coordinating Center of the SAE by telephone or confirmed e-mail. The NIDDK project officer will work with the Data Coordinating Center to transmit the SAE form and memo to all study centers and to the DSMB.

If the SAE is unexpected AND associated with a study drug, then the NIDDK project officer will notify the FDA no later than 15 days from the discovery of the SAE (no later than 7 days if the SAE is fatal or life threatening). The pharmaceutical manufacturer will also be notified. The clinical center investigator may also be responsible for completing an FDA MedWatch 3500 form.

The DSMB will review each SAE report and provide comments to the NIDDK project officer within one week of receipt of the report. If requested by any member of the DSMB, a teleconference will be scheduled to discuss the SAE and recommend any actions to the NIDDK sponsor.

The clinical center must submit to the NIDDK project officer and to the Data Coordinating Center a follow-up memo within one month of the SAE (and periodic updates if needed) to report the details of the disposition of the SAE. The NIDDK project officer will work with the Data Coordinating Center to distribute the follow-up memo to the clinical center and to the DSMB.

5.13. Procedures for unmasking treatment assignment

Treatment assignments are double masked throughout the study until all data collection for the PIVENS trial has been completed (i.e., after completion of the 24 week post trial Follow-up for all patients). Every effort will be made to maintain the masking throughout the study except in emergency situations. The code of specific pharmacological treatment will not be broken without the knowledge of the clinical center's principal investigator.

Unmasking of study medication will occur under the following conditions:

- **Severe allergic reaction (Stevens-Johnson Syndrome)**: Study medication is stopped indefinitely. The patient, primary care provider (PCP), and the investigator will be unmasked.

- **Pregnancy during the study**: Study medication 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.

- **Development of hepatotoxicity**: Study medication will be discontinued according to the guidelines in section 5.8.1.1. The patient, PCP, and the investigator will be unmasked.

- **New onset diabetes**: The patient will be referred to their PCP and managed according to the stepped care approach defined in section 5.8.1.2. The patient and PCP may be unmasked to the study medication but the investigator will continue to be masked.

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. Statistical design and analysis

6.1. Hypotheses

Primary hypotheses:

- In nondiabetic adult patients with NASH, improvement in insulin resistance over 96 weeks of treatment with pioglitazone, compared to treatment with placebo, will result in improvement in NASH disease activity as assessed by a histologic score comprised of steatosis, lobular inflammation, and cellular ballooning

- In nondiabetic adult patients with NASH, improvement in oxidative stress status over 96 weeks of treatment with vitamin E, compared to treatment with placebo, will result in improvement in NASH disease activity as assessed by a histologic score comprised of steatosis, lobular inflammation, and cellular ballooning

Secondary hypotheses:

- Pioglitazone and vitamin E are equally effective in achieving histologic improvement in nondiabetic patients with NASH

- Levels of proinflammatory cytokines, serum markers for fibrosis will decrease with treatment with pioglitazone and with vitamin E compared to treatment with placebo

- Nondiabetic adult patients with NASH have an impaired quality of life, and these scores will improve as their liver histology improves upon treatment with pioglitazone or vitamin E, compared to treatment with placebo

Other questions to be addressed:

- We predict that insulin resistance is common in nondiabetic adult patients with NASH and will be significantly related to waist-to-hip ratio, waist circumference, and hepatic histology

- We predict that degree of obesity will be significantly related to hepatic histology in nondiabetic patients with NASH and more severe obesity will be related to more advanced hepatic histology

- We predict that age will be significantly related to hepatic histology in nondiabetic adult patients with NASH and older individuals will have more advanced hepatic histology

- We predict that serum markers for fibrosis will be significantly related to hepatic histology in nondiabetic adult patients with NASH

- We predict worsening hepatic histology will be significantly related to a worsening in health-related quality of life in nondiabetic adult patients with NASH and more advanced histology will have worse health-related quality of life as measured by scores on the modified SF-36

- We predict that improvement in the measures of insulin resistance will be significantly related to improvements in liver histology and serum markers for fibrosis in adult nondiabetic patients with NASH

- We predict that therapy with vitamin E will improve insulin resistance in adult nondiabetic patients with NASH

- We predict that improvement in liver histology upon treatment with insulin sensitizers will be significantly related to improvements in waist-to-hip ratio and waist circumference in adult nondiabetic patients with NASH

6.2. Outcome measures

Primary outcome measure:

The primary outcome measure requires improvement in NASH activity after 96 weeks of treatment as determined by liver biopsies pre- and post-treatment. The measure is derived from changes from baseline to the end of treatment in the NASH CRN NAFLD/NASH activity score (NAS). The NAS ranges from 0 to 8 (highest activity) and is calculated as the sum of three components of the standardized histologic feature scoring system for liver biopsies:

NAS = Steatosis score (0-3) +
Lobular inflammation score (0-3) +
Hepatocyte ballooning score (0-2)

As noted earlier, a NAS of 5 or greater with each subscore at least 1 or a NAS of 4 with each subscore at least 1 and a finding of definite steatohepatitis by at least two of three NASH CRN pathologists is a requirement for enrollment into the trial.

The definition of improvement after treatment as the primary outcome measure requires the following three conditions:

- No worsening of the fibrosis feature score
- Improvement by at least 1 point in the hepatocyte ballooning feature score
- Either: (1) Improvement in NAS by 2 or more points spread across at least two of the NAS components, OR
  (2) Post-treatment NAS is 3 points or less

Secondary outcome measures:

- Change in NAS after 96 weeks of treatment compared to baseline NAS. Mean changes in NAS will be compared.

- Changes in fibrosis, steatosis, lobular inflammation, cellular ballooning and other specific features from the histologic scoring system after 96 weeks of treatment compared to baseline. Improvement in specific features will be defined as any improvement in the score. Mean changes will also be compared.

- Change in serum aminotransferase levels after 96 weeks of treatment compared to baseline. A biochemical response is considered a fall of ALT levels into the normal range. Patients are not required to have abnormal levels of aminotransferases in order to enter this trial as long as they meet histological and other clinical criteria. Therefore, patients with normal levels of transaminases may be enrolled. If an adequate number of such patients are enrolled, a subgroup analysis will be performed.

- Change in anthropometric measurements (weight, BMI, waist-to-hip ratio, waist circumference, triceps skin fold thickness and total body fat) after 96 weeks of treatment compared to baseline.

- Change in insulin resistance (assessed by HOMA), serum vitamin E levels, cytokines, fibrosis markers, and lipid profile after 96 weeks of treatment compared to baseline.

- Change in health-related quality of life (SF-36) after 96 weeks of treatment compared to baseline.

6.3. Statistical analysis

Primary hypotheses:

Statistical analyses for the two primary hypotheses will follow the intention-to-treat paradigm, which means that all randomized patients with baseline and 96 week liver biopsies will be included in the treatment group to which they were assigned. Any randomized patient who does not have the requisite biopsies will be accounted for and compared by assigned treatment group. Patients not able

to be included in the intention-to-treat analyses will be compared to those who are included with respect to demographic and other characteristics.

Since the primary outcome measure, defined in Section 6.2, is a binary indicator of improvement in histologic activity score 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 (66). Two P-values will be derived: one comparing proportions improved in the group assigned to pioglitazone 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.

Given the randomized design and adequate size planned for the PIVENS trial, it is unlikely that confounding of the treatment groups by covariates related to the change in histologic activity score will occur. However, if confounding should occur, logistic regression analyses with histologic improvement as the binary response and treatment group indicator and any suspected confounders as covariates will be carried out to determine the sensitivity of the primary P-value to confounding.

Secondary hypotheses:

The first secondary hypothesis, that pioglitazone and vitamin E are equally effective in achieving histologic improvement, involves the primary outcome measure. Equivalence will be assessed in two ways: (1) a formal test for equivalence, testing that the difference between proportions improved in the pioglitazone and vitamin E groups does not exceed 10% and (2) 95% confidence intervals on the difference in proportions improved.

In general, analyses for outcomes related to other secondary hypotheses will be conducted in two ways. Improvement will be analyzed both as a binary outcome (improved vs. not improved) and also in terms of the numerical change in the outcome. Binary outcomes will be compared using the uncorrected χ^2 test for 2x2 table. Numerical changes will be analyzed by descriptively comparing the between-treatment group differences in mean and median changes; P-values will be derived from Wilcoxon rank sum tests for comparison of the distribution of changes in each group. If concerns about confounding arise, logistic regression models for improvement outcomes and linear regression models for numerical change outcomes will be used to correct for the confounding. Analyses for secondary hypotheses will generally involve three separate analyses, one for each treatment group comparison: pioglitazone vs. placebo, vitamin E vs. placebo, and pioglitazone vs. vitamin E. No adjustments for multiple comparisons will be applied to the secondary hypotheses; however, any significant findings must be interpreted taking into account the strength of the finding and its biologic plausibility.

Other questions to be addressed:

A series of other questions of interest were listed in Section 6.1. Analyses related to these questions will involve a mix of appropriate exploratory and confirmatory analyses, similar, but not limited to those described for the primary and secondary hypotheses.

6.4. Missing data

The occurrence of missing data in this trial is expected to be low and, when present, is expected to be equally distributed across the 3 treatment groups. We estimate that careful selection of patients during the 6 months screening phase and the consent process should result in no more than 10% missing data from patients who drop out before completing the 96 week treatment period. In primary, intention-to-treat analyses, patients with missing data will be considered unimproved on the primary outcome measure.

The proportions with missing data will be compared across treatment groups using χ^2 tests. Non-significant P-values indicate that the data are missing at random (MAR). If this is the case, analyses of the non-missing data are not threatened. If the amount of missing data exceeds 10% and the data are MAR, then a variety of sensitivity analyses will be carried out to compare to the primary analysis using all available non-missing data: (1) compare pessimistic and optimistic imputations of the missing values, (2) correct for missing data using multiple imputation with 10 replicated samples, and (3) use mixed random effects logistic or linear regression models, depending on the type of outcome measure. Although unlikely in a large trial, it is possible that the missing data are not MAR and the missing data are non-ignorable. A few statistical methods are available when there are non-ignorable missing data and these would be employed; however; all such methods involve strong assumptions that cannot be verified from the available data.

6.5. Justification of sample size

The planned sample size for the PIVENS trial is 240 patients with equal allocation to each of the three treatment groups (80 per group).

We based the sample size estimates on a two-group, binomial comparison of the proportions of patients satisfying the primary outcome, improvement in the histologic activity score (defined in Section 6.2) over the course of treatment. Since PIVENS is a three group trial with two primary hypotheses, we assume that the two primary comparisons, pioglitazone vs. placebo and vitamin E vs. placebo, require the same sample size and reduce the type I error from 0.05 to 0.025 (Bonferroni correction). Expected proportions improved were approximated using pilot data from a 48-week pioglitazone study (Jay Hoofnagle and David Kleiner, personal communication) and from the placebo group in a two-year randomized trial of Urso vs. placebo (Keith Lindor, personal communication). Currently, there are no available data to estimate the response in vitamin E, which is assumed, for purposes of planning the trial, to be the same as for pioglitazone.

The sample calculations were performed using the nQuery Advisor 5.0 (67) software using the formula:

$$n = \frac{\left[z_{1-\alpha/2}\sqrt{2\bar{\pi}(1-\bar{\pi})} + z_{1-\beta}\sqrt{\pi_1(1-\pi_1)+\pi_2(1-\pi_2)} \right]^2}{(\pi_1-\pi_2)^2}$$

where,

n = sample size per group

π_1 = expected proportion improved in placebo group (assumed=0.14)

π_2 = expected proportion improved in pioglitazone or vitamin E group (0.40)

$\bar{\pi}$ = average of π_1 and π_2

α = Two-sided type I error (0.025, Bonferroni corrected for two comparisons)

β = Type II error (0.10; i.e., 90% power)

The number per group, using the above formula is 71. Inflating this number by the 10% expected missing data rate yields approximately 80 patients per group, or a total of 240 for the trial.

6.6. Interim analysis

An independent Data and Safety Monitoring Board (DSMB), appointed by the NIDDK, is responsible for approving the protocol for the PIVENS trial and for monitoring the accumulated interim data as the trial progresses to ensure patient safety and to review efficacy. The DSMB is a multidisciplinary group with a written charge provided by the NIDDK. The DSMB reports to the NIDDK, which will, in turn, communicate DSMB recommendations to the investigators, as appropriate. The DSMB meets in person to approve the protocol. After the trial commences, the DSMB meets twice a year to review data or other issues – once in person and once by telephone conference. The DSMB may request more frequent meetings if necessary to fulfill its charge. It may also request additional safety reports on a more frequent basis. For example, all serious adverse events are reported to the DSMB for their consideration and recommendations as they occur.

Interim data on safety measures requested by the DSMB are reviewed at each of the scheduled semi-annual full meetings. Two additional written safety reports will be reviewed by the DSMB between scheduled full meetings. Serious adverse events will be reviewed by the DSMB as they occur with the option of a teleconference discussion if any DSMB member so requests.

The DSMB will review quarterly reports by masked treatment groups of incident hepatotoxicities, as well as counts of patients who required more frequent liver function testing due to

rises in ALT levels of more than 1.5 times baseline ALT or beyond 250 U/L. The DSMB will also examine the trends in ALT or AST levels for each patient who experiences a rise in ALT.

The DSMB reviews one planned interim analysis of the primary outcome measure. O'Brien-Fleming statistical stopping guidelines for efficacy apply. This interim efficacy analysis will occur when approximately 50% of the data are complete or when approximately 120 of the 240 patients have completed baseline and 96 week biopsies.

The DSMB also reviews the overall progress of the trial in terms of recruitment and data quality and makes a formal recommendation to the NIDDK at the end of each scheduled meeting as to whether the trial should continue unmodified, continue with protocol modifications, or be stopped.

7. Human subjects issues

7.1. Overview

The study protocol, questionnaires, and consent forms will be submitted to each participating center's IRB. Sites which recruit patients will submit their recruitment materials to their IRB prior to use. A site may not initiate any patient contact about the PIVENS trial until the site has IRB approval for the trial. All study personnel must complete training in the Protection of Human Subjects per NIH guidelines. The proposed study anticipates recruiting a significant proportion of racial/ethnic minorities (African-Americans, Asian-Americans and Hispanics) as well as non-Hispanic white subjects.

7.2. Institutional Review board (IRB) approval

A site may not initiate patient activities in the PIVENS trial until the site has IRB approval for the trial. Consent forms must have IRB approval. Sites must provide the DCC with a copy of the initial IRB approval notice and subsequent renewals as well as copies of the IRB approved consent statements.

7.3. Informed consent

A prototype consent will be prepared for the trial for screening to determine eligibility with an affirmation of consent for randomization in the trial. Individual sites may add material but may not delete material thought to be necessary for informed consent. Clinics may reformat and reword information to conform to their local requirements. The patient must sign the consent to be eligible for the trial. The consent form will describe the purpose of the trial, the procedures to be followed, and the risks and benefits of participation. Copies of the signed consent forms will be given to the patient, and this fact will be documented in the patient's record.

7.4. Patient confidentiality

All laboratory specimens, study forms, reports, and other records that are part of the study data collection materials will be identified by coded number to maintain patient confidentiality. All records will be kept in locked file cabinets. All electronic records of study data will be identified by coded number. Clinical information will not be released without written permission of the patient, except as necessary for monitoring by the IRB. Consent procedures and forms, and the communication, transmission and storage of patient data will comply with individual site IRB and NIH requirements for compliance with The Health Insurance Portability and Accountability Act (HIPAA).

8. References

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9. Appendices

9.1. Participating centers

Clinical Centers

- Case Western Reserve University
- Indiana University
- Duke University
- St. Louis University
- University of California, San Diego
- University of California, San Francisco
- University of Washington
- Virginia Commonwealth University

Data Coordinating Center:

- Johns Hopkins University

National Institutes of Health:

- National Institute of Diabetes and Digestive and Kidney Diseases
- National Cancer Institute
- National Institute of Child Health and Human Development

9.2. Committees

- Steering Committee
- Executive Committee
- Database Committee
- Measures and Assessments Committee
- Pathology Committee
- Standards of Care Committee
- Pediatrics Committee
- Ancillary Studies Committee
- Presentations and Publications Committee
- Treatment Trial Protocol Committee
- Pilot and Feasibility Studies Committee

9.3. Data collection schedule

Assessment/Procedure	Screening visits			Follow-up visits Weeks from randomization															
	S1	**S2**	**RZ**	**2**	**4**	**8**	**12**	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**	**120**
Consent	X	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Baseline medical history	X	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Followup medical history	.	.	.	.	X	X	.	X	X	X	.	X	.	X	X	X	.	X	X
AUDIT, Skinner alcohol question	.	A	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Review of concomitant drugs	X	.	X	.	X	X	.	X	X	X	.	X	.	X	X	X	.	X	X
Review for adverse effects	.	.	.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Drug dispensing	.	.	X	.	X	X	.	X	X	X	.	X	.	X	X	X	.	.	.
Review of study drug adherence	.	.	.	.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	.
Physical exam (Detailed/Focused)	D	.	.	.	F	F	.	F	D	F	.	D	.	F	F	F	.	D	F
DEXA scan for body fat	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X	X
Liver biopsy	X	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X	.
Block 98 nutrition questionnaire	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
Functional activity questionnaire	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
HR-QOL (SF-36)	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
Liver symptom questionnaire	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
OGTT with insulin and C-peptide	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
Labs																			
Fasting glucose	X	.	.	.	.	.	.	.	X	.	.	.	.	.	X	.	.	.	.
Fasting lipid profile	X	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
CBC	X	.	.	.	.	.	.	.	X	.	.	X	.	.	.	.	.	X	X
Metabolic panel	X	.	.	.	.	.	.	.	X	.	.	X	.	.	.	.	.	X	.
Hepatic panel	.	X	.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GGT and prothrombin time	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	.
HbA1c	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	.
Pregnancy test (females)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Microalbuminuria	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	X
Serum, plasma for banking	.	X	.	.	.	.	.	X	.	X	.	X	.	X	.	X	.	X	X
Serum vitamin E (banked serum)	.	X	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.	X	.
Closeout form	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

Note: **Detailed (D) physical** includes measurement of height, weight, waist, and hips; vital signs (temperature, heart rate, respiratory 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, fetor).
Focused (F) physical includes measurement of height, weight, waist, and hips; vital signs (temperature, heart rate, respiratory rate, blood pressure); examination for scleral icterus and pedal edema and auscultation of heart and lungs.
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.
Lipid profile: total cholesterol, triglyceride, LDL, HDL.
Fasting visits: S1, S2, 16, 24, 32, 48, 64, 72, 80, 96, and 120.
Safety visits: 2, 12, 40, 56, and 88.

9.4. Whole blood draw schedule: mL of blood to be drawn at screening and follow-up visits

	Study visit (wk)																		
Procedure	**s1**	**s2**	**2**	**4**	**8**	**12**	**16**	**24**	**32**	**40**	**48**	**56**	**64**	**72**	**80**	**88**	**96**	**120**	**Total**
Fasting glucose	5	.	.	.	.	.	.	5	.	.	.	.	.	5	.	.	.	.	15
Fasting lipid	5	.	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	5	20
GTT w/insulin	.	25	.	.	.	.	.	.	.	.	25	.	.		.	.	25	25	100
CBC	5	.	.	.	.	.	.	5	.	.	5	.	.	.	.	.	5	5	25
Metabolic panel	5	.	.	.	.	.	.	5	.	.	5	.	.	.	.		5	.	20
Hepatic panel	.	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	85
GGT, PT/INR	.	5	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	.	15
HbA1c	.	5	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	.	15
Plasma: TGF-beta	.	5	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	5	20
Serum: fibrosis	.	10	.	.	.	.	.	.	.	.	10	.	.	.	.	.	10	10	40
Serum: Vit E	.	5	.	.	.	.	.	.	.	.	5	.	.	.	.	.	5	.	15
Serum: banking	.	25	.	.	.	.	20	.	20	.	25	.	20	.	20	.	25	20	175
Genetics	.	20	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	20
Other screening	40	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	40
Total	60	105	5	5	5	5	25	20	25	5	100	5	25	10	25	5	100	75	605

9.5. Glossary

3-NT	-	3-nitrotyrosine
AMA	-	antimitochondrial antibody
ANA	-	anti-nuclear antibody
AST	-	aspartate aminotransferase
ALT	-	alanine aminotransferase
anti-HBc	-	hepatitis B core antibody
anti-HBs	-	hepatitis B surface antibody
anti-HCV	-	hepatitis C virus antibody
BMI	-	body mass index (kg/m^2; calculated as the weight in kilograms divided by the square of the height in meters)
BUN	-	blood urea nitrogen
CHZ	-	chlorzoxazone
DCC	-	data coordinating center
DEXA	-	dual energy x-ray absorptiometry
DPP	-	Diabetes Prevention Program
DSMB	-	Data and Safety Monitoring Board
ERCP	-	endoscopic retrograde cholangiopancreatography
FDA	-	Food and Drug Administration
FFA	-	Free Fatty Acids
GGT	-	gamma glutamyl transferase
HBsAg	-	hepatitis B surface antigen
HCV	-	hepatitis C virus
HIPAA	-	Health Insurance Portability and Accountability Act
HOMA-IR	-	homeostasis model assessment method for insulin resistance (calculated as [fasting insulin (μU/ml) * fasting glucose (mmol/L)]/22.5)
HRQOL	-	health-related quality of life
INR	-	international normalized ratio for prothrombin time; $INR=(PT_{patient}/PT_{control\ mean})^{ISI}$, where ISI=International Sensitivity Index
MAR	-	missing at random
MRCP	-	magnetic resonance cholangiopancreatography
NAFL	-	nonalcoholic fatty liver
NAFLD	-	nonalcoholic fatty liver disease
NAS	-	NASH CRN NAFLD/NASH activity score
NASH	-	nonalcoholic steatohepatitis
NHANES	-	National Health and Nutrition Examination Survey
NIDDK	-	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	-	National Institutes of Health
OGTT	-	oral glucose tolerance test
PCOS	-	polycystic ovary syndrome
PCP	-	primary care provider

PIVENS	-	Pioglitazone vs vitamin E vs placebo for the treatment of nondiabetic patients with NASH trial
PPARγ	-	peroxisome proliferator-activated receptor-gamma
SAE	-	serious adverse event
SAM-e	-	S-adenosyl methionine
TNF	-	tumor necrosis factor
TZD	-	thiazolidinedione
TBARS	-	thiobarbituric acid reacting substances
UDCA	-	ursodeoxycholic acid (aka, URSO)
ULN	-	upper limit of normal
URSO	-	ursodeoxycholic acid (aka, UDCA)

9.6. Document history

PIVENS trial protocol (28 June 2004)

PIVENS trial protocol (20 June 2005)

Numerous editorial and wording changes were made to the following sections:

§ Design synopsis:
Added "upper arm circumference" to secondary outcome measures

§3.1 Design overview
- Changed "Patients will be asked to remain in the NAFLD Database with annual follow-up visits after the trial has ended." to "Patients will be asked to participate in the NAFLD Database with annual follow-up visits after the trial has ended."
- Changed "urine pregnancy test" to "pregnancy test" in this section and throughout; rationale was that some clinics may choose to do blood pregnancy tests

§4.4 Run-in period
- Changed "Any over-the-counter medication or herbal remedy that is being taken with an intent to improve hyperlipidemia will not be allowed for at least 3 months prior to randomization nor after randomization." to "Any over-the-counter medication or herbal remedy that is being taken with an intent to improve hyperlipidemia will not be allowed for at least 3 months prior to randomization and will be discouraged after randomization."

§5.2 Screening and baseline data collection
- Changed plasma glucose to serum glucose in this section and throughout

§5.2 Screening visit 1
- Added mid-upper arm circumference and respiratory rate to list of procedures
- Changed "ceruloplasmin" to "ceruloplasmin (if less than 40 years old)"
- Added iron overload to list of laboratory items
- Moved GGT and prothrombin time to the s2 visit
- Deleted globulin

§5.2 Screening visit 2
- Added GGT and prothrombin time to laboratory tests

§5.3 Randomization visit
- Deleted height, weight, vital signs, and brief interim medical history from the procedures to be completed at the randomization visit.
- Changed, "The medication assignment will not be generated unless the check finds that the

patient is eligible." to "The medication assignment will not be generated unless the check finds that the patient is eligible, and the clinic staff indicate that they want to randomize the patient."
- Changed "medication kit" to "medication bottles"
- Added option of sending study medication to clinical coordinator, if not using a research pharmacy

§ 5.4 Follow-up visits, Week 2 visit
- Deleted interim history, height, weight, vital signs, and focused physical examination

§ 5.4 Follow-up visits, Week 4 visit
- Changed "Interim history" to "Follow-up medical history" here and throughout
- Added interim drinking history
- Added respiratory rate to vital signs
- Added liver symptoms to items included in focused physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 8 visit
- Added interim drinking history
- Added respiratory rate to vital signs
- Added liver symptoms to items included in focused physical examination
- Added "Dispense study drug and reviw study drug adherence with patient"

§ 5.4 Follow-up visits, Week 12 visit
- Deleted Follow-up medical history
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 16 visit
- Added interim drinking history
- Added respiratory rate to vital signs
- Added liver symptoms to items included in focused physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 24 visit
- Added interim drinking history
- Added respiratory rate to vital signs
- Added waist and hip measurements, triceps skin fold, mid-upper arm circumference, and liver symptoms to list of procedures in detailed physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 32 visit
- Added interim drinking history
- Added respiratory rate to vital signs

- Added liver symptoms to items included in focused physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 40 visit
- Deleted Follow-up medical history
- Added "Review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 48 visit
- Added interim drinking history
- Added respiratory rate to vital signs
- Added waist and hip measurements, triceps skin fold, mid-upper arm circumference, and liver symptoms to list of procedures in detailed physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 56 visit
- Deleted Follow-up medical history
- Added "Review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 64 visit
- Added interim drinking history
- Added respiratory rate to vital signs
- Added liver symptoms to items included in focused physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 72 visit
- Added interim drinking history
- Added respiratory rate to vital signs
- Added liver symptoms to items included in focused physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 80 visit
- Added interim drinking history
- Added respiratory rate to vital signs
- Added liver symptoms to items included in focused physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 88 visit
- Deleted Follow-up medical history
- Added "Review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 96 visit
- Added interim drinking history

- Added respiratory rate to vital signs
- Added waist and hip measurements, triceps skin fold, mid-upper arm circumference, and liver symptoms to list of procedures in detailed physical examination
- Added "Dispense study drug and review study drug adherence with patient"

§ 5.4 Follow-up visits, Week 120 visit
- Added interim drinking history
- Added respiratory rate to vital signs

§ 5.5 Standardized questionnaires - Alcohol questionnaires
- Clarified that the interim alcohol questionnaire is included in the Follow-up medical history form, and is not a separate form

§ 5.7 Overview of scoring of liver biopsies
- Under (3) Fibrosis based upon Masson's trichrome stain as 0-4, added "perisinusoidal" to 1a and 1b
- Under (9), changed "Iron as 0 to 4" to "Hepatocellular iron grade based on iron stain as 0 (absent or barely discernible, 40x), 1 (barely discernable granules, 20x), 2 (discrete granules resolved, 10x), 3 (discrete granules resolves, 4x), and 4 (masses visible by naked eye)"

§ 9.3 Data collection schedule was revised

§ 9.4 Whole blood draw schedule was revised

§ 9.5 Glossary
- Added definition of AMA

PIVENS trial protocol (13 July 2006)
Following changes were made to sections below:

§ Design synopsis:
Deleted exclusion criteria - "Men of childbearing potential: unwillingness to use an effective form of birth control during the trial"

§ 4.2 Inclusion criteria:
Clarified wording on process for additional pathologist review in cases of NAS=4.

§ 4.3 Exclusion criteria:
Deleted Criterion #24. Men of childbearing potential: unwillingness to use an effective form of birth control during the trial

§ 5.4 Follow-up visits

Week 4 visit: added waist, hip measurements

Week 8 visit: added waist, hip measurements
Week 16 visit: added waist, hip measurements
Week 32 visit: added waist, hip measurements
Week 64 visit: added waist, hip measurements
Week 72 visit: added waist, hip measurements
Week 80 visit: added waist, hip measurements
Week 120 visit: added waist, hip measurements

§ 9.3 Data collection schedule was revised to include waist and hip measurements in focused physical examination

§ 6.2 Outcome measures
Clarified wording on process for additional pathologist review in cases of NAS=4

PIVENS Trial Protocol (18 April 2007)

§5.4 Follow-up visits
Week 120 visit: added DEXA scan for body composition; added blood draw for serum and plasma banking at central repository

§5.6 Specimen repository
added week 120 visit to blood collection schedule

§9.3 Data collection schedule: added DEXA scan, blood draw for serum and plasma banking, and closeout form at f120 visit

§9.4 Whole blood draw schedule: added blood draw for serum and plasma banking at f120 visit