THE COURSE OF SERUM ALANINE AMINOTRANSFERASE DURING PROLONGED ACETAMINOPHEN ADMINISTRATION

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The Course of Serum Alanine Aminotransferase During Prolonged Acetaminophen Administration

Thesis directed by Professor Richard Dart

ABSTRACT

Prolonged administration (>4 days) of therapeutic doses of acetaminophen causes an asymptomatic elevation of serum aminotransferase activity in healthy patients. While there is no systematic evidence that these elevations progress to liver injury, the widespread use of acetaminophen as a non-prescription analgesic make this a potential health hazard. The objective of this study is to describe the course of serum alanine aminotransferase (ALT) activity in healthy subjects administered 4 g/day of acetaminophen for a minimum of 16 days. The design is a randomized, blinded placebo controlled trial. Healthy adult volunteers with normal serum ALT were assigned to acetaminophen (1g four times daily) or placebo in a 4:1 ratio (to increase the probability of rare events in the acetaminophen group). Patients were monitored with laboratory testing every 3 days for a minimum of 16 days. At day 16, subjects who had an ALT that was less than 42 IU/L (the upper limits of the reference range) and who were within 10 iu/L of their day 0 ALT were completed. Subjects who had an ALT>10 IU/L on day 16 were continued on study drug and had continued ALT monitoring until they had 2 consecutive non-increasing ALT measurements. Subjects who had an ALT > 42 IU/L on day 16 were continued on study drug and had continued ALT monitoring until they had 2 consecutive non-increasing ALT

measurements less than 42 IU/L. The maximum duration of study drug administration was day 40. Our primary outcome was the number of subjects who did not meet stopping criteria by study day 40. A total of 205 acetaminophen and 47 placebo patients completed the study. One acetaminophen-treated subject (1/224, 0.5% 95% CI 0 to 2.5%) and no placebo (0/52 0% 95% CI 0 to 8.2%) treated subjects did not meet resolution criteria by day 40 of treatment. No subject had a change in liver function defined by elevation of the INR or bilirubin. Age, gender, race, ethanol use and baseline ALT were not associated with an increased risk of ALT elevation. ALT elevations during prolonged acetaminophen dosing appear to be self-limited and not accompanied by changes in liver function.

The form and content of this abstract are approved. I recommend its publication.

Approved: Richard C. Dart

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CHAPTER I

INTRODUCTION

Drug-induced liver injury (DILI) is the most common cause of acute liver failure in the United States.(1) Given the risk and morbidity of drug-induced liver injury, it is not surprising that DILI is also the most common reason that pharmaceuticals are not approved by the United States Food and Drug Administration and the most common reason that approved pharmaceuticals are removed from the market.(2) However, recent evidence has suggested that some pharmaceuticals may cause aminotransferase elevation suggesting liver injury but that these elevations are not accompanied by liver dysfunction, are not progressive and will reverse if treatment is continued.(3) As the mechanisms of acetaminophen toxicity are well understood, describing the course of aminotransferase changes for acetaminophen may provide insight that could be applied to other pharmaceuticals. This is important for drug safety and development as it may foster earlier recognition of medications causing serious liver injury while preventing premature abandonment of medications causing aminotransferase elevations but not liver injury.

One example of a medication that causes aminotransferase elevation and that seems rarely (if ever) to cause liver injury with therapeutic dosing is acetaminophen. Therapeutic doses of acetaminophen cause asymptomatic aminotransferase elevations in some people when administered for four or more days,(4-5) but there is no systematic evidence that suggests continued administration of doses in the therapeutic range will progress to clinical liver

injury. While there is general consensus based on clinical observations that these changes are self-limited and likely resolve if treatment is continued, the clinical course of these aminotransferase elevations is poorly characterized. The objective of this project is to describe the course of aminotransferase (serum alanine aminotransferase or ALT) change during prolonged administration of acetaminophen. The secondary objectives are to determine if these aminotransferase elevations are associated with liver dysfunction and to determine if these elevations are associated with demographic or clinical characteristics.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

Drug-induced liver injury is classically characterized as cholestatic, hepatocellular or mixed.(6) Cholestatic liver injury is defined by a predominant initial elevation of the alkaline phosphatase level. Hepatocellular injury is defined by predominant initial elevation of the alanine aminotransferase activity (alanine aminotransferase and aspartate aminotransferase- AST) and mixed hepatocellular injury is defined as elevation of both the alanine aminotransferase and alkaline phosphatase levels. While both ALT and AST have been used as markers of hepatic injury, ALT is considered more specific and is currently the preferred marker. Acetaminophen-induced liver injury is hepatocellular, and acetaminophen is considered the prototypical agent for study of hepatocellular injury. Marked elevation of the serum aminotransferase (greater than 1000 IU/L) is the hallmark of significant acetaminophen-induced liver injury.

The classic mechanism of acetaminophen-induced liver injury was defined by Mitchell in 1973.(7) Acetaminophen is oxidized by a specific hepatic cytochrome oxidase (CYP-2E1) to a reactive metabolite (N-acetylparaquinonimine or NAPQI). Under normal circumstances, this metabolite is detoxified by hepatic glutathione. However, in unusual circumstances such as overdose or starvation, glutathione may be depleted and the NAPQI binds to cysteine residues in hepatic proteins. If sufficient proteins are affected, mitochondrial injury and hepatic necrosis may occur. As the hepatocytes die,

they release ALT and AST into serum where their activity can be readily measured.

Acetaminophen overdose is a well-described cause of drug induced liver injury. In the early 1970s, Mitchell estimated that the minimum dose of acetaminophen required to cause liver injury in a healthy adult was approximately 15 gm as an acute ingestion.(7) The dose required to cause injury when taken over several days was more widely debated; a few retrospective studies and case reports suggested that therapeutic doses could cause liver injury in alcoholics. However, the lowest dose that would cause liver injury in healthy adults was always estimated to be well above the maximum recommended dose of 4 g/day.

This perspective changed when Watkins et al. reported that 76% of normal subjects taking 4 grams of acetaminophen daily for 10 days, either alone or in combination with an opioid, developed a serum ALT above the upper limits of normal (ULN).(8) ALT elevations were also reported for 38% of subjects in the placebo group. The highest recorded ALT was 574 IU/L (approximately 10 times the subject's baseline value). No subject developed symptoms of liver injury. Because the study medication was stopped if the subject had an ALT elevation greater than three times the upper limit of normal and stopped at 14 days for all subjects, this study does not provide much information on the course of ALT elevation with continued treatment.

Our group reported similar findings in healthy subjects who consumed 1 to 3 alcoholic beverages per day.(9) Participants were asked to take 1000 mg

acetaminophen four times per day or placebo for 10 consecutive days. One hundred participants completed the acetaminophen regimen and another 50 completed the placebo regimen. While the AST means were within the upper limits of normal for our laboratory (ULN) throughout the study with no significant difference between the groups, the ALT (a more sensitive marker of liver injury) measures indicated a different trend. Twenty-five of 150 participants (17%) had a baseline ALT measure within reference range and then experienced an ALT elevation above ULN at Day 4 and/or Day 11 (20 of 100 (20%) in acetaminophen group and 5 of 50 (10%) in placebo group; p=0.121). An additional 2 participants (1 in each group) had a baseline ALT above ULN but less than 50 IU/L with all subsequent ALT levels within reference range. The range of elevated ALT levels was 42 to 128 IU/L with only 3 of these elevations exceeding twice the ULN. The majority of these increases were reported on Day 11 of the study (the day following the last dose of medication).

Several other studies have also found asymptomatic ALT elevation with therapeutic doses of acetaminophen. These studies report ALT elevation above the ULN in 5-10% of participants treated with 4g/day of acetaminophen for up to 6 weeks.(10-13) However, these studies had infrequent sampling, so the course of ALT changes cannot be characterized.

The mechanism and clinical course of ALT elevation during therapeutic dosing is less clear. While ALT has diurnal variation of approximately 10% (14), no reports suggest variation that could account for ALT changes of the magnitude observed in acetaminophen treated subjects. The most readily

apparent mechanism for ALT elevation is increased death of hepatocytes. If this occurs, the most likely explanation is accelerated death of older hepatocytes which are less resistant to oxidative stress. However, other mechanisms have been proposed. These include immune-mediated reactions, increased release of ALT without hepatocytes death, prolongation of ALT half-life and an increase in enzymatic activity independent of serum ALT concentration. These mechanisms are best studied in animal models. However, the course of ALT must be defined in humans to have clinical relevance. Several prospective studies have described the onset of ALT elevation (see below) but there is very little data on the natural history of these elevations if acetaminophen is continued.(8-9, 15-16)

There are several important gaps in our knowledge about asymptomatic ALT elevations that occur with therapeutic acetaminophen dosing**.** In the studies of therapeutic acetaminophen described above (8-9, 15-16), more than 100 subjects developed an elevated ALT, but no subjects developed symptomatic liver injury, increased serum bilirubin or evidence of hepatic dysfunction. A systematic review of prospective medical literature found no reported cases of clinical hepatic injury, liver transplant or death due to therapeutic use of acetaminophen.(17) This suggests that isolated ALT elevation is not a hallmark of liver injury or impending liver injury. Senior et al. suggested that there may be an adaptive process that occurs with some medications. They point out that ALT elevations occur with the statins, tacrine and other medications but that these changes resolve when the medication is continued.(3) However, without

continuing the medications and systematically monitoring the ALT it will be impossible to determine if this occurs with acetaminophen.

There is some evidence suggesting that ALT elevations resolved. Parra et al. (12) performed a 4-week randomized, placebo-controlled study to determine the effect of 2 and 4 g/day of acetaminophen on the anticoagulant effects of participants taking warfarin. During this study they also measured serum ALT at baseline, 2 and 4 weeks. This study found a dose-dependent increase in ALT at 2 weeks that resolved by 4 weeks (Figure 2.1).

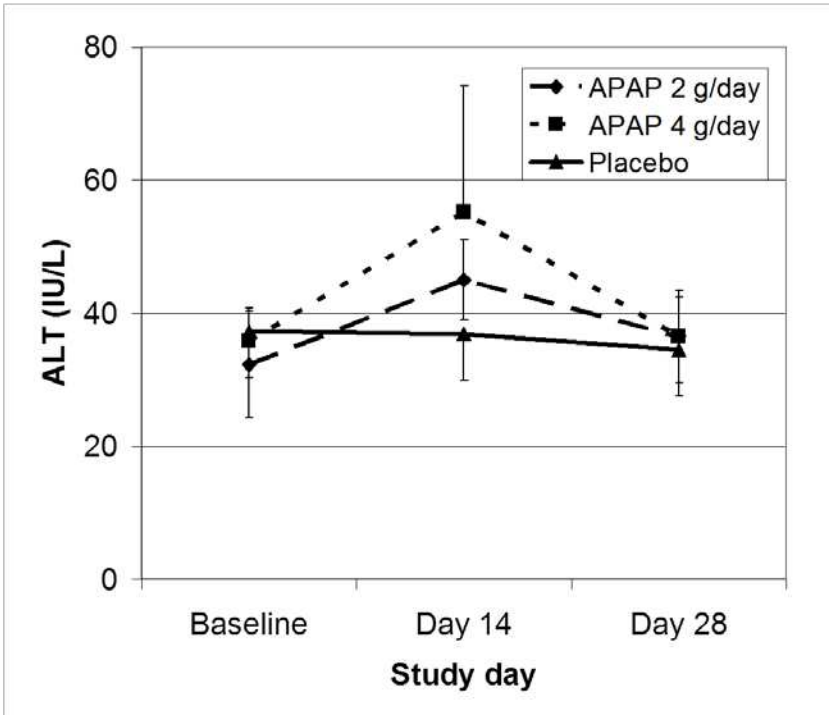

Figure 2.1. ALT at baseline, 14 and 28 days of acetaminophen administration. ALT – Alanine aminotransferase, APAP- Acetaminophen. Data from Parra et.al. 2007.(12)

No clinical trials have ever reported cases of therapeutic doses of acetaminophen causing clinical liver injury or liver failure. However, there are

anecdotal reports of individuals who develop liver failure while taking therapeutic doses of acetaminophen.(18) Furthermore, approximately 19% of cases of acute liver failure have evidence of acetaminophen exposure even though there is no history of acetaminophen exposure (19). This group may represent patients who were taking therapeutic doses but who did not give a history of acetaminophen use. While we believe it is is unlikely that there are cases of liver failure from therapeutic doses of acetaminophen, the only way to identify individuals who will go on to develop hepatic failure from therapeutic dosing is to prospectively dose subjects and follow the ALT until 1) resolution of the elevation or 2) the subject develops clinical liver injury.

The FDA Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation recognizes the importance of identifying medications that cause self-limited ALT elevation.(20) The monograph states *"Stopping a drug at the first hint of mild injury does not permit learning whether adaptation will occur, as it does for drugs such as tacrine that cause liver injury but do not cause severe drug-induced liver injury (DILI)."* Unlike previous guidelines which have focused solely on the degree of ALT elevation as a marker of DILI, the following criteria are suggested for stopping a medication in a clinical trial:(20)

- ALT or AST >8x upper limit of normal (ULN)
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (total bilirubin >2xULN or international normalized ratio (INR) >1.5)

- ALT or AST >3x ULN with the appearance of worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia.

In cases of less severe ALT elevation (> 3x ULN but not meeting other criteria) the guidelines recommend close observation defined as:

- Repeating liver tests two or three times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or study drug has been discontinued and subject is asymptomatic.
- Obtaining a more detailed history of symptoms and prior or concurrent diseases.
- Obtaining a history of concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g. INR).
- Considering gastroenterology or hepatology consultation.

By including these safety measures, our study will allow us to determine the course of acetaminophen-induced ALT elevations while ensuring the safety of the subjects. Subjects who meet the criteria for drug-induced liver injury can be treated with N-acetylcysteine, an extremely effective antidote for acetaminophen poisoning.

Several factors may contribute to the variability in response to acetaminophen. The most commonly cited factor is ethanol consumption, which has been suggested to increase the susceptibility to acetaminophen by increasing the formation of NAPQI through CYP-2E1 induction (21) and decreasing the detoxification capacity by depleting glutathione(22). Another potential risk factor is obesity, which is associated with increased serum ketones (another CYP2E1 inducer), fatty liver disease and hepatic oxidative stress.(23) Finally, one study found that Hispanic subjects were twice as likely to have an ALT elevation greater than 3 times the upper limit of normal while administered 4 grams of acetaminophen per day(8), and another study found an association between ALT elevations and variation in CD44 in mice suggesting genetic or social factors may alter the risk of elevations.(15) These characteristics may represent special risk factors for acetaminophen induced aminotransferase elevations and require further study.

This topic is clinically relevant because acetaminophen is widely used and there are many people who use it daily and for more than 4 days. Over 60 million Americans consume acetaminophen each week.(1) Until recently, the maximum recommended non-prescription dose of acetaminophen was 4g/day. Recently, one manufacturer of non-prescription products has changed their label to recommend a maximum of 3g/day. Still, many consumers take 4g/day for longer than 4 days. There are several use patterns of acetaminophen; one-time use for a minor symptom that resolves, consistent use over several days for recurrent or persistent symptoms, and long-term use for chronic daily symptoms. While the

safety of short term and intermittent use has been established, there are growing concerns that use longer than 10 days causes serum ALT elevation (see below). A review of the 2003 National Health and Nutrition Examination Survey found that 20% of Americans reported taking an analgesic nearly every day for as long as a month at some point in their life.(2) Three percent of Americans take acetaminophen on a daily basis for a month or more.

In addition to nonprescription acetaminophen products, patients with chronic pain will take acetaminophen in narcotic combination products for a prolonged period. Approximately 17 million people will use a mild strength opioid (such as codeine, oxycodone or hydrocodone) each month, and the majority of these opioids will be acetaminophen combination products. The median duration of use for codeine and codeine derivative products is 27 days, and 25% will use these products for more than a year. The recommended dose of these products would result in individuals ingesting up to 4 gm of acetaminophen per day.

Given the number of people who use acetaminophen for prolonged periods of time, even a very low rate of liver failure from therapeutic doses of acetaminophen (a non-lifesaving medication) would have public health implications. While it is not feasible to conduct a formal trial to detect very rare events like liver failure, ALT elevations (which may be a precursor of liver injury) are frequent and easily detected. Therefore our study will follow the ALT of subjects taking therapeutic doses of acetaminophen to determine if these elevations resolve while acetaminophen dosing is continued

The focus of this project is detecting changes in serum aminotransferase activity in subjects taking administered acetaminophen for several days. Therefore relevant publications will describe clinical trials where acetaminophen is administered and aminotransferase activity is monitored. A search of the National Library of Medicine using PubMed was conducted on 8.20.12 using the search terms outlined in Table 2.1 to identify relevant studies.

Table 2.1. Search terms and results of PubMed search.

	Term	Limits	Returned
1	Acetaminophen		15884
2	Acetaminophen	MeSH	12766
3	Acetaminophen	MeSH + Clinical Trial+ Human	1957
4	Alanine aminotransferase		34678
5	Aspartate aminotransferase		30676
6	4 or 5		46949
7	3+(4 or 5)		25
8	Liver failure	Clinical Trial+ Human	2332
9	3+8		22

Review of abstracts identified 9 trials where the primary outcome was serum aminotransferases activity.(8-10, 15-16, 24-27) Two studies measuring the effect of acetaminophen on warfarin-induced anticoagulation reported ALT.(12, 28) In addition to the studies identified during the search, 2 clinical trials of acetaminophen for osteoarthritis,(11, 13) and one study that measured the effect of acetaminophen on blood pressure(29) were identified during review of bibliographies. These studies are summarized in Table 2.2.

Table 2.2. Studies of therapeutic acetaminophen dosing that report changes in alanine aminotransferase (ALT). APAP=acetaminophen, IBU=ibuprofen, nl= normal range, Plcbo=placebo, NR= not reported. *Crossover study- all patients received acetaminophen in 1 phase.

Author Year	Population	N	APAP dose	Duration of treatment	Outcome Acetaminophen Group	Peak ALT APAP	Outcome Comparison Group	Peak ALT Comparison
Bradley 1991	Osteoarthritis patients	61 APAP 62 IBU 1.2 61 IBU 2.4	4 g/d 1.2 g/d 2.4 g/d	4 weeks	11/59 inc>10U/L	NR	4/62 (IBU1.2) 6/61 (IBU 2.4)	NR
Kwan1999	Healthy Volunteers	20*	4 g/d	14 days each arm	NR (≥ 2 subjects had ALT>ULN	116 IU/L	NR (Plcbo)	NR
Kuffner 2001	Alcoholic volunteers	118 APAP 112 Plcbo	4g/d	2 days	Mean ALT 40	182 IU/L	Mean ALT 42 (Plcbo)	196 IU/L
Pincus 2001	Osteoarthritis patients	210*	4 g/d	6 weeks each arm	5% AST>nl	NR	11% AST>nl (misprostol/ diclofenac)	NR
Watkins 2006	Healthy Volunteers	106 APAP 39 Plcbo	4 g/d	10 days	76%ALT>nl	636 IU/L	38% > nl (Plcbo)	>80 but <120 IU/L
Heard 2007	Healthy moderate drinkers	100 APAP 50 Plcbo	4 g/d	10 days	20 % ALT>nl	128 IU/L	10% >nl (Plcbo)	47 IU/L
Kuffner 2007	Alcoholic volunteers	258 APAP 114 Plcbo	4 g/d	3 days	9% ALT> 3x nl	312 IU/L	7% 3x nl (Plcbo)	288 Iu/L
Parra 2007	Warfarin users	12 4g 12 2g 12 Plcbo	2 and 4 g/d	4 weeks	2g/d ALT inc 4 IU/L 4g/d ALT inc 8 IU/L	NR	No change in ALT (Plcbo)	NR
Temple 2010	Healthy volunteers	24	4,6,8 g/d	3 days	4g/d ALT inc 1.5 IU/L 6g/d ALT inc 2 IU/L 8g/d ALT inc 5 IU/L	1 x ULN		
Bartels 2008	Alcoholic volunteers	40	3.9 g/d	4 days	Mean ALT 37	NR	Mean ALT 36 (Plcbo)	Bartels 2008
Dart 2010	Alcoholic volunteers	74 APAP 68 Plcbo	4 g/d	5 days	54% ALT> nl	238 IU/L	38% ALT>nl (Plcbo)	249 IU/L
Heard 2010	Healthy non-drinkers	24 APAP	4 g/d	10 days	58% ALT>nl	136 IU/L	None	None
Harrill 2010	Healthy Volunteers	49 APAP 10 Plcbo	4 g/d	7 days	63% ALT> nl	460 IU/L	NR (Plcbo)	NR
Sudano 2010	Volunteers with coronary disease	37 *	4g/d	14 dags	Mean ALT increased 5.2 IU/L	NR	NR	

These studies demonstrate that ALT elevations occur when therapeutic doses of acetaminophen are administered for more than 4 days. However, the majority are two weeks or shorter so they do not demonstrate resolution. The longer duration studies were designed to evaluate other outcomes. While they do not show any evidence of acute liver injury, they did not measure ALT frequently and therefore cannot fully characterize the changes in ALT over the course of the study.(11-13, 28)

CHAPTER III

STUDY OBJECTIVES AND METHODS

The broad objective of this project is to evaluate the safety of long-term use of acetaminophen. To achieve this objective, this project has three specific aims.

Specific Aim 1: To characterize the course of aminotransferase activity during prolonged therapeutic acetaminophen administration. Specifically, to determine the proportion of patients treated with long-term acetaminophen who develop persistent ALT elevations.

Many patients who take acetaminophen for more than 4 days will have a rise in their serum alanine aminotransferase (ALT), but it is not known if these elevations will progress to clinical liver injury. Previous studies have not intensely monitored the changes in ALT beyond 10 days, but suggest that clinical evidence of liver injury does not occur and in the unlikely event that it does occur, it resolves. By continuing to administer therapeutic doses of acetaminophen to subjects who have ALT elevation, we can determine when the ALT elevation resolves.

Hypothesis 1: We hypothesize that no subject will have an ALT elevation that does not resolve on therapy. If we have zero subjects who have ALT elevations beyond the extended dosing period (zero numerator), our study design will allow us to conclude that the actual rate of elevations that do not resolve on therapy is less than 1.5 %.

Specific Aim 2: To determine if ALT elevations are associated with changes in liver function.

Aminotransferase elevations are considered evidence of hepatic injury, but hepatic injury without clinical evidence of hepatic dysfunction appears to have little clinical significance for drug induced liver injury. Asymptomatic aminotransferase elevations that are not accompanied by changes in hepatic function have been described for several medications. Given the long-term safety of acetaminophen, it is likely that subjects who develop ALT elevations will not have evidence of hepatic dysfunction.

Hypothesis 2: We hypothesize that no subjects who experience an elevation of ALT will have an elevation in serum bilirubin to greater than 2x the upper limit of normal or an elevation of INR to greater than 1.5.

Specific Aim 3: To compare demographic and clinical characteristics of subjects who have aminotransferase elevation during prolonged acetaminophen administration to subjects who do not have elevations.

The rate of aminotransferase elevation in clinical trials varies from 20 to 80%.(4, 8-10, 12, 15-16) It is likely that susceptible subjects have identifiable characteristics. These may be related to genetic or environmental factors. We have previously shown that ALT elevation occurs in both males and females at a roughly similar rate.(9, 16) However, there may be differences between ethnic groups. Watkins suggested an increased rate in some Hispanic populations, so this study includes a stratified enrollment plan which will include at least 20% Hispanic subjects to further investigate this potentially high risk population.(8)

This is an exploratory aim without an explicit comparison, so there is no specific hypothesis. We will compare demographic and clinical characteristics of the elevation groups in an exploratory analysis to identify demographic or laboratory findings that suggest an increased rate of ALT elevation. The findings of this study may be used to identify potential high risk groups that can then be included in future controlled trials.

The study design is a randomized, controlled trial comparing placebo to acetaminophen. The study allocation is 4 acetaminophen subjects to 1 placebo subject. Subjects will be healthy adult volunteers. The following participant groups were excluded from enrollment:

1) History of acetaminophen ingestion on any of the four days preceding study enrollment
2) Measurable serum acetaminophen level at time of enrollment
3) Viral markers of Hepatitis B or C , or viral markers of Hepatitis A with an ALT level greater than ULN during screening laboratory testing
4) Serum ALT or AST level greater than ULN at Screening or Day 0
5) Total bilirubin level greater than ULN at Screening or Day 0
6) INR level greater than ULN at Screening
7) Alkaline phosphatase level greater than ULN at Screening
8) Platelet count less than 125,000/mL at Screening
9) Known cholelithiasis
10) Positive pregnancy test at Screening (female participants only)

11) History of consuming more than an average of 3 alcohol containing drinks daily over the preceding 2 weeks
12) History of consuming 3 or more alcohol containing drinks on any given day during the 2 weeks prior to study enrollment
13) New prescription medication started within the previous 30 days
14) Currently taking isoniazid
15) Currently taking warfarin
16) Currently adheres to a fasting type diet as determined by self-report
17) Currently has anorexia nervosa as determined by self-report
18) Participant is clinically intoxicated, psychiatrically impaired or unable to give informed consent for any reason
19) Known hypersensitivity or allergy to acetaminophen

As the primary objective of the study was descriptive, we powered the study to determine the proportion of subjects who did not resolve by study day 40. The sample size is based on the requirement that the upper 95% confidence limit for p be less than 0.015. This is equivalent to requiring that the probability of observing all zeros is less than or equal to 0.025 when the true underlying probability of persistent ALT elevation is 0.015. This results in the equation: $Pr(0 \text{ events} \mid p=0.015) = (1-0.015)N = 0.025$ so $N=\ln(0.025) / \ln(0.985) = 244$. Therefore to adequately power our study, we will require 244 subjects be assigned to receive acetaminophen therapy in order to ensure that our probability of mistakenly accepting the null hypothesis is 0.025 when p=0.01. We are assuming a 15% screen failure and a 15% dropout rate and with a 4:1

acetaminophen: placebo assignment we anticipate enrolling approximately 425 subjects.

The intervention includes a dosing protocol was for a minimum of 16 days for all patients. Figure 2.1 shows subject flow and decision points for the study. Subjects who had an ALT elevation (defined below) will go on to the Extended Dosing Period. A 4:1 randomization scheme was used to increase the number of subjects exposed to acetaminophen 4 g/day who develop asymptomatic serum ALT elevation and therefore go on to the Extended Dosing Period (see definition of extended dosing period outline below).

Participants were provided with study drug and instructed to take two 500 mg tablets four times per day with 4-hour intervals between doses. This dosing regimen is consistent with the Food and Drug Administration (FDA) approved daily dose for acetaminophen.

Laboratory testing was performed throughout the study. Including Day 0, all participants completed a total of 7 study visits (Days: Screening, 0, 4, 7, 10, 13 and 16). A hepatic function panel and INR were measured at each study visit. If a subject's ALT level at Day 16 was either above ULN (47 IU/L) or more than 10 IU/L greater than their Day 0 ALT, they entered into the Extended Dosing Period. This value for ALT change was selected because fewer than 5% of placebo-treated subjects had changes of greater than 10 IU/L during our previous study. This portion of the study is to determine if the ALT elevation resolves while the subject continues to take acetaminophen. During the Extended Dosing Period the subject continued taking study medication and had

additional laboratory testing every 3 days until the ALT elevation has resolved (resolution defined in the following paragraph).

For participants whose ALT was greater than the ULN at Day 16, resolution was defined as two consecutive ALT measures within the reference range. For participants whose ALT was within reference range but greater than 10 IU/L above Day 0 ALT activity at Day 16, resolution was defined as two consecutive non-increasing ALT measures within the reference range. The Extended Dosing Period continued until resolution or Day 40, whichever occurred first. Once the ALT elevations resolved, the participant completed the trial. If a subject had an ALT level either above ULN or greater than 10 IU/L above their Day 0 ALT activity at Day 40, they discontinued the study medication and were monitored every 3 days until the ALT elevation resolved or until study day 49, whichever occurred first. If a subject's ALT elevation persisted beyond study day 50, the participant was referred to a hepatologist unrelated to this study. Study flow is shown in Figure 3.1.

The dependent variables were the ALT after day 0, INR after day 0 and bilirubin after day 0. We selected the ALT as the aminotransferase of interest because it is more liver-specific than the AST. The independent variables of interest were age, gender, race, self-reported ethanol consumption (yes/no), Day 0 ALT, study group. The primary outcome was the proportion of subjects who do not meet resolution criteria by study day 40.

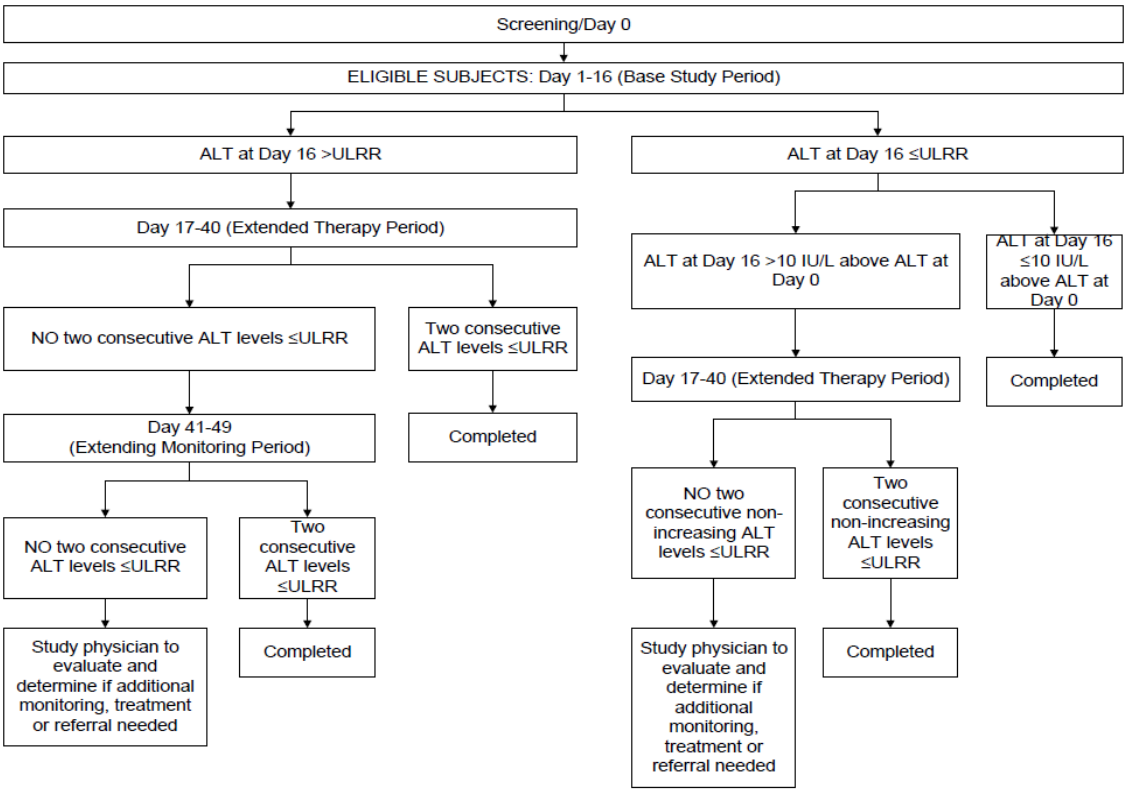

Figure 3.1. Subject flow through the study.

The secondary outcomes were: 1) The proportion of subjects who develop an abnormal INR or serum bilirubin greater than the upper limit of normal for our laboratory; 2) The proportion of subjects who go into the extended dosing period (extenders); 3) The proportion of subjects who experience an increase in ALT more than 10 IU/L above baseline (bumpers). This cutoff was selected as it was the upper limit of the increase noted in the placebo group in our previous study (and therefore represents expected variation if there is no effect of acetaminophen); and 4) The proportion of subjects who experience an increase in ALT more than 1.5 times baseline (responder). This cutoff was used by Harrill et al.(15)

Our statistical analysis plan for describing the change in ALT during continued acetaminophen dosing was to plot the ALT values for each subject over the course of the study. Descriptive statistics for the ALT values of the acetaminophen and placebo groups for each outcome were determined at each time point. The proportion of subjects (with 95% confidence intervals) was determined using the binomial approximation for each of the following outcomes: 1) ALT elevation not resolve by study day 40, 2) have an elevated INR or bilirubin, 3) enter the extended dosing period, 4) have an ALT greater than 10 IU/L above baseline and 5) have an ALT greater than 1.5 times their baseline values. We characterized the age, sex, race, ethnicity, baseline ALT, and average daily alcohol consumption of subjects meeting each of outcomes and subjects who do not meet these outcomes with descriptive statistics. Finally, the demographic and clinical characteristics listed above were compared between subjects meeting the outcomes and those not meeting the outcomes.

CHAPTER IV

RESULTS

The study was stopped after 398 subjects were consented due to slow enrollment. Of these 398 subjects, 122 were excluded at screening or at baseline and 276 subjects started the protocol, 24 withdrew during the study and 252 subjects completed the study. The disposition of patients is shown in Figure 4.1. The characteristics of withdrawn subjects for each disposition at baseline are shown in Tables 4.1 to 4.5.

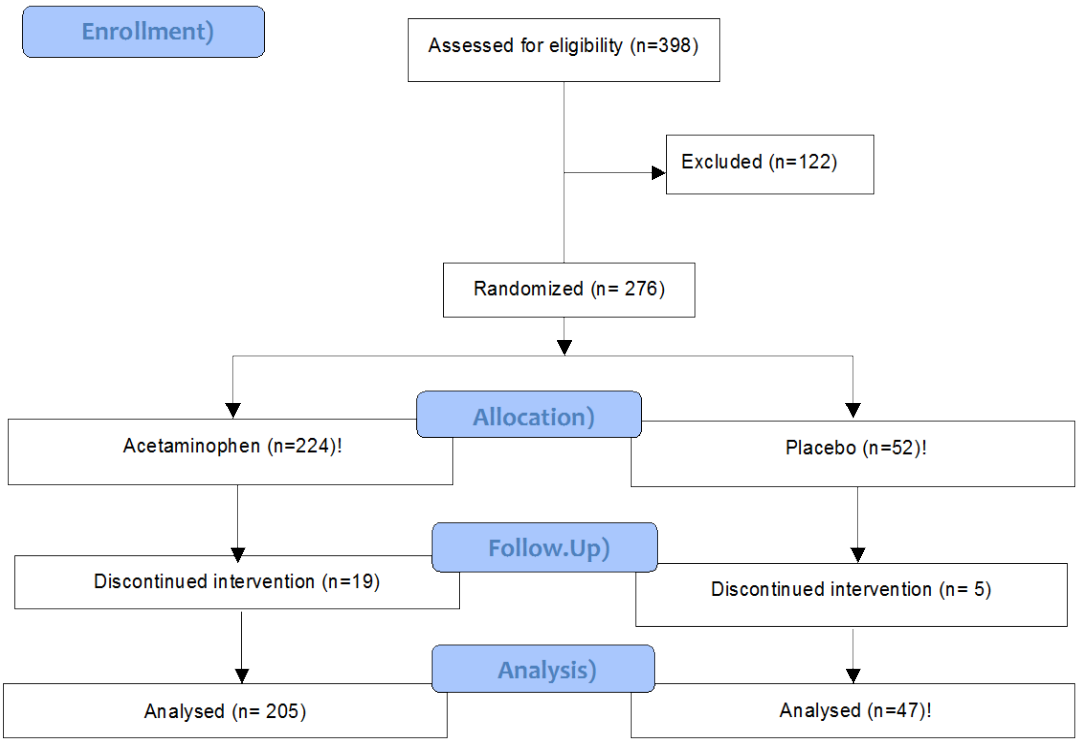

Figure 4.1. Subject disposition during the study.

Table 4.1. Characteristics of subjects who withdrew consent after randomization and prior to receiving study drug.

	Placebo (n=0)	Acetaminophen (n=4)
Age (years)		33.5 (24 to 44)
Male		0 (0%)
Race		
White		4 (100%)
Hispanic		0
Black		0
Other/Mixed		0
Body Mass Index		24.3 (19.6 to 31.7)
Alcohol use		4 (100%)
Screening ALT		18.5 (14 to 31)
Peak ALT		21 (14 to 36)
Final ALT		17.5 (11 to 30)

Table 4.2. Characteristics of subjects who were withdrawn from study due to non-compliance.

	Placebo (n=2)	Acetaminophen (n=4)
Age (years)	24 (22 to 26)	33 (21 to 41)
Male (%)	2 (100%)	4 (100%)
Race		
White	1 (50%)	2 (50%)
Hispanic	0	1 (25%)
Black	1 (50%)	1 (25%)
Other/Mixed	0	0
Body Mass Index	26.3 (22.7 to 29.9)	28.2 (19.5 to 62.1)
Alcohol use	3 (75%)	2 (100%
Screening ALT	16 (13 to 19)	16 (12 to 21)
Peak ALT	18 (13 to 23)	19.5 (17 to 26)
Final ALT	16 (11 to 21)	19 (13 to 20)

Table 4.3. Characteristics of subjects who were removed for an adverse effect (adverse effects are described in detail below).

	Placebo (n=1)	Acetaminophen (n=4)
Age (years)	34	24 (20 to 49)
Male (%)	0	3 (75%)
Race		
White	0	3 (75%)
Hispanic	0	1 (25%)
Black	0	0
Other/Mixed	1 (100%)	0
Body Mass Index	25.3	22.3 (16.7 to 29.6)
Alcohol use	1 (100%)	3 (75%)
Baseline ALT	17	16.5 (11 to 34)
Peak ALT	17	21.5 (15 to 35)
Final ALT	15	19 (15 to 28)

Table 4.4. Characteristics of subjects who were removed at the subject's request.

	Placebo (n=2)	Acetaminophen (n=6)
Age (years)	46 (44 to 48)	27 (20 to 38)
Male (%)	0	2 (33%)
Race		
White	0	1
Hispanic	1	1
Black	1	2
Other/Mixed	0	2
Body Mass Index	25.9 (25.6 to 26.2)	26.2 (20.7 to 30.7)
Alcohol use	1 (50%)	4 (66%)
Baseline ALT	16.5 (14 to 19)	15.5 (9 to 29)
Peak ALT	17 (15 to 19)	40 (13 to 120)
Final ALT	15 (15 to 15)	34.5 (13 to 54)

Table 4.5. Characteristics of subjects removed for other reasons.

	Placebo (n=0)	Acetaminophen (n=1)
Age (years)		24
Male (%)		1
Race		
White		
Hispanic		
Black		1
Other/Mixed		
Body Mass Index		27.8
Alcohol use		1
Baseline ALT		21
Peak ALT		41
Final ALT		29

The ALT values of subjects who were withdrawn prior to receiving study drug, for non-compliance, for adverse events other than stopping criteria and for other reasons were all lower than the upper limit of the reference range. Two subjects who withdrew from the study at their request (Table 3.4) had an ALT greater than the upper limit of the reference range. One subject had a peak ALT of 120 on study day 10 that had decreased to 67 IU/L on day 28 when the subject stopped taking acetaminophen and withdrew. A follow-up ALT off medication on study day 30 was 54 IU/L. A second subject had an ALT of 36 on study day 16, stopped taking study medication on study day 18 and had an ALT of 119 IU/L on study day 21. His ALT decreased to 46 IU/L on study day 24 while not taking acetaminophen. The reasons subjects who withdrew at their own request gave for leaving the trial were: inconvenience/too much time required to participate (4 acetaminophen); no transportation (1 acetaminophen, 1 placebo); moved out of state (1 acetaminophen); no reason given (1 placebo).

Among completed subjects, study drug compliance was high in both groups. The median proportion of days where completed subjects documented taking all pills on time was 0.9 (IQR 0.8 to 0.9). Two APAP subjects were inadvertently discontinued at day 16: a 28 year old female with a baseline ALT of 16 IU/L, and a peak ALT of 37 IU/L and a 31 year old female with a baseline ALT of 12 IU/L and a peak of ALT 24 IU/L. The baseline characteristics of the subjects completing the trial are shown in Table 4.6.

Table 4.6. Characteristics of subjects completing the trial.

	Placebo (n=47)	Acetaminophen (n=205)
Age (years)	32 (19.0, 64.0)	34 (18.0 to 74.0)
Male	12 (25.5%)	57 (27.8%)
Race		
White	35 (74.5%)	142 (69.3%)
Hispanic	4 (8.5%)	33 (16.1%)
Black	4 (8.5%)	13 (6.3%)
Other/Mixed	4 (8.5%)	17 (8.3%)
Body Mass Index	23.8 (17.3, 48.8)	25.8 (16.5, 53.5)
Self reported ethanol use	35 (74.5%)	167 (81.5%)
Baseline ALT	17.0 (11.0, 47.0)	19.0(9.0, 44.0)

The course of serum ALT activity for placebo-treated subjects (Figure 4.2) and acetaminophen-treated subjects (Figure 4.3) are shown below. For Specific Aim 1, we hypothesized that no subject would have an ALT elevation that did not resolve by study day 40. One acetaminophen-treated subject (1/224, 0.5% 95% CI 0 to 2.5%) and no placebo (0/52 0% 95% CI 0 to 8.2%) treated subjects did not meet resolution criteria by day 40 of treatment. As the 95% confidence interval for the rate of non-resolvers among acetaminophen-treated subjects

includes 1.5%, we must reject Hypothesis 1. The course of the ALT for the subject who did not resolve is shown in Figure 4.4.

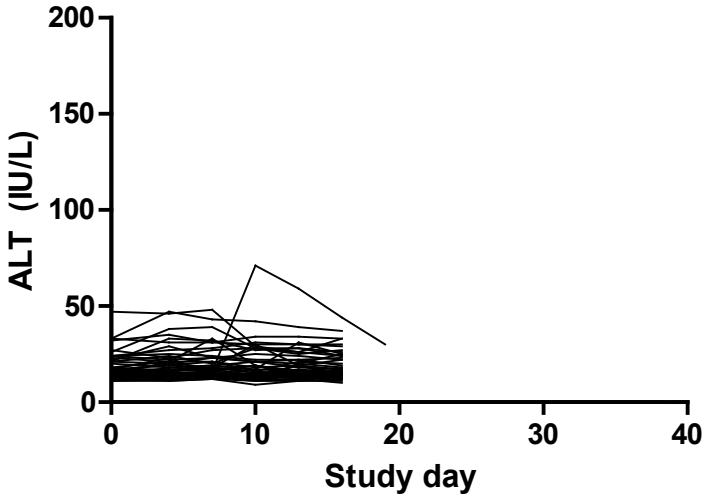

Figure 4.2. ALT of subjects receiving placebo.

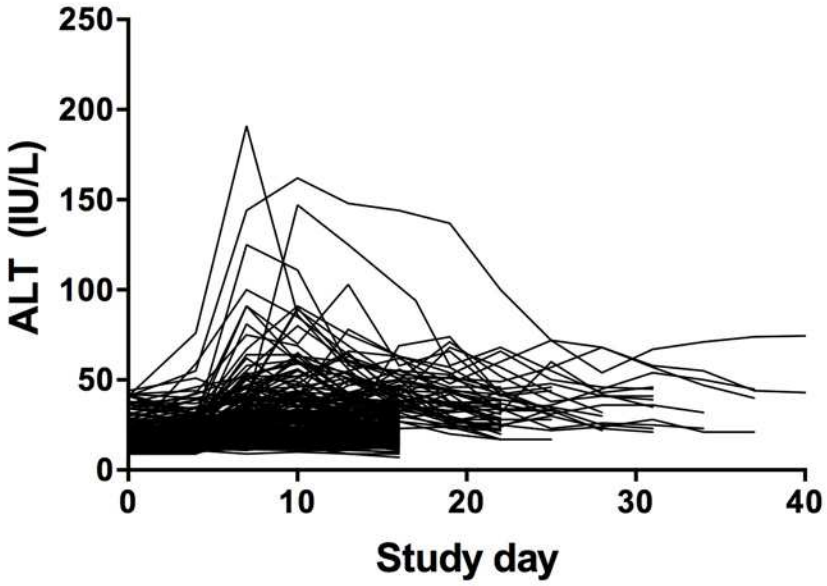

Figure 4.3. ALT of subjects receiving 4 g/day of acetaminophen.

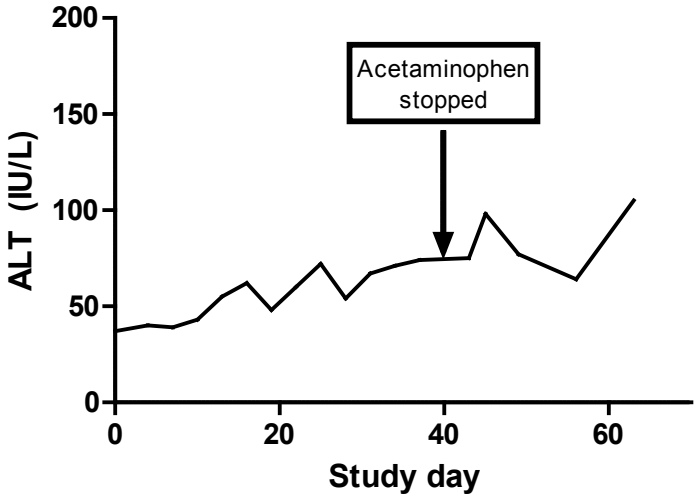

Figure 4.4. Detailed course of ALT for single acetaminophen subject who did not resolve.

For Specific Aim 2 we hypothesized that no subjects who experience an elevation of ALT will have an elevation in serum bilirubin to greater than twice the upper limit of normal or an elevation of INR to greater than 1.5. No subjects in either the acetaminophen group (0/205 0% 95% CI 0 to 2.0%) or placebo group (0/52) developed an INR>1.5 or a total serum bilirubin>2x the upper limit of normal at any point during the study. This suggests that the rate of drug-induced liver injury that occurs in healthy adults taking 4g/day of acetaminophen is unlikely to be more than 2% and that the observed ALT elevations are not clinically significant injury.

Specific Aim 3 was an exploratory aim to investigate differences in clinical characteristics of subjects who experienced ALT elevations. As this was an exploratory aim, we categorized ALT elevation using several definitions.

1) Extenders: Subjects who had an ALT>10IU/L above baseline OR 47 IU/L at day 16

2) Responders (as defined by Harrill(15)): Subjects who have peak ALT more than 1.5 times their baseline ALT.
3) Bumpers: Subjects who have a peak ALT more than 10 IU/L above their baseline.

Extenders had similar characteristics to non-extenders in both treatment groups (Table 4.7). In logistic regression, treatment group was associated with increased odds of entering the extended period and age, sex, race, baseline ALT and alcohol use were not associated with entering the extended dosing period (Table 4.8).

A significant minority of placebo subjects (13.5%, 95% CI 6.4 to 25.6%) and almost half (42.4%, 95% CI 36.1 to 49.0) of the acetaminophen group had an increase in ALT> 10 IU/L above their baseline ALT (Bumpers). The upper limit for the 95% CI of the 90% tolerance interval for ALT increase for placebo treated subjects was 22.5 IU/L. However, when the one outlier was excluded, the 95% CI of the 90% tolerance interval was 13.5 IU/L. This is similar to our predicted value of 10 IU/L that was used to define bumping. The characteristics of bumpers and non-bumpers were similar (Table 4.9), and the adjusted odds ratio for bumper vs non-bumper are shown in table 4.10.

Table 4.7. Characteristics of extenders and non-extenders for acetaminophen and placebo treated subjects.

	Acetaminophen		Placebo	
	Extenders	Non-extenders	Extenders	Non-extenders
N	48	157	1	46
Age (years)*	32.0 (25.0 to 52.0)	34.0 (27.0 to 46.0)	32.0	32.5 (25.0 to 46.0)
Male	12 (25.0%)	45 (28.7%)	1 (100%)	11 (23.9%)
Race				
White	28 (58.3%)	114 (72.6%)	1 (100%)	34 (73.9%)
Hispanic	11 (22.9%)	22 (14.0%)	0 (0.0%)	4 (8.7%)
Black	4 (8.3%)	9 (5.7%)	0 (0.0%)	4 (8.7%)
Other/Mixed	5 (10.4%)	12 (6.4%)	0 (0.0%)	4 (8.7%)
BMI*	26.6 (21.7 to 31.2)	25.8 (22.0 to 30.9)	20.3	23.9 (21.3 to 29.1)
Alcohol use	35 (72.9%)	132 (84.1%)	0 (0.0%)	35 (76.1%)
Baseline ALT*	20.0 (16.0 to 25.5)	19.0 (15.0 to 22.0)	17.0	18.5 (14.0 to 21.0)

*Values for age, BMI and baseline ALT are median and interquartile range.

Table 4.8. Adjusted odds ratios entering extended dosing period.

Variable	Odds Ratio	95% Confidence interval
Female	1.47	0.64 to 3.73
Race		
Caucasian	1.0	Reference
Black	1.71	0.47 to 6.26
Hispanic	1.86	0.79 to 4.40
Other	1.85	0.58 to 5.91
Age (per yr)	1.01	0.98 to 1.04
Baseline ALT (Per IU/L)	1.03	0.98 to 1.08
BMI	0.98	0.93 to 1.04
Ethanol use	1.81	0.81 to 4.1

Table 4.9. Characteristics of subjects who had ALT elevation >10 IU/L above baseline compared to those who did not.

	Acetaminophen		Placebo	
	Bumper	Non-Bumper	Bumper	Non-Bumper
N	90	115	7	40
Age (years)*	36.0 (25.0 to 50.0)	34.0 (27.0 to 44.0)	32.0 (27.0 to 57.0)	32.5 (25.0 to 41.5)
Male	26 (28.9%)	31 (27.0%)	2 (28.6%)	10 (25.0%)
Race				
White	57 (63.3%)	85 (73.9%)	7 (100%)	28 (70.0%)
Hispanic	16 (17.8%)	17 (14.8%)	0 (0.0%)	4 (10.0%)
Black	9 (10.0%)	4 (3.5%)	0 (0.0%)	4 (10.0%)
Other/Mixed	8 (8.9%)	9 (7.8%)	0 (0.0%)	4 (10.0%)
BMI*	25.9 (21.8 to 30.0)	25.8 (22.0 to 31.0)	22.6 (20.3 to 26.2)	23.9 (21.2 to 29.4)
Alcohol use	73 (81.1%)	94 (81.7%)	6 (85.7%)	29 (72.5%)
Baseline ALT*	19.0 (16.0, 22.0)	19.0 (15.0, 23.0)	20.0 (17.0, 26.0)	16.0 (13.0, 21.0)

*Values for age, BMI and baseline ALT are median and interquartile range.

Table 4.10. Adjusted odds ratios for developing an ALT increase >10 IU/L above baseline value.

Variable	Odds Ratio	95% Confidence interval
Female	1.03	0.53 to 2.03
Race		
Caucasian	1.0	Reference
Black	3.56	1.02 to 12.45
Hispanic	1.45	0.66 to 3.2
Other	1.40	0.50 to 3.96
Age (per yr)	1.02	0.99 to 1.04
Baseline ALT (Per IU/L)	1.01	0.97 to 1.05
BMI	0.98	0.93 to 1.03
Ethanol use	0.90	0.42 to 1.92

When we categorized the subjects as responders (peak ALT>1.5 times baseline value), the proportion of placebo meeting the definition decreased (3.5% 95% CI (6.4 to 25.6%)) while the proportion of acetaminophen subjects increased

slightly (45.5% (39.1 to 52.0%)) compared to the definition of a 10 U/L increase. However, there were still no clear differences in the characteristics of responders and non-responders (Table 4.11 and 4.12).

4.11. Characteristics of Responders and Non-responders in both treatment groups.

	Acetaminophen		Placebo	
	Responders	Non-responders	Responders	Non-responders
N	95	110	7	40
Age (years)*	36.0 (26.0 to 50.0)	34.0 (27.0 to 44.0)	32.0 (25.0 to 50.0)	32.5 (26.0 to 45.0)
Male	23 (24.2%)	34 (30.9%)	1 (14.3%)	11 (27.5%)
Race				
White	61 (62.4%)	81 (73.6%)	7 (100%)	28 (70.0%)
Hispanic	17 (17.9%)	16 (14.5%)	0	4 (10.0%)
Black	7 (7.4%)	6 (5.5%)	0 (0.0%)	4 (10.0%)
Other/Mixed	10 (10.5%)	7 (6.4%)	0	4 (10.0%)
BMI*	25.7 (21.8 to 30.0)	26.0 (22.0 to 31.2)	22.1 (20.3 to 22.9)	24.4 (21.2 to 29.8)
Alcohol use	77 (81.1%)	90 (81.8%)	6 (85.7%)	29 (72.5%)
Baseline ALT*	18.0 (15.0 to 22.0)	20.0 (16.0 to 24.0)	17.0 (13.0 to 20.0)	16.5 (14.0 to 22.5)

*Values for age, BMI and baseline ALT are median and interquartile range.

Adverse effects were common in both the placebo and acetaminophen groups. Overall, 62.3% of subjects had adverse events (65.6% in acetaminophen, 48.1% in placebo). The majority of adverse events were rated as unrelated or possibly related to study drug. The most common adverse effects probably related to acetaminophen were gastrointestinal (nausea, vomiting or abdominal pain). There were no serious adverse effects or deaths.

Table 4.12. Adjusted odds ratios for developing a peak ALT> 1.5 times the baseline value.

Variable		Odds Ratio	95% Confidence interval
Female		1.21	0.61 to 2.39
Race			
	Caucasian	1.0	Reference
	Black	1.80	0.54 to 5.99
	Hispanic	1.67	0.75 to 3.69
	Other	1.81	0.63 to 5.24
Age (per yr)		1.02	1.00 to 1.05
Baseline ALT (Per IU/L)		0.96	0.92 to1.00
BMI		0.99	0.94 to 1.04
Ethanol use		0.89	0.42 to 1.88

CHAPTER V

DISCUSSION AND CONCLUSIONS

Administration of acetaminophen for more than 4 days causes asymptomatic ALT elevations in a substantial number of healthy subjects. Subjects with elevated ALT have no change in INR or bilirubin, so these ALT elevations are not associated with hepatic dysfunction.

We have demonstrated that acetaminophen-induced ALT elevations do not progress and will resolve if therapy is continued in almost all subjects. The peak and decline occurred within the first 2 weeks of administration for most subjects, and the majority of the remaining subjects had a peak and decline within 3 weeks. Only 1/205 (0.5%) subject did not resolve on therapy and the course of ALT in this subject (continued elevation after more than 20 days off study drug) is not consistent with our current understanding of acetaminophen-induced liver injury. Unfortunately, the subject did not undergo a full evaluation for other causes and we cannot exclude acetaminophen as a cause for the elevations we observed.

The risk of having an ALT elevation that does not meet stopping criteria at day 16 was similar across gender, age, race, baseline ALT and alcohol use. Similarly, the risk of having a peak more than 50% above baseline value was not associated with any of the clinical or demographic variables evaluated. One previous study had suggested an increased risk for Hispanic subjects.(8) While the proportions of Hispanic subjects who went into the extended dosing period (33%), were responders (54%) and were bumpers (48%) were all slightly higher

than the proportions of Caucasians in these groups (20%, 41%, and 40% extenders responders and bumpers), the differences were not statistically significant and the magnitude of the absolute risk difference was less than 15% in all cases. This suggests that while there may be a slight increase in risk for ALT elevation for subjects with Hispanic ethnicity, ethnicity is not the major determinant of susceptibility. We also found a threefold increase in the risk of having an ALT increase more than 10 IU/L for subjects of African descent compared to Caucasians, but there was no increased risk for being an extender or responder.

While the exact mechanism of ALT elevation from therapeutic doses of acetaminophen is not clear (it may be due to hepatocyte death, apoptosis or changes in the clearance or activity of the enzymes or some other mechanism), in the clinical setting, elevation of ALT is considered a marker of liver injury. The lack of relationship between baseline ALT and degree or duration of elevation during this study is clinically relevant because liver disease is considered a risk factor for acetaminophen-induced liver injury. While we excluded patients with serologic or self-reported liver disease, it is likely that our population included subjects with mild (undiagnosed) liver disease such as non-alcoholic hepatic steatosis (NASH). Approximately 20% of the U.S. population has some degree of NASH, and often the only manifestation is mild elevation of the aminotransferases that may remain within the normal range.(30) The lack of correlation between baseline ALT and subsequent changes in ALT suggests that patients with NASH (or other mild liver disease) are not at increased risk for liver

injury from acetaminophen. As NASH is highly correlated with obesity (30), the lack of association between ALT elevation and body mass index further supports that patients with NASH are not at increased risk of injury from therapeutic doses of acetaminophen.

Ethanol use is a theoretical risk factor for liver injury from acetaminophen. Ethanol induces CYP 2E1, the enzyme that oxidizes acetaminophen, and increases the formation of NAPQI.(31) While studies of recently abstinent alcoholics and moderate ethanol users have suggested that this theoretical risk is not real,(5) our study is the first to show the effects of prolonged acetaminophen dosing on serum aminotransferase activity are similar between ethanol users and non-drinkers suggesting that ethanol consumption does not increase the risk of liver injury from therapeutic doses of acetaminophen. One potential limitation is that we relied on self-reported ethanol use. This has the potential to introduce misclassification bias for ethanol use. However, there is no reason to suspect systematic differences in reporting ethanol use between those with and without ALT elevations, so it is unlikely that the misclassification would alter our conclusions.

This project has several clinical implications. First, as noted in previous studies, acetaminophen use should be considered in the differential diagnosis of modest elevation of the serum ALT. Clinicians may defer work up of modest ALT elevations in patients who are using therapeutic doses of acetaminophen and instead opt for repeating the measurements to document resolution in 1-2 weeks.

Second, our study suggests that the ALT elevations are self-limited, resolve on therapy and that they do not progress to clinically significant liver injury. This is consistent with the observation that many patients use acetaminophen or acetaminophen-containing products for years without progressive liver injury. Approximately 20% of US adults reported prescription or nonprescription non-narcotic analgesic use on a frequent basis, that is nearly every day for as long as a month, at some point during their lifetime.(32) National survey data suggest that 2% of women age 22 to 51 years and 5.5% of women age 52 to 72 years used acetaminophen at least 6 days/week during the past year.(33) A review of clinical trials that included 1530 subjects taking 3.9 to 4 g/day of acetaminophen for between 4 weeks and 12 months identified no cases of liver failure.(34) As noted in the introduction, the best controlled evidence that these ALT elevations resolve (prior to the current study) was reported by Parra during a study designed to measure the effect of acetaminophen on warfarin-induced anticoagulation.(12) While this was a small study (only 24 acetaminophen treated patients), there was a dose-dependent elevation in the mean ALT reported at day 14 that had resolved by day 28. Placebo treated patients had no change in ALT. The time course and degree of elevation (mean ALT increase of 20 IU/L) are consistent with the changes observed in the current trial.

There are numerous examples of medications that are reported to cause elevation of serum aminotransferase activity that resolve if administration is continued. Commonly recognized examples include the statins, isoniazid, tacrine

and amiodarone.(35-38) While there are rare reports of progressive liver injury with these medications, they frequently cause aminotransferase elevations that do not progress to liver injury. Heparin is an example of a medication that commonly causes ALT elevations but has never been reported to cause liver failure.(35, 39) Finally, even a "fast food" diet may cause aminotransferase elevations within 1 week but has never been documented to cause liver failure.(40) This study demonstrates that acetaminophen should be added to the list of medications that cause self-limited aminotransferase elevations.

The final implication is that the ALT elevations are generally modest but are frequent. The range of ALT elevations observed in this study are similar to other studies in ambulatory patients treated for more than 4 days.(9, 11-13, 16, 28) In these trials, most subjects had a peak ALT less than twice baseline, and only one subject had a peak ALT above 200 IU/L. The degree of elevation is lower than the changes observed in inpatient studies where more than half of subjects had peak ALT that was at least twice baseline ALT and the highest reported ALT was more than 500 IU/L.(8, 15) Not surprisingly, the proportion of subjects meeting each outcome for ALT elevations varied with the definitions. In our study, we believe that an increase of 10 IU/L is the most valid definition as the upper limit of the 95% CI for change in ALT in the placebo group was 10.5 IU/L. Approximately 20% of placebo subjects met this definition while 15% of placebo subjects had a peak ALT>1.5 times baseline (the definition used by Harrill(15)).

The proportion of subjects experiencing ALT elevations in our placebo group was consistent with our previous work.(9) We observed a relatively low incidence of ALT elevations (approximately 10% had a peak ALT above the upper limit of the reference range). Other studies have reported that approximately 40% of placebo subjects had an ALT elevation above the upper limits of the reference range and 20% had a peak ALT at least twice their baseline value.(8, 15) The most likely explanation for the difference in magnitude and rate of the ALT elevations observed in these two studies is the study setting. While our study was an outpatient trial, these two studies (8, 15) were conducted on a clinical research unit. It is possible that the lower rate in our study is due to non-compliance, while all subjects on the inpatient unit would have received every dose. Our subjects documented excellent compliance in their study diary, but as we did not directly observe dosing, we cannot completely exclude this possibility. A second potential explanation is that residence in a research unit causes elevation of the serum ALT. This is supported by the relatively high rate of ALT elevations observed in the placebo group (38%) of Watkins' study. One previous study has suggested that residence in a research unit results in ALT elevations. In 2000, Narjes summarized the changes in serum aminotransferase observed in 220 placebo treated subjects hospitalized during 29 Phase 1 trials.(41) They noted only 3% of placebo outpatient subjects developed an abnormal ALT while 12% of placebo inpatient subjects met this criteria. The mean AST increased 8 IU/L and the mean ALT increased 17 IU/L. Obviously, a simple additive effect would not explain the full difference in the magnitude of the

elevations between the highest values reported by Watkins's and the highest values observed in outpatient studies. However, this may reflect an interaction between inpatient admission and acetaminophen that produces a multiplicative effect. This could be clinically relevant when hospitalized patients are administered acetaminophen. However, one clinical trial did not report any significant effect when post-operative patients (who would be at very high risk) were administered therapeutic acetaminophen doses.(42)

There are several strengths to this study. First, the study used frequent monitoring to detect elevations and resolution that may have been missed in previous studies. This allows a very precise description of the course of aminotransferase activity during the first 16 days of acetaminophen use. As non-prescription acetaminophen is not labeled for use beyond 10 days without consulting a healthcare provider, most prior studies have stopped prior to documenting resolution of the elevations. Our results are reassuring in that the vast majority of elevations are minimal and resolve quickly even if acetaminophen is continued.

The limitations of this study include the use of healthy volunteers without evidence of liver disease or heavy ethanol use. It is possible that people with these characteristics may respond differently. Another limitation is that some subjects may only experience aminotransferase elevations after 16 days of acetaminophen dosing. We also only followed subjects to the resolution of their elevations. It is possible that subjects may have subsequent elevations if

acetaminophen dosing was continued. These patients may also have a different course than the subjects observed in this study.

The major limitation to the internal validity of our results is the use of self-reported dosing. While we documented very high compliance, it is possible that subjects did not accurately record their medication use. This could lead us to underestimate the actual rate of missed doses and to mischaracterize the course of aminotransferase changes related to acetaminophen use. However, non-institutionalized subjects reflect the real-world circumstances of the vast majority of acetaminophen users. Another limitation is that our sample size would not allow us to detect rare events. While our study was large and randomized, it is worth noting that we had a baseline imbalance in some of the potential characteristics that may be related to our outcome. First, there was a numerically higher proportion of Hispanics in the acetaminophen group than in the placebo group. However, this should increase the precision of our estimate for developing ALT abnormalities and increase the probability of detecting uncommon events in this putative high risk group. A second major difference in baseline characteristics was that placebo patients had a higher median BMI than acetaminophen subjects. This would decrease the precision of our rate estimate and could decrease our ability to detect rare events. However, when BMI was evaluated as a continuous variable, there was no relationship between BMI and any outcome. Finally, in our exploratory analysis several characteristics had trends toward significant association with being an extender, responder or bumper. While the absolute risk difference between ethnic groups was small

(<20%) and not statistically significant in this sample, larger samples would be required to definitively determine if there are differences in the rate of elevation among these groups.

In conclusion, a significant number of subjects administered 4 g/day of acetaminophen develop mild elevation of serum aminotransferase activity. These elevations are not accompanied by changes in liver function (as measured by INR and serum bilirubin) and resolve in the vast majority of subjects within 3 weeks. The risk of elevation does not differ by demographics, baseline aminotransferase activity or ethanol use. This study suggests that the aminotransferase elevations observed during long-term treatment with acetaminophen have minimal clinical implications.

REFERENCES

1. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology. 2005 Dec;42(6):1364-72.

2. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 2003 Jul 31;349(5):474-85.

3. Senior JR. Monitoring for hepatotoxicity: what is the predictive value of liver "function" tests? Clin Pharmacol Ther. 2009 Mar;85(3):331-4.

4. Heard K. Asymptomatic alanine aminotransferase elevations with therapeutic doses of acetaminophen. Clin Toxicol (Phila). 2011 Feb;49(2):90-3.

5. Rumack B, Heard K, Green J, Albert D, Bucher-Bartelson B, Bodmer M, et al. Effect of Therapeutic Doses of Acetaminophen (up to 4 g/day) on Serum Alanine Aminotransferase Levels in Subjects Consuming Ethanol: Systematic Review and Meta-analysis of Randomized Controlled Trials. Pharmacotherapy. 2012 Jul 31.

6. Navarro VJ, Senior JR. Drug-related hepatotoxicity. N Engl J Med. 2006 Feb 16;354(7):731-9.

7. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther. 1973;187(1):211-7.

8. Watkins PB, Kaplowitz N, Slattery JT, Colonese CR, Colucci SV, Stewart PW, et al. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial. JAMA. 2006 Jul 5;296(1):87-93.

9. Heard K, Green JL, Bailey JE, Bogdan GM, Dart RC. A randomized trial to determine the change in alanine aminotransferase during 10 days of paracetamol (acetaminophen) administration in subjects who consume moderate amounts of alcohol. Aliment Pharmacol Ther. 2007 Jul 15;26(2):283-90.

10. Bartels S, Sivilotti M, Crosby D, Richard J. Are recommended doses of acetaminophen hepatotoxic for recently abstinent alcoholics? A randomized trial. Clin Toxicol (Phila). 2008 Mar;46(3):243-9.

11. Bradley JD, Brandt KD, Katz BP, Kalasinski LA, Ryan SI. Comparison of an antiinflammatory dose of ibuprofen, an analgesic dose of ibuprofen, and acetaminophen in the treatment of patients with osteoarthritis of the knee. N Engl J Med. 1991 Jul 11;325(2):87-91.

12. Parra D, Beckey NP, Stevens GR. The effect of acetaminophen on the international normalized ratio in patients stabilized on warfarin therapy. Pharmacotherapy. 2007 May;27(5):675-83.

13. Pincus T, Koch GG, Sokka T, Lefkowith J, Wolfe F, Jordan JM, et al. A randomized, double-blind, crossover clinical trial of diclofenac plus misoprostol versus acetaminophen in patients with osteoarthritis of the hip or knee. Arthritis Rheum. 2001 Jul;44(7):1587-98.

14. Rivera-Coll A, Fuentes-Arderiu X, Diez-Noguera A. Circadian rhythms of serum concentrations of 12 enzymes of clinical interest. Chronobiol Int. 1993 Jun;10(3):190-200.

15. Harrill AH, Watkins PB, Su S, Ross PK, Harbourt DE, Stylianou IM, et al. Mouse population-guided resequencing reveals that variants in CD44 contribute to acetaminophen-induced liver injury in humans. Genome Res. 2009 Sep;19(9):1507-15.

16. Heard KJ, Green JL, Dart RC. Serum alanine aminotransferase elevation during 10 days of acetaminophen use in nondrinkers. Pharmacotherapy. 2010 Aug;30(8):818-22.

17. Dart RC, Bailey E. Does therapeutic use of acetaminophen cause acute liver failure? Pharmacotherapy. 2007 Sep;27(9):1219-30.

18. Prescott LF. Therapeutic misadventure with paracetamol: fact or fiction? Am J Ther. 2000 Mar;7(2):99-114.

19. Khandelwal N, James LP, Sanders C, Larson AM, Lee WM. Unrecognized acetaminophen toxicity as a cause of indeterminate acute liver failure. Hepatology. 2011 Feb;53(2):567-76.

20. (CBER) USDoHaHSFaDACfDEaRCCfBEaR. Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation Rockville MD2009 [cited 2012 August 24]; Available from: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

21. Oneta CM, Lieber CS, Li J, Ruttimann S, Schmid B, Lattmann J, et al. Dynamics of cytochrome P4502E1 activity in man: induction by ethanol and disappearance during withdrawal phase. J Hepatol. 2002 Jan;36(1):47-52.

22. Lauterburg BH, Velez ME. Glutathione deficiency in alcoholics: risk factor for paracetamol hepatotoxicity. Gut. 1988 Sep;29(9):1153-7.

23. Corcoran GB, Wong BK. Obesity as a risk factor in drug-induced organ injury: increased liver and kidney damage by acetaminophen in the obese overfed rat. J Pharmacol Exp Ther. 1987 Jun;241(3):921-7.

24. Dart RC, Green JL, Kuffner EK, Heard K, Sproule B, Brands B. The effects of paracetamol (acetaminophen) on hepatic tests in patients who chronically abuse alcohol - a randomized study. Aliment Pharmacol Ther. 2010 Aug;32(3):478-86.

25. Kuffner EK, Dart RC, Bogdan GM, Hill RE, Casper E, Darton L. Effect of maximal daily doses of acetaminophen on the liver of alcoholic patients: a randomized, double-blind, placebo-controlled trial. Arch Intern Med. 2001 Oct 8;161(18):2247-52.

26. Kuffner EK, Green JL, Bogdan GM, Knox PC, Palmer RB, Heard K, et al. The effect of acetaminophen (four grams a day for three consecutive days) on hepatic tests in alcoholic patients--a multicenter randomized study. BMC Med. 2007;5:13.

27. Temple AR, Lynch JM, Vena J, Auiler JF, Gelotte CK. Aminotransferase activities in healthy subjects receiving three-day dosing of 4, 6, or 8 grams per day of acetaminophen. Clin Toxicol (Phila). 2007;45(1):36-44.

28. Kwan D, Bartle WR, Walker SE. The effects of acetaminophen on pharmacokinetics and pharmacodynamics of warfarin. J Clin Pharmacol. 1999 Jan;39(1):68-75.

29. Sudano I, Flammer AJ, Periat D, Enseleit F, Hermann M, Wolfrum M, et al. Acetaminophen increases blood pressure in patients with coronary artery disease. Circulation. 2010 Nov 2;122(18):1789-96.

30. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002 Apr 18;346(16):1221-31.

31. Thummel KE, Slattery JT, Ro H, Chien JY, Nelson SD, Lown KE, et al. Ethanol and production of the hepatotoxic metabolite of acetaminophen in healthy adults. Clin Pharmacol Ther. 2000 Jun;67(6):591-9.

32. Paulose-Ram R, Hirsch R, Dillon C, Losonczy K, Cooper M, Ostchega Y. Prescription and non-prescription analgesic use among the US adult population: results from the third National Health and Nutrition Examination Survey (NHANES III). Pharmacoepidemiol Drug Saf. 2003 Jun;12(4):315-26.

33. Curhan GC, Bullock AJ, Hankinson SE, Willett WC, Speizer FE, Stampfer MJ. Frequency of use of acetaminophen, nonsteroidal anti-inflammatory drugs, and aspirin in US women. Pharmacoepidemiol Drug Saf. 2002 Dec;11(8):687-93.

34. Kuffner EK, Temple AR, Cooper KM, Baggish JS, Parenti DL. Retrospective analysis of transient elevations in alanine aminotransferase during long-term treatment with acetaminophen in osteoarthritis clinical trials. Curr Med Res Opin. 2006 Nov;22(11):2137-48.

35. Watkins PB, Zimmerman HJ, Knapp MJ, Gracon SI, Lewis KW. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. JAMA. 1994 1994 Apr 6;271(13):992-8.

36. Chalasani N, Aljadhey H, Kesterson J, Murray MD, Hall SD. Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. Gastroenterology. 2004 2004 May;126(5):1287-92.

37. Pollak PT, Shafer SL. Use of population modeling to define rational monitoring of amiodarone hepatic effects. Clin Pharmacol Ther. 2004 2004 Apr;75(4):342-51.

38. Nolan CM, Goldberg SV, Buskin SE. Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. JAMA. 1999 Mar 17;281(11):1014-8.

39. Olsson R, Korsan-Bengtsen BM, Korsan-Bengtsen K, Lennartsson J, Waldenstrom J. Serum aminotransferases after low-dose heparin treatment. Short communication. Acta Med Scand. 1978;204(3):229-30.

40. Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindstrom T, Nystrom FH. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. Gut. 2008 May;57(5):649-54.

41. Narjes H, Nehmiz G. Effect of hospitalisation on liver enzymes in healthy subjects. Eur J Clin Pharmacol. 2000 Jul;56(4):329-33.

42. Mitchell SJ, Hilmer SN, Murnion BP, Matthews S. Hepatotoxicity of therapeutic short-course paracetamol in hospital inpatients: impact of ageing and frailty. J Clin Pharm Ther. 2011 Jun;36(3):327-35.

APPENDIX A

HUMAN SUBJECTS PROTOCOL

AMINOTRANSFERASE TRENDS DURING PROLONGED THERAPEUTIC ACETAMINOPHEN DOSING

COMIRB #06-1265

Full Protocol Version 016
Version Date 22 March 2011

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Study Objectives
The broad, long term objectives of this project are to evaluate the safety of long-term use of acetaminophen.

Specific Aim1: To characterize the course of aminotransferase levels during prolonged acetaminophen administration. *Specifically to determine the proportion of patients treated with long-term acetaminophen that develop persistent ALT elevations.*
Many patients who take acetaminophen for more than 4 days will have a rise in their serum alanine aminotransferase (ALT), but it is not known if these elevations will progress to clinical liver injury. Previous studies have not intensely monitored the changes in ALT beyond 10 days, but suggest that clinically evidence of liver injury does not occur and in the unlikely event that it does occur, it resolves. By continuing to administer ***therapeutic*** doses of acetaminophen to subjects who have ALT elevation, we can determine when the ALT elevation resolves.

Hypothesis: We hypothesize that less than 1.5% of acetaminophen treated subjects will develop ALT elevations that do not resolve on therapy.

Let p be the proportion of subjects treated with long-term acetaminophen (4g/day average for dosing period duration) that develop persistent ALT elevations. The null hypothesis is H0: p=0 and the alternative hypothesis is Ha: p=0.015. If we find that 0/244 patients who receive acetaminophen have elevation of their ALT that persists beyond the Extended Dosing Period we will accept the hypothesis that fewer than 1.5% of subjects will have ALT elevation that do not resolve on treatment.

Specific Aim 2: To compare demographic and clinical characteristics of subjects who have aminotransferase elevation during prolonged acetaminophen administration to subjects who do not have elevations.
The rate of aminotransferase elevation in clinical trials varies from 20 to 80%. It is likely that susceptible subjects have identifiable characteristics. These may be related to genetic or environmental factors. We have previously shown that the elevation occurs in both males and females. Other researchers have suggested an increased rate in some Hispanic populations. This study will have sufficient subgroups to compare rates across several demographic and clinical factors. We will also compare genotypes of relevant cases.

Hypothesis: This is an exploratory aim without an explicit hypothesis. We will compare demographic and clinical characteristics of the elevation groups in an exploratory analysis to identify demographic or laboratory findings that suggest an increased rate of ALT elevation. The findings of this study may be used to identify potential high risk groups that can then be included in future controlled trials.

Specific Aim 3: To describe the drug metabolism genotypes and changes in serum cytokines in subjects who have aminotransferase elevation and to compare these changes to subjects who do not have aminotransferase elevations. The cytokine comparison will also compare changes in both groups with changes in placebo treated subjects.
Hepatic adaptation (ALT elevation that resolves on treatment) is well described for several medications including statins, isoniazid and tacrine (see Background). However, some subjects may not adapt and go on to liver failure. In this preliminary study, we will characterize the genotype of drug metabolism pathways and the cytokine response of any subject who does not have resolution of ALT elevation while on therapy or who meets stopping criteria. We will then perform a nested case-control study to compare these characteristics to age, gender and ethnicity of matched controls who 1) do not have ALT elevation and 2) have ALT elevation that resolve on therapy.

Hypothesis: This is an exploratory aim. Our hypothesis is that we will find increased odds of ALT elevation in subjects with specific pharmacogenomic profiles. The prevalence of these profiles in the community has not been adequately described to allow us to perform formal sample size calculations. There will be no formal statistical testing. If we can identify pharmacogenomic profiles that appear to have increased odds of ALT elevation, we will use these to screen subjects for future trials to confirm or refute these preliminary findings in a controlled trial. The findings of this study may be used to identify potential high risk groups that can then be included in future controlled trials.

Specific Aim 4: To determine the proportion of subjects that have detectable serum acetaminophen-cysteine adducts (APAP-CYS) concentrations 1, 2, and 3 days (Study Days 2, 3, and 4) after starting the maximum recommended dosing of acetaminophen (4 g/ day). Dosing with acetaminophen causes the production of APAP-CYS during metabolism in the liver, and their presence can indicate potential hepatic necrosis.

Hypothesis: This is an exploratory aim. Our hypothesis is that APAP-CYS concentrations will be detectable as early as day 1 of dosing.

Background
As described in detail below, acetaminophen use is common and many consumers take 4g/day for longer than 4 days. The use of 4g/day of acetaminophen for more than 4 days causes an asymptomatic ALT elevation in some people. This elevation most likely resolves while continuing treatment, but it is possible that some individuals may go on to develop clinical liver injury. By carefully following healthy subjects who are taking the maximal daily dose of acetaminophen, we can safely determine if the ALT elevation resolves or progresses to clinical liver injury. If a subject develops clinical liver injury we can

intervene before irreversible injury occurs. This research will have important clinical and research implications.

Acetaminophen is widely used and there are many people who use it daily and for more than 4 days

Over 60 million Americans consume acetaminophen each week[1]. There are several use patterns of acetaminophen; one time use for a minor symptom that resolves, consistent use over several days for recurrent or persistent symptoms, and long-term use for chronic daily symptoms. While the safety of short term and intermittent use has been established, there are growing concerns that use longer than 10 days causes serum ALT elevation (see below). A review of the 2003 National Health and Nutrition Examination survey found that 20% of Americans reported taking an analgesic nearly every day for as long as a month at some point in their life[2]. Three percent of Americans take acetaminophen on a daily basis for a month or more.

In addition to nonprescription acetaminophen products, patients with chronic pain will take acetaminophen in narcotic combination products for a prolonged period. Approximately 17 million people will use a mild strength opioid (such as codeine or hydrocodone) each month, and the majority of these will be in an acetaminophen combination product. The median duration of use for codeine and codeine derivative products is 27 days, and 25% will use these products for more than a year. The recommended dose of these products would result in individuals ingesting up to 4 gm of acetaminophen per day.

These studies suggest that there are millions of people taking the maximum daily dose of acetaminophen for more than 4 days.

Taking acetaminophen for more than 4 days causes asymptomatic elevation of the serum ALT in some people.

While it is well-known that acetaminophen overdose may result in hepatotoxicity as well as liver failure and death if untreated, the safety of therapeutic use of acetaminophen has been demonstrated in several large studies[3-6]. Despite a long record of safety, recent publications indicate that therapeutic doses of acetaminophen can be associated with asymptomatic elevations of aminotransferase activity greater than the upper limit of normal (ULN) in some subjects. Watkins et al[7] reported that 76% of normal subjects taking 4 grams of acetaminophen daily for 10 days, either alone or in combination with an opioid, developed a serum ALT above the ULN. ALT elevations were also reported for 38% of subjects in the placebo group.

Our group has recently reported similar findings in healthy subjects who consumed 1 to 3 alcoholic beverages per day[8]. Participants were asked to take study medications (1000 mg acetaminophen four times per day or placebo) for 10 consecutive days. One hundred participants completed the acetaminophen regimen and another 50 completed the placebo regimen. While the AST means

were within ULN throughout the study with no significant difference between the groups, the ALT measures indicated a different trend. A total of 25 of 150 participants (17%) had a baseline ALT measure within reference range and then experienced an ALT elevation above ULN at Day 4 and/or Day 11 (20 of 100 (20%) in acetaminophen group and 5 of 50 (10%) in placebo group; p=0.121). An additional 2 participants (1 in each group) had a baseline ALT above ULN but less than 50 IU/L with all subsequent ALT levels within reference range. The range of elevated ALT levels was 42 to 128 IU/L with only 3 of these elevations exceeding twice the ULN. The majority of these increases were reported on Day 11 of the study (the day following the last dose of study medication).

Several other studies have also found asymptomatic ALT elevation with therapeutic doses of acetaminophen. These studies report ALT elevation above the ULN in 5-10% of participants treated with 4g/day of acetaminophen for up to 6 weeks. Unfortunately, these studies had infrequent sampling, so the course of ALT changes cannot be characterized.

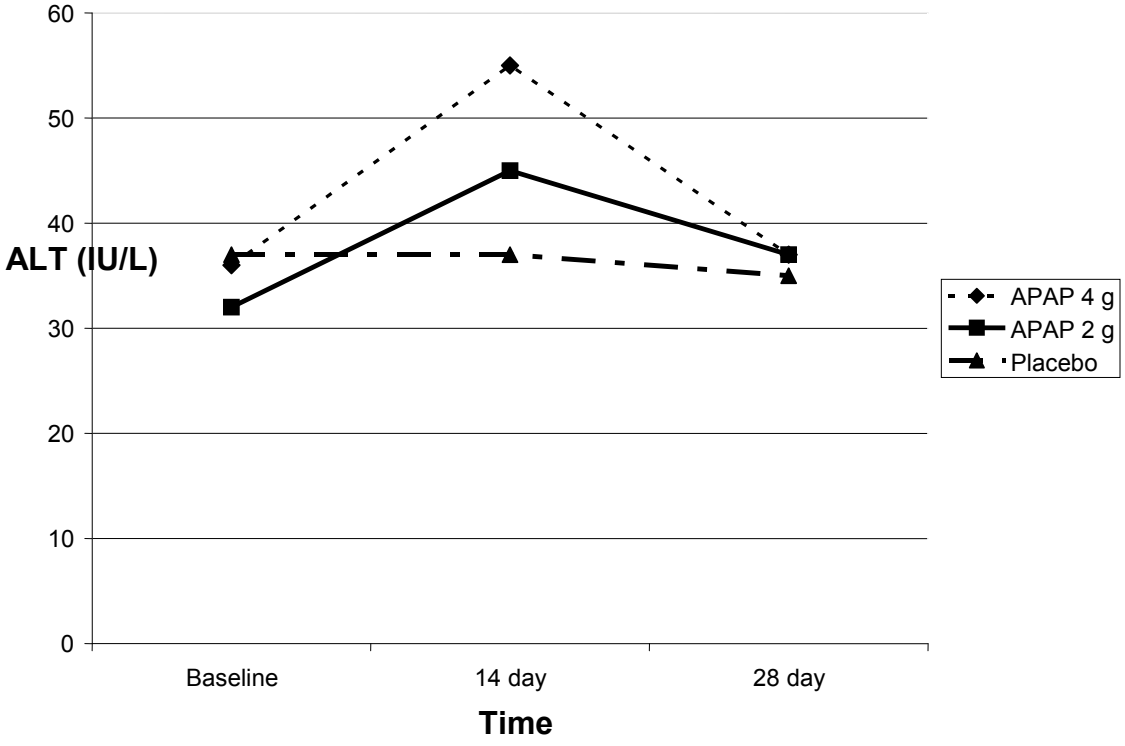
It is clear that the maximum daily dose of acetaminophen causes asymptomatic ALT elevation in some subjects. However, the clinical course and implications of these elevations are not clear. Without systematic study, it is impossible to determine if clinical liver injury may occur.

Although use for several days may cause ALT elevation, studies suggest that long-term use does not progress to clinical liver injury or failure.
In the studies of therapeutic acetaminophen described above, there were more than 100 subjects who developed abnormal ALT, but no subjects developed symptomatic liver injury, increased serum bilirubin or evidence of hepatic dysfunction. A systematic review of prospective medical literature found no reported cases of clinical hepatic injury, liver transplant or death due to therapeutic use of acetaminophen[9]. *This suggests that ALT elevation is not a hallmark of liver injury or impending liver injury.* Senior et al have suggested that there may be an adaptive process that occurs with some medications. They point out that ALT elevations occur with the statins, tacrine and other medication but that these changes resolve when the medication is continued. However, *without continuing the medications and systematically monitoring the ALT it will be impossible to determine if this occurs with acetaminophen.*

There is evidence suggesting that ALT elevation due to therapeutic acetaminophen administration may resolve while therapy is continued.

Recently, Parra et al[10] performed a 4-week randomized, placebo-controlled study to determine the effect of 2 and 4 g/day of acetaminophen on the INR of participants taking warfarin. During this study they also measured serum ALT at baseline, 2 and 4 weeks. This study found a dose-dependent increase in ALT at 2 weeks that resolved by 4 weeks. The results of this study are shown in Figure 1.

Figure 1. ALT at baseline, 14 and 28 days. Data from Parra et.al 2007[10].



The alternative hypothesis is that acetaminophen-induced liver injury will progress to liver failure.

No systematic studies have detected cases of therapeutic doses of acetaminophen causing clinical liver injury or liver failure. However, there are anecdotal reports of individuals who develop liver failure while taking therapeutic doses of acetaminophen[11]. Furthermore, the cause of liver failure was not determined in 17% of cases evaluated in the Acute Liver Failure (ALF) Study

Group[12]. While unlikely, it is conceivable that some of these cases could be occult, subacute acetaminophen poisoning. In fact, a follow-up study suggested that 19% of patients with ALF of indeterminate cause had evidence of acetaminophen exposure. This group may represent patients who did not give a history of acetaminophen use because they were taking therapeutic doses. *While we believe this is unlikely, the only way to identify individuals who will go on to develop hepatic failure from therapeutic dosing is to prospectively dose subjects and follow the ALT until 1) resolution of the elevation or 2) the subject develops clinical liver injury.*

The forthcoming FDA publication entitled Drug-Induced Liver Injury: Premarketing Clinical Evaluation[13] recognizes the importance of identifying medications that cause self-limited ALT elevation. The monograph states "*Stopping a drug at the first hint of mild injury does not permit learning whether adaptation will occur, as it does for drugs such as tacrine that cause liver injury but do not cause severe drug-induced liver injury (DILI)."* Unlike previous guidelines which have focused on the degree of ALT elevation as a marker of DILI, the following criteria have been proposed for stopping a medication.

- ALT or AST >8x upper limit of normal (ULN)
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (total bilirubin >2xULN or international normalized ratio (INR) >1.5)
- ALT or AST >3x ULN with the appearance of worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia

In cases of less severe ALT elevation (> 3x ULN but not meeting other criteria) the guidelines recommend close observation defined as

- Repeating liver tests two or three times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or study drug has been discontinued and subject is asymptomatic.
- Obtaining a more detailed history of symptoms and prior or concurrent diseases.
- Obtaining a history of concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g. INR).
- Considering gastroenterology or hepatology consultation.

By including these safety measures, our study will allow us to determine the course of acetaminophen-induced ALT elevations while ensuring the safety of the subjects. Subjects who meet the criteria for drug-induced liver injury can be

treated with N-acetylcysteine, an extremely effective antidote for acetaminophen poisoning.

The most appropriate study population is healthy subjects who are not regularly taking acetaminophen.
There are several populations we could evaluate in this study. One population would be patients already using acetaminophen therapeutically. However, this group may systematically exclude individuals at risk for acetaminophen-induced liver injury. Studying subjects who are taking acetaminophen would self-select out any person who could not tolerate acetaminophen. It is possible that these individuals are the very group who are sensitive to acetaminophen-induced ALT elevation. Furthermore, patients who are taking acetaminophen long-term may already be adapted to acetaminophen which would prevent us from monitoring them during the adaptation phase. By using normal subjects who are not regularly taking acetaminophen, we will study the group that is truly at risk for injury if they were to begin using acetaminophen daily.

By monitoring ALT frequently, we can identify subjects who are progressing towards clinical liver injury and intervene
Although several studies have reported no evidence of progression of ALT elevation to clinical liver injury during prolonged administration of therapeutic doses of acetaminophen, it is a reasonable concern that this could happen during our study. However, this protocol has several measures that will allow us to identify progression early and intervene BEFORE clinical liver injury develops.

First, all subjects will be closely monitored for biochemical indicators and symptoms of liver injury. Subjects will have an ALT, total bilirubin, and INR measured every 3 days. In addition, a research team member will contact the subject every 3 days and conduct a structured interview to assess for symptoms of clinical liver injury. Using the FDA proposed guidance, clinical trial material (CTM) will be withdrawn immediately if a subject develops any one of the stopping criteria listed below (subject treatment in the event of meeting stopping criteria is outlined in the next paragraph):

- ALT or AST >8x ULN
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (TBL >2xULN or INR >1.5)
- ALT or AST >3x ULN with the appearance of worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia.

With this highly structured protocol, we will be able to detect liver injury early and intervene.

If a participant develops clinical liver injury, we will implement two interventions. The first will be stopping the CTM. In Watkins[7] study that first reported acetaminophen induced ALT elevation, the authors reported that ALT decreased

when acetaminophen was stopped. Similarly, in a preliminary study of non-drinkers treated with 4 g/day of acetaminophen, we found that 10/12 subjects who had ALT elevation had a decreasing ALT within 24 hours of stopping acetaminophen and all subjects had a falling ALT within 11 days of stopping acetaminophen. These findings suggest that stopping acetaminophen is sufficient to prevent the progression of ALT elevation.

While we believe that stopping acetaminophen is a sufficient intervention for preventing progressive ALT elevations, these subjects will be evaluated for treatment with N-acetylcysteine. N-acetylcysteine is a very effective therapy for treatment of acetaminophen toxicity. NAC decreases mortality and improves liver function. As the efficacy of NAC is time dependent[14], by intervening at an earlier point (prior to hepatic failure), we can further assure the safety of our participants. Finally, acetaminophen induced liver injury is completely reversible so even in the worst case scenario, permanent effects are not expected.

By characterizing the genotype and cytokine response of subjects who do not have resolution of their ALT elevation, we can potentially identify subjects at risk for liver failure from prolonged acetaminophen use.
We will characterize the inflammatory response (cytokines) and drug metabolism genotype of subjects who do not have resolution of their ALT elevation while on drug. These subjects will be compared to age, gender and ethnicity matched controls in nested case-control design to identify risk factors for failure to adapt to acetaminophen use. These characteristics can potentially be used to identify high-risk subjects and will serve as preliminary data for future studies to determine if there are subjects who may develop clinical liver injury from therapeutic use of acetaminophen.

We will also be looking to characterize when Acetaminophen-Cysteine (APAP-CYS) is detectable in serum during short term dosing with acetaminophen.
In addition to causing elevations in ALT levels in some people, dosing with acetaminophen causes the production of acetaminophen-cysteine adducts (APAP-CYS) as acetaminophen is metabolized in the liver and converted to N-acetyl benzoquinoneimine (NAPQI), which binds to cysteine residues in liver proteins. Previous studies have shown that these adducts shortly precede hepatic necrosis following acetaminophen overdose. In cases where acetaminophen may be a primary cause for hepatotoxicity leading to liver injury but no acetaminophen concentrations are detected in the serum, an alternative test measuring APAP-CYS could serve as an indicator of acetaminophen exposure and overdose.

Recent work done at the Rocky Mountain Poison and Drug Center demonstrated that APAP-CYS are formed even at the recommended dosages of acetaminophen. Concentrations of APAP-CYS were evident in the majority of subjects taking the maximum permitted daily dose of acetaminophen for five

days and ten days, suggesting that adducts are a sign of acetaminophen exposure, and not necessarily overdose. This study will allow us to assess the development of adducts in serum after a short-term therapeutic course of acetaminophen, as currently the time required for development of detectable concentrations of APAP-CYS in the serum is not known.

In summary

- Acetaminophen use is common and many individuals take 4g/day for longer than 4 days.
- The use of 4g/day of acetaminophen for more than 4 days causes an asymptomatic ALT elevation in some people.
- This elevation most likely resolves while continuing treatment, but it is possible that some subjects may go on to develop clinical liver injury.
- By carefully following healthy subjects who are taking the maximal daily dose of acetaminophen, we can safely determine if the ALT elevation resolves or progresses to clinical liver injury. If a participant develops clinical liver injury we can intervene before irreversible injury occurs.
- This study may identify factors for subjects who may be at increased risk to develop clinical liver injury from therapeutic doses of acetaminophen.
- Findings of this study will assist with characterization and timing of adducts development, and may support the use of APAP-CYS assay methods in determining whether a patient with acute liver damage had ingested an excessive overdose of acetaminophen.

Methods

Informed consent will be obtained from all participants. Screening and Day 0 activities will include a structured medical history and a review of systems. All eligible subjects will complete the following screening laboratory testing:

- Viral hepatitis screening (HAV Ab, HBsAG, and HCV Ab)
- Hepatic function panel (total protein, albumin, total & direct bilirubin, alkaline phosphatase, aspartate aminotransferase (AST) and ALT)
- Triglycerides (fasting)
- Complete blood count (CBC)
- International normalized ratio (INR)
- Serum acetaminophen
- Serum pregnancy test (female subjects only)
- Serum acetaminophen-protein adduct sample
- Reference tube of frozen serum

Results of hepatitis testing will be reported to the Colorado Department of Public Health & Environment as required by law. This screening will aid the investigator in identifying participants with underlying liver conditions that could alter serum ALT activity. Participants with viral markers of Hepatitis B or C and participants with viral markers of Hepatitis A with ALT levels above the ULN will be excluded from the study at screening, prior to CTM administration.

Subjects will be informed of eligibility to enter the Base Study Period. Day 0 study visits will be scheduled within 1 week of screening visits.

Eligible subjects (based upon inclusion and exclusion criteria outlined below) will be randomly assigned to either an acetaminophen treatment group or placebo control group (4:1 randomization) and will begin taking CTM on Day 1. The dosing protocol will continue for a minimum of 16 days for all patients. Subjects who have an ALT elevation (defined below) will go on to the Extended Dosing Period. A 4:1 randomization scheme will increase the number of subjects exposed to acetaminophen 4 g/day who develop asymptomatic serum ALT elevation and therefore go on to the Extended Dosing Period (see below). This group of subjects will ultimately provide the most important outcome, so we want to ensure an adequate sample size for this population. Participants will be provided CTM with instructions to take 2 tablets 4 times per day with 4 hour intervals between doses. The average daily dose over the study period will be 4 g/day; this dosing regimen is consistent with the Food and Drug Administration (FDA) approved daily dose for acetaminophen.

Laboratory testing will be done throughout the study (Appendix A). Including Day 0, all participants will complete a total of 10 study visits (Days Screening, 0, 1, 2, 3, 4, 7, 10, 13 and 16). Blood samples will be collected at each study visit. On study visit Day 4 a whole blood sample will be drawn for potential genotyping. The total amount of blood to be collected during the 16 day study period is approximately 16 tablespoons. Up to an additional 2 to 11 tablespoons may be collected during the Extended Dosing Period. (See below). A hepatic function panel will be measured at each study visit.

An acetaminophen-protein adduct sample will be collected at each study visit during the Base Study Period, and at every other visit (Days 19, 25, 31, and 37) during the Extended Dosing Period. The acetaminophen-protein adduct test is an experimental assay that quantifies the formation of protein adducts with acetaminophen. The clinical significance of adduct formation is unknown at this time. A frozen reference sample will also be collected at each study visit. The frozen reference samples taken at each blood draw will be kept in a secured freezer at the RMPDC. These samples may be used for additional acetaminophen or hepatic testing in the future. The samples will be labeled with protocol number, subject number, date/time of collection and day of study participation.

Triglyceride testing will occur at the Screening, Day 10 and Day 16 Visits. Fasting will be required for 9-12 hours prior to these blood draws based on the validity of the clinical lab test for triglycerides. Subjects will be instructed to fast for 12 hours prior to these visits. Instances of less than 9 hours of fasting will be recorded as protocol deviations.

Samples will be stored for future cytokine and genotype analysis using the system described in the above section. Our plan is to characterize the cytokine response and drug metabolism and transporter genes of subjects who have ALT elevations that do not resolve and compare these to a subset (3:1 control to responder) of matched age, sex and ethnicity controls who 1) do not have ALT elevation and 2) who have ALT elevation that resolves. Cytokine analysis will be performed to characterize changes in inflammatory cytokines as secondary liver injury may be caused by an over-response of the inflammatory system to the initial injury. This testing will be performed in the Proteomics Core and will include TNF alpha, IL-6, IL-13 and other inflammatory cytokines. Drug metabolism genotyping will be performed to determine if genetic factors are associated with liver failure to adapt to acetaminophen dosing. We will perform the genotyping for multiple relevant genes using available technology. For example, the one potential system would be the Affymetrix Drug Metabolism Enzymes and Transporter method. This system includes 172 markers involved in drug metabolism, including several genetic variants of CYPP450 enzymes, non-CYP enzymes that metabolize medications (such as glutathione-s-transferase) and drug transporters that may be involved in acetaminophen metabolism. However, this is a rapidly changing field, and it is possible that this product may not be available (or a better product may become available). We therefore propose to use the most relevant system available for the evaluation of drug metabolism pathways at the time of the analysis. Ultimately, we hope to perform genotyping samples from all subjects and will then compare subjects who have ALT elevation to subjects who do not have ALT elevation. Ultimately, these tests may identify subjects at risk for liver injury from therapeutic doses of acetaminophen.

If a subject's ALT level at Day 16 is either above ULN or more than 10 IU/L greater than their Day 0 ALT, they will be entered into the Extended Dosing Period (Appendix A and B). This value for ALT change was selected because fewer than 5% of placebo-treated subjects had changes of greater than 10 IU/L during our previous study. This portion of the study is to determine if the ALT elevation resolves while the subject continues to take acetaminophen. The Extended Dosing Period will involve continuation of CTM and additional laboratory testing every 3 days until the ALT elevation has resolved (resolution defined in the following paragraph). This Extended Dosing Period will allow investigators to evaluate aminotransferase trends while on continued therapy.

For participants whose ALT is greater than the ULN at Day 16, resolution is defined as two consecutive ALT measures within the reference range. For

participants whose ALT is within reference range but greater than 10 IU/L above Day 0 at Day 16, resolution is defined as two consecutive non-increasing ALT measures within the reference range. The Extended Dosing Period will continue until resolution or Day 40, whichever occurs first. Once the ALT elevations have resolved, the participant will have completed the trial. If a subject has an ALT level either above ULN or greater than 10 IU/L above their Day 0 ALT at Day 40, they will be instructed to discontinue CTM and we will continue to monitor the subject every 3 days until the ALT elevation resolves or until study day 49, whichever occurs first. In the unlikely event that a subject's ALT elevation persists beyond study day 50, the participant will be referred to a hepatologist unrelated to this study. (Appendix B)

A participant may request to withdraw from the study at any time. If this occurs the subject will discontinue the CTM immediately. Regardless of previous ALT level, at least one additional ALT level will be obtained within five days of withdrawal from all prematurely withdrawn subjects who have ingested at least one dose of CTM. Additional hepatic function testing will be completed at least weekly at the discretion of the study physician. Follow-up ALT levels do not need to be obtained from subjects who withdraw from the study prior to first dose of CTM.

Participants will be given a study diary in which to record actual times of CTM ingestion, concomitant medications, alcohol intake and physical activity. Each participant will be instructed to avoid ingestion of any acetaminophen outside of the study protocol for any reason. Ingesting acetaminophen other than CTM during study participation will result in participant withdrawal. We will provide a list of types and common brand names of medications that contain acetaminophen as listed on MedLine Plus Health Information – A Service of the US Library of Medicine and the National Institutes of Health[15] (Appendix C) as well as a phone number to call if the participant has questions. Subjects requiring analgesic or antipyretic medication will be instructed to contact study personnel for an individual consultation on the most appropriate therapy. All concomitant medications will be recorded in the subject diary.

CTM is dispensed on Days 0, 7 and 16, and on Day 28 as necessary (based on participation in the Extended Dosing Period). On Day 0, CTM for Day 1 – Day 8 will be dispensed (total of 64 pills). On Day 7, CTM for Day 9 – Day 16 will be dispensed (total of 64 pills). On Day 16, CTM for Day 17 – Day 28 will be dispensed (total of 96 pills). On Day 28, CTM for Day 29 – Day 40 will be dispensed (total of 96 pills) to subjects still active in the Extended Dosing Period of the study. All subjects will be asked to return unused CTM upon completing their participation in the study.

Diaries will be dispensed to all subjects on Days 0, 7 and 16. Extended Dosing Period diaries will be dispensed to subjects on Day 28 as necessary to subjects

still active in the Extended Dosing Period of the study. Collection of diaries will occur throughout the study following their completion. All subjects will be asked to return outstanding study diaries upon completing their participation in the study.

Review of subject diaries and CTM dosing will occur at each study visit. Proper completion of the study diaries, as well as proper dosing with CTM will be confirmed by study staff. Subjects with incorrect diary completion or CTM dosing discovered at study visits will be re-counseled on correct dosing and diary completion instructions.

Safety and compliance will be monitored throughout the study period. Non-compliance with CTM dosing defined as either 1) more than two consecutive missed doses, or 2) more than six missed doses total will result in study withdrawal. These criterions apply to the Base Study Period (Days 1 – 16) only. The participant will be contacted by phone between all study visits to ensure participant safety, compliance and completion of the study diary as well as to schedule study visits. If the participant is unavailable by phone, a voice message will be left instructing the participant to contact study personnel as soon as possible. The participant will be asked about adverse events at each point of contact (visits and phone calls). These events will be recorded in the Case Report Form (CRF). Spontaneous reports of adverse events will also be recorded. Laboratory studies and subject symptoms will be monitored in a timely manner throughout the study. Study personnel will be available to all participants 24 hours a day via cellular phone. Unscheduled study visits may occur as warranted upon spontaneous reports of safety concerns. Physicians from the Medical Toxicology Division of the RMPDC will be available 24/7 for any subject who requires clinical evaluation.

If at any time after Day 0 a participant experiences any one of the following, they will be instructed to immediately discontinue CTM, will enter the ALT elevation protocol (Appendix D) which allows for additional testing to ensure participant safety and to investigate other potential sources for the elevation, and will be evaluated for n-acetylcysteine therapy:

- ALT or AST >8x ULN
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (total bilirubin >2xULN or INR >1.5)
- ALT or AST >3x ULN with the appearance of worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia.

These stopping criteria are based on the FDA draft Guidance for Industry regarding Drug-Induced Liver Injury: Premarketing Clinical Evaluation[13].

Participants meeting the stopping criteria will continue to be monitored and/or

referred appropriately by study physicians until the ALT elevation resolves. This may require additional laboratory testing or other assessments beyond the outlined schedule based upon the discretion of the study physicians monitoring the participant. Each subject will be individually evaluated for treatment with n-acetylcysteine, the effective antidote for acetaminophen toxicity.

Subjects with onset of a minor illness alone will not be withdrawn from the study. These participants will be instructed to contact study personnel for individual consultation with the study physician. The study physician will determine the appropriate course of therapy (if any) and review each concomitant medication with the subject to prevent the use of any acetaminophen containing products during the study. Such conditions will be listed in the adverse event log and will be considered during statistical analysis of the trial. If the study physician feels the safety of the subject is compromised by continuation in the study, the subject will be instructed to discontinue the CTM immediately. Regardless of previous ALT level, at least one additional ALT level will be obtained within five days of withdrawal from all prematurely withdrawn subjects who have ingested at least one dose of CTM. Additional hepatic function testing will be completed at least weekly at the discretion of the study physician. Follow-up ALT levels do not need to be obtained from subjects who withdraw from the study prior to first dose of CTM.

Statistical Analysis Plan

Primary Analysis

If the observed proportion of persistent ALT elevations in the treated group is greater than zero then we will reject the null hypothesis that the true underlying proportion of persistent elevations is 0. In this case we will present the observed proportion and the 95% confidence interval.

Secondary Analyses

We will use two sample t-tests and chi-square tests (as appropriate) to compare Day 0 demographic and clinical characteristics between subjects with and without persistent ALT elevations. All tests will be two-sided and use a p-value of 0.05 to determine if group differences are statistically significant. We will also provide estimated group difference and the corresponding 95% confidence interval.

We will use two-sample t-tests to compare the maximum change in cytokine within and between groups at 16 days. We will compare at 16 days since all subjects will have data up to this timepoint (except for missing data). We will compare proportions of patients with genotypes between the elevation, non-elevation and control groups using a X^2 or Fischer exact test. Because the number of genes to be tested is large (on the order of 300), we will adjust p-values for multiple comparisons to control the proportion of false positive tests. We will evaluate whether the cytokine data requires transformation. An adjusted p-value of 0.05 will be used to determine statistically significant differences. We

will also graphically display the profile of mean values at each timepoint for both groups along with standard error bars.

Recruiting methods
Advertisements approved by the governing institutional review board (COMIRB) will be posted locally, including the Denver Health and University of Colorado campuses pending institutional approval. If additional recruitment is warranted, a COMIRB approved advertisement will also be posted in regional and or local newspaper(s). Interested volunteers will contact study personnel for additional information and to schedule a study visit.

Protected Health Information (PHI), such as name, date of birth and telephone number, will be used to schedule participants and to conduct laboratory testing.

Consent procedures
Study personnel that have completed the required COMIRB training will obtain written informed consent from all study participants. Study personnel will be adequately trained to explain the study, answer questions and document the consent process. Subjects will be informed about the study's purpose and be required to provide written consent before enrollment in the study. Subjects will be told during the consent process that in previous studies, elevation of the liver enzymes, which may be a sign of liver injury, occurred in approximately 20% to 80% of participants. Subject comprehension will be assessed by asking the participant to describe the study in his/her own words. This consent will be obtained in a quiet, unhurried setting. A copy of the consent form will be given to all subjects.

Authorization procedures
Written authorization from the subject for the use and disclosure of PHI as related to this research study will be obtained at the time of consent. Study personnel conducting the subject enrollment will obtain this authorization. The subject will sign an approved HIPAA authorization form and will receive a copy of the signed and dated form. Research staff will protect the privacy of PHI through the use of secured facilities and servers.

Treatment, intervention or observation
Participants in the acetaminophen treatment group will be given therapeutic doses of acetaminophen (4 g/day) on each study day. Participants in the placebo group will be given placebo tablets to ingest on the same schedule. All CTM will be indistinguishable from one another. Study personnel will not know the study group assignment of the participants.

Participants will be randomized at a ratio of 4:1, acetaminophen to placebo. This randomization scheme was chosen to maximize the number of participants

receiving active drug. This will allow the aminotransferase trends to be better characterized in the acetaminophen treatment group.

Inclusion and exclusion criteria

Inclusion Criteria

Subjects of any gender or ethnic background who are age 18 years or older.

Exclusion Criteria

The following participant groups will be excluded from enrollment:

1) History of acetaminophen ingestion on any of the four days preceding study enrollment
2) Measurable serum acetaminophen level at time of enrollment
3) Viral markers of Hepatitis B or C , or viral markers of Hepatitis A with an ALT level greater than ULN during screening laboratory testing
4) Serum ALT or AST level greater than ULN at Screening or Day 0
5) Total bilirubin level greater than ULN at Screening or Day 0
6) INR level greater than ULN at Screening
7) Alkaline phosphatase level greater than ULN at Screening
8) Platelet count less than 125 10^9/L at Screening
9) Known cholelithiasis
10) Positive pregnancy test at Screening (female participants only)
11) History of consuming more than an average of 3 alcohol containing drinks daily over the preceding 2 weeks
12) History of consuming 3 or more alcohol containing drinks on any given day during the 2 weeks prior to study enrollment
13) New prescription medication started within the previous 30 days
14) Currently taking isoniazid
15) Currently taking warfarin
16) Currently adheres to a fasting type diet as determined by self report
17) Currently has anorexia nervosa as determined by self report
18) Participant is clinically intoxicated, psychiatrically impaired or unable to give informed consent for any reason
19) Known hypersensitivity or allergy to acetaminophen

Selection of study population

The study population will consist of healthy volunteers age 18 years and older. Both male and female subjects from diverse ethnic backgrounds will be enrolled. Enrollment will be monitored to ensure a minimum of 30 subjects are enrolled in each of the following four strata: Hispanic females, Hispanic males, non-Hispanic females, non-Hispanic males. This population will provide a diverse sample for generalization of study results and provide adequate data for sub-analyses.

Subject accrual

No more than 600 volunteers will be screened for participation with a goal of 300 (240 acetaminophen: 60 placebo) completed subjects. We estimate approximately 15% of subjects to be ineligible for the trial at screening and

another 15% to withdraw from the trial after at least one dose of CTM. Based upon the results from the Moderate Drinker trial (COMIRB #02-999)[8], we expect at least 48 subjects (20%) in the acetaminophen treatment group and 6 (10%) in the placebo group to develop an ALT level greater than ULN at some point during the initial 10 days of the study.

The sample size was determined using the following calculations to address the hypothesis for Specific Aim 1.

Sample Size:

The sample size is based on the requirement that the upper 95% confidence limit for p be less than 0.015. This is equivalent to requiring that the probability of observing all zeros is less than or equal to 0.025 when the true underlying probability of persistent ALT elevation is 0.015.

$Pr(0 \text{ events} \mid p=0.01) = (1-0.01)^N = 0.025 \quad \Rightarrow \quad N=\ln(0.025) / \ln(0.985) = 244$

Thus, we require subjects be assigned to receive acetaminophen therapy in order to ensure that our probability of mistakenly accepting the null hypothesis is 0.025 when p=0.01.

We have included a placebo group in this study to allow us to compare our findings to those reported in other trials. Because there is substantial variation in the rate of ALT elevations reported in placebo treated patients, it will be difficult to interpret our rate of ALT elevation in treated patients without knowing the rate in placebo patients. For example, in our previous study we found a rate of ALT elevation of 20% in acetaminophen patients and a rate of 10% in placebo subjects. Another study found ALT elevation in 40% of placebo subjects and 80% in acetaminophen subjects. Thus the ratio of ALT elevation in treatment to placebo is 2:1 in both studies. Without knowing the placebo rate the studies appear to have very different results.

For the placebo group we would like a precision of +/- 7% around our point estimate for the percent of subjects who will have an ALT measured above the upper limit of normal. Based on our previous work we expect that 10% of placebo treated subjects will have this finding.

Estimated duration of the study

Estimated duration of the study is twenty four months. The time required for the first study visit is approximately 40 minutes. All subsequent visits are expected to require approximately 10 minutes. A minimum of ten study visits will be required for participants who complete the trial. Subjects who participate in the Extended Dosing Period will attend an additional two to eight study visits, each requiring approximately 10 minutes. Follow-up beyond the Extended Dosing Period will only be necessary in the event of unresolved ALT elevations at study day 40.

Examinations, laboratory tests, procedures and follow-up visits

Appendix A outlines the study requirements including physical examinations, laboratory testing, study visits and study documentation.

Appendix B includes a flow-chart that maps a participant's experience throughout the course of the study, including the progression through the Extended Dosing and Extended Monitoring Periods.

Subjects with onset of a minor illness alone will not be withdrawn from the study. These participants will be instructed to contact study personnel for individual consultation with the study physician (top of page 7 of protocol). The study physician will determine the appropriate course of therapy (if any) and review each concomitant medication with the subject to prevent the use of any acetaminophen containing products during the study. Such conditions will be listed in the adverse event log and will be considered during statistical analysis of the trial. If the study physician feels the safety of the subject is compromised by continuation in the study, the subject will be instructed to discontinue the CTM immediately. Regardless of previous ALT level, at least one additional ALT level will be obtained within five days of withdrawal from all prematurely withdrawn subjects who have ingested at least one dose of CTM. Additional hepatic function testing will be completed at least weekly at the discretion of the study physician. Follow-up ALT levels do not need to be obtained from subjects who withdraw from the study prior to first dose of CTM.

Drugs, devices or instruments to be used
A needle and test tubes will be used for each blood draw. Participants will be instructed to take the CTM (1000 mg of acetaminophen or placebo) every four hours for four doses on each study day. The maximum recommended daily dose of acetaminophen is 4 grams.

Protected Health Information
Research personnel will collect the following PHI at the time of enrollment and during the study:

- Name and contact information
- Demographic information
- Diagnoses
- Medical history (including alcohol use) and physical examination
- Laboratory results from blood tests
- Surveys and questionnaires
- Research visit records
- Portions of previous medical records relevant to this study

The principal investigator and/or his staff may make the PHI collected during this research project available to the Food and Drug Administration or other regulatory agencies, the COMIRB, the sponsor and the Data Safety Monitoring Board for the purpose of evaluating the research project. All documents

containing PHI will be kept in a secured location at RMPDC and all electronic data will be stored on a secure server.

The subject name, identification number, gender and date of birth may also be shared with the collaborating laboratories in order to process blood samples. A unique identifier will be used to de-identify blood samples as much as possible prior to sending to the collaborating laboratories. All laboratory results will be reported to RMPDC using this unique identifier. This information is detailed in the HIPAA Authorization Form B: Enrollment into Research. Participants may request access to their PHI by submitting a written request to the principal investigator of this study (Kennon Heard, MD).

Data and Safety Monitoring Plan

1) The primary study objective is to characterize the course of aminotransferase levels during prolonged acetaminophen therapy. This will be done by evaluating visual representations of the data and by characterizing the group of participants who develop ALT elevations. The secondary study objective is to compare the proportion of subjects in each group (placebo and acetaminophen) that have an ALT measure greater than ULN at the end of acetaminophen treatment. This analysis will be done using a Chi-Square analysis (or Exact test as needed). All data analysis will be performed by the Denver Health Rocky Mountain Poison and Drug Center (RMPDC).

2) The RMPDC research staff will monitor adverse events at study visits and during phone contacts. All adverse events will be recorded in the appropriate place in the CRF. The DSMB will review serious adverse events and will be responsible for evaluating the events for changes in the risk level of the study. Adverse event reporting to COMIRB, the Clinical Translational Research Center (CTRC) and the sponsor will be done according to the standards of each institution. Serious adverse events will be reported to COMIRB within 5 business days of occurrence. A more detailed adverse event reporting plan is outlined in Appendix E.

   Serum acetaminophen concentrations obtained after the screening visit will not be provided to study personnel. Rather, these levels will be monitored by the laboratory with critical values reported to the study team. All other laboratory values will be monitored by study personnel blinded to treatment group assignment. Based upon previous studies, it is likely that a placebo treated subject will enter the Extended Dosing Period, therefore eliminating the risk of unblinding based on laboratory values.

3) Some minor side effects of acetaminophen have been occasionally reported and have included nausea, vomiting, lightheadedness and rash. ALT elevations that do not require the discontinuation of CTM will be reviewed at the scheduled DSMB meetings. ALT elevations that meet the

criteria for discontinuation of CTM will be immediately reported to the DSMB for review. Participants will report all adverse events to study personnel during study visits, scheduled phone calls or by using the on-call phone number listed in the study diary. A study physician will monitor all adverse events and will be available for immediate consultation.

4) Interim analysis will be conducted once 150 subjects have completed the study. This trial will be stopped if any of the following occur:
    a. One or more deaths are reported that are possibly related to CTM
    b. One or more subjects develop fulminant hepatic failure that is possibly related to CTM
    c. One or more subjects shows evidence of functional impairment of the liver
    d. More than five subjects meet the criteria for discontinuation of CTM
    e. Upon agreement of DSMB recommendation based upon rates of adverse events or other safety data

5) There will be a DSMB to oversee this study (details listed below).

6) The project coordinator will oversee all study activities, including protocol adherence and completion of CRFs.

7) Secure facilities (locked file cabinets) and secure servers will be used to insure confidentiality of all participant data and study records.

8) Descriptive and non-parametric analysis will be used to analyze the data.

Data Safety Monitoring Board (DSMB)
There will be an independent DSMB responsible for data and safety monitoring to ensure the safety of the participants and the integrity of the data. A DSMB charter will be drafted and approved by the investigators and the DSMB prior to participant enrollment. The DSMB will be composed of:

- Gregory Bogdan, PhD, toxicologist, not directly affiliated with the study (CHAIR)
- Lisa Forman, MD, hepatologist, not directly affiliated with the study
- Tammi Schaeffer, DO, medical toxicologist and emergency medicine physician, not directly affiliated with the study
- Becki Bucher-Bartelson, PhD, biostatistician, not directly affiliated with the study operations

To ensure the safety of subjects, we have planned several early meetings of the DSMB to allow early identification of safety concerns. Scheduled DSMB meetings will occur at the following milestones:

- after the completion of the first 25 participants
- after the completion of the first 75 participants

- after the completion of the first 150 participants (interim analysis)
- after the completion of the first 300 participants
- at the end of the study

Unscheduled DSMB discussions may occur if additional safety data becomes available that warrants review (i.e. in the event of a serious adverse event). These discussions may take place via a conference call or meeting. The DSMB chair will determine when such discussions are warranted.

COMIRB, the sponsor and the DSMB will receive immediate notice of any serious adverse events. Scheduled DSMB meetings will be held according to the milestones listed above and unscheduled meetings may be held in the case of an emergent event (i.e. report of a serious adverse event that requires group discussion on potential safety concerns). A DSMB summary report will be provided following each meeting (scheduled and unscheduled). Summary reports will reflect the DSMB's discussion of adverse events and the safety of individual subjects and will be signed by the DSMB chair.

The Principal Investigator will respond to identified problems in a DSMB report with an action plan and follow-up. The action plan will be submitted to COMIRB along with the DSMB report if possible, and any necessary amendments to the protocol or consent form. If a delay in implementing the action plan is expected, a letter from the investigator will accompany the DSMB report explaining the situation.

Risks

Subjects

Subjects in the treatment group will receive a therapeutic dose of 1000 mg acetaminophen every four hours for four doses daily on each study day. The labeled use of acetaminophen allows for a maximum of four grams per day for 10 days. The dosing period is longer than that on the approved label. Uncommon risks of acetaminophen include nausea, vomiting, lightheadedness, rash and allergic reaction. Minor ALT elevations are expected based upon recent publications[7,10]. However, as noted above, Parra[10] documented resolution of this ALT elevation for subjects on treatment. Additionally, multiple randomized clinical trials of subjects of various ages and ethnic backgrounds demonstrated that the risk of drug induced liver injury is extremely rare. A systematic review of the literature reveals that 30,865 subjects have received therapeutic dosing of acetaminophen without a single incident of fulminant hepatic injury[9].

Taking CTM doses less than four hours apart may result in supratherapeutic levels of serum acetaminophen. All instances of doses of CTM taken less than 3.5 hours apart will be documented as a protocol violation.

Subjects in the placebo group will not receive any active medication. Minimal risk is associated with the ingestion of placebo tablets.

There is only slight discomfort associated with blood venipuncture. A hollow needle will be placed in the subject's arm for taking blood samples. This will be left in for the duration of the time it takes to extract the required blood samples needed for the study (approximately 45 seconds). When the needle goes into a vein, it hurts for a short time. In about one in 10 cases a small amount of bleeding under the skin will produce a bruise. The risk of a blood clot forming in the vein is about one in 100, while the risk of infection or significant blood loss is one in 1000. Approximately 2 tablespoons of blood will be removed at each blood draw. A total of 16 tablespoons will be taken for research purposes during the Base Study Period. An additional 2 to 11 tablespoons may be taken during the Extended Dosing Period.

Violation of privacy and loss of confidentiality are both risks to which research participants are exposed. The possibility of these risks increases when protected health information is collected.

Hepatitis test results will be reported to the Colorado Department of Public Health and Environment as required by law. This information may affect a participant's ability to obtain insurance.

Although there are no systematic data that suggest alcohol consumption can predispose subjects taking therapeutic doses of acetaminophen to liver injury, the current label suggests patients contact their healthcare providers if they drink 3 or more alcoholic beverages every day. Therefore, subjects will be advised to avoid consuming 3 or more alcohol containing drinks daily during the study period.

There is a risk that a woman with child bearing potential may become pregnant during the study. Acetaminophen is considered safe in pregnancy (FDA Pregnancy classification A). However, any subject who becomes pregnant during the study will be removed from the study.

The study may include risks that are unknown at this time.

Investigators/Institutions
A risk of infection (needle stick or eye splash) exists with any blood draw. Proper phlebotomy techniques will ensure minimal risk to the investigator.

Benefits
This study is designed for the researcher to learn more about aminotransferase trends during prolonged therapeutic acetaminophen use. This study is not designed to treat any illness or to improve the participant's health.

Funding

The source of funding for this project is McNeil Consumer Healthcare. The McNeil Research Endowment in Clinical Analgesia at Denver Health Rocky Mountain Poison & Drug Center is chaired by Richard Dart, MD, PhD (co-investigator). There will be no costs to the subjects. Participants will be compensated $10.00 per day for each study day completed. Maximum compensation for the Base Study Period is $180.00 In addition, maximum compensation for the Extended Dosing Period is $240.00 An additional $10.00 will be paid for each follow up visit beyond the Extended Dosing Period, if applicable. If a subject leaves the study early, or needs to be taken out of the study, they will only be paid for the days that they have completed.

Special consent issues
No special consent issues have been identified.

Biological Specimens/Genetic Testing
Participants may have genetic testing to characterize their drug metabolizing and transporter enzymes. Please see Appendix Q of the COMIRB application for details.

All leftover serum from study samples ("reference samples") will be kept in a secured freezer at the RMPDC. These samples may be used for either validation of laboratory results or additional acetaminophen or hepatic testing in the future. For example, these samples may be used to help validate another experimental hepatic related test such as the protein-adduct assay. The samples will be labeled with unique protocol number, subject number, date/time of collection and day of study participation. Only the RMPDC will have access to these samples. Participants may request their samples to be withdrawn by submitting a written request to the principal investigator of this study (Kennon Heard, MD). Blood sample banking is required for study participation.

References

1. Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, Mitchell AA. Recent patterns of medication use in the ambulatory adult population of the United States: The Slone survey. Journal of the American Medical Association 2002; 287:337-344.

2. Paulose-Ram R, Hirsch R, Dillon C, Gu Q. Frequent monthly use of selected non-prescription and prescription non-narcotic analgesics among U.S. adults. Pharmacoepidemiol Drug Saf 2005; 14:257-66.

3. Case JP, Baliunas AJ, Block JA. Lack of efficacy of acetaminophen in treating symptomatic knee osteoarthritis: a randomized, double-blind, placebo-controlled comparison trial with diclofenac sodium.[see comment]. Archives of Internal Medicine 2003; 163:(2)169-78.

4. Golden HE, Moskowitz RW, Minic M. Analgesic efficacy and safety of

nonprescription doses of naproxen sodium compared with acetaminophen in the treatment of osteoarthritis of the knee. Am J Ther 2004; 11:(2)85-94. 1075-2765. Golden, H.E., Rush-Presbyterian-St Luke's, Chicago, Illinois, USA.

5. Pincus T, Koch G, Lei H, Mangal B, Sokka T, Moskowitz R, et al. Patient Preference for Placebo, Acetaminophen (paracetamol) or Celecoxib Efficacy Studies (PACES): Two randomised, double blind, placedo controlled, crossover clinical trials in patients with knee or hip osteoarthritis. Ann. Rheum. Dis. 2004; 63:(8)931-939. 0003-4967. Pincus, T., Div. of Rheumatology and Immunology, Vanderbilt Univ. School of Medicine, 203 Oxford House, Nashville, TN 37232-4500, United States.

6. Temple AR, Benson GD, Zinsenheim JR, Schweinle JE. Multicenter, randomized, double-blind, active-controlled, parallel-group trial of the long-term (6-12 months) safety of acetaminophen in adult patients with osteoarthritis. Clinical Therapeutics 2006; 28:1-14.

7. Watkins PB, Kaplowitz N, Slattery JT, Colonese CR, Colucci SV, Stewart PW, et al. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial. JAMA 2006; 296:87-93.

8. Heard K, Green JL, Bailey JE, Bogdan GM, Dart RC. A randomized trial to determine the change in ALT during 10 days of acetaminophen administration in subjects who consume moderate amounts of alcohol. Alimentary Pharmacology & Therapeutics 2007; 26:283-90.

9. Garcia Rodriguez LA, Gonzalez-Perez A. Long-term use of non-steroidal anti-inflammatory drugs and the risk of myocardial infarction in the general population. BMC Med 2005; 3:17

10. Parra D, Beckey NP, Stevens GR. The Effect of Acetaminophen on the International Normalized Ratio in Patients Stabilized on Warfarin Therapy. Pharmacotherapy 2007; 27:675-683.

11. Krahenbuhl S, Brauchli Y, Kummer O, Bodmer M, Trendelenburg M, Drewe J, et al. Acute liver failure in two patients with regular alcohol consumption ingesting paracetamol at therapeutic dosage. Digestion 2007; 75:232-7.

12. Davern TJ 2nd, James LP, Hinson JA, Polson J, Larson AM, Fontana RJ, et al. Measurement of serum acetaminophen-protein adducts in patients with acute liver failure.[see comment][erratum appears in Gastroenterology. 2006 May;130(6):1933]. Gastroenterology 2006; 130:(3)687-94.

13. Drug Induced Liver Injury Network, U. S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for Industry Drug Induced Liver Injury: Premarketing Clinical Evaluation [Web Page]. 2007 Oct; Accessed 2008 Jan 17. Available at: http://www.fda.gov/cder/guidance/index.htm.

14. Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985) [see comments]. New England Journal of Medicine 1988; 319:(24)1557-62.

15. MedMaster Patient Drug Information, Bethesda (MD): American Society of Health-System Pharmacists, Inc.; ©2004. Acetaminophen: Brand Names [Web Page]. 2007 Mar 1; Accessed 2007 Jun 14. Available at: http://www.nlm.nih.gov/medlineplus/druginfo/medmaster/a681004.html#brand-names.

Appendix A. Schedule of Events

Table 1. Schedule of Events

SCHEDULE OF EVENTS	Screening (S) & Base Study Period (Study Days)																Extended Therapy Period (Study Days)																								
	S	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Informed Consent	■																																								
Study Eligibility	■																																								
Medical History & Review of Systems	■																																								
Nutritional History	■																																								
Serum Pregnancy Test (female only)	■																																								
Viral Hepatitis Screen[a]	■																																								
INR	■	■				■			■			■			■			■			▫			▫			▫			▫			▫			▫			▫			▫
Serum Acetaminophen[b]	■		■	■	■							■						■																							▫
Complete Blood Count	■																																								
Triglycerides (fasting)	■											■						■																							
Hepatic Function Panel[c,d]	■	■	■	■	■	■			■			■			■			■			▫			▫			▫			▫			▫			▫			▫			▫
Acetaminophen-Protein Adduct Sample	■	■	■	■	■	■			■			■			■			■			▫						▫						▫						▫			
Banked Genotyping sample						■																																			
Banked Reference Sample	■	■	■	■	■	■			■			■			■			■			▫			▫			▫			▫			▫			▫			▫			▫
Study Medications			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫
Participant Contact by Phone								■			■			■			■			▫			▫			▫			▫			▫			▫			▫			▫	
Adverse Event Recording		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫	▫

[a]Viral hepatitis screen includes: HAV Ab, HBsAG, and HCV Ab. [b]Serum acetaminophen levels at Screening, Day 10 ,Day 16 and Day 40 will be viewed by laboratory personnel for safety monitoring. Study personnel will be notified of any critical values but will otherwise not receive results until subject has completed the trial. [c]Hepatic function panel includes: total protein, albumin, total & direct bilirubin, alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). [d]If a subject withdraws from the study for any reason, at least one additional ALT level will be obtained within five days of withdrawal regardless of the previous ALT level.

Subject Initials_____

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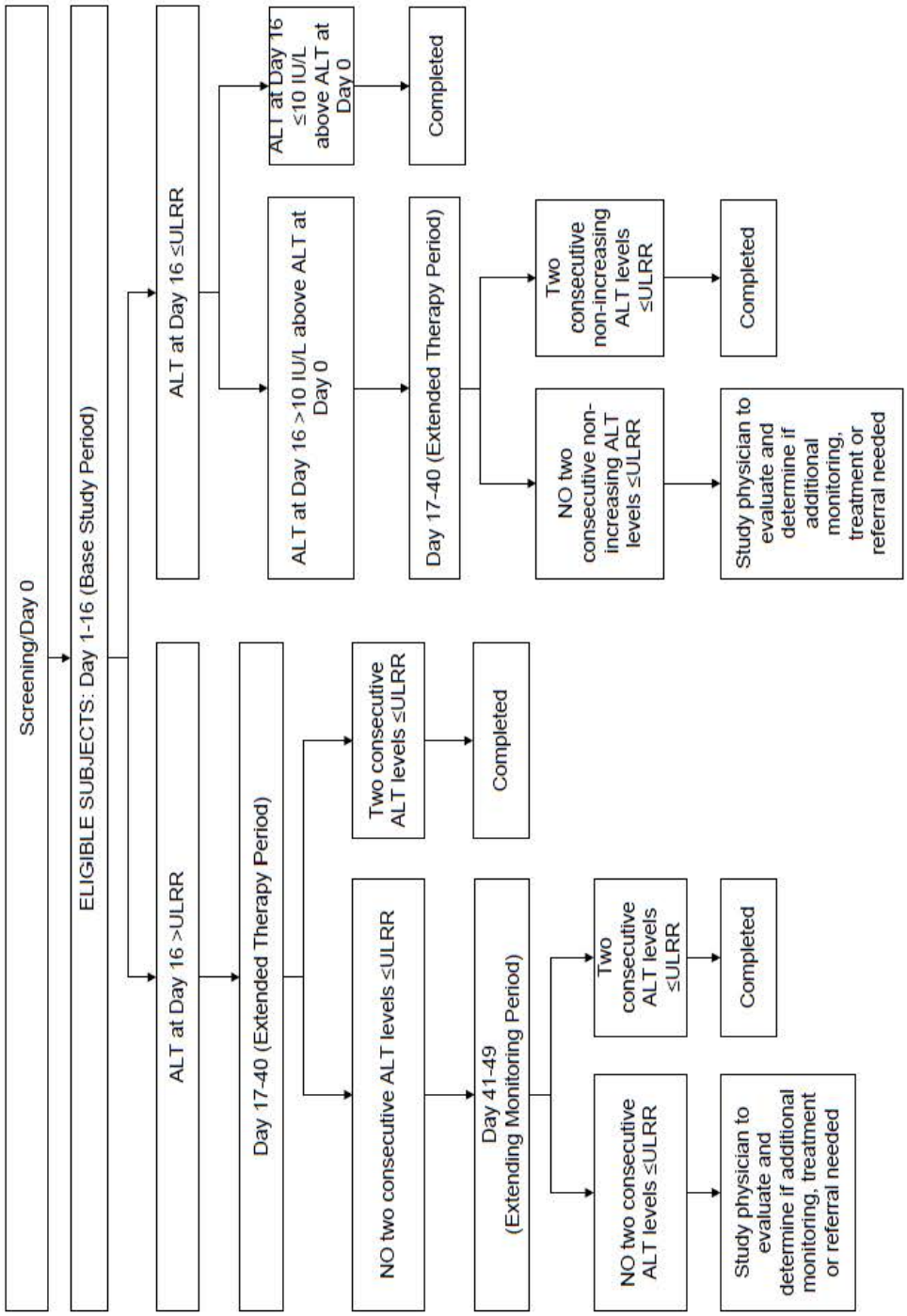
[a]Viral hepatitis screen includes: HAV Ab, HBsAG, and HCV Ab. [b]Serum acetaminophen levels at Screening, Day 10, Day 16 and Day 40 will be viewed by laboratory personnel for safety monitoring. Study personnel will be notified of any critical values but will otherwise not receive results until subject has completed the trial. [c]Hepatic function panel includes: total protein, albumin, total & direct bilirubin, alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). [d]If a subject withdraws from the study for any reason, at least one additional ALT level will be obtained within five days of withdrawal regardless of the previous ALT level.

Appendix B. Study Schematic



Appendix C. Brand names and type of products that contain acetaminophen

Brand names of acetaminophen

- Acephen®
- Anacin® Aspirin Free Maximum Strength Tablets®
- Capital® and Codeine
- Endocet®
- Excedrin P.M.® Caplets®
- Excedrin P.M.® Geltabs®
- Excedrin P.M.® Tablets
- Excedrin® Extra-Strength Caplets®
- Excedrin® Extra-Strength Tablets
- Excedrin® Migraine Caplets®
- Excedrin® Migraine Geltabs
- Excedrin® Migraine Tablets
- FeverAll® Children's
- FeverAll® Infants'
- FeverAll® Junior Strength
- Gelpirin®
- Genapap®
- Genapap® Children's
- Genapap® Drops Infant's
- Genapap® Extra Strength Caplets®
- Genapap® Extra Strength Tablets
- Genapap® Gel-Coat Caplets®
- Genebs®
- Genebs® Extra Strength Caplets®
- Genebs® Extra Strength Tablets
- Goody's® Extra Strength Tablets
- Goody's® Fast Pain Relief Tablets
- Goody's® Headache Powders
- Liquiprin® Drops
- Roxicet®
- Supac®
- Tylenol®
- Tylenol® Arthritis Pain Extended Relief Caplets®
- Tylenol® Meltaways Children's
- Tylenol® Concentrated Drops Infant's
- Tylenol® Extra Strength Adult
- Tylenol® Extra Strength Caplets®
- Tylenol® Extra Strength Gelcaps®
- Tylenol® Extra Strength Geltabs®
- Tylenol® Extra Strength Tablets
- Tylenol® Meltaways Junior Strength
- Tylenol® Suspension Children's
- Tylenol® with Codeine Elixir
- Tylenol® with Codeine No. 3
- Tylenol® with Codeine No. 4
- Tylox®
- Vanquish® Caplets®
- Wygesic®

Brand names of acetaminophen combination products

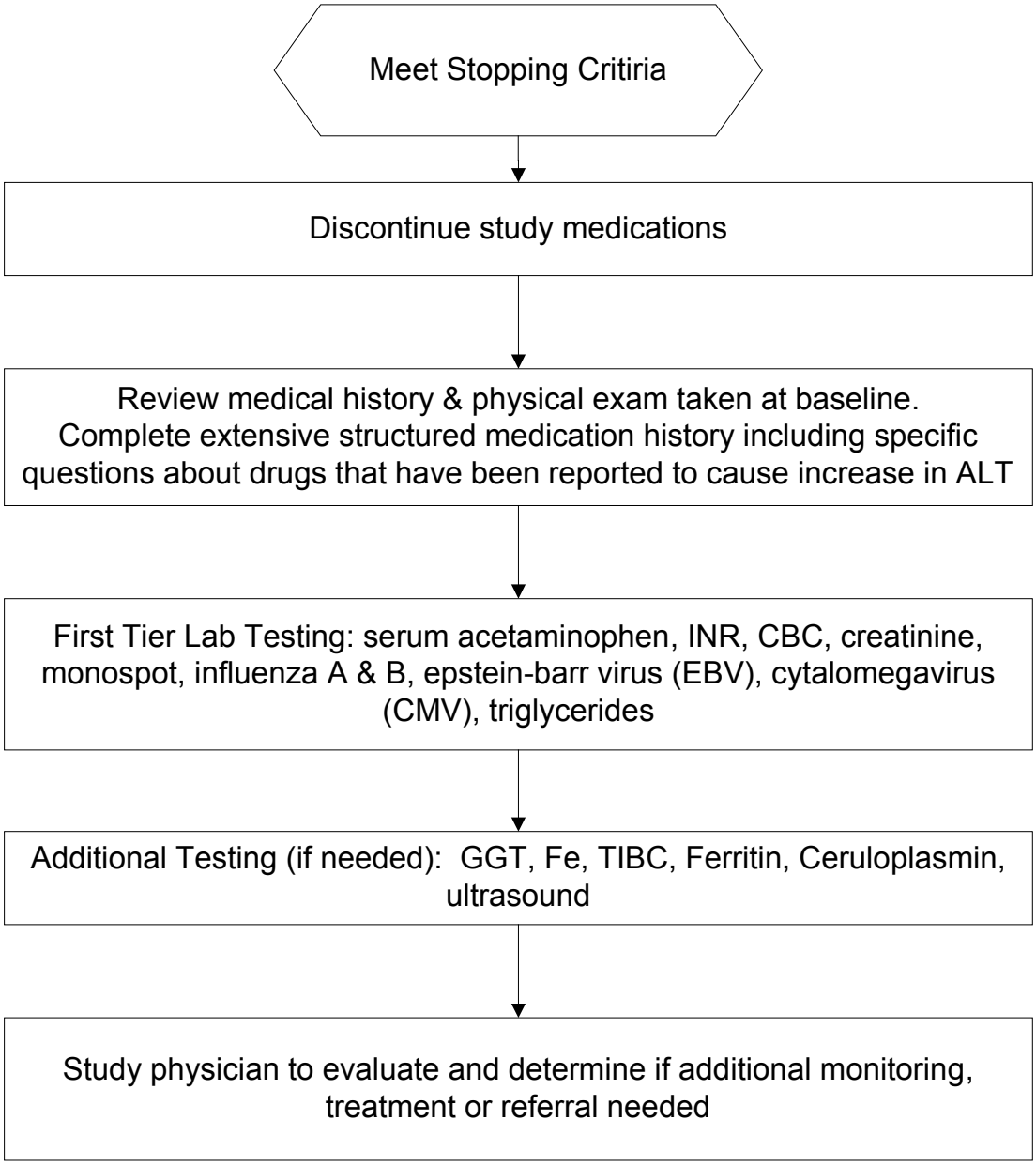
- Allerest® No Drowsiness containing Acetaminophen and Pseudoephedrine Hydrochloride
- Axocet® containing Acetaminophen and Butalbital
- Benadryl® Severe Allergy and Sinus Headache Maximum Strength Caplets® containing Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride
- Bupap® containing Acetaminophen and Butalbital
- Dristan® Cold No Drowsiness Formula Maximum Strength Caplets® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Duradrin® containing Acetaminophen, Dichloralphenazone, and Isometheptene Mucate
- Excedrin® Aspirin-Free Caplets® containing Acetaminophen and Caffeine
- Excedrin® Aspirin-Free Geltabs® containing Acetaminophen and Caffeine
- Excedrin® Quicktabs® containing Acetaminophen and Caffeine
- I.D.A.® containing Acetaminophen, Dichloralphenazone, and Isometheptene Mucate
- Midol® Menstrual Formula Maximum Strength Caplets® containing Acetaminophen, Caffeine, and Pyrilamine Maleate
- Midol® Menstrual Formula Maximum Strength Gelcaps® containing Acetaminophen, Caffeine, and Pyrilamine Maleate
- Midol® PMS Maximum Strength Caplets® containing Acetaminophen, Pamabrom, and Pyrilamine Maleate
- Midol® PMS Maximum Strength Gelcaps® containing Acetaminophen, Pamabrom, and Pyrilamine Maleate
- Premsyn PMS® Caplets® containing Acetaminophen, Pamabrom, and Pyrilamine Maleate
- Sedapap® containing Acetaminophen and Butalbital
- Sinarest® No Drowsiness Tablets containing Acetaminophen and Pseudoephedrine Hydrochloride
- Sine-Off® Maximum Strength No Drowsiness Formula Caplets® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Sinutab® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Sinutab® Sinus Medication Maximum Strength Without Drowsiness Tablets containing Acetaminophen and Pseudoephedrine Hydrochloride
- Sominex® Pain Relief Formula containing Acetaminophen and Diphenhydramine Hydrochloride
- St. Joseph® Cold Tablets for Children containing Acetaminophen and Phenylpropanolamine Hydrochloride
- Sudafed® Sinus & Headache Caplets® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Sudafed® Sinus & Headache Maximum Strength Tablets containing Acetaminophen and Pseudoephedrine Hydrochloride
- Tylenol® Allergy Sinus NightTime Maximum Strength Caplets® containing Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride
- Tylenol® Cold Decongestant and Fever Reducer Concentrated Drops Infant's containing Acetaminophen and Pseudoephedrine Hydrochloride
- Tylenol® Flu NightTime Maximum Strength Gelcaps® containing Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride
- Tylenol® PM Extra Strength

Brand names of acetaminophen combination products

- Midol® Teen Menstrual Formula Caplets® containing Acetaminophen and Pamabrom
- Midrin® containing Acetaminophen, Dichloralphenazone, and Isometheptene Mucate
- Ornex® Caplets® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Ornex® Maximum Strength Caplets® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Pamprin® Maximum Pain Relief Caplets® containing Acetaminophen, Magnesium Salicylate, and Pamabrom
- Pamprin® Multi-Symptom containing Acetaminophen, Pamabrom, and Pyrilamine Maleate
- Percogesic® containing Acetaminophen and Phenyltoloxamine Citrate
- Percogesic® Extra Strength Caplets® containing Acetaminophen and Diphenhydramine Hydrochloride
- Phrenilin® containing Acetaminophen and Butalbital
- Phrenilin® Forte containing Acetaminophen and Butalbital
- Tylenol® PM Extra Strength Caplets® containing Acetaminophen and Diphenhydramine Hydrochloride
- Tylenol® PM Extra Strength Gelcaps® containing Acetaminophen and Diphenhydramine Hydrochloride
- Tylenol® PM Extra Strength Geltabs® containing Acetaminophen and Diphenhydramine Hydrochloride
- Tylenol® Sinus Geltabs® Maximum Strength Tablets containing Acetaminophen and Pseudoephedrine Hydrochloride
- Tylenol® Sinus Medication Maximum Strength Geltabs® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Tylenol® Sinus Medication Maximum-Strength Caplets® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Tylenol® Sinus Medication Maximum-Strength Gelcaps® containing Acetaminophen and Pseudoephedrine Hydrochloride
- Tylenol® Women's Caplets® containing Acetaminophen and Pamabrom
- Ultracet® containing Acetaminophen and Tramadol Hydrochloride
- Women's Tylenol® Menstrual Relief Caplets® containing Acetaminophen and Pamabrom

Last Revised - 07/01/2006
http://www.nlm.nih.gov/medlineplus/druginfo/medmaster/a681004.html#brand-names

Appendix D. Alanine Aminotransferase (ALT) Elevation Protocol



Appendix E. Adverse Experience Reporting

An adverse event is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study agent(s), whether or not related to the study agent(s). For each subject, adverse events occurring after informed consent is obtained should be recorded. Recording should be done in a concise manner using standard, acceptable medical terms. The adverse event recorded should not be a procedure or a clinical measurement (i.e., a laboratory value or vital sign) but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Preexisting conditions that worsen in severity or frequency during the study should also be recorded (a preexisting condition that does not worsen is not an adverse event). Further, a procedure or surgery is not an adverse event; rather, the event leading to the procedure or surgery is considered an adverse event.

If, in the Investigator-Sponsor's judgment, a clinically significant worsening from Day 0 is observed in any laboratory or other test parameter (e.g., electrocardiogram (ECG), angiogram), physical exam finding, or vital sign, a corresponding clinical adverse event should be recorded. If a specific medical diagnosis has been made, that diagnosis should be recorded. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, "hepatitis" and not "elevated liver function tests" should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an adverse event, using appropriate medical terminology (e.g., thrombocytopenia, peripheral edema, QT prolongation).

A serious adverse event is any adverse event occurring at any dose that:

1. Results in death
2. Is life-threatening
3. Requires inpatient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability/incapacity (i.e., a substantial disruption in a person's ability to conduct normal activities of daily living)
5. Is a congenital anomaly/birth defect

In addition, an important medical event that may not result in death, be life-threatening, or require/prolong hospitalization may be considered a serious adverse event when, based on appropriate medical judgment, it may jeopardize the subject and/or may require medical or surgical intervention to prevent one of the outcomes listed above.

An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the current labeling for the product. A reasonably related adverse event is one that is, in the opinion of the investigator, possibly, probably or definitely related to study agent. Any serious adverse event, regardless of relationship to the study agent, must be reported to the Company immediately (with causality assessment). A serious adverse event must be reported if it occurs during a subject's participation in the study (whether

receiving study agent or not) or within thirty (30) days of receiving the last dose of study agent, whichever is longer. A MedWatch Form must be completed and faxed to the Company within one business day of observation or notification of the event. Any serious adverse event that is ongoing when a subject completes his/her participation in the trial must be followed until any of the following occurs:

- The event resolves or stabilizes;
- The event returns to Day 0 condition or value (if a Day 0 value is available);
- The event can be attributed to agent(s) other than the study agent, or to factors unrelated to study conduct.

APPENDIX B

INFORMED CONSENT FORM

Colorado Multiple Institutional Review Board
SUBJECT CONSENT FORM
Protocol Number: 06-1265

AMINOTRANSFERASE TRENDS DURING PROLONGED THERAPEUTIC ACETAMINOPHEN DOSING

Principal Investigator: Kennon Heard, MD
Version: 016
Version Date: 22 March 2011

Project Description

You are being asked to participate in a research study on the safety of acetaminophen (Tylenol®). Acetaminophen is commonly used to treat fever or pain. Your body clears acetaminophen by processing it in the liver. It has been reported that between 20% and 80% of people who take acetaminophen for more than 4 days will develop an abnormal liver test. It is unknown why this happens in some people and not others. Because there are large numbers of people that take acetaminophen for extended periods of time, it is important to find out when the liver tests return to normal. The Food and Drug Administration has recently released a draft document that states the importance of studying drugs that cause abnormal liver tests to make sure these tests return to normal, if the drug is continued.

The objective of this study is to monitor liver function tests (blood levels of an indicator of liver function) of healthy people taking the maximum permitted daily dose of acetaminophen compared to people taking placebo (inactive pills) for 16 to 40 days. Those people that have normal liver tests after 16 days will have completed their part of the study. People who have abnormal liver function tests at 16 days will continue taking acetaminophen or placebo, and have their liver tests monitored closely until they return to normal for up to an additional 24 days. This is to (1) make sure these tests return to normal and (2) determine when these tests return to normal while still taking acetaminophen or placebo. If at any time the liver tests indicate anything more than a minor increase, you would be immediately told to stop taking the study drug. The intent of this form is to educate you about this study's risks, benefits, and design.

This study is being conducted at Denver Health Medical Center, University of Colorado Hospital, and University of Colorado at Boulder Clinical and Translational Research Center. Up to 600 local subjects will be enrolled in this

research study.

Procedures
If you agree to participate and provide informed consent, you will undergo a Screening visit that consists of: medical history, questions about any symptoms you have, your alcohol use history, and blood tests to measure liver enzymes to verify that you are eligible for the study (Table 1). You will be required to fast for 12 hours prior to the blood-draw at your screening visit. You will be screened for viral hepatitis and any positive results will be reported to the Colorado Department of Public Health & Environment as required by law. If these tests are positive you will not continue participation in the study. You will be informed of these results.

If you are eligible to continue in the study, you will be placed by chance into a research group by a procedure similar to the toss of a coin. Neither you nor the study physician will be able to decide to which group you are assigned. You will have a 4 in 5 chance of being assigned to the acetaminophen group. You will have a 1 in 5 chance of being assigned to the placebo group (a placebo is an inactive substance). Neither you nor the study physician will know which group you have been assigned.

Base Study Period: You will be instructed to begin taking the study medication on Day 1. Study medication will be provided. You will take two caplets four times per day. Each dose should be taken at least four hours apart. You will be given a study diary to record the exact times you ingest your study medication doses. If you miss a dose, please call the number in your study diary for further instruction.

Laboratory testing will be done every day from Day 0 through Day 4, and every 3 days after Day 4. You will be required to fast 12 hours prior to study visits on Days 10 and 16. If your liver tests are normal on Day 16, you will have completed the study (Table 1). No further study visits or testing is needed.

Extended Dosing Period: If on Day 16 your liver tests are abnormal or slightly elevated since you began the study, you will continue into the Extended Dosing Period (Day 17 up to Day 40). The Extended Dosing Period involves continuation of study medication, and additional laboratory testing every 3 days until the abnormal level has come down. You will be closely monitored during this period to ensure your safety. Continuing to take the drug is important to determine if the liver test will return to normal while still taking the drug.

You will be told to stop taking study medication if your laboratory measures start increasing too much or if the study physician thinks it is necessary.

You should not take any additional acetaminophen for any reason during the study. Taking more acetaminophen could cause you to have an acetaminophen overdose. We will provide a list of types and common brand names of medications that contain acetaminophen as well as a phone number to call if you

have questions. If you require additional medication for pain or fever call the phone number on your study diary for treatment options. You need to write down all medications you take in your study diary.

You should avoid drinking more than 3 alcoholic beverages on any day during the study.

You will be asked to maintain a study diary with the following information:

- Medication log which records all prescription and over-the-counter medications
- Medication log of study medication
- Alcohol log
- Physical activity log

You will be contacted by phone between all study visits to remind you of study requirements and to schedule study visits. If you are unavailable by phone, we will leave you a voice message requesting you to contact study personnel at the phone number in the study diary.

Additional Consent for Blood Samples

We will also obtain some extra blood that we will freeze and save ("reference sample"). The reference samples taken at each blood draw (1 teaspoon per collection) will be kept in a secured freezer at the Denver Health Rocky Mountain Poison and Drug Center (RMPDC). These samples may be used for additional acetaminophen or hepatic testing in the future. You may withdraw these samples at any time by sending a written request to Dr. Kennon Heard, 777 Bannock St MC0180, Denver CO 80204.

Please read each sentence below and think about your choice. After reading each sentence, mark "yes" or "no" and initial. If you have questions, please talk to your doctor or nurse. You may not take part in the study if you do not agree to the storage and future use of your blood and tissue samples.

1. I give my permissions for my blood to be kept by Denver Health Rocky Mountain Poison and Drug Center (RMPDC), for use in future research.

   _____YES _____NO Initials _____

2. I give my permissions for my blood samples to be used for research about other health problems (for example: causes of heart disease, osteoporosis, diabetes).

   _____YES _____NO Initials _____

3. I give my permission for my study doctor (or someone he or she chooses) to contact me in the future to ask me to take part in more research.

   _____YES _____NO Initials _____

Sometimes blood samples are used for genetic research (about diseases that are passed on in families). Even if your blood samples are used for this kind of research, the results will not be told to you and will not be put in your health records. The samples will not be identified with your personal information. Your blood samples will only be used for research and will not be sold. However, the research done with your samples, derivatives or extracts may help to develop new products or ideas in the future. There is no plan for you to share in any financial gains.

Discomfort and Risks
Uncommon risks of acetaminophen include nausea, vomiting, lightheadedness, rash and allergic reaction.

Taking your study medication doses less than four hours apart may result in increased levels of acetaminophen in your blood.

There is a possibility that your liver function tests will not return to normal, for which you will be closely monitored. If at any time after baseline your blood results go above a safe limit, a study physician will be consulted and additional testing will be provided. You will be instructed to discontinue taking study medication. Additional follow-up may be required if the study physician deems it necessary. If you test positive for Hepatitis in this study, we must report your name to the Colorado Department of Public Health and Environment. Finding out that you have Hepatitis may make it hard for you to get insurance.

Approximately 2 tablespoons of blood will be removed by putting a needle into your vein. This is the standard method used to obtain blood for tests. You will feel pain when the needle goes into the vein. A bruise may form at the site. A total of 16 tablespoons will be taken for research purposes during the Base Study Period. An additional 2 to 11 tablespoons may be taken during the Extended Dosing Period. A maximum of 28 tablespoons may be taken for research purposes over the course of this study.

There is a risk that a woman with child bearing potential may become pregnant during the study. Acetaminophen is considered safe in pregnancy. However, any subject who becomes pregnant will be removed from the study. The study may include risks that are unknown at this time.

Benefits
This study is designed for the researcher to learn more about how acetaminophen effects liver enzymes. This study is not designed to treat any illness or to improve your health. Also, there are risks as mentioned in the Discomforts and Risks section above.

Cost to Subject
There is no cost to you for participating in this study. There will be no charge for procedures or drugs required by the study.

Subject Payment
You will be paid $10 per day for your involvement in the study. Maximum compensation for the Base Study Period is $180.00. In addition, maximum compensation for the Extended Dosing Period is $240.00. You will be paid an additional $10.00 for each follow up visit beyond the Extended Dosing Period, if applicable. If you leave the study early, or if we have to take you out of the study, you will be paid only for the days you have completed. It is important to know that payments for participation in a study are taxable income.

Who is paying for this study?
This research is being funded by McNeil Consumer Healthcare, who manufactures Tylenol ® (acetaminophen).

Voluntary Participation and Study Withdrawal
Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you do not take part in the study, your doctor will still take care of you. You will not lose any benefits or medical care to which you are entitled.

If you chose to take part, you have the right to stop at any time. If you stop the study before you have completed all study activities and you have taken at least one dose of the study medication, you will be asked to provide one additional blood sample within five days of stopping to ensure your safety. If there are any new findings during the study that may affect whether you want to continue to take part, you will be told about them.

If you miss too many doses of study medication during the Base Study Period you will be withdrawn from the study. You will also be withdrawn from the study if you take any additional acetaminophen during the course of the study.

The study doctor may decide to stop your participation without your permission, if he or she thinks that being in the study may cause you harm, or for any other reason. Also, the sponsor may stop the study at any time.

Invitation for Questions
The researcher carrying out this study is Kennon Heard, MD. You may ask any questions you have now. The main person to talk to if you have questions about this study is Dr. Kennon Heard. His phone number is (303) 389-1264. You can also talk to a Subject Advocate at the General Clinical Research Center (GCRC)/ the Clinical Translation Research Center (CTRC). The phone number there is (720) 848-6662. You will be given a copy of this form to keep. If you have questions regarding your rights as a research subject, please call the Colorado Multiple Institutional Review Board (COMIRB) office at (303) 724-1055.

Confidentiality
We will try to keep your medical records confidential, but it cannot be guaranteed. Records that identify you (including your medical records) and the consent form signed by you, may be looked at by the following people:

- Federal agencies that oversee human subject research
- Colorado Multiple Institutional Review Board
- The investigator and research team for this study
- The sponsor or an agent for the sponsor
- Regulatory officials from the institution where the research is being conducted, to ensure compliance with policies or monitor the safety of the study

The results of this research may be presented at meetings or published articles. However, your name will be kept private. You will be asked to sign a separate authorization form. This form will explain who will have access to your protected health information.

A description of this clinical trial will be available on http://www.Clinical Trials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Injury and Compensation
You should inform your care provider(s) if you decide to participate in this research study. If you have questions about injury related to the research, you may call Kennon Heard, MD at (303) 389-1264 and/or your private physician. Kennon Heard, MD should be informed about any injury you experience while you take part in this study.

If you are hurt by this research, we will give you medical care. The investigator will determine if your injury or illness is research-related. The term “research-related injury” means physical injury caused by drugs or procedures required by the study which are different from the medical treatment you would have received if you had not participated in the trial. Medical treatment will be provided at no cost to you or your insurance company for a research-related injury. The cost of

this care will be paid for by Denver Health – Rocky Mountain Poison and Drug Center.

Authorization
I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I know that being in this study is voluntary. I choose to be in this study. I know I can stop being in the study and I will still get the usual medical care. I will get a copy of this consent form.

_____
Printed Name of Study Participant

_____	_____	_____
Signature	Date	Time (24h clock)

_____	_____
Signature of Person Conducting Review of Consent	Date

_____	_____
Investigator Signature	Date