Hepatic Steatosis Snap Shot

Sites Participating: All sites

Principal Investigator: Anna S.F. Lok, MD (University of Michigan)

Co-Investigators: Zachary Goodman, MD; Joel Greenson, MD; Jay Everhart, MD; Raymond Chung, MD

Study Name: HEPATIC STEATOSIS IN CHRONIC HEPATITIS C: RISK FACTORS AND ROLE ON THE DEVELOPMENT OF HEPATIC FIBROSIS

Separate Consent Form: No

Withdrawal Form: No

Eligible Patients: Lead-in, Randomized, W20 Responders, Sustained Virologic responders and Breakthrough/Relapser
(Express patients are not eligible)

Visit Schedule (additional data/specimen and forms for AS)

Note: "**X**" means all participating sites take part.

Lead-In Phase

Visit Number ➔	Form #	S00	W00	W02	W04	W08	W12	W16	W20	W24
Fasting Insulin seq.121	Excel file		**X**							
Glycosylated Hemoglobin	121	**X**								

Randomized Phase

Visit Number ➔	Form #	R00	M12	M18	M24	M30	M36	M42	M48	M54
Fasting Insulin seq. 121	Excel file				**X**					
Glycosylated Hemoglobin	121									

Hepatic Steatosis in Chronic Hepatitis C:
Risk factors and role in the development of hepatic fibrosis

Principal Investigator: Anna S.F. Lok, MD

Co-Investigators: Zachary Goodman, MD, Joel Greenson, MD, James Everhart, MD, Raymond Chung, MD

SPECIFIC AIMS:

The central hypothesis of this ancillary study is that hepatic steatosis plays a key role in the development of fibrosis and cirrhosis in patients with chronic hepatitis C. In addition, we hypothesize that both viral and non-viral factors are involved in the development of hepatic steatosis in patients with chronic hepatitis C, and the benefits of long-term interferon therapy is in part mediated via reduction of hepatic steatosis. The specific aims of this study are:

1. To determine the prevalence of hepatic steatosis in patients with chronic hepatitis C.

2. To determine if there is a correlation between hepatic steatosis and hepatic fibrosis in patients with chronic hepatitis C.

3. To identify viral and non-viral risk factors for hepatic steatosis in patients with chronic hepatitis C.

4. To identify metabolic predictors of week 20 and sustained virologic response

5. To determine the effects of long-term interferon therapy on hepatic steatosis in patients with chronic hepatitis C.

6. To determine the effects of virologic clearance on insulin resistance in patients with chronic hepatitis C.

RELATIONSHIP OF PROPOSED STUDY TO NIH HALT-C TRIAL

The objective of the HALT-C trial is to determine if long-term interferon therapy can reduce the risk of progression to cirrhosis, decompensated liver disease and/or hepatocellular carcinoma (HCC) in patients with chronic hepatitis C who failed to respond to previous interferon therapy. The main goal of this ancillary study is to determine if hepatic steatosis plays a role in the development of fibrosis and cirrhosis in patients with chronic hepatitis C. This study will also examine risk factors for hepatic steatosis in patients with chronic hepatitis C. This ancillary study will help to improve our understanding of the mechanisms of hepatic fibrogenesis in patients with chronic hepatitis C and is therefore directly linked to the objective of the HALT-C trial. Most of the work involved in this study is related to the main protocol. The only additional elements involve simple clinical assessments such as waist circumference, one time testing for fasting insulin level, and grading of liver biopsies for hepatic steatosis. The latter component will be performed when liver biopsy slides (H&E) are graded for hepatic inflammation.

BACKGROUND AND SIGNIFICANCE

Macrovesicular steatosis is one of the characteristic histologic features of chronic hepatitis C. Hepatic steatosis has been reported in 31-72% patients with chronic hepatitis C [1-4], the wide range in prevalence being related to differences in patient selection in particular alcohol consumption, and the lack of standardization of grading systems for steatosis.

Steatosis is a well-recognized feature of alcoholic liver disease and non-alcoholic steatohepatitis (NASH) [5]. Non-alcoholic fatty liver disease (NAFL) which includes steatosis, steatohepatitis, and cirrhosis is now considered to be the most prevalent form of liver disease in the United States [6]. The

etiology of NASH is unknown, but it is frequently found in association with female gender, obesity, diabetes mellitus and hypertriglyceridemia [7,8]. Recent studies however, found that a significant proportion of patients with NASH do not have any identifiable risk factors [9,10]. These data suggest that other as yet unknown risk factors may be involved in NASH.

The cause of hepatic steatosis in patients with chronic hepatitis C is unclear and may be related to both viral and non-viral factors. A recent study found that transgenic mice that express HCV core gene developed progressive hepatic steatosis and subsequently HCC [11,12]. Laboratory studies also showed that HCV can elicit free radical mediated lipid peroxidation, possibly by affecting iron storage within the hepatocyte, which may in turn promote fat deposition [13]. These data suggest that HCV may play a direct role in the development of hepatic steatosis, and hepatic steatosis may be a precursor of HCC. However, other studies indicate that non-viral factors are also important. Two recent studies found a good correlation between hepatic steatosis and body mass index [14,15]. Many patients with chronic hepatitis C have a long history of alcohol consumption. It is conceivable that past or current alcohol consumption may play an important role in the development of hepatic steatosis in patients with chronic hepatitis C. HCV infection has been linked to a broad spectrum of extrahepatic diseases including diabetes mellitus [16,17]. The co-existence of diabetes in a high proportion of patients with chronic hepatitis C may contribute to the frequent detection of hepatic steatosis. Thus, multiple risk factors, viral and non-viral, may be involved in the development of hepatic steatosis in patients with chronic hepatitis C but the relative importance of these factors has not been properly examined.

Fatty liver was previously thought to be a benign condition with a very low risk of progression [18,19]. However, recent studies found that 15-50% patients with NAFL had fibrosis or cirrhosis at presentation [5,7-9,20,21] and up to 40% patients developed progression of fibrosis during follow-up. It is therefore possible that the frequent presence of steatosis in patients with chronic hepatitis C may contribute to the development of fibrosis and cirrhosis. This hypothesis was supported by a recent study of 148 consecutive patients with chronic hepatitis C demonstrating a significant correlation between steatosis and fibrosis [14].

In summary, current data indicate that hepatic steatosis is common in patients with chronic hepatitis C but the exact prevalence and severity remains to be determined. Recent studies suggest that both viral and non-viral factors may be involved in the development of hepatic steatosis in patients with chronic hepatitis C but the relative importance of these factors is not known. It has been postulated that necroinflammation and fibrosis in NASH occurs as a result of two hits [22]. The first hit is related to hyperinsulinemia due to insulin resistance causing increased free fatty acids leading to accumulation of lipids in hepatocytes. The second hit occurs when there is oxidative stress which can be induced by endotoxins, alcohol, iron and possibly HCV. In patients with chronic hepatitis C, obesity, alcohol consumption and diabetes may provide the first hit, and alcohol, iron and HCV may provide the second hit.

Emerging evidence suggests that metabolic factors play an important role not only in hepatitis C disease progression, but also in response to antiviral therapy. Increased BMI and hepatic steatosis have been associated with diminished responses to interferon and ribavirin therapy (Bressler et al, Hepatology 2003; 38:639; Kaserer K et al, Histopathology 1998;32:454). Further, weight loss has been associated with improved steatosis and fibrosis in persons with chronic HCV (Hickman IJ, Gut 2002;51:89). The relationship of these metabolic factors and insulin resistance with response to PEG-IFN and ribavirin therapy has not been explored. We hypothesize that hepatic steatosis and increased insulin resistance independently predict nonresponse to PEG-IFN and ribavirin therapy for chronic HCV. While other baseline factors, including BMI and weight were included in the original analysis of the HALT-C lead in patients by Shiffman et al (Gastro 2004; in press), we plan to focus on metabolic factors. The HALT-C cohort offers the advantage of a histologically uniform group that minimizes the confounding effect of fibrosis in this analysis.

Accumulating evidence has also identified a link between HCV infection and the development of diabetes. The mechanism of this link appears to be an independent association between HCV infection and insulin resistance (Aytug S, et al, Hepatology. 2003; 38:1384-92; Delgado A, et al, Transplantation in press 2004). Recent work by us (Delgado A, et al) has demonstrated that insulin resistance in post-liver transplant recipients is correlated linearly with HCV viral load. A recent study from a transgenic mouse model has also demonstrated that HCV core protein expression can lead to insulin resistance in the absence of significant liver pathology, implying that viral infection itself can produce insulin resistance in addition to any effects produced by accompanying inflammation and fibrosis (Shintani et al, Gastro 2004; 126:840). Because the HALT-C trial represents a relatively uniform group with respect to histology, and all patients were treated with the same lead-in antiviral therapy, this group represents a unique cohort to test the hypothesis that virologic clearance is associated with improvements in insulin resistance.

This ancillary study will examine the prevalence and severity of hepatic steatosis in a large cohort of patients with chronic hepatitis C. Viral and non-viral risk factors for hepatic steatosis will be assessed prospectively. Hepatic steatosis will be graded using a standardized scoring system and will be correlated to semi-quantitative (Ishak) and quantitative (morphometry) assessment of hepatic fibrosis. The effects of interferon therapy on hepatic steatosis will also be determined. The advantages of utilizing the HALT-C trial for our proposed ancillary study include the large sample size, long duration of follow-up and the inclusion of an untreated control group. Because this is a prospective study, alcohol consumption can be more reliably assessed. Detailed and accurate quantification of past and current alcohol consumption is a major focus of the HALT-C trial. Life-time and ongoing alcohol consumption will be determined using the Skinner survey. Other risk factors that have not been examined in previous studies such as fat distribution and hyperinsulinemia will be evaluated. This trial will enable us to examine the prevalence and impact of hepatic steatosis in patients with chronic hepatitis C both cross-sectionally and longitudinally. The longitudinal part of the study will allow us to determine if hepatic steatosis in patients with hepatitis C fluctuate over time and to identify factors that correlate with these changes. It will also determine if changes in hepatic steatosis correlate with changes in hepatic fibrosis further supporting a role of hepatic steatosis in fibrogenesis. Long-term interferon therapy can potentially decrease steatosis by inducing weight loss or increase steatosis by elevating serum triglyceride level. This study will determine the net effects of long-term interferon therapy on hepatic steatosis in patients with chronic hepatitis C.

MATERIALS AND METHODS

Inclusion/Exclusion:
All Lead-in patients enrolled in the NIH HALT-C trial will be studied. This study will enroll approximately 1000 patients with chronic hepatitis C who have significant fibrosis (Ishak score of 3-6). Patients with other causes of liver disease will be excluded from this study. Express patients are not eligible for the Steatosis Ancillary Study.

Patients will receive 6 months of combination therapy of interferon and ribavirin during the lead-in phase. Patients who remain viremic at treatment week 20 will be eligible for continued participation in this trial. These patients will be randomized to continue treatment with interferon alone for 3.5 years or no treatment. All patients will have repeat liver biopsies at the end of years 2 and 4.

This ancillary study will consist of a **cross-sectional** and a **longitudinal part.** In the following pages:
♦ Indicates additional data/sample collection for this ancillary study.

Schedule of Visits and Specimen Collection:

Cross-sectional Study – All tests performed at Screening (S00) or Baseline (W00).

This study will include all patients who remain eligible for continued participation in the NIH HALT-C trial after the lead-in phase. Baseline clinical data will be collected for all enrolled patients. Tests for insulin level and grading of liver biopsies for hepatic steatosis will not be performed until all patients have completed the lead-in phase to determine who will remain in the trial.

During the screening phase, clinical data will be collected and blood tests will be performed as part of the main protocol. Fasting blood glucose and triglyercide levels will be checked. Patients with known diabetes will be tested for glycosylated hemoglobin (HbA1c). These procedures are part of standard care of hepatitis C patients evaluated for interferon treatment and will be performed as part of the main protocol. An additional sample of blood will be collected for insulin level. Baseline liver biopsies will be scored for inflammation, fibrosis and iron. These assessments will be performed as part of the main protocol. The biopsies will additionally be graded for hepatic steatosis.

Baseline clinical data will be collected during the screenings or at baseline on:

a) Sex, age, ethnicity, Form #1, Trial ID Assignment.

b) Total body fat estimated by body mass index (BMI). BMI will be derived from body height and weight, Form #11, Physical Exam.

c) ♦ Visceral fat estimated by waist circumference taken with a cloth tape at the level of the umbilicus, with the patient supine, Form #11, Physical Exam (See Appendix A for directions).

d) Quantitative lifetime alcohol consumption and alcohol consumption during the year prior to entry into the Trial based on Skinner survey, Form #41.

e) ♦ History of diabetes, Form #3, Screening Medical History Interview. Current use of diabetic medications, Form #3, Screening Medical History Interview, and Form # 7, Baseline Medications Interview.

f) History of hyperlipidemia, Form #3, Screening Medical History Interview. Current use of lipid lowering medications, Form #3, Screening Medical History Interview, and Form #7, Baseline Medications Interview.

g) ♦ History of morbid obesity, Form #3, Screening Medical History Interview and Form #146, Weight History. Previous treatment for obesity such as jejunoileal bypass, Form #3, Screening Medical History Interview.

h) ♦ Concomitant medications that may induce hepatic steatosis, Form #7, Baseline Medications Interview.

Laboratory tests during the screening phase (S00):

a) Liver panel including AST and ALT, Form #35, Screening Visit 2 Local Lab.

b) HCV genotype, Form #33, Central Lab - HCV Genotype.

c) HCV RNA level, Form #31, Central Lab – HCV RNA.

d) Serum iron/TIBC, Form #30, Local Lab.

e) ♦ Fasting blood glucose, Form #30, Local Lab, and HbA1c for known diabetics, Form #121, Glycosylated Hemoglobin.

f) Fasting triglyceride, Form #30, Local Lab.

g) ♦ Fasting insulin level during the baseline visit (W00). Fasting insulin level will be tested at the Michigan Diabetes Center. Fasting insulin results will be sent to NERI as an excel file.

Baseline liver biopsies will be assessed by the central Pathology Committee for:

a) Hepatic inflammation according to Ishak score.

b) Hepatic fibrosis according to Ishack score as well as computerized morphometry after staining with sirrus red.

c) Hepatic iron content according to the method determined by the Pathology Committee.

d) ♦ Hepatic steatosis – the same H&E slide used for grading hepatic inflammation will be used to quantify hepatic steatosis. Hepatic steatosis will be scored based on percent of hepatocytes with fat. Scores will range from 0 to 4+: 0 (none), 1+ (1-5%), 2+ (5-33%), 3+ (33-67%), and 4+ (>67%).

Longitudinal study: This study will include all patients who completed the trial and have repeat liver biopsies at the end of years 2 and 4.

Serial clinical data will be collected on:

a) Body weight at each visit, Form #11, Physical Exam. This will permit changes in total body fat or BMI to be determined.

b) ♦ Waist circumference will be determined at the end of year 2 (M24) and 4 (M48) to assess changes in visceral fat, Form #11, Physical Exam.

c) Quantitative alcohol consumption during the study will be assessed every 6 months, Form #42, Alcohol Use Questionnaire.

d) ♦ New diagnosis of diabetes, and/or hyperlipidemia during the study, Form #30, Local Lab, and Form #10, Study Visit.

e) ♦ Concomitant medications that may induce hepatic steatosis, Form #12, Medications Interview.

Serial laboratory tests will be performed per protocol:

a) Liver panel at each visit, Form #30, Local Lab.

b) HCV RNA every 3 months, Form #31, Central Lab – HCV RNA.

c) Fasting blood glucose every 6 months, Form #30, Local Lab.

d) Fasting triglyceride level every 6 moths, Form #30, Local Lab.

e) ♦ Fasting insulin level at the end of year 2 (M24) Excel file, with Fasting Insulin results.

Liver biopsies obtained at the end of year 2 (M24) and year 4 (M48) will be assessed for hepatic inflammation, fibrosis, iron and steatosis.

To address aim 6, all non-diabetic patients with TW 20 response followed until TW 60 will be included. A group of matched (BMI, race, HCV genotype) non-diabetic controls with no virologic response at TW20 will also be studied if a difference in IR is detected at TW 60 between patients with SVR and relapsers.

1. Serial clinical data at baseline, TW20, TW 48, and TW 60 for patients with TW20 virologic response, and data at baseline and TW20 for matched controls.

2. Serial laboratory tests: liver panel, HCV RNA, glucose, triglycerides as per protocol. Fasting insulin levels at TW00 and TW 60 for patients with TW20 response.

If results show a difference in IR at TW60 between patients with SVR and relapsers, fasting insulin levels at TW20 will also be tested in study subjects and matched controls.

Specimen Collection, Handling, and Shipping:

- **Blood collection and processing for <u>insulin assays</u> at baseline and at year 2 (M24)**

1. Collect blood in a red top tube – 7mls (Do <u>NOT</u> use tubes that have EDTA coating).

2. Allow the blood to clot for at least 30 minutes – this does not necessarily have to be on ice if not available (blood can be allowed to clot for up to 2-2.5 hours).

3. Spin the tube for 10-15 minutes at 3000 RPMs.

4. Aliquot serum into two tubes with 1 ml in each tube. Use standard 2 ml aliquot tubes supplied by the Repository These tubes should be labeled with the aliquot tube labels supplied by BBI and NERI. The specimens for this study will have a sequence number of 121 + 122. Collection of these specimens should be recorded on the appropriate Aliquot Form for this patient's visit-Form #72-Lead in Phase Aliquot Form for W00 specimen and Form #73-Randomized Phase Aliquot form for M24 specimen.

5. Freeze at -80^0C until shipment to the Central Repository. These specimens should be included in standard frozen specimen shipments to the Repository.

6. Frozen serum aliquots will be batch shipped to the University of Michigan Diabetes Research and Training Center from the Central Repository for insulin testing. Baseline (W00) frozen serum samples will be sent after all patients have completed the week 24 (W24) visit; only specimens from patients who have been randomized will be sent. Frozen serum specimens from month 24 (M24) will be shipped after all patients have completed the month 24 (M24) visit.

7. Shipments can be received weekdays. The repository will ship the samples to:

   4321 Med Sci I
   Box 0601
   1150 West Medical Center Drive
   Ann Arbor, MI 48109-0601

   Principal Investigator Anna Lok, MD: Phone: 734-936-4780Fax: 734-615-3855

8. Pamela Richtmyer should be notified of these shipments. Phone: 734-615-0158
   Email: pater@med.umich.edu

9. After each batch of insulin test results is obtained, the results will be recorded on Form #120, Fasting Insulin and data entered into the Trial Data Management System.

Assay procedures: A solid-Phase, chemiluminescent enzyme immunometric assay for the determination of serum insulin levels was developed for the IMMUOLITE automated immunoassay analyzer. In the 60-minute assay procedure, patient serum incubates with an immobilized monoclonal anti-insulin antibody and an enzyme-labeled polyclonal antibody.

- **Glycosylated Hemoglobin**
  1. Local labs will be responsible for running glycosylated hemoglobin tests on patients with known diabetes.

  2. Blood drawn for glycosylated hemoglobin can be collected in the same tube as for fasting glucose and triglycerides. These three tests can be run from 5 mls of whole blood.

  3. Preparation of the blood for the tests should be done according to the local lab's guidelines.

  4. Record the results of the glycosylated hemoglobin testing on Form #121, Glycosylated Hemoglobin. This form should be data entered into the Trial Data Management System at each clinical center.

  5. The normal range for glycosylated hemoglobin is 4-10%.

- **Liver Biopsy**
  All biopsies collected, as part of the main protocol should be processed according to main HALT-C Trial protocol. Computerized morphometry for hepatic fibrosis will be performed at AFIP under Dr. Goodman's supervision.

- **Visceral fat**
  Estimated by waist circumference taken with a cloth tape at the level of the umbilicus, with the patient supine. (See Appendix A for directions.) Waist circumference will be recorded at screening (S00), Month 24, and Month 48 on Form #11, Physical Exam.

DATA ANALYSES

This study will include all patients that qualify for continued participation in the HALT-C trial after the lead-in phase. We plan to include all patients because most of the data required will be collected as part of the main trial with negligible additional work. Hourigan et al demonstrated a significant correlation between hepatic steatosis and fibrosis in their study on 148 patients [14]. Our study will have 3-4 times more patients. Thus, we should have no problem in showing a correlation if it exists. We have included all patients in the HALT-C trial as the effects of interferon on hepatic steatosis is unknown and a larger sample size may be needed to demonstrate an effect. In the single published report on 154 patients with hepatitis C, the proportion of patients with steatosis before and after 6 months of interferon monotherapy was unchanged [3] but the degree of steatosis was not compared and the duration of treatment may not be too short to demonstrate an effect.

Cross-sectional study

The following analyses will be performed:

1. Prevalence of hepatic steatosis: Grades 0, 1, 2, 3, and 4 will be determined for all baseline liver biopsies.

2. Hepatic steatosis grades will be correlated with hepatic fibrosis scores and morphometric assessments.

3. The following risk factors for hepatic steatosis will be assessed individually. In addition, multivariate analysis will be performed to identify independent risk factors that correlate with hepatic steatosis.

   - Host factors: age, gender, ethnicity.
   - Obesity: weight, total body fat (BMI), visceral fat (waist circumference).
   - Metabolic factors: presence or absence of diabetes, hyperlipidemia, and hyperinsulinemia.
   - Alcohol consumption: life time alcohol consumption and consumption within the year prior to the liver biopsy.
   - Viral factors: HCV genotype, HCV RNA level.
   - Hepatic inflammation: ALT level and hepatic inflammation grade.
   - Iron: serum iron, TIBC and hepatic iron score.

   Risk factor data such as BMI, life time alcohol consumption, diabetes status etc. will be coded as categorical or continuous variables as appropriate. These will be evaluated against the degree of steatosis (5 levels) and dichotomous collapsed steatosis codes (such as <33% vs ≥33%) using the Cochran-Mantel-Haenszel chi-square test with one degree of freedom. Multivariate analysis will be performed using logistic regression analysis. These risk factor variables and the steatosis levels will similarly be evaluated against the fibrosis scores (6 levels). Because fibrosis will also be quantified morphometrically, it will also be analyzed as a dependent variable by analysis of variance.

4. These risk factors and their relationship to week 20 and sustained virologic response will also be assessed.

Univariate analysis will be carried out using categorical or continuous variables as appropriate.These will be categorized against dichotomous (W20 responder v nonresponder, SVR v no SVR (all W20 nonresponders will be categorized as no SVR)) variables using the Cochran-Mantel-Haenszel chi-square test with one degree of freedom. Multivariate analysis will be performed on significant variables identified in univariate analysis by logistic regression.

Longitudinal study

The following analyses will be performed:

1. Prevalence of hepatic steatosis in year 4 biopsies will be compared to the year 2 and baseline biopsies of all patients and separately for the treated patients and controls.

2. Changes in hepatic steatosis between year 4 and baseline biopsies will be compared for the entire cohort of patients, and separately for the treated patients and controls.

3. Correlation between changes in hepatic steatosis with changes in hepatic fibrosis.

4. Correlation between changes in hepatic steatosis and changes in other risk factors for hepatic steatosis such as BMI, alcohol consumption, triglyceride level, hepatic inflammation, and hepatic iron content.

5. Correlation between changes in insulin resistance (measured using the homeostasis model assessment (HOMA)) from baseline to week 60 of the lead-in patients and virologic response or relapse.

We will compare HOMA-IR (calculated from fasting insulin and glucose levels) from subjects at entry into the lead in phase of HALT-C and compare week 60 samples from among responders (HCV RNA undetectable) and relapsers (HCV RNA +). Because of inherent insulin resistance among diabetic patients, we will confine the analysis to nondiabetic subjects (about 80%) of those with W60 samples. This would comprise about 250 subjects with available W60 sera. These would be evenly divided between responders and relapsers (125 in each group). Changes in HOMA-IR would be calculated between the responder and relapser groups. An advantage of studying W60 sera is that any confounding effects of PEGIFN and RBV on IR will be minimized, including weight loss on therapy. If differences can be discerned between the 2 groups, then it will also be of interest to perform W20 determinations among responders and nonresponders in a larger number of subjects. A secondary analysis would include assessment of the correlation between HOMA-IR and HCV RNA levels at baseline for all subjects and at W60 for relapsers, as well as correlation of virologic response or relapse with fasting glucose levels and HgbA1c in those persons with diabetes or impaired glucose tolerance (about 20% of the group).

PITFALLS AND LIMITATIONS

This study does not involve any additional invasive tests or time commitment from the trial participants. Most of the data required will be collected as part of the main trial, so we do not anticipate there will be any problem in conducting this study. It is possible that steatosis may have no role in the development of fibrosis/cirrhosis in patients with chronic hepatitis C. However, results from studies in patients with NASH and the recent report of Hourigan support that steatosis may be important in hepatic fibrogenesis. Patients in this study will all have significant fibrosis (Ishak score $\geq$3) and non-response to prior interferon therapy. Results of our study may not necessarily apply to other patients with chronic hepatitis C. However, the patients we will be studying are at greatest risks of developing fibrosis and cirrhosis, and any factor that can promote fibrosis should be easily demonstrated in this study.

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Appendix A: Directions for determining waist circumference

1. Record waist circumference at screening (S00), month 24 (M24) and month 48 (M48) visits. This will be recorded on Form #11, Physical Exam.

Waist Circumference Measurement Instructions

- To define the level at which waist circumference is measured, a bony landmark is first located and marked.
- The patient stands, and the examiner, positioned at the right of the patient, palpates the upper hipbone to locate the right iliac crest. (See figure below.)
- Just above the uppermost lateral border of the right iliac crest, a horizontal mark is drawn, and then crossed with a vertical mark on the midaxillary line.
- The measuring tape is placed in a horizontal plane around the abdomen at the level of this marked point on the right side of the trunk.
- The plane of the tape is parallel to the floor, and the tape is snug, but does not compress the skin.
- The measurement is made at normal minimal respiration.
- Record the circumference in units (cm or in) that were used on the measuring instrument. It is not necessary to record both centimeters and inches.
- Round centimeters to the nearest cm. Round inches to the nearest inch.