

Non-Alcoholic Fatty Liver Disease

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At the University of Michigan, he is the Principal Investigator for the NIH-funded Virahep-C study in addition to studies on mechanisms of insulin resistance in hepatitis C and the role of insulin sensitizers in chronic hepatitis C infection. He is also the Principal Investigator for several studies in NAFLD including an NIH-funded study of PPAR alpha agonist fenofibrate in patients with non-alcoholic steatohepatitis (NASH). In addition, he is also a co-investigator for 3 NIH-funded studies including the study of insulin resistance in hepatitis C, role of leptin replacement in patients with NASH and the DILIN (Drug Induced Liver Injury Network) study.

Good morning. This is the Focused Clinical Update Session on Non-alcoholic Fatty Liver Disease (NAFLD). Non-alcoholic fatty liver disease starts with patients who initially develop hepatic steatosis. The most common risk factor, as you know, is the presence of metabolic syndrome which is common in the United States population. Individual risk factors include obesity, diabetes and hyperlipidemia (more likely hypertriglyceridemia vs. hypercholesterolemia). Once steatosis develops, this can lead to development of inflammation and fibrosis referred to as NASH (non-alcoholic steatohepatitis). Here is where some of the abstracts we will be discussing today come in looking at what puts someone at risk for NAFLD. We know that patients with NASH tend to have more progression of their underlying liver disease and ultimately develop fibrosis and cirrhosis. A question we always ask is whether we need to biopsy patients with NAFLD. There's a bit of a bias depending on who you ask – many say yes, biopsy these patients because we do come across patients who have moderate to severe disease. I'm even seeing pediatric patients who are in their teens and early 20's who have cirrhosis. These are children who have been obese all their lives. Some of them have very severe liver disease but with minimal or only modestly elevated aminotransferase levels. You have to keep that in mind. On the other side, we see several hundred patients each year in our clinics. Obviously, it's very difficult to biopsy all these patients. The question is "Can we somehow characterize these patients so that we know who is more likely to have moderate or severe disease and maybe just biopsy those patients?" We also need to ask ourselves "What do we have available for treatment of NAFLD?" Again there's a bit of a bias to the answers. We'll talk about what is currently being recommended and some of the new therapies that are being tested later in the session.

What are the complications of having metabolic syndrome in addition to being at risk for NAFLD? As you know, the presence of metabolic syndrome is a huge cardiovascular risk, so there's now a big move to treat these patients with lipid lowering agents, the most common being statins. Are statins safe in patients with NAFLD? We'll touch upon that in some of the abstracts. There is now data to suggest that statins may actually have another important role. Some statins and some of the other lipid lowering agents act as antioxidants – there is some data showing that they actually improve insulin resistance (IR). IR seems to

be the key factor that bridges everything – risk for steatosis, risk for NASH and also risk for some of the cardiovascular complications like hypertension and coronary artery disease. If you can improve insulin sensitivity, you can potentially improve all these factors. Some of the lipid-lowering agents are very useful in decreasing the hepatic triglyceride content; they improve hypertriglyceridemia and this in turn seems to improve IR. It's kind of a cycle – improve one, you improve the other. So there's a lot of interest now in lipid lowering agents as therapeutic agents for NAFLD as well. The way we're thinking about some of these drugs has really changed over the last few years.

There's a lot of literature on studies trying to look at whether we can predict who is at risk for NASH and also predict more severe liver disease including fibrosis based on serological markers. People have looked at several things such as fibrosis markers, DHEA, and gene polymorphisms. Basically, the question is "Are there some target genes that, when they change, actually put someone at a higher risk of more severe liver disease?"

The first abstract is on the role of DHEA (dehydroepiandrosterone) in NAFLD. DHEA is basically an adrenal steroid and there is some data to show that it is anti-diabetic and it may also improve IR and obesity. So where does DHEA come in, in terms of NASH?

Abstract 224655: "Low circulating DHEA levels in severe non-alcoholic fatty liver disease – A potential metabolic basis of disease progression"

Severe NAFLD is characterized by oxidative stress, IR and progressive fibrosis, all of which can be modulated by DHEA. This particular study asked the question, "Can we, based on DHEA levels, differentiate patients with mild, moderate (simple steatosis and NASH), and severe NAFLD (NASH with fibrosis stage 3-4)?" This is an impressive study. They had 78 patients with NAFLD and 44 controls. They do not go into detail about what these other diseases are, though in the results they say they compare NAFLD with cholestatic liver disease. I'm not sure that all 44 patients had cholestatic liver disease, which may be important because patients with cholestatic liver disease rarely have steatosis, and as far as we know, IR does not seem to play much of a role in this setting. Thus patients with cholestatic liver disease are reasonable controls. Serum levels of DHEA were obtained at the time of biopsy. Fifty-three had just simple steatosis and 25 had NASH. They were also interested in looking at fibrosis as well: stage 0-2 versus 3-4 (with 3-4 being bridging fibrosis and cirrhosis). They found that overall patients with stage 3-4 were older. Patients with lower DHEA levels (so DHEA being protective), have greater association of liver disease. They used a cut off level of 0.45 mcg/dl, which appeared to separate patients with moderate to severe disease (fibrosis stage 3-4) from those with mild disease. Again, they don't tell us how they got to this cut off – is this a median level? It's not entirely clear. Every patient in the study who had stage 3 and stage 4 had DHEA level less than 0.45 mcg/dl. If you look at the figure, there are several patients who had mild disease and also had low levels. So, it's not sensitive and it's not specific in that manner. However, if you had a higher level, it was very useful as a negative predictor. If you had high levels, you can confidently say, at least on this data, that you don't have severe disease. I think there is a typo in the abstract because they talk about greater than 0.45, I think it should be less than 0.45 and then everything makes sense.

It's unclear what exactly the role of DHEA is but there's a lot of data in animal models supporting the fact that it improves IR as I mentioned. That's where most of the interest is. Insulin appears to be pro-fibrogenic. When we talk about risk factors for steatosis, NASH, inflammation, fibrosis – insulin seems to play a key role and anything which decreases IR may improve these conditions. When we talk about someone having IR, they tend to have high levels of insulin circulating throughout the day. For any given

carbohydrate meal or intake, if one has IR, their body has to produce much, much higher levels of insulin to get the same glycemic effect compared to someone without IR. It may be that DHEA is actually regulating some of the genes which have expression of some of these pathways.

In this abstract, if you look at the DHEA levels with age, the peak levels are in the 20's and 30's and they start going down. We know that age is an important factor; so the older you are the more likely you are to have severe disease. Part of this is that it is an age factor or disease factor and nothing to do with the fact that the lower levels are actually causing it – it could be the other way around. That is that DHEA levels decrease with age and this might put an older person at risk for more severe liver injury from steatosis. There is also some data in humans that DHEA can actually improve central obesity. Whether this leads to improvement in NAFLD/NASH related liver injury by decreasing influx of fatty acids into the liver is not known.

Abstract 223230: “The functional gene polymorphisms for the pathogenesis of non-alcoholic steatohepatitis”

This study looks at genetic influences on NASH pathogenesis in a Japanese population. This paper looked at four candidate genes. One is microsomal triglyceride transfer protein (MTP) which increases triglyceride export from the hepatocyte. There's also some data to show that MTP may also improve or have some role in mitochondrial beta oxidation of the fat that's stuck in the hepatocytes in someone with fatty liver disease. Next is polymorphisms in the β -3 adrenergic receptor, the third one is manganese superoxide dismutase. Polymorphisms in the manganese superoxide dismutase gene decrease the transport of manganese to the mitochondria and there's some theory to support that it might actually decrease mitochondrial oxidation of the fat in the hepatocytes. The last gene is interleukin-1. All of these are somehow believed to be promoting fatty acid oxidation or inflammation in the setting of NASH. Sixty-three patients with biopsy proven NASH and 150 healthy controls had blood samples analyzed by polymerase chain reaction (PCR). You have to be careful when you interpret these data. There are a lot of candidate genes which have been studied and this study looked at only four. They looked at patients who had NASH and what they found was changes in these alleles were found in patients with more steatosis, more severe NASH and more fibrosis. They found a clear difference whether you have severe disease versus not quite severe disease. We also have to keep in mind that this was a study from Japan. A lot of these studies come from different places and if you look at a lot of these data, we have not seen the same results in this country compared to other countries like Japan – so you don't know if it's a population based effect. It's really unclear at this point. Another marker which has gotten a lot of attention recently is adiponectin. Low adiponectin is associated with IR. Also, low adiponectin appears to be associated with more transport of visceral fat to the liver.

The next few abstracts relate to Fibroscan[®] which is basically an ultrasound with low frequency waves. It measures the elasticity of the liver. The liver is one big elastic unit so the more fibrosis there is the less elastic it is, the less stretchable it is – the Fibroscan[®] takes into consideration this concept. It emits sensitive, low frequency ultrasound waves and brings a signal back (calculated as kilopascals) and gives you an idea of how elastic the liver is. The higher the kilopascals, the less elastic it is. The less elastic suggests more fibrosis. They make sure that the region of interest is well into the liver so they're not really looking at the surface or the capsule. As you know, the capsule is very fibrotic. They try to make sure that the region of interest is well into the hepatic parenchyma. Most of the work so far has been done in patients with chronic hepatitis C.

There's been a concern when you have a morbidly obese patient with high BMI, if it can actually create bounce back on the scan. I am not very familiar with this. It's not routinely available in the U.S. yet, only in parts of Europe. One paper is actually from the U.S. but its routine use is not approved in the U.S.

Abstract 220604: “Efficacy of non-invasive hepatic fibrosis quantification by liver elasticity measurement in nonalcoholic steatohepatitis (NASH) - Comparison of ultrasonic transient elastography and histopathological diagnosis”

The standard assessment of NASH progression still relies on histopathological diagnosis by liver biopsy, but is an invasive test and assessment of progression over time (treatment) is difficult as it will involve doing repeated liver biopsies. The aim of this study was to compare hepatic fibrosis in NASH patients using 2 methodologies: the Fibroscan[®] to measure elasticity score (ES) and histopathological findings on liver biopsy. This abstract doesn't give much data. Thirty eight NASH patients had their elasticity score (ES) compared to fibrosis stage on their liver biopsy as measured by the Brunt classification. Six controls were assessed. There was no difference in controls and early fibrosis stage 1 and 2 but at stage 3 and 4, the elastometer showed clear differences ($p < .05$). Basically they found an incremental decrease in the elasticity, especially in stage 3 and stage 4 which were significantly different from the other patients suggesting that you may be able to pick patients who have more severe liver disease with this technique. These are small numbers of patients. The advantage of the Fibroscan[®] is that it takes less than 10 minutes – so, it is quick and avoids the complications of biopsy. Unfortunately, this abstract doesn't give much detail. For example, in terms of the Fibroscan[®] cut offs, what was the difference between the stages?

Abstract 217667: “Risk factors and hepatic elastography (Fibroscan) in the prediction of hepatic fibrosis in non-alcoholic steatohepatitis”

This is a multicenter study from France and the U.S. (Boston). Patients (N=29) either had pathological confirmation of NASH in the absence of serological evidence of chronic hepatitis and negligible alcohol intake (<20g/week) or pathologically confirmed cryptogenic cirrhosis with obesity and diabetes mellitus (DM). Fibrosis was staged using a modified Brunt staging system from F0-4, with F0=no fibrosis, F1=sinusoidal fibrosis, F2=portal fibrosis, F3=bridging fibrosis and F4=cirrhosis. Demographics, body mass index, presence or absence of diabetes, AST/ALT ratio, AST to platelet ratio index (APRI) and Fibroscan[®] (results measured in kilopascals) were performed on all patients. Statistical analysis included single and multiple step-wise logistic regression to identify predictors of stage >F2 NASH, and ROC curves were calculated for prediction of significant fibrosis (>F2). All the factors they evaluated have been suggested as important in terms of disease progression. In this study all these factors were individually predictive of fibrosis. However, in multivariable analysis, only liver stiffness (OR= 1.15, P=0.0016) and age (OR=1.052, P=.03) predicted fibrosis and these are not high odds ratios.

Hepatic stiffness cut off at 10 kilopascals had sensitivity of 88% and sensitivity of 72% to differentiate fibrosis greater than 2 – patients with more severe liver disease. The argument for differentiating stages of fibrosis is that if we have patients with more severe liver disease, perhaps we can target these patients for specific treatments or try to be more aggressive with their management. In addition, if you have someone with bad liver disease, especially cirrhosis, it has implications for screening for hepatocellular carcinoma. We have data to suggest that patients with cryptogenic cirrhosis (most of them are thought to have NAFLD as the etiology for their cirrhosis) are less frequently screened for hepatocellular carcinoma compared to patients with viral hepatitis-related cirrhosis, for example. They tend to present much later, with larger tumors and more advanced disease. The Fibroscan[®] appears then to have good sensitivity and specificity for predicting advanced stage of fibrosis. Its place in clinical practice is still awaiting more study.

Abstract 223277: “The PPAR- α agonist Wy-14643 prevents development of nonalcoholic fatty liver disease in mice heterozygous for a mitochondrial trifunctional protein defect”

There’s a lot of data looking at peroxisomal proliferator activated receptor gamma (PPAR- γ) agonists (the glitazones such as pioglitazone and rosiglitazone). They have been shown to improve histologic features of NASH. There’s a large, multicenter NIH study (NASH Clinical Network) ongoing now looking at pioglitazone in patients with NASH. There’s some interest in PPAR alpha (PPAR- α) which also seems to be quite important in terms of regulating genes that play a key role in fatty acid oxidation. There’s some data to suggest that gene polymorphisms associated with PPAR- α are increased in patients with NASH, suggesting that PPAR- α function is increased in fatty liver disease. There are lipid lowering agents such as fenofibrate that are PPAR- α agonists – this agent appears to have a very potent action in decreasing intracellular lipid (hepatic triglycerides). This drug is available in the market. In animal models, PPAR- α agonists have been shown to improve insulin sensitivity as well. So you’ll probably hear more about PPAR- α agonists though they are not as potent insulin sensitizers as PPAR gamma agonists. One of the side effects of PPAR- α agonist is weight gain despite the fact that they decrease hepatic fat – there is increased mobilization of the fat to the periphery.

This paper looks at the PPAR- α agonist Wy-14643. There are several studies that have looked into this before. These authors have actually shown that this drug improves some of these features of fatty liver in mouse models of hepatic steatosis – these mice were administered Wy-14643 and what they found in their mice was they can actually prevent steatosis with this agent. This is not a product which is available to study in humans yet. I wanted to bring this out to show that there are other agents that are being looked at in NASH using other available PPAR- α agonists such as fenofibrate (trade name in the U.S. is TriCor[®]). We are currently doing a randomized, placebo-controlled study of fenofibrate in patients with NASH at our institution. When we try to recruit patients for our study, our patients always ask me, “If it’s available, why can’t I just take it?” We don’t even know if it works. There’s a lot of theoretical suggestion in animal models that it helps, we don’t actually know how good it is in humans and that’s one of the reasons we’re doing the study.

What about metformin (trade name in U.S. is Glucophage)? Metformin improves hepatic glucose and does have insulin sensitizing properties although it is not as potent an insulin sensitizer as the glitazone agents. The advantage of metformin is it also suppresses appetite in some patients, so metformin is actually being used in morbidly obese patients who are insulin resistant, hoping to get some weight loss in these patients

The next few studies look at the lipid lowering agents. The first one is probucol. There has been at least one study that I know of looking at probucol for treatment of NASH. ProbucoL as you know is a lipid-lowering agent and it is also an antioxidant. There’s a lot of interest in antioxidants, in NASH, and there’s some data to show that probucol improves liver enzymes.

Abstract 215858: “Changes in liver histology after one year of treatment with probucol in nonalcoholic steatohepatitis”

This study is a small study raising the awareness that some of the lipid lowering agents may be useful. They had histology before and at the end of one year in eight patients. ProbucoL dose is 500 mg per day, the dose used in a previous study as well. They looked at ALT, weight, liver histology. At the end of one year, ALT clearly decreased. Weight went down a bit. Anytime patients are in studies, there’s always a motivation to change their lifestyle habits, so I suspect you will see some decrease in weight in a lot of these patients. If you look at most of the agents which have been looked at in histology with NASH, one

thing you'll consistently see is decreased steatosis. It doesn't take much to decrease steatosis in the liver. A bit of weight loss can do that. It's always hard to interpret small studies to determine how much weight loss is contributing to decrease in steatosis. They didn't see much change in fibrosis - again, when you have such small numbers of patients and take into consideration the variability, the sampling, and consider also that a year is probably not long enough to see that, it is not surprising. They looked at overall NASH score, which also decreased on treatment with probucol. Anytime you decrease one or two components, you will get a decrease in the overall score as well. Hopefully, there will be larger studies with this agent - to show that this agent is efficacious in NASH.

Abstract 221706: “*Lovastatin is not hepatotoxic to patients with pre-existing liver disease*”

This study looks at the safety of lovastatin. I'm sure you get calls all the time about the safety of 'statins' in patients with liver disease. As you know, there was a large study from Indianapolis a few years ago where they looked at three different statins - that study showed that statins are generally safe in patients with underlying liver disease with no significant increased risk of hepatotoxicity.

This was also another large retrospective study and it is from Kaiser Foundation in Northern California. It is a retrospective study from 1994 to 2004. Their primary endpoint was to see if these patients developed hepatotoxicity (see abstract). The secondary endpoint was to see what determined the development of more severe liver toxicity - so ALT greater than three times normal plus any development of cirrhosis, liver failure. You have to realize these are just large databases. You don't have individual patient profiles. Even if you say someone's ALT is abnormal, you don't know if it's from fatty liver, hepatitis C or etiologies like that. They think that a lot of these patients were actually fatty liver patients.

They had almost 14,000 patients who were exposed to lovastatin and almost 80,000 who were not on lovastatin - these are huge numbers. What they found was the overall prevalence of toxicity was very low. It was very small, reiterating the fact that these classes of agents are generally safe, whether patients have underlying liver disease or no underlying liver disease. In fact, patients on lovastatin were less likely to develop study endpoints once again suggesting that these drugs are safe if not even decrease liver enzymes. They found that if you are on lovastatin, it is more likely that you're going to find less of the toxicity. The way they define their primary outcome, hepatotoxicity is actually less in patients with lovastatin suggesting yes, it's definitely safe. The question is how are these agents working? Are they working directly or are they working indirectly? If you decrease the lipid profiles, you know that it influences insulin sensitivity and you could argue that it might be improving the liver enzymes and liver injury as well. Some of these agents may have direct impact in the hepatocyte. But again they're not making any interpretation that is actually useful because we don't have details on this patient population. At least these medications are very safe and definitely not a contraindication. Did they look at a subgroup with a pre-existing known liver disease? They did not and that is a problem. It was a problem with the study from Indianapolis as well. They did not know the etiology of the abnormal liver enzymes. There is one study that I know of from China which looked at statins specifically in patients with viral hepatitis C and they showed that these agents are safe in this setting.

Abstract 213327: “*Safety and efficacy of pravastatin 80 mg/day in 320 hypercholesterolemic patients with compensated chronic liver disease (CLD): Results of a prospective, randomized, double-blind, placebo-controlled study*”

This is the first prospective, placebo-controlled trial of a statin in patients with CLD. They had two aims. From the cardiovascular standpoint (the indication was high LDL) - how effective is it in reducing LDL?

The second question was what is the incidence of toxicity? These were pre-selected patients with well-compensated liver disease, eligible for this study based on their cardiovascular risk. Two-thirds of the patients had fatty liver, one quarter had hepatitis C and the rest had a spectrum of CLD. This was a classic, randomized placebo controlled study and what they found was that there was a statistically significant lowering of the LDL and total cholesterol in the pravastatin group compared to the placebo controls. With regard to toxicity, there was no statistically significant difference in the ALT end points; they looked at how many had more than two times normal or doubling of baseline ALT. This was actually the same in pravastatin compared to placebo. They also looked at time to onset of ALT elevations and there was no difference whether you were on the drug or the placebo.

Current recommendations are to follow these patients on the statin drugs by following the enzymes on a frequent basis. Some people say every three months. There's a move to actually take that recommendation away and suggest that you really don't have to test periodic liver enzymes in these patients unless there is good reason to like development of symptoms.

Abstract 223068: "Pravastatin, a HMG-CoA reductase inhibitor, attenuates hepatic oxidative stress to retard liver fibrogenesis of nonalcoholic fatty liver diseases in an in vivo study: An evidence for therapeutic potential of statin on nonalcoholic steatohepatitis"

This abstract is also on pravastatin. This was in a mouse model, fed an MCT (medium-chain triglyceride) diet, which develops fatty liver. They were given diet with or without pravastatin. They were interested in several things. They were interested in looking at the lipid profile, the hepatic injury (histology with fibrosis) and also mRNA expression of TGF- β , which as you know is for fibrosis. They are also looking at markers for oxidative stress. Basically, what they found was on all these accounts, pravastatin did better to improve histology and decrease oxidative stress in this animal model, suggesting again that there could be a role for statins as long term treatment for NASH.

Abstract 205975: "Moderate alcohol drinking may not be the risk factor for fatty liver in health checkup of middle-aged Japanese"

This is very interesting. As you know, we always tell our liver patients not to drink alcohol. We actually tell patients sometimes "If you have liver disease, not even one drink a day." We are actually writing a paper on a prospective study which looked at factors that predict more severe liver disease in patients with fatty liver. We looked at lifetime alcohol consumption in these patients. We were surprised at what we found. Patients who had a higher lifetime alcohol consumption had less severe liver disease. This sounds contradictory, but you have to keep in mind that when we diagnose patients as having NAFLD – these patients are not drinking excessively at that time but they may have consumed enough alcohol in the remote past. The definition of non-alcoholic fatty liver disease is based on recent history unless the alcohol history is significant with development of complications attributable to alcohol or history of dependency. If they say, "Look I used to drink 20 years ago when I was a student," and they have not been drinking recently and they meet all the other criteria, you label them as having NAFLD. Our interest was to look at the influence of lifetime alcohol use on histological severity in NAFLD. It's kind of a tough sell to tell people that alcohol may be protective. There are several recent studies in the literature suggesting that alcohol may actually improve metabolic syndrome. This may explain the findings.

In the current abstract, the investigators looked at the effect of alcohol specifically in patients with fatty liver. Their aim was to determine whether alcohol drinking was a risk for fatty liver (diagnosed by ultrasound). They had more than 50,000 patients. They had two components – cross-sectional and also a longitudinal study up to five years. They don't define in the abstract what the alcohol consumption was for occasional or daily drinkers. They also looked at men versus women. They followed them prospectively

for five years. They looked at what the average alcohol consumption was for occasional and daily drinkers.

Overall fatty liver prevalence was about 17%. That is similar to what we always think of as the prevalence of fatty liver in the U.S. at least based on some studies - close to 20%. The prevalence was much higher in men versus women. Among non-drinkers, occasional drinkers and daily drinkers, the prevalence of fatty liver was 28%, 27%, and 18% respectively in men. Among women, the numbers were 12%, 8% and 5% and this was significant. They also looked at who developed a fatty liver over the five-year period. In the non-drinkers and occasional alcohol group, it was 13% versus 17% and among the daily drinkers it was 9%. They don't give us a p-value on this one. How do we interpret this data? If you are someone who drinks on a regular basis, you are less likely to have fatty liver. There may be a threshold, however, beyond which, the cumulative amount of alcohol consumption may indeed lead to more severe liver disease. Another way to look at this is that as fatty liver disease advances with development of significant fibrosis/cirrhosis, the amount fat decreases. If this is the case, the story is obviously different.

This is only one part of the story. What they don't tell us, at least in the prospective, is what happened to other features of the metabolic syndrome. What was their waist hip ratio or waist circumference? What was their insulin sensitivity? We don't know all that information. Were the patients who were drinking on a regular basis less likely to have features of metabolic syndrome compared to the population who was not drinking as much? We don't know this information and this would be important to better understand the significance of these results.

These abstracts are interesting as they approach several aspects of NASH research and hopefully we will learn more in the future to better diagnose severity and manage patients with NASH. We usually encourage patients to lose weight. It's how you word it. When you tell a patient to just 'lose weight' that tends to discourage patients. If you recommend following a low glycemic index diet (something I do), that sounds kind of sexy – the idea being to recommend a diet that has lower carbohydrate content that is preferable also less processed along with high fiber content. I find that when patients are referred to a dietitian with the goal of a low glycemic index diet, it helps a lot. You can tell a patient to cut down on carbs and exercise more, but we don't spend enough time to take a good history of what the patients are eating. If we tell them what to eat or not eat based on what their routine is, they may do better. Just by cutting down carbs, you lose weight and what regular exercise does is maintain that. What the dietitian does is actually look at a patient's day-to-day dietary intake and they modify that. They ask them to switch this food with that food and I find that beneficial for the patients. I see a lot of physicians that are routinely treating NAFLD patients with vitamin E, which has antioxidant properties although there is no solid data to support its use. As I mentioned earlier, metformin is being used in obese patients with metabolic syndrome. So far the data on metformin has not been very convincing. We don't know that it actually improves histology in NASH. It does appear to lower the liver enzymes but from a histologic standpoint, it doesn't seem to do a whole lot but there are ongoing studies with this agent. I think we should wait until we know more data, particularly about safety, before we start prescribing glitazones. Until we get more data on efficacy and safety of the long-term use of glitazones in patients with NASH (NASH Clinical Network), I do not recommend prescribing these agents although I suspect they will prove to be safe and effective as therapeutic agents. Until then, what do you do? The focus is not so much trying to tell the patient to become thin. I think you can twist it in a way to say that the goal is make them more insulin sensitive. You might have better success with this approach. You work with the intention of improving their insulin sensitivity and decreasing the features of metabolic syndrome. You tell patients that insulin resistance is very important not only from a liver standpoint, but also from a cardiovascular standpoint. We recently showed (publication in press) that among patients with NAFLD, the presence of metabolic syndrome is associated with a greater degree of histologic severity compared to those without metabolic syndrome – again suggesting that it's very important to focus our efforts in helping our patients

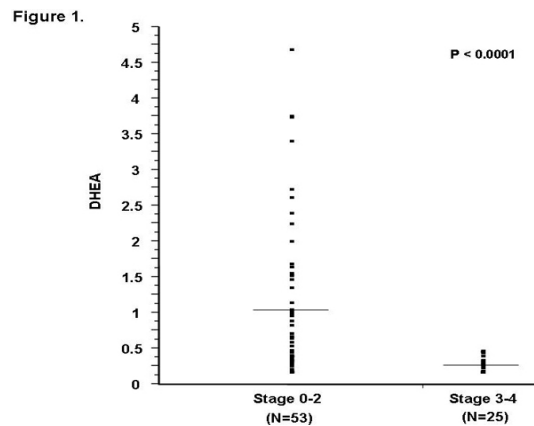
from the point of correcting metabolic syndrome and improving insulin resistance. In this way, you are not only trying to focus on the liver but you are also trying to focus on the cardiovascular standpoint and that is always more stimulating for the patient.

Thank you.

Abstracts Discussed

224655: Low Circulating DHEA Levels in Severe Non-alcoholic Fatty Liver Disease - A Potential Metabolic Basis of Disease Progression. Michael R Charlton, Paul Angulo Hernandez, Phunchai Charatcharoenwitthaya, Lawrence Burgart, Samer Gawrieh, Keith Lindor, Kimberly Viker, Anuradha Krishnan

Background: The biological basis of variability in histological progression of NAFLD is not known. More severe non-alcoholic fatty liver disease (NAFLD) is characterized by oxidative stress, insulin resistance and progressive fibrosis, phenomena which can be modulated by dehydroepiandrosterone (DHEA). Aim: We evaluated whether circulating DHEA levels vary between and/or can be used to identify and differentiate patients with mild (simple steatosis and NASH with fibrosis stage 0-2) and more severe (NASH with fibrosis stage 3-4) NAFLD. Methods: Serum samples were obtained prospectively at the time of liver biopsy in 78 patients with NAFLD and 44 controls with other forms of liver disease. Serum levels of DHEA were measured by ELISA. Model building was carried out using logistic regression techniques. Results: DHEA levels in patients with NAFLD are shown in figure 1. All patients with more severe NASH (n=25) had low plasma levels of DHEA (<0.45microg/dl). A model incorporating DHEA (stratified into <0.45 microg/dl >0.45 microg/dl), patient age (<45 vs. >45) and gender (M vs. F) was found to exhibit the greatest area under the receiver operator curve (AUC=0.87). A DHEA level of >0.45 microg/dl alone (a level associated with hypoadrenalism) had both sensitivity and negative predictive value of 100% in distinguishing between mild and severe NAFLD. Specificity was 58.5%, and positive predictive value was 47.8%. A relationship between histological severity of NAFLD and DHEA levels was not seen in the control group with cholestatic liver diseases. Conclusions: Patients with more severe NAFLD, as indicated by the presence of NASH with advanced fibrosis stage, can be identified with a high degree of accuracy using a simple model. These data provide novel evidence for relative DHEA deficiency in patients with histologically progressive NASH.



223230: The Functional Gene Polymorphisms for the Pathogenesis of Non-Alcoholic Steatohepatitis. Akira Hirose, Toshiji Saibara, Chikako Namikawa, Yasuko Nozaki, Masafumi Ono, Akemi Yoshioka, Masaya Takahashi, Naoaki Akisawa, Shinji Iwasaki, Saburo Onishi

BACKGROUND/AIMS: The pathogenesis of non-alcoholic steatohepatitis (NASH) is poorly understood. The aim of this study was to examine genetic influences on NASH pathogenesis in Japanese population. METHODS: Blood samples from 63 patients with biopsy-proven NASH and 150 healthy controls were analyzed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Four functional polymorphisms were studied: the -493 G/T polymorphism in the promoter of microsomal triglyceride transfer protein (MTP), the 190 T/A polymorphism in β 3-adrenergic receptor, which results in Trp64Arg (W64R) amino acid replacement, the 1183 T/C polymorphism in the mitochondrial targeting sequence of manganese superoxide dismutase (MnSOD), and the -511 T/C polymorphisms in promoter sequence of the interleukin-1 β . RESULTS: NASH patients had a much higher incidence of the MTP gene G allele (P=0.001) and of the G/G genotype (P=0.002) compared to the controls. Fat occupied more area in liver lobules and the stage of NASH was advanced in patients with the G/G genotype than in patients with G/T genotype (P=0.04). β 3-adrenergic receptor R allele frequency (P=0.01) and the R/- (W/R and R/R) genotype frequency (P=0.008) were significantly higher in NASH patients than those in control subjects. Obesity, hypertriglyceridemia and hyperinsulinemia were associated with NASH patients with the R/- genotype (P<0.001), whereas a decrease in insulinogenic index was associated with NASH patients with the W/W genotype (P<0.01). NASH patients also had a higher incidence of the MnSOD T/T genotype (P=0.016). Interleukin-1 β -511 T allele frequency (P=0.0001) and the T/T genotype frequency (P=0.00001) were significantly higher in NASH patients than those in control subjects. CONCLUSION: The G allele in the MTP promoter leads to decreased MTP transcription, less export of triglyceride from hepatocytes, and greater intracellular triglyceride accumulation. The R allele in the β 3-adrenergic receptor leads to reduce

energy expenditure. The T allele in MnSOD mitochondria targeting sequence leads to less transport of MnSOD to the mitochondria. The T allele in the interleukin-1 β promoter leads to the development of inflammatory changes in the liver. Therefore, this study confirmed that the contribution of hepatic steatosis, obesity, glucose intolerance, hypertriglyceridemia and inflammation of the liver influenced by the functional gene polymorphisms for the development of NASH in the Japanese population.

217667: Risk Factors and Hepatic Elastography (Fibroscan) in the Prediction of Hepatic Fibrosis in Non Alcoholic Steatohepatitis. *Thomas B Kelleher, Chelsea MacFarlane, Victor de Ledinghen, Michel Beaugrand, J Foucher, Laurent Castera, M Ziol, N Ganne, Farnan Rory Afdhal Nezam*

Background: NASH affects up to 20% of the US population. Despite its increasing prevalence a liver biopsy remains essential to confirm clinical suspicion and determine the degree of fibrosis or indeed the presence of cirrhosis. In this study we evaluated clinical and biochemical risk factors for NASH fibrosis and compared them to hepatic stiffness (elastography) as determined by Fibroscan. Methods: Enrolled patients (n=129) all had pathological confirmation of NASH in the absence of serological evidence of chronic hepatitis (autoimmune and viral) with negligible alcohol intake (<20g/week) or had pathologically confirmed cryptogenic cirrhosis with obesity and diabetes mellitus (DM). Fibrosis was staged using a modified Brunt staging system from F0-4 with , F0 = no fibrosis, F1 = sinusoidal fibrosis, F2 = portal fibrosis, F3 = bridging fibrosis and F4 = cirrhosis. Demographics, BMI, presence of diabetes, AST/ALT ratio, AST to platelet ratio index (APRI) and Fibroscan (results in kilopascals KPa) were performed on all patients. Statistical analysis included single and multiple step-wise logistic regression to identify predictors of stage >F2 NASH and ROC curves were calculated for prediction of significant fibrosis (>F2). Results: 129 patients enrolled in three tertiary referral Hepatology centers (US and France). Patient characteristics and results are shown in table 1. Single logistic regression suggested that age, DM, AST/ALT ratio and hepatic stiffness were predictive of fibrosis but stepwise multivariate analysis showed only liver stiffness (OR 1.149; p = 0.0016) and age (OR 1.052; p = 0.031) remained predictive of fibrosis. Hepatic stiffness cutoff of 10 KPa had a sensitivity of 88% and specificity 72% for significant fibrosis (>F2). Conclusions: Obesity (BMI up to 40) is not a contraindication to measuring liver stiffness, which is highly predictive of fibrosis in NASH patients. Liver stiffness represents an excellent initial screening test for NASH.

	Fibrosis F0-F1 (n=64)	Fibrosis F2-F4 (n=65)
Mean Age (range)	50 (25-78)	57 (21-81)
BMI (range)	29 (18-41)	30 (22-40)
DM	8 (12%)	24 (37%)
F0/F1	26/38	0
F2/F3/F4	0	27/12/26
AST/ALT ratio	0.68 +/-0.4	0.92 +/- 0.5
APRI	0.72 +/-1.17	0.84 +/-0.46
Hepatic Stiffness (range)	6.92 (3.4-20)	18.83 (4.4-75)
AUC ROC (95% CI)		
AST/ALT	0.67 (0.58-0.77)	
APRI	0.73 (0.61-0.84)	
Hepatic Stiffness	0.86 (0.79-0.92)	

220604: Efficacy of Non-invasive Hepatic Fibrosis Quantification by Liver Elasticity Measurement in Nonalcoholic Steatohepatitis (NASH) -Comparison of Ultrasonic Transient Elastography and Histopathological Diagnosis. *Yoshitaka Fukuzawa, Tomohiko Ohashi, Eiji Matsumoto, Ken Sato, Minoru Ayada, Naoki Hotta, Akihiko Okumura, Tetsuya Ishikawa, Shinichi Kakumu*

[Background and Aim] The global & golden standard for NASH progression still relies on histopathological diagnosis by liver biopsy, but since this is an invasive test, it causes a large amount of physical and mental stress for patients (particularly elderly patients) and assessment of progression over time (treatment) is difficult. The aim of this study was to compare NASH patients' hepatic fibrosis evaluation using an elastometer to measure elasticity score (ES), with a histopathological diagnosis using a liver biopsy, and examine the efficacy of both methods. [Subjects and Methods] We measured the ES of all NASH patients (n=38) using an elastometer (Fibroscan, ver.3 EchoSens, Paris, France) applying a low-frequency shear wave-elastography. The patients laid on a bed on their backs and the location of measurement was determined with B-mode ultrasound. The elasticity

was automatically calculated in the apparatus and the data were shown as Kilo Pascal (KPa). Measurements were taken 5-10 times for each case and we obtained the average value. We comparatively examined the correlation between the measurement values and hepatic fibrosis progression (Fibrotic Staging, by Brunt classifications) including the control group (n=6). [Results] Normal control group (group C, n=6) (Mean±SD, 3.5±1.3), group S0 (n=8) (3.9±1.5), group S1 (n=9) (6.8±2.3), group S2 (n=8) (8.4±3.5), group S3 (n=7) (35.4±7.8), group S4 (n=6) (59.1±18.8). No significant difference was shown between group C and group S0, but together with the progression of fibrosis, clear significant differences were evident between groups (P< 0.05). This was particularly notable in the shift in progression from S1-S2 to S3-S4. Further, the average measurement time for each case was (7.8±2.8) minutes, a significantly shorter time than that with liver biopsy (P< 0.05). [Conclusion] We have shown that using ES obtained by an elastometer, it is possible to assess the progression of hepatic fibrosis in NASH non-invasively, simply, quickly and quantitatively, and it is extremely effective for long-term monitoring, including therapy. It is considered effective (promising) as an alternative test for NASH patients for whom a liver biopsy is not possible, periodically or frequently.

223277: The PPAR- α Agonist Wy-14643 Prevents Development of Nonalcoholic Fatty Liver Disease In Mice Heterozygous for a Mitochondrial Trifunctional Protein Defect. *Jamal A Ibdah, Peter Perlegas, Hermina Borgerink, J. Mark Cline*

Background and Aims: We reported previously that aging mice heterozygous for a mitochondrial trifunctional protein (MTP) defect develop insulin resistance and hepatic steatosis (*Gastroenterology* 128:1381-90, 2005). MTP is the enzyme complex that catalyzes the last 3 steps of long chain mitochondrial β -oxidation. The peroxisomal proliferator activated receptor (PPAR- α) regulates the gene expression of mitochondrial fatty acid oxidation enzymes and is activated by the peroxisomal proliferator Wy-14643. We hypothesized that treatment of the heterozygous (MTP $^{+/-}$) mice with Wy-14643 enhances mitochondrial fatty acid oxidation and prevents development of hepatic steatosis. **Methods:** A study group of 3-month old male mice (12 MTP $^{+/-}$ and 12 MTP $^{+/+}$ mice) were fed a diet containing the peroxisome proliferator Wy-14,643 (0.05% w/w diet) and a control group of 3-month old male mice (12 MTP $^{+/-}$ and 12 MTP $^{+/+}$ mice) were fed a control chow diet. All mice were sacrificed after 12 months of dietary treatment and liver sections were evaluated using routine histology and Oil Red O fat stain. Scores of 0 to 3 were assigned to hepatic steatosis. Serum insulin levels were also measured. **Results:** On the chow diet, MTP $^{+/-}$ mice developed mild but significant hepatic steatosis (score 1+ steatosis) while MTP $^{+/+}$ had no or minimal hepatic steatosis. There was no steatosis noted in the liver sections obtained from the MTP $^{+/-}$ or MTP $^{+/+}$ mice treated with the Wy-14,643 diet. However, the Wy-14643 diet caused development of hepatic tumors in both MTP $^{+/-}$ and MTP $^{+/+}$ mice, a known effect of Wy-14643 use in mice. Gross and histological examination revealed that 75% and 25% of the MTP $^{+/-}$ mice developed significant hepatic adenomatous and hepatocellular carcinomatous lesions, respectively, compared to 33% and 17% in the MTP $^{+/+}$ mice. The MTP $^{+/-}$ mice on the control chow diet had hyperinsulinemia (2.31 \pm 0.45 ng/ml, mean \pm SD) compared to MTP $^{+/-}$ on the Wy-14,643 diet (1.1 \pm 0.8 ng/ml, mean \pm SD, P<0.001), suggesting that the Wy-14,643 diet prevents development of hyperinsulinemia in the MTP heterozygous mice. Insulin levels in the MTP $^{+/+}$ mice on the chow and Wy-14,643 diets were 0.95 \pm 0.63 and 1.3 \pm 0.6 ng/ml, respectively. **Conclusion:** The PPAR- α agonist Wy-14643 prevents development of hepatic steatosis in the MTP $^{+/-}$ mice suggesting that enhancement of mitochondrial fatty acid oxidation prevents development of NAFLD.

215858: Changes in Liver Histology After One Year of Treatment with Probucol in Nonalcoholic Steatohepatitis. *Shahin Merat, Mohsen Aduli, Rozana Kazemi, Masoud Sotoudeh, Reza Malekzadeh*

Background and Aims: Nonalcoholic steatohepatitis (NASH) is rapidly becoming a major health problem in many communities. No effective medical treatment has been yet approved for this entity. It appears that oxidative damage is an important step in the pathology of NASH. Probucol is a lipid lowering agent with significant antioxidant effects. We have previously shown its effectiveness in normalizing liver enzymes in NASH patients. In the current study, we report liver histology changes after using probucol for one year in 8 patients. **Material and Methods:** Eight cases of biopsy proven patients with NASH were included. Viral and autoimmune hepatitis and other liver disorders were ruled out. None of the patients used alcohol. The patients were given 500 mg probucol daily for 1 year. Aminotransferases, lipid profile and weight changes were evaluated during the treatment. Liver biopsies were performed before treatment and after one year. The biopsies were staged using a modification of the system developed by Brunt et al. According to this modification, proposed by Sotoudeh et al. in *DDW* 2004, steatosis, hepatocyte ballooning, lobular inflammation and portal inflammation were scored from zero through three. Fibrosis was staged from zero through four. The paired-samples T-test was used to compare variables before and after treatment. **Results:** The patients completed one year of treatment and underwent the second liver biopsy. At the end of therapy the mean alanine aminotransferase and aspartate aminotransferase levels decreased from 94 and 55 to 41 and 26 respectively (p=0.012 and 0.011 respectively). The mean weight of patients decreased by 2.9 kg and the mean body mass index by 0.67 kg/m², none reached statistical significance. The pathologic grade of liver histology decreased from 7.4 to 5.6 (p=0.021), the stage changed from 1.13 to 1.25 (p=0.76), and the total score decreased from 8.5 to 6.9 (p=0.048). No adverse drug effects were observed. **Conclusion:** Probucol is effective in normalizing aminotransferase levels in patients with NASH. It also significantly

reduces the histologic grade of steatohepatitis after one year of treatment. We failed to demonstrate statistically significant improvement in histologic stage.

221706: Lovastatin is Not Hepatotoxic to Patients with Pre-Existing Liver Disease. *Andrew L Avins, Michelle M Manos, Theodore R Levin, Lynn M Ackerson, Wei K Zhao, Rosemary C Murphy, Douglas J Watson, Peggy May T Hwang, Amy R Replogle, Jeffrey G Levine*

BACKGROUND: Data in the literature addressing the risk of adverse hepatic outcomes associated with statin use among patients with pre-existing liver disease are limited. In addition, the effect of statins on lipid metabolism may have beneficial effects among patients with certain hepatic diseases. **METHODS:** This was a retrospective cohort study. Subjects were adult members of the Northern California Kaiser Permanente Medical Care Program from January 1994 through June 2004 who had at least two alanine aminotransferase (ALT) elevations 6-18 months apart, a medical-record diagnosis of liver disease, or chronic hepatitis B or hepatitis C infection. Patients were excluded if they used a statin medication within one year of study entry. The primary endpoint was defined as concurrent serum ALT >3 times the upper limit of normal, total bilirubin >2 times the upper limit of normal, and alkaline phosphatase <1.5 times the upper limit of normal (modified Hy's Rule). Secondary endpoints included any ALT >3 times the upper limit of normal, cirrhosis, and liver failure. For univariate analyses, follow-up time was partitioned into lovastatin-exposed and unexposed time and rates of each endpoint were calculated and attributed to the appropriate exposure time. Multivariate analyses were conducted with Cox proportional hazards models. **RESULTS:** The study group consisted of 13,492 patients with lovastatin exposure and 79,628 patients without lovastatin exposure during the follow-up period. In univariate analyses, exposure to lovastatin was associated with strong and significant reductions in all primary and secondary endpoints, including Hy's Rule (Relative Rate (RR)=0.29, 95% Confidence Interval (CI): 0.14 to 0.60), elevated ALT (RR=0.55, 95% CI: 0.47 to 0.65), cirrhosis (RR=0.25, 95% CI: 0.19 to 0.35), and liver failure (RR=0.21, 95% CI: 0.14 to 0.31). Results were similar when adjusted for age and gender in multivariate analyses. Rates of statin prescription were similar across subgroups defined by hierarchical categories of liver-disease certainty, suggesting that confounding by contraindication was not a likely explanation for these results. **CONCLUSION:** Exposure to lovastatin was associated with a large and significant decrease in adverse hepatic endpoints, possibly mediated through an effect on liver disease by lovastatin's alteration of lipid metabolism. These results are not consistent with an increased risk of lovastatin hepatotoxicity in patients with pre-existing liver abnormalities.

213327: Safety and Efficacy of Pravastatin 80mg/day in 320 Hypercholesterolemic Patients with Compensated Chronic Liver Disease (CLD): Results of a Prospective, Randomized, Double-blind, Placebo-controlled Study. *James H Lewis, Mary Jean Fusco, Jeffrey R Medoff, Mary Ellen Mortensen, Steven Zweig*

HMG-CoA reductase inhibitors are associated with mild elevations in liver-associated enzymes (LAEs). In pts in clinical trials of cholesterol reduction using pravastatin (Prava) with ALT values up to 1.5X ULN at entry, no significant hepatic events were reported (Circulation 2002;105:2341), and retrospective studies suggest the safe use of statins in pts with elevated LAEs (Gastroenterology 2004;126:1287). Herein we present the first prospective study of a statin in hypercholesterolemic pts with known CLD. **METHODS:** Screened adult pts with >6mo of well-compensated CLD of any type and LDL cholesterol >100mg were eligible for statin treatment. The primary efficacy endpoint was the percentage (%) change from baseline to wk 12 of LDL-C and the primary safety endpoint was the % of pts with at least one ALT >2X for those with a normal baseline or a doubling of ALT for those with elevated baseline ALT (up to 5X) within 36wk of once daily dosing with Prava 80mg. Several CLD were represented, the most common being NAFLD (64%) and chronic hepatitis C (24%). Other CLD included PBC, PSC, Wilson dis., autoimmune hepatitis, and hemochromatosis. IRB approval and permission was obtained for all subjects. Enrollment was in a ratio of 1:1 Prava vs placebo (PBO). **RESULTS:** Prava pts had a clinically significant reduction in total cholesterol (20%), LDL-C (31%) and TGs (9%) compared to PBO by wk 12 (3%, 3%, and 6% increase respectively; p<0.0001 for all 3 comparisons). The % fall in LDL from baseline at wk 12 for Prava-treated pts was similar for those with mild (100-130mg/dL), moderate (130-160mg/dL) and severe LDL (>160mg/dL) at baseline (29.1%, 32.3% and 33% respectively). Among 160 pts on Prava, only 12 (7.5%) met the ALT safety endpoint vs 20 (12.5%) of 160 PBO recipients (p=NS). There was no statistically difference in ALT endpoints at any of the cumulative visits from wk 1 to 36 vs PBO, and the # pts having sustained ALT elevations for at least 2 consecutive wks was comparable (5% vs 7% PBO, p=NS). The time to onset of elevated ALT was also not different between groups (median for both >36wk log rank p=NS). The cumulative # of events in the Prava group regardless of ALT at baseline also was not different from PBO. One pt with NAFLD in the PBO group had transient bilirubin >2X ULN w/o ALT elevation that resolved. **CONCLUSIONS:** This prospective RCT of Prava 80mg/day was not associated with any excess of ALT or bilirubin elevations compared to PBO in a variety of CLDs in hypercholesterolemic pts over 36 wks and produced a significant reduction in total cholesterol, LDL-C and TGs, thereby supporting its safety and efficacy in this setting.

223068: Pravastatin, a HMG-CoA Reductase Inhibitor, Attenuates Hepatic Oxidative Stress to Retard Liver Fibrogenesis of Nonalcoholic Fatty Liver Diseases in an In Vivo Study: An Evidence for Therapeutic Potential of Statin on Nonalcoholic Steatohepatitis. *Motoki Inoue, Susumu Tazuma, Hideyuki Hyogo, Daisuke Komichi, Toshiya Kobuke, Michihiro Nonaka, Keiko Iwamoto, Yoshitaka Nabeshima, Tomokazu Ishitobi, Takeyoshi Ajima, Daiki Miki, Hiroya Yamamoto, Kazuaki Chayama*

BACKGROUND/AIMS: Nonalcoholic fatty liver diseases (NAFLD) is often associated with metabolic syndrome, characterized by obesity, diabetes, hyperlipidemia and hypertension with insulin resistance as a key pathogenic factor. Its progress to cirrhosis, liver failure and hepatocellular carcinoma is of clinical importance. However, the therapeutic strategy of this disease is not yet well-defined. In this regard, we recently reported that atorvastatin, a HMG-CoA reductase inhibitor, improves liver dysfunction as well as serum lipid levels in nonalcoholic steatohepatitis (NASH) patients (ref. *AHJ*; 46: 292-293, 2005), although the underlying mechanism is yet to be established. Thus, the aim of this study was to confirm the therapeutic effects of statin in NASH model, and to explore the mechanism of such an action with a special attention to oxidative stress. **METHODS:** As a NAFLD model, male C57BL/6J mice were fed a methionine and choline deficient (MCD) diet with or without a HMG-CoA reductase inhibitor, pravastatin (10, 100 mg/kg/day) for 8 weeks (n=15 each), and another 15 mice were fed with a normal chow as a control. After 1, 4 and 8 weeks on the MCD diet, mice were sacrificed for following analyses. 1. Serum lipids, total cholesterol (TC) and triglycerides (TG), were enzymatically measured. 2. Hepatic injury was assessed by pathohistology (Hematoxylin-Eosin staining) and serum levels of releasing enzymes (AST, ALT, GGT). 3. Hepatic fibrosis was assessed by histology (Azan staining) along with hepatic mRNA expression of transforming growth factor (TGF) β and α -smooth muscle actin (α SMA) by real time PCR. 4. Oxidative stress was assessed by hepatic lipid peroxidation by thiobarbituric acid reactive substances (TBARS). **RESULTS:** 1. MCD diet caused NAFLD-like liver damages in histology and serum parameters, but no drastic change was found in serum lipids. 2. In contrast, pravastatin improved liver steatosis, inflammation and fibrosis, but unchanged serum parameters. 3. Pravastatin repressed hepatic TGF β mRNA expression on week 1, although hepatic TGF β and α SMA mRNA expression were elevated on the MCD diet regardless of administration of pravastatin throughout the experimental period. 4. Hepatic TBARS was increased by MCD diet time-sequentially, but this was attenuated by pravastatin in a dose-dependent manner. **SUMMARY AND CONCLUSIONS:** 1. Pravastatin improved liver steatosis and fibrosis in NASH model, suggesting its therapeutic potential. 2. Such an action of pravastatin seemingly resulted from attenuating oxidative stress, because of repressed TGF β expression at an early stage.

205975: Moderate Alcohol Drinking May Not Be the Risk Factor for Fatty Liver in Health Checkup of Middle-aged Japanese. *Tamaki Yamada, Mitsuru Fukatsu, Sadao Suzuki*

(Background/Aim) Fatty liver (FL) associated with multiple metabolic disorders was reported to be caused by obesity and alcohol drinking, while alcohol consumption is associated with a lower prevalence of metabolic syndrome (*Diabetes Care* 2004;27:2954-59. *Obes Res* 2004;12:1375-85). Therefore, we aimed to determine whether alcohol drinking may be the risk factor for FL by cross-sectional and longitudinal manners in health checkup of the apparently healthy middle-aged Japanese. (Methods) 1) Cross-sectional study: 55,615 participants without hepatitis virus who received health checkup from 1999 to 2003 (men: 29,101. 49.3 \pm 11.8, y.o., women: 26,514. 48.7 \pm 11.4 y.o.) were enrolled and data at the first visit during this period were used to determine characteristics of drinking habits and the related factors of FL diagnosed by ultrasonography. 2) Longitudinal study: 9,156 participants of both 1998 and 2003 without hepatitis virus (men: 4,750. 49.1 \pm 9.6 y.o., women: 4,406. 48.7 \pm 8.9 y.o.) were enrolled to determine the risk factors for newly diagnosed FL by ultrasonography in 2003. Multiple logistic regression analyses were performed to determine alcohol drinking as the risk factor for FL in men and women, adjusted for age, body mass index (BMI), and smoking in the cross-sectional study and these factors and BMI increase for 5 years in the longitudinal study. (Results) 1) The overall percentages of occasional and daily drinkers were 33.6 and 45.3% in men and 31.3 and 8.9% in women, respectively. The average alcohol consumptions in the occasional and daily drinkers were 53.8 and 218.7 g/week in overall, 58.3 and 222.9 g/week in men, and 48.1 and 194.2 g/week in women, respectively. The prevalence of FL were 17.2, 23.5, and 10.3% in overall, men, and women. The prevalence of FL in non-, occasional, and daily drinkers were 27.9, 27.3, and 18.6% in men and 12.2, 7.9, and 5.3% in women, respectively (p<0.01 for both). Occasional and daily drinking were negatively associated with FL in both sexes (men: occasional, OR:0.86, 95%CI:0.80-0.94. daily, OR:0.56, 95%CI:0.51-0.60. women: occasional, OR:0.78, 95%CI:0.71-0.87. daily, OR:0.57, 95%CI:0.46-0.69). 2) FL was newly developed in 13.3, 17.2, and 9.1% of overall, male, and female participants in 5 years, respectively. Daily drinking was negatively associated with FL in men (daily, OR:0.77, 95%CI:0.62-0.96). (Conclusions) In health checkup of middle-aged Japanese, the prevalence of FL was significantly lower in the alcohol drinkers of both sexes and daily alcohol drinking was negatively associated with FL in men. Our findings suggested that moderate alcohol drinking may not be the risk factor for FL in these participants.