SUPPLEMENTAL MATERIALS AND METHODS:

Small Dense LDL

Small dense LDL cholesterol (sdLDL-C) in serum was measured by the homogenous quantitative assay kit (Denka Seiken, Japan). Percent small-dense LDL was subsequently calculated from sdLDL-C and LDL-cholesterol. VLDL, LDL and HDL particle number and size were measured by proton NMR spectroscopy at Liposcience (Raleigh, NC).

Lipoprotein particle size and number

VLDL, LDL and HDL particle number and size were measured by proton NMR spectroscopy at Liposcience (Raleigh, NC) as described previously (1-3). Briefly, the NMR method uses the characteristic signals (methylene and methyl shift) originated from lipoprotein subclasses of different size as the basis of their quantification. Each subclass signal represents the number of terminal methyl groups on the lipids contained within the particle. Cholesterol esters and triglycerides in the particle core each contribute 3 methyl groups, and phospholipids and unesterified cholesterol in the surface shell each contribute 2 methyl groups. The methyl NMR signal emitted by each subclass serves as a direct measure of the concentration of that subclass.

HDL2 Cholesterol

HDL2-C was calculated indirectly by subtracting high density lipoprotein 3-cholesterol (HDL3-C) from total high density lipoprotein cholesterol (HDL-C). Precipitation of apoB-containing lipoproteins and HDL2 was performed as previously described. Briefly, 20 µL of HDL3 reagent containing 19.1 mg/mL of

50 kDa molecular weight dextran sulfate (Cat#00501, Warnick reagent, Spanish Fork, UT 84660 USA) and 1.95 mol/L of MgCl2 (Cat. #BDH0244, VWR, USA) was added to 200 µL of serum. After a quick vortex, the sample was incubated at room temperature for 10 min, followed by centrifugation at 1000 x g for 15 min. The clear supernatant was then assayed for total cholesterol using a Roche Modular P automated chemistry analyzer.

Apolipoprotein B:

ApoB was measured on Roche P modular system according to the kit catalogue No 03032639 122 and associated package insert No 03252728001v12.

Apolipoprotein A-I:

ApoA-I was measured on Roche P modular system according to the kit catalogue No 03032612 122 and associated package insert No 03252701001v10.

HDL-C

HDL-C was measured on Roche P modular system according to the Randox kit catalogue No CH 2655 and associated package insert.

LDL-C

LDL-C was measured on Roche P modular system according to the Randox kit catalogue No CH 2656 and associated package insert.

ALT:

ALT was measured on Roche P modular system according to the kit catalogue number 11876805 216 and associated package insert No 11965328001V13.

***Ex vivo* studies of lipid synthesis:**

Liver biopsy samples were obtained by percutaneous methods according to established protocols during elective cholecystectomy. The samples were collected in Dulbecco's eagle media and divided using aseptic techniques into co-culture plates and incubated at 37°C. The liver tissue was incubated with a fixed concentration of palmitate/cyclodextrin (100µM of palmitate) and acetate (50µM). Individual liver samples were exposed to ^{14}C palmitate (1 µCi), ^{3}H acetate (10 µCi) as well as both together. The use of ^{14}C and ^{3}H at a ratio of 1:10 was based on need to separate the counting windows for tritium and carbon when used together. Cellular lipids were extracted from the liver tissue following overnight incubation using methods described by Folch et al (4). Lipid classes were separated using thin layer chromatography. Individual bands corresponding to the known lipid components were cut off the plate and placed into scintillation vials into which scintillation cocktail was added and radioactivity measured by scintillation counting. The incorporation of radiolabeled ^{14}C and ^{3}H into ketone bodies was assessed by measuring radioactivity of acid soluble medium by the method described(5).

In initial studies, tissue viability was confirmed up to 24 hours by lactate dehydrogenase and potassium release in to the medium. Also, time course studies were done and maximal uptake was seen after 6 hours of incubation with labeled substrates. All studies for this paper were therefore done at a 6-hour time point. Appropriate preliminary studies to set counting windows, account for counting geometry and document count recovery was also performed to validate

the methodology. Nuclear extracts, western blots, and bile acid analyses were peroformed as described previously (30).

SUPPLEMENTAL RESULTS:

	Correlation Coefficient (R)	*P*-value
STEATOSIS		
ALT	0.281	.02
VLDL-P	0.347	.031
Homocysteine	-0.245	.04
Insulin	0.251	.02
LOBULAR INFLAMATION		
Apolipoprotein-A1	-0.249	.04
ApoB:ApoA1 ratio	0.361	.003
Glucose	0.303	.01
HDL-C	-0.323	.005
Insulin	0.217	.05
LDL-P	0.329	.008
Small dense LDL-C	0.285	.019
Percent sdLDL-C	0.243	.047
Triglycerides	0.221	.05
CYTOLOGIC BALLOONING		
HDL-C	-0.229	.047
Insulin	0.211	.06
FIBROSIS		
Age	0.381	<.001
ALT	-0.275	.021
FFA	0.233	.05
Homocysteine	0.262	.029
HDL-C	-0.243	.031
HDL-Particle concentration	-0.375	.002
Insulin	0.283	.01
LDL-C	-0.145	.02
Total Cholesterol	-0.271	0.016

Supplemental Table 1: Correlation between histological parameters associated with nonalcoholic fatty liver disease (NAFLD) and serum atherogenic profile.

References

1. Otvos JD, Jeyarajah EJ, Bennett DW. Quantification of plasma lipoproteins by proton nuclear magnetic resonance spectroscopy. Clin Chem 1991; **37**(3): 377-386.

2. Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. Clin Chem 1992; **38**(9): 1632-1638.

3. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. Clin Lab 2002; **48**(3-4): 171-180.

4. FOLCH J, LEES M, SLOANE STANLEY GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957; **226**(1): 497-509.

5. Bruce JS, Salter AM. Metabolic fate of oleic acid, palmitic acid and stearic acid in cultured hamster hepatocytes. Biochem J 1996; **316 (Pt 3)**(Pt 3): 847-852.