

## SUPPLEMENTAL MATERIAL AND METHODS

### Measurement of lipid/lipoprotein (LDL, HDL, and VLDL) particle size and their concentrations

**Small dense LDL-C and %sdLDL:** Small dense LDL cholesterol (sdLDL-C) in serum was measured by the homogenous quantitative assay kit (Denka Seiken, Japan) (1). Percent small-dense LDL was subsequently calculated from sdLDL-C and LDL-cholesterol

### **VLDL, LDL, and HDL size and particles numbers by nuclear magnetic resonance (NMR):**

VLDL, LDL and HDL particle number and size were measured by proton NMR spectroscopy at Liposcience (Raleigh, NC) as described previously (2-4). 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 is 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 (5). Briefly, 20  $\mu$ L 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 MgCl<sub>2</sub> (Cat. #BDH0244, VWR, USA) was added to 200  $\mu$ L 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.

	Age Group 1 (18-39 years)	Age Group 2 (40-59)	Age Group 3 (60+ years)	P for trend
<b>DEMOGRAPHICS</b>				
N	650	2854	3019	--
Age (years)	33±5	51±5	70±7	--
Gender (%male)	54	55	49	--
BMI (kg/m <sup>2</sup> )	29.7±6.7	30.2±5.9	29.1±5.4	<.0001
ALT (IU/L)	28±18	28±15	23±12	<.0001
AST (IU/L)	26±22	26±12	25±10	.0004
<b>TRADITIONAL FACTORS</b>				
HDL-C (mg/dL)	50±12	51±14	52±15	.003
LDL-C (mg/dL)	104±31	102±32	89±32	<.0001
Triglycerides (mg/dL)	148±149	144±155	127±73	<.0001
Total cholesterol (mg/dL)	187±43	187±43	173±44	<.0001
<b>INSULIN RESISTANCE</b>				
Hemoglobin A1c (%)	5.5±1.1	5.9±1.2	6.0±1.0	<.0001
Free Fatty Acids (mmol/L)	0.62±0.30	0.65±0.27	0.67±0.26	<.0001
Glucose (mg/dL)	98±33	106±38	110±33	<.0001
Insulin (uU/mL)	16.4±23.7	15.1±19.0	14.1±14.9	.006
<b>VLDL</b>				
Apolipoprotein-B (mg/dL)	91±25	92±26	83±25	<.0001
VLDL-P (nmol/L)	4.9±10.2	4.1±8.2	2.8±4	<.0001
VLDL size (nm)	47.6±7.1	47.5±6.5	46.8±5.6	.0002
<b>LDL</b>				
LDL-P (mg/dL)	1552±568	1594±559	1432±526	<.0001
%sdLDL-C	26.6±9.7	28.0±11.2	27.2±8.1	.0003
sdLDL-C (mg/dL)	27.8±13.5	28.5±13.7	24.2±11.4	<.0001
sdLDL-P (nmol/L)	812±509	836±502	747±430	<.0001
<b>HDL</b>				
Apolipoprotein-A1 (mg/dL)	142±28	148±29	151±31	<.0001
HDL2-C (mg/dL)	11.8±5.7	11.8±5.9	12.8±6.2	<.0001
<b>MISCELLANEOUS</b>				
Lp(a) (mg/dL)	36.6±42.8	37.7±44.1	40.2±46.8	.053
ApoB:ApoA1 ratio	0.67±0.23	0.65±0.26	0.57±0.21	<.0001
NAFLD liver fat score	-0.403±3.774	-0.614±2.62	-0.91±2.351	<.0001
<b>INFLAMMATORY</b>				
Fibrinogen (mg/dL)	390±90	411±93	444±100	<.0001
hs-C-reactive protein (mg/L)	3.36±5.16	3.45±5.83	3.72±7.86	.23
Lp-PLA2 (ng/mL)	136±44	134±43	136±45	.19
Myeloperoxidase (pmol/L)	464±163	452±148	478±180	<.0001
<b>METABOLIC</b>				
Homocysteine (umol/L)	10.3±4.3	11.7±4.9	13.5±5.2	<.0001
Folate	11.5±4.4	11.5±4.4	13.3±5	<.0001
TSH (uIU/mL)	2.21±4.01	2.28±4.53	2.46±3.98	.36
Vitamin D (ng/mL)	30.7±14.2	33.5±14.1	35±14.6	<.0001

**Supplemental Table 1:** Impact of age on serum atherogenic profile. Atherogenic profile was better in individuals 60 years or older compared to the younger cohorts. All data presented as mean ± S.D.

	ALT Q1	ALT Q2	ALT Q3	ALT Q4	P for trend
<b>DEMOGRAPHICS</b>					
N	1342	1794	1638	1724	
Age (years)	60±16	60±13	58±12	54±12	<.0001
Gender (%male)	28	43	62	70	<.0001
BMI (kg/m <sup>2</sup> )	28.4±6.3	29.2±5.8	29.8±5.4	30.8±5.5	<.0001
ALT (IU/L)	12±2	18±2	25±2	43±14	----
AST (IU/L)	18±4	21±4	25±5	33±13	<.0001
<b>TRADITIONAL FACTORS</b>					
HDL-C (mg/dL)	54±15	54±14	51±14	48±13	<.0001
LDL-C (mg/dL)	96±32	96±32	95±33	98±34	.08
Triglycerides (mg/dL)	118±96	122±86	142±149	158±131	<.0001
Total cholesterol (mg/dL)	181±42	181±42	179±45	181±45	.30
<b>INSULIN RESISTANCE</b>					
Hemoglobin A1c (%)	5.8±1.1	5.8±1.0	6.0±1.2	6.0±1.1	.007
Free Fatty Acids (mmol/L)	0.62±0.26	0.65±0.26	0.65±0.26	0.69±0.27	<.0001
Glucose (mg/dL)	104±35	104±30	108±38	110±37	<.0001
Insulin (uU/mL)	11.7±13.7	13±14.8	14.8±14	18.7±21.4	<.0001
<b>VLDL</b>					
Apolipoprotein-B (mg/dL)	86±25	86±24	88±26	91±27	<.0001
VLDL-P (nmol/L)	2.3±4.3	2.7±5.5	3.7±6.2	5.0±9.1	<.0001
VLDL size (nm)	45.4±5.6	46.1±5.7	47.8±6	48.8±6.5	<.0001
<b>LDL</b>					
LDL-P (mg/dL)	1446±529	1472±523	1519±550	1602±577	<.0001
%sdLDL-C	24.7±7.1	25.7±7.5	28.7±11.9	30.5±9.9	<.0001
sdLDL-C (mg/dL)	23.7±10.6	24.6±11	27.2±13.4	29.7±14.5	<.0001
sdLDL-P (nmol/L)	684±436	721±430	826±470	911±503	<.0001
<b>HDL</b>					
Apolipoprotein-A1 (mg/dL)	152±32	152±30	148±29	144±29	<.0001
HDL2-C (mg/dL)	13.6±6.2	12.9±6.1	11.5±5.7	11.2±5.9	<.0001
<b>MISCELLANEOUS</b>					
Lp(a) (mg/dL)	43.5±46.8	41.3±47.8	38.2±44.6	33.2±41.3	<.0001
ApoB:ApoA1 ratio	0.59±0.22	0.59±0.20	0.61±0.26	0.67±0.25	<.0001
NAFLD liver fat score	-1.85±2.16	-1.16±2.25	-0.62±2.10	0.50±3.25	<.0001
<b>INFLAMMATORY</b>					
Fibrinogen (mg/dL)	443±105	432±101	418±95	407±89	<.0001
hs-C-reactive protein (mg/L)	4.3±7.5	3.5±6.7	3.3±6.2	3.1±5.2	<.0001
Lp-PLA2 (ng/mL)	138±45	135±44	132±42	134±44	.0089
Myeloperoxidase (pmol/L)	479±170	465±180	452±153	468±156	.0014
<b>METABOLIC</b>					
Homocysteine (umol/L)	12.9±5.8	12.3±5.4	12.2±5	12.1±4.2	<.0001
Folate	11.3±4.2	12.9±4.9	13.0±5.2	13.2±4.9	<.0001
TSH (uIU/mL)	2.7±6.2	2.2±3.1	2.3±5.1	2.2±1.5	.072
Vitamin D (ng/mL)	31.8±14.1	35±14.5	34.6±14.3	33.6±14.4	<.0001

**Supplemental Table 2:** Atherogenic variables by quartiles of ALT in 6,498 subjects with no known chronic medical problems. All data presented as mean ± S.D.