Wednesday, July 30, 2014

Poster Session: 9:30 AM - 5:00 PM

Lipids/Lipoproteins

B-087

A study of the difference in the Request of laboratory lipid metabolism tests in Primary Care in Spain

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BACKGROUND: To compare the inter-practice variability in lipid metabolism laboratory tests requested by General Practitioners (GPs) in Spain, according geographic and hospital characteristics, using appropriateness indicators, to try to ascertain the degree in requesting appropriateness.

METHODS: We obtained the number of serum cholesterol (Chol), HDL-cholesterol (HDL-chol) and tryglicerides (Tryg) requested by GPs for the year 2012 from 76 laboratories at different hospitals from diverse regions across Spain. Every patient seen in any primary care center (PCC) of any of these 76 health departments, regardless of the reason for consultation, gender or age, was included in the study.

Two types of appropriateness indicators were calculated: every test requests per 1000 inhabitants and ratio of related tests requests (HDL-chol/Chol, Tryg/Chol). The indicators results obtained in different location and for type of management were compared.

RESULTS: In total GPs requested 16013622 laboratory lipid metabolism tests in year 2012 in a Spanish population (17679195 inhabitants) that is almost half of the whole country population. Chol, HDL-chol and Tryg per 1000 inhabitants indicators results ranged from 106.3 to 550.7; 20.4 to 417.5 and from 94.0 to 439.2 respectively. The variability of HDL-chol/Chol, Tryg/Chol indicators results was also considerable, and ranged from 0.19 to 1.00 and from 0.54 to 1.00 respectively.

There were significant differences according to hospital setting in tests requests per 1000 inhabitants. In rural location Chol, HDL-chol and Tryg were higher. However, no significant differences according to hospital setting in related tests requests indicator results were detected.

In relation to institution management, no significant differences were obtained.

DISCUSSION: The high variability observed is difficult to explain by differences in patient case mix between regions.

CONCLUSION: There is a need to design and establish strategies from laboratory in consensus with requesting clinicians to improve lipid metabolism tests appropriate use and hence clinical decision making.

B-089

Lipoprotein A Levels in Individuals with Type 2 Diabetes Mellitus Attending Tribhuvan University Teaching Hospital ,Nepal

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Background: Type-2 diabetes mellitus is a common disease, affecting a large proportion of individuals worldwide. It is also recognized as an independent risk factor for cardiovascular diseases. Various markers for assessing risk for cardiovascular diseases are used in clinical laboratory. One of the emerging marker in this regard is lipoprotein a, elevated level of which is regarded to be associated with increased risk of atherosclerosis and thrombotic diseases. This study intends to assess the level of lipoprotein a in diabetic patients.

Methods: The study included 204 patients with type 2 diabetes mellitus and 204 age and sex matched controls. Lipoprotein a levels were measured and comparison was done between the lp(a) levels in diabetic patients and control.

Result: Mean serum Lp(a) levels in diabetes mellitus patients was 44.2±35.8 mg/dl, which was significantly higher when compared to control group (mean 21.1±11.2 mg/dl, $p < 0.05$).

Conclusion: The result of present study indicates that levels of Lp(a) are increased in patients with type 2 diabetes mellitus.

B-091

High-fat diet and lipid profile.

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The importance of diet in maintaining health is widely accepted and recognized. Diet lipid profile is important to prevent chronic diseases and improve the quality of life of individuals. The objective is to analyze the effect of high-lipid diet from different sources, on triglycerides (TG), total cholesterol (TC), noHDL cholesterol and fatty acid profile in serum of growing rats. Weanling Wistar rats were fed during 10 days with 40% dietary fat provided: by butter (B group); by olive oil (O group) and by high oleic oil (AO group). Control group (C) received normocaloric diet according to AIN´93. Diets fatty acid profiles were determined by gas chromatography (GC); ω6/ω3 and unsaturated/saturated (PUFA/SFA) ratios of diets were calculated. Serum levels of TG and TC were determined by enzymatic-colorimetric method and fatty acid profile was determined by GC. The statistical analysis used Bartlett's test, followed by one-way analysis of variance (ANOVA) and Dunnett as post test (*p<0.01). RESULTS: diets: ω6/ω3 ratio: B=5,6/1; O=49,6/1; AO=86/1; C=9/1; PUFA/SFA, B=0.06; O=1,36; AO=0,72; C=3,89. Serum (mean±SD mg/dL) TG B=113.0±31.2*; O=77.6±12.1; AO=67.0±15.9 C=59,1±14,8; TC B=89.2±10.1*; O=73.1±7.3; AO=71.0±10.6 C=62.1±13.6 Fatty acids profile expressed as area%±SD were:

	B	O	AO	C
Palmitic	21,2±2,5	15.7±1,7	13.5±0.7	17.3±1,4
Oleic	19.1±5.2 *	22.0±5.1 *	33.0±4.8 *	10,6 ±2,0
Linoleic	8.9±1.8 *	11.8±2.8 *	8.9±1.0 *	19.0±3,5
α-Linolenic	0.4±0.1*	0.5±0.2*	0.3±0.1*	1.2±0.3
Araquidonic	6.36±1.45	8.15±1.97	9.48±1.73	8.59±2.15
EPA	0.93±0.82	0.67±0.30	0.88±0.19	0.83±0.44
DHA	1.22±0.30	0.74±0.20*	0.83±0.18*	1.25±0.24

TG and TC levels in B group were statistically higher compared to C. Experimental groups showed higher serum oleic acid levels with lower α-linolenic and linoleic acids levels compared to C. This fact would exacerbate the route of the ω9 family and decreases essential fatty acids. It seems that the sources of dietary lipids provoked changes in serum fatty acid profile levels, but not in response to the high fat percentage.

Supported by UBACyT 20020120200068

B-093

Comparison of a Direct Enzymatic Assay and Polyacrylamide Tube Gel Electrophoresis for Measurement of Small Dense Low-Density Lipoprotein Cholesterol

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Background: Small-dense low density lipoprotein cholesterol (sdLDL-C) has been linked to the progression of cardiovascular disease. We compared two methods for determination of sdLDL-C: a direct enzymatic method (sdLDL-EX) and polyacrylamide tube gel electrophoresis (sdLDL-PGE). In addition, we evaluated the associations of these lipid measures with other atherosclerosis-related markers.

Methods: A total of 242 outpatients (age more than or equal to 19 years old) were recruited. All blood samples (excluding those with triglycerides over 400 mg/dL) were analyzed for lipid profile with sdLDL-PGE (Quantimetrix Lipoprint™, CA) and sdLDL-EX assay (Denka Seiken, Japan) an enzymatic-surfactant-based assay. We also evaluated the following atherosclerosis-related markers: apolipoprotein A-I (apoA-I), apoB, glucose, hemoglobin A1c (HbA1C), high-sensitivity C-reactive protein (hsCRP), creatinine, cystatin C, and vitamin D. The sdLDL-PGE method

separates the intermediate density lipoprotein (IDL) particles into three midbands (MID-A to C) and the LDL particles into seven subfractions (LDL-1 to 7); the sdLDL-PGE result is calculated as the sum of cholesterol concentrations from LDL3 to LDL7.

Results: The mean age of the patients (58 males and 184 females) was 54.5 years. The regression equation between the sdLDL-PGE (x) and sdLDL-EX assay (y) was $y_{mg/dL} = 0.748x + 26.14$, $r = 0.713$. The sdLDL-EX assay yielded higher measured sdLDL-C concentrations than the sdLDL-PGE assay (33.06±12.79 *vs*. 9.3±12.19 mg/dL, P <0.001); however, the absolute difference between two methods did not significantly correlate with the average sdLDL-C concentration ($R^2 = 0.005$, P = 0.290). sdLDL-C as measured with the sdLDL-EX assay exhibited significant positive correlations with VLDL, MIDC, MIDB, and LDL2 (all P <0.001), which have been suggested as atherogenic lipoproteins, but did not correlate with the less atherogenic lipoproteins MIDA (P = 0.891) and LDL1 (P = 0.604). The sdLDL-EX and sdLDL-PGE methods yielded similar patterns of correlation between sdLDL-C and atherosclerosis-related markers: positive correlations with TG, TC, LDL-C, apoB, glucose, and HbA1C but inverse correlations with HDL-C and vitamin D. However, there was no significant correlation between sdLDL-C and apoA-I, hsCRP, or creatinine levels with either method.

Conclusion: The direct enzymatic assay for sdLDL-C correlated well with the assay based on polyacrylamide gel electrophoresis. The enzymatic method appears to measure cholesterol in a boarder range of atherogenic lipoprotein particles than PGE, and thus contribute in directing specific interventions of cardiovascular prevention. Because the direct enzymatic assay can be automated, it can be used as a routine method to assess small dense LDL cholesterol.

B-094

Performance Evaluation of a New Ready-to-use Liquid Triglycerides Assay on the High-Throughput ADVIA Chemistry Systems

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Background: Measurement of serum triglycerides is an important component to determine lipid status of a patient. Increased serum triglycerides are risk factors for cardio vascular disease. Serum triglycerides are also used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders. The automated ADVIA® Clinical Chemistry Systems currently have a Siemens serum triglycerides assay that requires manual mixing of two reagent components before use on the system. An improved assay, Triglycerides_2 (TRIG_2), using ready-to-use liquid reagents, is under development. Furthermore a new concentrated reagent (TRIG_c) was developed to be automatically diluted on-system to provide larger number of tests per kit for high-volume users. The objective of this study was to evaluate the performance of both new assays on the ADVIA Chemistry Systems.

Methods: In the ADVIA Chemistry TRIG_2 and TRIG_c assays, sample is diluted and reacted with a single reagent for 5 minutes. The lipase in the reagent hydrolyzes triglycerides into glycerol, which is then converted into glycerol-3-phosphate by glycerol kinase, the latter then being oxidized to H_2O_2 by glycerol oxidase. The H_2O_2 is colorimetrically (at 505 nm) detected by a Trinder's reaction. The triglyceride concentration in a sample is determined from a linear calibration curve using Siemens ADVIA Chemistry Calibrator. The performance evaluation in this study included precision, interference, linearity, and correlation with a commercially available triglyceride (TGL) assay run on the Dimension® XPAND system. Data were collected for all ADVIA Chemistry Systems (ADVIA 1200, ADVIA 1650, ADVIA 1800, and ADVIA 2400), which use the same ADVIA Chemistry TRIG_2 or TRIG_c reagent packs, calibrators, and commercial controls.

Results: The imprecision (total %CV) of the new ADVIA Chemistry assays with two-level commercial controls and two serum pools ranging from ~90 to ~500 mg/dL (n = 80) on all ADVIA Chemistry Systems (1200/1650/1800/2400) was ≤2.1% (for both TRIG_2 and TRIG_c). The analytical ranges of the new assays are from 10 - 550 mg/dL (extendable to 1100 mg/dL by auto-dilution). The assays correlated well with the Dimension TGL assay: TRIG_2 = 0.94 [TGL] + 4.4 and TRIG_c = 0.93 [TGL] + 4.3 (r = 0.99, n = 101; sample range: 20-540 mg/dL for both). The new assays demonstrated no interference at a triglycerides level of ~150 mg/dL with unconjugated or conjugated bilirubin (up to 15 mg/dL), hemoglobin (up to 500 mg/dL), and ascorbic acid (up to 3 mg/dL). Minimum on-system stability for both was 60 days (with reagent blanking every 14 days).

Conclusion: The data demonstrates good performance of the TRIG_2 and TRIG_c assays on the high-throughput ADVIA Chemistry Systems from Siemens Healthcare Diagnostics.* Under development. Not available for sale in the USA.

B-095

Comparison of equations for the calculation of LDL-C in hospitalized patients

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Background: Prediction of cardiovascular disease (CVD) mortality is dependent on the calculation of low density lipoprotein-cholesterol (LDL-C). The Friedewald equation is the most widely used formula to calculate LDL-C but is less accurate in patients with comorbidities and extreme lipid values. Several novel formulae have been reported to outperform the Friedewald formula over a wide-range of lipid levels.
Methods: This study was a retrospective evaluation of lipid profiles in 14219 patients in South Africa, from 1 January 2013 to 30 June 2013. We evaluated four formulae (Friedewald, Chen, de Cardova, Hattori) and compared these to our direct measurement of LDL-C, (total cholesterol) TC, (HDL-cholesterol) HDL-C using Beckman reagents and instruments (Beckman Coulter). Linear regression and ROC analysis were performed.

Results: Average age of the population was 52 years (39% male, 61% female, mean LDL-C 2.9 mmol/l±1.15 SD). Directly measured LDL-C highly correlated with non-HDL (r=0.93; 95% CI 0.926-0.933). The de Cardova formula showed a high correlation with directly measured LDL-C (r=0.90 p<0.001), comparable to Friedewald calculated values for directly measured LDL-C (r=0.95 p<0.001). The de Cardova formula was favorable in some ranges of HDL, TC and the lowest TG range (r=0.97 p<0.001) but performed least well compared with three other LDL calculations (AUC=0.8331). The Chen formula performed better than Friedewald (AUC=0.9049). The Hattori formula outperformed all formulae including Friedewald over various ranges of lipid values (AUC=0.9097, Figure 1).
Conclusions: We confirm that the de Cardova formula could replace directly measured LDL-C if validated in the laboratory. In contrast to several recent findings, we show favorable correlations of this formula with Friedewald at extreme TG values. However, the Hattori formula appears to be the best for application in hospitalized patients, even at extreme lipid value **Figure 1 ROC analysis of Friedewald, Chen, de Cardova(nwLDL-C), Hattori calculations**

B-097

Comparison of Lipoprotein (a) methods using two commercially available immunoturbidimetric methods on an automated chemistry analyzer.

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Background: Lipoprotein(a) is an LDL-like particle that can vary in size and components based upon the variable number of kringle IV repeats present in apolipoprotein(a). Plasma Lipoprotein (a) levels are static in an individual and can help with assessing an individual's risk of developing atherosclerotic plaques and coronary artery disease. Previously we ran the Diasorin SPQ II immunoturbidimetric method on a Roche Cobas Fara.

Objective: To compare two commercially available immunoturbidimetric user-defined methods for measuring Lipoprotein (a) on an automated chemistry analyzer to the method used by our reference laboratory.

Methods: Method#1 (Kayama Biomedical Company K-Assay Lp(a) immunoturbidimetric assay) and Method #2 (Pointe Scientific, Inc. Lp(a) immunoturbidimetric assay) were evaluated sequentially on the same Beckman UniCel DxC 800 Synchron chemistry analyzer. Each method was performed per manufacturer's instructions. The methods were evaluated for accuracy, precision, linearity and patient sample comparisons were performed. The medical decision point of 30mg/dL was used to assess patient sample results between the assays. We used 37 frozen Lithium Heparin plasma samples ranging from below the reportable range for either assay up to above the reportable range for the current method.

Results: Both assays were linear from across the reportable range with the Method#2 showing a negative bias at values above 90mg/dL (ranging from 2.4-9 mg/dL). Accuracy was within the allowable error limit of 10mg/dL for both methods. Assay variation was assessed using the same material and similar between methods, %CV were 2-5% for Method#1, and 3-12% for Method #2. Recovery of QC material was lower in Method#2 than in Method#1. The patient samples results were similar with a mean biases of 4.97mg/dL for Method #1 and -7.02mg/dL for Method #2. In method #1 two samples had a bias > 10mg/dL, both were above 60mg/dL. Method #2 had seven samples with a bias> 10mg/dL, four between 30 and 60 mg/dL and three above 60 mg/dL.

Conclusion: Both assays were comparable to the reference method. Method #1 yielded results for the QC material and samples more similar to historical values.

B-098

Lipid hydroperoxides in apolipoprotein E-containing high-density lipoprotein

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Background: Higher levels of high-density lipoprotein (HDL)-cholesterol have been associated with lower risk of coronary heart disease. It is noteworthy that the low level of HDL-cholesterol remains predictive risk of cardiovascular disease (CVD) even when low-density lipoprotein (LDL)-cholesterol concentration has been kept to low level by the treatment. Some cholesteryl ester transfer protein (CETP) inhibitors are currently undergoing clinical evaluation. In fact, CETP inhibitors increase HDL-cholesterol levels; however, the mortality rate of CVD has not largely changed. It indicates that the HDL levels do not necessarily reflect its functions. So it is important to estimate the quality of HDL together with its quantity. It is well known that CETP inhibitors raise apolipoprotein (apo) E-containing HDL, a minor subpopulation of HDL. Therefore we investigated the qualitative evaluation of apoE-containing HDL, especially antioxidant ability estimated by containing lipid hydroperoxides (LOOH) level.

Methods: HDL (1.063<d<1.210 g/mL), isolated from serum obtained from 10 healthy volunteers by ultracentrifugation, was separated into apoE-containing and apoE-deficient HDL by Heparin-Sepharose choromatography. The compositions of HDL were determined by Lowry's Method (protein) and enzymatic test kits (cholesterol, phospholipids, and triglyceride). Before and after the oxidation by $CuSO_4$, the concentration of LOOH was measured by the ferrous oxidation in Xylenol Orange (FOX) assay using triphenylphosphine to get higher specificity for LOOH. Non-denaturing gel electrophoresis was performed for analyzing distributions of apoE and particle sizes. Surface charge was characterized by lipoprotein electrophoresis using agarose-gel.

Results: The particle size of apoE-containing HDL, developed by CBB-R250 staining and immunoblotting using anti-apoE antibody, was larger than apoE-deficient HDL. The relative electrophoretic mobilities were obviously small in apoE-containing HDL on agarose gel electrophoresis pattern. Concentrations of LOOH in apoE-containing HDL and apoE-deficient HDL were 22.3 and 1.2 nmol/mg protein, respectively. After the oxidation by $CuSO_4$, LOOH levels were increased to 153.8 and 294.0 nmol/mg protein in apoE-containing HDL and apoE-deficient HDL, respectively.

Conclusion: ApoE-containing HDL which is known to increase by CETP inhibitors is different in the particle size and surface charge from apoE-deficient HDL. ApoE-containing HDL has extremely higher level of LOOH than apoE-deficient HDL. This suggests that apoE-containing HDL might protect LDL from early and weak oxidation due to its higher susceptibility to oxidation or its rapid ability to accept LOOH from oxidized LDL. However, the total capacity of antioxidant ability per unit protein mass could be smaller than apoE-deficient HDL, suggesting that the increase of apoE-containing HDL did not simply reflect the increase of antioxidant ability.

B-099

LDL Subfractions Analysis in Pro-atherogenic Dyslipidemia

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Background: Early recognition of pro atherogenic risk factors is important for prevention and treatment of coronary artery disease (CAD). The NCEP ATP III guidelines identified LDL cholesterol (LDL-C) as the primary target for CAD therapy and risk assessment. New ACC/AHA guidelines replaced traditional lipid risk factors with a 10 year ASCVD risk calculator weighing heavily on non-lipid risk factors, ignoring a large body of evidence clearly recognizing specific dyslipidemic profiles with increased CAD risk. Numerous studies clearly demonstrate that small dense LDL, VLDL remnants and IDL are independently atherogenic while large buoyant LDL, HDL and possibly large VLDL may not be. Exclusion of such evidence could result in patient misclassification possibly leading to under or overtreatment of individuals. In this study, pro atherogenic lipoprotein subfractions were measured using the Quantimetrix Lipoprint LDL system, *(Quantimetrix Corporation, Redondo Beach, CA)*. The test yields critical information for early detection of individuals at risk or with existing CAD and allowing for a more individualized implementation of treatment.

Objective: Demonstrate the benefit of measuring the atherogenic LDL subfractions with the comprehensive analysis on the Lipoprint LDL system and assist clinicians in identifying, stratifying and customizing treatment for those at risk.

Methods: Lipid profiles for a total of 273 recruited subjects were determined by testing their total cholesterol, triglycerides, LDL-C and HDL-C using standard clinical methods. Subjects were segregated into two groups, "normolipedemic" and " dyslipidemic" based on ATP III desirable lipids status. Cholesterol levels in the lipoproteins subfractions, large VLDL, Mid-C (VLDL remnants), Mid-B (large IDL), Mid-A (small IDL), LDL-1 and LDL-2 (large buoyant LDL), LDL-3 to LDL-7 (small dense LDL) and HDL were also measured in both groups using the Quantimetrix Lipoprint LDL system, a linear polyacrylamide gel electrophoresis method. Results from the traditional lipid profile were compared to the lipoprotein subfraction profiles obtained by Lipoprint.

Results: The lipid test results, mean and range, for the 273 study subjects were: total cholesterol 196 (104 - 319) mg/dL, triglycerides 96 (25 - 345) mg/dL, LDL-C 117 (58 - 215) mg/dL and HDL-C 55 (26 - 137) mg/dL. Out of the 273 total subjects, 133 (49%) were classified normolipidemic according to the ATP III lipid guidelines while140 (51%) had at least one parameter outside the recommendations. LDL subfractions analysis by the Lipoprint system revealed that 17 (13%) out of the 133 previously classified "normal" subjects had cholesterol levels outside the 95 % confidence interval range for a given LDL subfraction. Of the 141 "dyslipedemic subjects," 69 (49%) had a normal LDL subfraction distribution. Lower levels of large buoyant LDL-1 were observed in many of the dyslipidemic subjects.

Conclusions: Clinical studies identify small dense LDL, VLDL remnants and IDL subfractions independently associated with increased CAD risk above other lipid factors. Measurement of these highly atherogenic lipoprotein subfractions as demonstrated by the Lipoprint system could be a better predictor of CAD risk than measurement of other traditional lipid risk factors.

B-100

Cell Surface Re-Engineering by Lipid Anchoring Approach

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Background: Many of the biological processes such as cell-cell adhesions, extracellular/ intracellular communications occurring on the cell surface are governed and guided by the cell surface receptors. Cell surface is a platform for introduction of various biomolecules such as proteins, carbohydrates, etc., that may further improve the potentiality of the cells. Lipidation of cell membranes is one such approach which plays an important role in many biological applications such as drug/gene delivery and serves as biomimicking models. Introduction of chemoselective functional groups via bio-orthogonal copper-free click chemistry at the cell surface further facilitates for many cellular modifications and enables for rapid and efficient cell surface labeling. Lipids can be introduced on to the cell membrane in different forms such as liposomes, micelles for efficient delivery of drug/gene or other bioactive molecules of interest.

Objective: To study and evaluate the potential anchoring effects of phospholipid (DSPE-PEG2000-DBCO) and cholesterol (CHOL-PEG2000-DBCO) based lipids on cell surface for cell surface re-engineering.

Methods: To investigate the lipid anchor incorporation effects on cell membranes, different concentrations of biotin conjugated anchor lipids were prepared by reacting N3-Biotin with anchor lipids namely DSPE-PEG2000-DBCO and CHOL-PEG2000 -DBCO via Copper free click chemistry for 1hr at RT, PBS buffer pH 7.4. The obtained conjugated anchor lipids at varying concentrations were incubated with raw 267.4 cells for different incubation times ranging from 5- 20 mins at 37°C. The lipid conjugated cells were further labeled with Streptavidin-FITC for 5 mins and the effects were examined using Confocal microscopy and Flow cytometry.

Results: Confocal microscopy and flow cytometry data suggests that 5 mins of incubation of the anchor lipid (CHOL-PEG2000 -DBCO, 5 μM) with cells is enough to see its incorporation into the cells; however a higher fluorescent intensity signal was observed at 20 mins indicating more lipid incorporation. The confocal microscopy data clearly depicts the intact incorporation of CHOL-PEG2000 -DBCO -biotin conjugate into the cell membrane without any internalization. In comparison at 20 mins, for (DSPE- PEG2000 -DBCO, 5 μM) there was decreased fluorescent signal and seen from both the cell membrane and cytoplasm indicating internalization of this conjugated lipid. This data shows the different effects of phospholipid and cholesterol based anchor lipids on cell membrane and thus can be used for introduction or transport of various drug/gene/carbohydrates/biomolecules for cell surface re-engineering purposes.

Conclusion: CHOL-PEG2000-DBCO was shown to rapidly incorporate into the cell membrane within 5 mins (20 mins being the optimum incubation time) and it was visibly evident that there is no internalization of the lipid into the cytoplasm,

unlike the DSPE-PEG2000-DBCO. Moreover the fluorescent signal intensity for DSPE based anchor lipid was very weak when compared to CHOL anchor lipid. This comparative study of lipid anchoring via copper free Click Chemistry suggests that they can serve for potential in vivo cell surface re-engineering applications.

B-101

CORRELATION BETWEEN GLYCATED HEMOGLOBIN AND SERUM LIPIDS IN TYPE 2 DIABETICS IN EASTERN LIBYA.

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Background: Diabetes mellitus with its accompanying vascular complications is on a rise globally and a similar trend has been observed in Libya too. Early detection, effective monitoring and timely management is important to control this growing problem. Various studies have shown dyslipidemia as a significant risk factor of atherosclerosis leading to vascular complications in diabetes mellitus. HbA1c as a marker of long term glycemic control in diabetics is an established fact. The present study is aimed at correlating HbA1c with lipid parameters in blood to understand its role as a marker of dyslipidemia.

Materials and methods: Sixty subjects in the age group ranging from 40 to 70 years have been recruited from Seventeenth February Teaching Hospital, Al- Baida for the study, twenty controls with no history of diabetes, twenty recently diagnosed diabetics under treatment and twenty old cases of diabetes mellitus who have suffered an episode of coronary artery disease (CAD) or cerebrovascular accident (CVA). Venous samples were drawn after an overnight fast for glucose, glycated hemoglobin, total cholesterol, triacylglycerol and HDL cholesterol and these tests were performed using authenticated kits and Cobas integra 400 analyzer. LDL cholesterol was calculated using Friedwald's formula.

Results: Slightly high levels of fasting blood glucose ($p=0.03$), Glycated hemoglobin ($p=0.02$), high levels of triglycerides ($p<0.001$) and low levels of HDL cholesterol ($p<0.001$) were observed in diabetics under treatment when compared with controls, but total cholesterol and LDL cholesterol showed no difference. However, diabetics with complications showed higher levels of fasting blood glucose ($p<0.0001$), glycated hemoglobin ($p<0.0001$), total cholesterol ($p<0.001$), triglycerides ($p<0.001$), low density lipoprotein cholesterol ($p<0.001$), and low HDL cholesterol ($p<0.0001$). There is positive correlation between glycated hemoglobin and serum triglycerides and inverse correlation with HDL cholesterol in both the diabetic groups, though not strong in recently diagnosed diabetic patients under treatment. A strong correlation was observed between glycated hemoglobin and total cholesterol and LDL cholesterol only in the diabetic group with coronary artery and cerebrovascular complications.

Conclusion: The present study has shown a significant correlation between glycated hemoglobin and serum total cholesterol and LDL cholesterol; the dyslipidemia risk factors causing atherosclerosis in diabetic mellitus not under control. Hence measurement of glycated hemoglobin is significant as a dual marker not only to monitor long term glycemic control but also in predicting dyslipidemia in type 2 diabetes mellitus.

B-102

CARDIOVASCULAR RİSK FACTORS İN PATIENTS WITH HEMODIALYSIS:PARAOXONASE AND HYPERHOMOCYSTEINEMIA

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Increased risk of cardiovascular disease (CVD) has been recognized as an important cause of morbidity and mortality in chronic renal failure (CRF). Hyperhomocysteinemia has also been accepted as an independent risk factor for CVD. Paraoxonase (PON1) is an enzyme with antioxidant activity, which circulates in plasma attached to HDL.

The aim of this study was to investigate the risk of CVD in CRF by depending on lipid/lipoprotein profiles, homocysteine and PON1 activity in plasma of hemodialyzed patients.

MATERIAL and METHODS

Subjects: Total of 42 patients undergoing hemodialysis (HD), and 43 healthy volunteers were included the study. The clinical data of patients and the controls are summarized in Table 1.

Table 1:Clinical features of the study groups

	Control	Hemodialysis
Number of participants	43	42
Sex (M/F)	21/22	21/21
Age (years)	24-61	16-64
Mean age(months)	34,7±10,7	36,9±13,3
Dialysis duration (months)	-	8-108
Mean dialysis duration (months)	-	36,6±22,2

Methods: Fasting blood samples obtained from study groups were drawn into anticoagulant-free tubes and centrifuged at 2,000 g for 10 min. Serum samples were used for the measurements of triglyceride (TG), total cholesterol (TC), LDL-C, HDL-C, and homocysteine levels,arylesterase activity and also paraoxonase activity.
RESULTS

There was no significant difference in age and sex distribution between the study groups.When compared to controls increased plasma TG but decreased TC,HDL-c and LDL-C levels were observed HD patients, even though all lipid/lipoprotein levels were in normal laboratory range.Significantly decreased PON1 and arylesterase activities, but increased homocysteine values were found in HD patients than those of control

DISCUSSION Patients with CRF, especially HD patients, experience excessively high cardiovascular morbidity and mortality.increased TG, but decreased TC, HDL-C and LDL-C levels , and also decreased PON1 and arylesterase activities observed in HD patients in the present study were in agreement with many previous reports. That increased homocysteine levels in the present study have supported the previous studies , and homocysteine may be suggested as an independent risk factor for CVD in HD patients.İn conclusion, as reflected by decreased PON1 and increased homocysteine levels, CVD risk might be increased in HD pat ents.

B-103

The usefulness of non-HDL cholesterol in less resourced countries

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Background: Common characteristic features of diabetic dyslipidemia are the elevation of plasma triglycerides and triglyceride-rich VLDL cholesterol, reduced HDL cholesterol, and an increased number of small dense LDL cholesterol particles (1). Although LDL cholesterol is not typically elevated in patients with diabetes, the changes in LDL cholesterol composition that can accompany the disease make the LDL cholesterol exceptionally atherogenic (2,3).

Aim: This study primarily aims to determine and compare the power and influence of non-HDL cholesterol and LDL cholesterol, in predicting coronary heart disease among diabetic versus non-diabetic adult Ghanaians.

Methods: A cross-sectional study was performed on 302 subjects who consisted of 154 previously diagnosed diabetes patients and 148 non- diabetics. BMI, WC, blood pressure (BP), total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), fasting glucose, high-sensitivity C-reactive protein (hs-CRP), adiponectin and resistin were measured.

Results: The mean age was 52.2 (±8.7). There was a higher (negative) correlation between adiponectin and non-HDL cholesterol ($r=-0.5756$; $p<0.0001$) than adiponectin and LDL cholesterol ($r=-0.5152$; $p<0.0001$). Higher positive correlation was observed with resistin and non-HDL cholesterol ($r=0.5756$; $p<0.0001$) than resistin and LDL cholesterol ($r=0.5494$; $p<0.0001$). Similar patterns of correlation were observed with hs-CRP, blood pressure and 10 year cardiovascular disease risk.

Conclusion: This study contributes to the existing body of literature by suggesting that easily calculated non-HDL cholesterol is superior to LDL cholesterol in cardiovascular disease risk assessment. The use of non-HDL would also be more practical and reliable target for lipid lowering therapy in less resourced country like Ghana

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B-104

Comparison of the Vantera NMR Analyzer Tests for Lipid and Lipoproteins to Conventional Enzymatic Assays on the Siemens Dimension Vista Chemistry Analyzer

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Objective: Traditional lipid cardiovascular biomarkers are largely based on the cholesterol content of the major lipoprotein fractions. Lipoproteins, however, have a polydisperse size distribution and their association with cardiovascular disease has been shown to vary not only based on their cholesterol content but also on their size and particle count. The Vantera analyzer uses NMR technology to quantify not only lipid concentrations but also size and particle counts of lipoproteins. In this study, we evaluated several lipid and lipoprotein assays on the Vantera and compared the results to conventional enzymatic assays for lipids and lipoproteins, as measured on the Siemens Vista. **Methods:** The Vantera has 3 FDA approved tests: Triglycerides (TG), HDLc and LDL particle number (LDLp). We measured these tests, as well as several other lipid and lipoprotein test parameters, in 450 patients with a wide variety of lipid disorders by the Vantera and Vista. LDLc was calculated by the Friedewald equation, using total cholesterol (TC) from either the Vantera or the Vista as indicated. **Results:** The results from the two assay systems show good correspondence (refer to Table below). Similar calculated LDLc results were also obtained, using either Vantera TG and HDLc plus Vista TC (LDLc1) or all Vantera parameters, including TC (LDLc2), when compared to LDLc calculated with Vista parameters. In addition, we compared the measured LDLc on Vantera (mLDLc) with the calculated LDLc from Vista.

	TC	TG	HDLc	LDLc1	LDLc2	mLDLc
Deming Slope	0.96	0.89	0.88	1.13	1.09	1.17
Intercept	2.71	1.69	6.42	-0.23	3.30	3.59
R^2	0.93	0.92	0.93	0.93	0.85	0.87

We also validated the lipoprotein particle count and size measurements for the major lipoprotein classes by showing that the calculated lipoprotein core volumes matched the measured core lipids, namely cholesteryl esters and TG ($R^2 = 0.91$). **Conclusions:** The Vantera NMR analyzer generates comparable lipid and lipoprotein results to traditional methods, while at the same time yielding additional cardiovascular risk information related to lipoprotein particle count and size.

B-105

Establishment of Reference Range for HDL Subfractions in Japanese Population with a New Automated Assay

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Background: Measurement of high-density lipoprotein (HDL) 2 and HDL3 subfractions might be more useful for evaluating coronary risk than total HDL-cholesterol (C). However, methods of measuring HDL2 and HDL3 are quite laborious for general clinical use and thus so far it has been difficult to establish reference ranges. Recently, we have succeeded in establishing a fully automated homogeneous assay for HDL3-C. We carried out a study to establish a reference range for HDL subfractions in Japanese population using our novel homogeneous assay for HDL subfractions.

Methods: HDL-C and HDL3-C were measured by our homogeneous assays on a Hitachi 917 automated clinical chemistry analyzer (Hitachi). HDL2-C was calculated as the difference between total HDL-C and HDL3-C. Subjects were recruited in Japan. 670 volunteers who did not have a history of CAD/CHD were invited to participate. Subjects were partitioned according to the following four parameters: male, female, young (male: equal or younger than 45 y, female: equal or younger than 55 y) and old (male: older than 45 y, female: older than 55 y). Data from the stratified subjects were analyzed for their distribution characteristics with the Shapiro-Wilk test, and estimated values were computerized. Based on distribution characteristics and independency, an appropriate test was chosen to compare the distribution of the variables among sub-groups.

Results: The Shapiro-Wilk test revealed that all sub-groups were nonparametric. Therefore, the Wilcoxon rank-sum test was used to compare the distribution of variables among sub-groups for HDL3-C, HDL2-C, total HDL-C, and HDL2-C/HDL3-C ratio. Females had significantly higher HDL3-C ($p < 0.01$), HDL2-C ($p < 0.001$), total HDL-C ($p < 0.001$), and HDL2-C/HDL3-C ratios ($p< 0.001$) compared to males. Regarding age, HDL2-C ($p = 0.72$) and total HDL-C ($p = 0.62$) did not show any significant differences, whereas older group showed higher HDL3-C ($p < 0.001$) and lower HDL2-C/HDL3-C ratios ($p < 0.05$) than younger group. Consequently, only sex difference was found in HDL2-C, whereas both sex and age differences were identified in HDL3-C.

Conclusion: Our analysis suggests that HDL subclasses can identify the CHD risk more accurately than total HDL-C.

B-106

Revising the Lipid Profile (LP): Evaluation of the Apo-A1 and Apo-B Assays on the Vitros-5600 [V-5600] Analyzer

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Backgroundt: In view of recent literature we are considering revision of the VAMC LP to consist only of apo-B, apo A1 and a calculated ratio, The initial step was to assess the properties of the apo-A1 and apo-B assays on our V-5600 analyzers (OCD: Parsippany, NJ).

Methods: On day 1 apo-A1 and apo-B were assayed once on each of 3 V-5600 analyzers in 36 specimens on which an LP had been requested. On the same day aliquots were sent to a referral laboratory [RL] (ARUP; Salt Lake City, UT) for assay of apo-A1 and apo-B using immuno-nephelometry and after 24 hour refrigerated storage the apo-A1 and apo-B assays were re-run on one of our V-5600 analyzers. All in-house assays were done according to manufacturer's instructions.

Results: The TC and TG concentration ranges were [99, 298 mg/dL] and [56, 746 mg/dL.] respectively. Data suggested that median within machine, between day CVs for apo-A1 (1%) and apo-B (0.65%) were significantly less than between machine, within day CVs [2.17 and 1.36%; p<.002 for both analytes, Mann-Whitney test]. Between-machine within day CVs were an increasing function of concentration for apo-A1 and fit by the equation: **CV (%) = 0.78 + 0.000089 [apo-A1]^2** while for apo-B the corresponding CV has a minimum at a concentration of 100 mg/dL. Assuming RL apo-A1 and apo-B assays to be done the following day we compared the second day V-5600 assays (Y) to the RL assay (X) using Deming regression with results: **Y = 24.42** (14, 34.8) **+ 0.716** (0.64, 0.79) **X** and **Y = 7.38** (1.78, 13) **+ 0.994** (0.935, 1.05) **X for apo-A1 and apo-B respectively.** With respect to classification into accepted risk categories [<80, 80-119 and 120 mg/dL.] the V-5600 and RL apo-B assays place 32/36 (89%) patients into the same risk category and the remaining 4 are one category apart.

Conclusions: The current data indicates acceptable precision parameters for the V-5600 apo-A1 and apo-B assays. Significant proportional and constant bias in the V-5600 apo-A1 assay relative to the RL assay indicates that results for this assay remain method dependent. This appears less problematic for the apo-B assay where only constant bias is noted. V-5600 and RL Apo-B assays are in satisfactory agreement with respect to risk classification..

B-108

Determination of reference intervals for LDL and HDL cholesterol subclasses and their clinical relevance in acute coronary syndrome

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Background: To evaluate the performance of a newly developed assay system in quantifying small-dense LDL cholesterol (sdLDL-C) and HDL3 cholesterol (HDL3-C), we collected blood from 2041 control subjects during an annual health check-up conducted at the Kansai Medical University.

Methods: Exclusion criteria included hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg), a high body mass index (BMI ≥ 30 kg/m2), or a decrease in the estimated glomerular filtration rate (≤60 ml/min), HbA1c (≥6.6%), LDL (40-160 mg/dL), AST (≥ 60 U/L), ALT (≥ 70 U/L), or triglyceride (TG) (>250 mg/dL) levels. As a result, samples from 1412 subjects (average age 34 ± 10 years; 293 men and 1119 women) were used for further analysis. Twenty samples collected from acute coronary syndrome (ACS) patients during their first visit to our emergency department were used to examine the clinical utility of the novel assay.

LDL-C, HDL-C, sdLDL-C, and HDL3-C were simultaneously measured using a homogenous assay system (Denka-Seiken Co., Ltd.). Large buoyant (lb) LDL-C levels were calculated by subtracting sdLDL-C values from LDL-C values. Similarly, HDL2-C levels were calculated by subtracting HDL3-C from HDL-C. Samples were centrifuged within an hour of collection, and the serum was stored at 4°C until measurement, which was done within 8 hours of collection. Reference intervals were

determined for 4 groups (based on gender and age). This was determined using the normalized data generated from the Box-Cox power transformation model, and the 95% confidence interval was calculated using a parametric method.

Results: Coefficient of variance values for the intra- and inter-assay variation in LDL-C, HDL-C, sdLDL-C and HDL3-C were less than 6.13%. LDL-C, sdLDL-C, lbLDL-C, HDL-C, HDL2-C, and HDL3-C values positively correlated with age in both men and women. LDL-C and lbLDL-C in men, and LDL-C, sdLDL-C, and lb LDL-C in women positively correlated with BMI, but HDL-C and HDL2-C in both groups negatively correlated with this parameter. sdLDL-C and HDL3-C in men, and HDL-C, HDL2-C, and HDL3-C in women positively correlated with alcohol consumption, while this negatively correlated with lbLDL-C in men.

The sdLDL-C reference interval was the lowest in younger women (13-22 mg/dL) and the highest in older men (16-53 mg/dL). The lb-LDL-C interval was the highest in older women (49-119 mg/dL), and the lowest in younger women (43-111 mg/dL). Reference intervals for HDL2-C and HDL3-C were the highest in older women (29-77 mg/dL and 19-36 mg/dL, respectively), and the lowest in young men (22-66 mg/dL and 17-30 mg/dL).

In ACS patients, both sdLDL-C and lb-LDL-C values were higher while HDL2-C and HDL3-C values were significantly lower than those in the control group. In relation to ACS, a multiple logistic regression analysis revealed significant odds ratios in age (1.3), lbLDL-C/sdLDL ratio (26.4) and sdLDL-C/HDL2-C ratio (269.7).

Conclusion: These findings suggest that the LDL-C and HDL-C subclasses may be useful biomarkers in predicting cardiovascular complications in patients with atherosclerosis.

B-109

Overexpression Of Del-1, An Oxidized LDL Blocking Protein, Suppressed Atherogenesis In Mice Without Lowering Oxidized LDL Concentration, But with Reducing LOX-1 Ligand Containing ApoB Activity (LAB).

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Background: Oxidized LDL (oxLDL) is implicated in the pathogenesis of atherosclerosis. However, measurement of circulating oxLDL concentration often fail to provide the accurate state of atherosclerosis or the risk of atherothrombotic diseases such as myocardial infarction and ischemic stroke. To solve this problem, we have devised a novel ELISA assay system based on the binding of modified LDL to an oxidized LDL receptor LOX-1, rather than determining the concentration of a specific epitope of anti-oxLDL antibody. LOX-1 binding activity of apoB-containing lipoprotein was designated as LAB. LAB well predicted the risk of coronary artery disease and ischemic stroke (Inoue, Clin Chem 2010), and reflected the intimal thickening of carotid artery (Okamura, Atherosclerosis 2013).

Aim: To understand the reason why the receptor-based assay has been superior to the antibody-based assay in evaluating the progression of atherosclerosis and the risk of atherosclerosis-related diseases.

Methods and Results: We found that Del-1 selectively bound to oxLDL but not to native LDL, leading to the inhibition of the uptake of DiI-labeled oxLDL (DiI-oxLDL) via oxLDL receptors including LOX-1, SR-A, CD36, and SR-B; but not to the inhibition of DiI-labeled native LDL uptake via LDL receptor expressed in COS-7 cells. We also found that Del-1 inhibited DiI-oxLDL uptake by cultured human umbilical vein endothelial cells (HUVEC) and THP-1-deribed macrophages. Furthermore, Del-1 suppressed oxLDL-dependent signal transduction in LOX-1 expressing CHO cells and in HUVEC. Del-1 also suppressed oxLDL-induced secretion of endothelin-1 in HUVEC.

To examine in vivo effects of Del-1 on atherogenesis, we established Del-1 transgenic mice (Del-1Tg), and fed their males high-fat diet along with control wild-type mice (WT) (n=6 each) for 20 weeks from the age of 24 weeks. Oil red O-positive atheromatous area at aortic roots dramatically decreased in Del-l Tg compared with WT (3.1±1.4 vs. 17.7±2.0 % of aortic roots area, P<0.001). Reflecting the antiatherogenic effects, plasma LAB activity was significantly decreased in Del-1Tg compared with WT mice (13.3±4.3 vs. 106.2±20.1 ng/ml, P<0.05), while oxidized LDL concentration determined by conventional antibody-based assay did not differ between Del-1Tg and WT (698.9±34.4 vs. 741.9±46.0 nmol/ml). Other lipid parameters except triglycerides were not different. Plasma tryglyceride concentration in Del-1Tg was slightly lower than that in WT.

Conclusion: In the presence of Del-1, an oxLDL blocking protein, the oxLDL concentration determined by conventional anti-oxLDL antibody-based assay dissociates from atherogenicity, while LAB well associated with it. This might be a reason why circulating LAB activity better reflects the state and the risk of atherosclerotic diseases. This might be a reason for LAB activity reflects the progression and the risk of atherosclerotic disease better than oxLDL concentration determined by anti-oxLDL antibody.

B-110

Hypertriglyceridemia is a major contributor of high small dense LDL level in patient with metabolic syndrome

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Background: Patients with metabolic syndrome (MetS) have shown higher small dense low density lipoprotein cholesterol (sdLDL-C) level than healthy controls. However, which component of MetS made the largest contribution to an increase in sdLDL-C has not fully determined. We aimed to determine major contributing component of MetS to high sdLDL-C concentration and sdLDL-C/LDL-C ratio.

Methods: Four hundred and forty seven subjects (225 men; 222 women) with MetS were randomly selected from the Korean Metabolic Syndrome Research Initiatives-Seoul cohort study. Age and sex-matched 360 healthy controls (181 males and 179 females) were also randomly selected from the same cohort.

Results: When we compared means of sdLDL-C concentration between subgroups divided according to whether subjects met or not met each MetS component in patients with MetS (Table 1), significant difference in sdLDL-C concentration was found only between subgroups divided according to whether subjects met or not met triglyceride (TG) criteria. For healthy control, there were significant differences in sdLDL-C concentration according to the presence or absence of TG and waist circumference components. We observed similar pattern for sdLDL-C/LDL-C ratio. Pearson correlation analysis showed total cholesterol, LDL-C, and TG showed relatively strong correlations with sdLDL-C concentration (r = 0.730, 0.508 and 0.543 respectively for men; 0.748, 0.692 and 0.653 respectively for women), whereas only TG maintained a strong correlation with sdLDL-C/LDL-C ratio (r = 0.789 for men and 0.745 for women). In multiple regression analysis, we found TG level was a significant determinant of sdLDL-C concentration and sdLDL-C/LDL-C ratio.

Conclusion: Among five MetS components, only the abnormal TG level worked as a differing factor of sdLDL-C concentration and sdLDL-C/LDL-C ratio, and which results were reproducible in both genders with or without MetS. Our results also supported a hypothesis that atherogenic effect of hypertriglyceridemia could be partially mediated by elevated sdLDL related to high TG.

Table 1. Differences of sdLDL-C between subgroups divided by whether met or not met each component

Components	Male (n = 406)			Female (n = 401)		
	Component -	Component +	*p* value	Component -	Component +	*p* value
Patients with metabolic syndrome						
Waist circumference	48.66 ± 18.9	48.77 ± 18.93	0.9691	43.08 ± 12.55	44.88 ± 18.06	0.5891
Triglyceride	30.44 ± 11.22	51.78 ± 18.18	< 0.0001	33.06 ± 11.93	47.72 ± 17.3	< 0.0001
High density lipoprotein cholesterol	49.95 ± 20.26	47.69 ± 17.6	0.3709	46.75 ± 20.86	43.71 ± 15.64	0.2344
Blood pressure	50.44 ± 19.09	48.39 ± 18.87	0.5400	46.76 ± 16.3	43.77 ± 17.74	0.2473
Fasting blood sugar	49.78 ± 17.65	46.95 ± 20.85	0.2796	44.34 ± 16.49	45.05 ± 18.72	0.7673
Healthy controls						
Waist circumference	35.55 ± 15.66	42.72 ± 18.33	0.0463	26.81 ± 12.58	33.24 ± 17.17	0.0147
Triglyceride	29.7 ± 12.01	48.23 ± 15.72	< 0.0001	24.54 ± 9.63	47.44 ± 16.96	< 0.0001
High density lipoprotein cholesterol	35.71 ± 16.46	41.13 ± 13.39	0.1195	27.21 ± 13.33	31.35 ± 15.05	0.1137
Blood pressure	36.09 ± 15.96	38.65 ± 17.36	0.4559	28.1 ± 13.76	26.86 ± 13.72	0.7384
Fasting blood sugar	36.53 ± 16.14	32.17 ± 19.11	0.6437	28.00 ± 13.72	-	-

B-111

Comparison of Lipoprotein Profile Analysis by Nuclear Magnetic Resonance (NMR) and Agarose Gel Electrophoresis.

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Objective: Lipoprotein analysis by agarose gel electrophoresis is useful for identifying rare genetic lipid disorders and confirming the level of the major lipoprotein fractions. The NMR Vantera method can also be used for quantifying lipoprotein subfractions, size and particle counts but the two methods have not been compared. **Methods:** Serum samples from 250 patients with a wide variety of lipid disorders were analyzed on the Sebia lipoprotein gel electrophoresis system and the Vantera NMR system. The major lipoprotein fractions (alpha-HDL, pre-beta-VLDL and beta-LDL) were quantified by densitometric scanning of the electrophoresis gel; these values were divided into tertiles and compared to the NMR particle count (HDL-P, VLDL-P and LDL-P). The speed of migration of each fraction on electrophoresis was characterized

and catergorized into tertiles (fast, medium, slow), then compared to lipoprotein particle size (HDL-Z, VLDL-Z and LDL-Z). Data was analyzed by ANOVA to identify differences between the methods.

Results: Results from the particle count by NMR analysis showed the same trend as did quantification of the major bands by lipoprotein electrophoresis.

		Electrophoresis Grouping Tertiles		
NMR particle count (mean)		low	medium	high
HDLp (μmol/L)	p<0.001	27.8±0.9	33.1±0.9	36.2±0.9
VLDLp (nmol/L)	p<0.001	26.9±4.4	36.4±4.4	84.8±4.4
LDLp (nmol/L)	p<0.001	1056±62.1	1154±60.7	1490±62.6

Although ANOVA showed a significant correlation (p<0.006) between size of lipoproteins with migration speed by electrophoresis, the results were not always consistent between the three fractions, particularly for VLDL. Using a novel method for displaying NMR results, we also show that the pattern of NMR results typically overlap with the interpretation of the lipoprotein phenotype based on electrophoresis for disorders, such as Type I, IIa, III hyperlipidemia and LCAT Deficiency. **Conclusions:** Because the analysis by NMR and electrophoresis depend on different physical properties of lipoproteins, there was not always a complete concordance of the results. However, in general NMR analysis of lipoproteins usually leads to the same clinical interpretation of the lipoprotein phenotype as electrophoresis and provides additional information that may be potentially useful for assessing cardiovascular risk.

B-112

A serum oxidized high-density lipoprotein marker and its association with the smoking status in males

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Background: High-density lipoprotein (HDL) particle, whose major protein is apolipoprotein A-I (apoA-I), protects against atherosclerosis via its anti-oxidant properties. The oxidative modification of apoA-I is associated with dysfunctional HDL. Cigarette smoking, a major atherosclerotic risk factor and a precipitating condition leading to oxidative stress, is often accompanied by low HDL-cholesterol levels in the circulation. However, the adverse effects of smoking on atherosclerosis remain incompletely understood with regard to dysfunctional HDL, and easy biomarkers for smoking-related HDL modifications are needed. A new assay we developed for oxidized apoA-I, oxHDL, may be a suitable marker, since we have found high oxHDL levels under some oxidative stress conditions. The aim of this study was to investigate the association between the oxHDL levels and the smoking status in males.

Methods: A total of 260 Japanese males (mean age, 61 years) were consecutively recruited from general health check-ups. The subjects who had a history of cardiovascular disease, had been diagnosed with metabolic syndrome or received lipid-modulating drugs were excluded. The smoking status was self-reported. Clinical data, including serum lipid levels, were obtained from subjects in a fasted state. The serum oxHDL levels were quantified using a sandwich ELISA system, which utilizes monoclonal antibodies prepared by immunization with H_2O_2-oxidized human apoA-I.

Results: The mean/median levels of the relevant variables were as follows: low-density lipoprotein cholesterol, 3.0 mmol/L; triglycerides, 1.0 mmol/L; HDL-cholesterol, 1.5 mmol/L; oxHDL, 221 U/mL and oxHDL/HDL-cholesterol ratio, 3.9. Compared to non-smokers (n = 188), current smokers (n = 71) tended to exhibit higher oxHDL levels (217 versus 239 U/mL) and lower HDL-cholesterol levels (1.6 versus 1.5 mmol/L), while current smokers showed significantly higher oxHDL/HDL-cholesterol levels (3.9 versus 4.1, $p < 0.05$). The difference in the oxHDL/HDL-cholesterol levels remain significant after adjusting for age, body mass index, blood pressure, other lipids and glucose levels. Moreover, a significant inverse correlation ($r = 0.3$, $p < 0.05$) was found between the oxHDL/HDL-cholesterol levels and the Brinkman index (number of cigarettes smoked per day × number of years of the habit) in current smokers.

Conclusion: The present findings suggest that smoking may independently and oxidatively modify HDL particles, thus leading to dysfunctional HDL in males. The oxHDL/HDL-cholesterol ratio may therefore be useful for assessing the atherosclerotic burden in relation to the smoking status.

B-113

Effects of body weight on low-density lipoprotein and high-density lipoprotein subclasses assessed by homogenous small-dense low-density lipoprotein and high-density lipoprotein 3 cholesterol assays

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Background: The incidence of overweight has been increasing in epidemic proportions, and it adversely increases the prevalence of most cardiovascular (CV) diseases. Obesity results in diverse changes in laboratory parameters such as triglyceride (TG) levels, high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively) levels, and glucose metabolism due to insulin resistance. Of these, HDL-C and LDL-C levels play a key role in the development of CV diseases; as such, they are very valuable biomarkers for the prediction of future CV events. Both lipoproteins have subclasses: small dense LDL (sdLDL) and large buoyant LDL (lbLDL) and HDL2 and HDL3, which may be superior biomarkers to LDL and HDL, respectively. However, assaying these subclasses using ultracentrifugation, electrophoresis, or high-performance liquid chromatography is not easy, requires significant time, and commonly produces inaccurate, quantitative results.

Methods: A homogenous assay system for sdLDL-C and HDL3-C has recently become available, and it is reported to have favorable performance (Clinical Chemistry 57:57-65, 2011; Clin Chim Acta. 427:86-93, 2014). In the present study, using these homogenous assay systems, we explored the relationship between body weight and LDL and HDL subclass concentrations. The levels of lbLDL-C and HDL2-C were calculated by subtracting those of sdLDL-C and HDL3-C from the levels of LDL-C and HDL-C, respectively. Data are expressed as means ± standard deviation.

The enrolled subjects were women aged 34 ± 10.5 years (20-64 years) (n = 1,276) working in our hospital, and informed consent was obtained at the annual health checkup during blood sampling.

Results: Body mass index (BMI) was significantly correlated with both lbLDL-C ($r = 0.2691$, $p < 0.01$) and sdLDL-C ($r = 0.3223$, $p < 0.01$). Similarly, waist circumference (cm) was significantly correlated with lbLDL-C ($r = 0.2638$) and sdLDL-C ($r = 0.3315$). In contrast, BMI was negatively correlated with HDL2-C ($r = -0.2715$, $p < 0.01$) but not HDL3-C ($r = -0.0173$). Waist circumference was significantly correlated with HDL2-C ($r = -0.2771$) but not HDL3-C ($r = -0.0686$). Multiple regression analysis with age, systolic blood pressure, alanine aminotransferase, TG, cystatin-C, C-reactive protein, and HDL-C and LDL-C subclasses as independent variables revealed that these parameters were independently correlated with BMI. The lbLDL-C and sdLDL-C values were similarly independently correlated with BMI. Both HDL2 and HDL3 were independently correlated with BMI, but the t-value of HDL2 was much greater than that of HDL3. When waist circumference was used as the dependent variable, it was also independently correlated with the above parameters. The t-value of HDL2-C (8.231) was the highest, followed by those of lbLDL-C (4.549), sdLDL-C (2.931), and HDL3-C (2.361).

Conclusion: The present findings are in contrast to those of earlier studies in which sdLDL-C increased and HDL3-C decreased with weight gain and changes in both lbLDL-C and HDL2-C were more closely related to body weight differences. These findings also indicate that these LDL-C and HDL-C subclasses assessed using the homogenous method may be novel predictive markers for atherosclerosis.

B-114

Effect of SAA on the structure and measurement method of HDL

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Background: Serum amyloid A (SAA), which is one of the acute phase proteins, is commonly found in high-density lipoprotein (HDL) in the circulation. SAA is known to become a major HDL protein component in the acute phase due to a displacement of apolipoprotein AI (apoAI). Consequently, this remodeling could affect HDL metabolism, however the actual influences have not been fully elucidated. In this study, we focused on the structural differences between SAA containing HDL and normal HDL. In addition, the effect of attached SAA on the values of HDL-cholesterol (HDL-C) measurement was estimated.

Methods: HDL (d=1.063-1.210 g/mL) isolated from the patients with or without inflammation was characterized by agarose gel electrophoresis for analyzing the surface charge and by nondenaturing gel electrophoresis for analyzing particle size

and distribution of apoAI and SAA. HDL-C concentrations of the patients with various SAA levels were analyzed by two methods. One is the homogeneous method using α-cyclodextrin sulfate which is commonly used in clinical laboratories, and the other is the ultracentrifugation method as a reference method. SAA was measured by latex agglutination-turbidimetric immunoassay method.

Results: The increase of serum SAA levels induced the decrease of HDL mobility on agarose gel electrophoresis patterns. In the nondenaturing gel electrophoresis, HDLs obtained from the patients with low serum SAA levels were separated to two distinct particles, HDL_2 and HDL_3. On the other hand, HDLs obtained from the patients with high serum SAA levels indicated two kinds of typical patterns; one was characterized as the additional band at the intermediate particle size between HDL_2 and HDL_3, and the other was characterized as two bands extremely larger size than HDL_2 and smaller size than HDL_3. SAA was identified in the additional band for the former and in the larger band for the latter. HDL-C concentrations measured by the homogeneous method were highly correlated with those by the ultracentrifugation method in both the patients with low (SAA≤8 μg/mL, n=94) and high (8<SAA≤4762 μg/mL, n=154) SAA levels. Although no significant difference was observed in the regression lines of both groups, the ratios of HDL-C concentrations obtained by the ultracentrifugation method to those by the homogeneous method showed a tendency to be higher in the patients with acute inflammation.

Conclusion: Our data indicated that the large amount of SAA attached to HDL during inflammation and changed in the surface charge and the particle size of HDL. However, no definite relevance between serum SAA level and HDL particle size was observed. A good correlation between the homogeneous method and the ultracentrifugation method could be explained by the assay principle of the homogeneous method used, in which total cholesterol is measured after inhibition of enzymatic reaction against lipoproteins (mainly VLDL and LDL) except HDL by α-cyclodextrin sulfate. It suggests that the values obtained by the homogeneous method used here are probably not affected by a change in the structure of HDL.

B-115

Accuracy based proficiency test for triglyceride in South Korea

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Background: When laboratory test results are not standardized, a different result may be obtained for the same clinical sample. In this study we performed two trials of accuracy based proficiency test for triglyceride measurement among 53 candidate laboratories and assessed current performance of the routine measurement for triglyceride in Korea. .

Methods : A total of 6 levels of commutable frozen serum pools were prepared as secondary reference materials for triglyceride measurement according to CLSI 37-A. Test results from ReCCS were regarded as target reference values for group using glycerol blanking method, and values from CDC for the other group using method without glycerol blanking. For each trial, 3 levels of pooled serum were sent to participating laboratories and imprecision, bias and total error for each trial were calculated.

Results: The bias of 18 laboratories (34%) using enzymatic method with glycerol blank ranged from -7.62% to -1.47%, and that of 35 laboratories (66%) using enzymatic method without glycerol blanking ranged from -9.09% to 1.67%. Coefficient variations (CVs) ranged from 3 to 5% for each level of reference materials but did not show significant difference between the two groups. When total error (≤15%) was used for acceptability criteria, all of the results from 53 laboratories were acceptable. However, when inaccuracy criteria (≤±5%) is used, unacceptable rates for the 1st and 2nd trial were 33% and 50% in the group using glycerol blank method, 15% and 63% in the group using without glycerol blanking method respectively.

Conclusions : Through accuracy based proficiency test, comparison to the target value determined by a reference measurement procedure allows both an absolute and relative performance yardstick for laboratories using different measurement procedures. And data obtained from this proficiency test made a footstep for further national laboratory standardization for triglyceride.

Table 1. Accuracy based proficiency test for triglyceride among 53 participating laboratories

	TG(mg/dL)	CFS 11302	CFS 11303	CFS 21301	CFS 12-1-1	CFS 12-2-3	CFS 12-2-4
Reference	CDC (total glyceride)	186.38	139.55	231.19	154.73	90.01	239.43
	ReCCS (triglyceride)	175.20	128.50	221.90	144.40	87.10	236.60
Without glycerol blanking (N=35)	Mean(n=35)	181.37	133.28	227.78	148.03	83.15	231.46
	Max	197.17	143.83	248.17	164.17	93.00	255.67
	Min	170.83	121.83	214.83	140.83	78.17	220.00
	Range (Max-Min)	26.33	22.00	33.33	23.33	14.83	35.67
	SD	6.02	4.51	7.46	4.86	3.09	7.63
	%CV	3.3	3.4	3.3	3.3	3.7	3.3
With glycerol blanking (N=18)	Mean(n=18)	177.63	128.42	225.60	147.65	79.18	233.48
	Max	187.67	138.83	246.00	159.33	85.50	251.17
	Min	161.00	117.00	205.00	135.67	74.17	211.33
	Range (Max-Min)	26.67	21.83	41.00	23.67	11.33	39.83
	SD	7.80	5.77	11.01	6.01	2.73	9.35
	%CV	4.4	4.5	4.9	4.1	3.4	4.0

B-117

The association between lipoprotein(a) levels and coronary heart disease risk in different ethnic groups: results from the Multi-Ethnic Study of Atherosclerosis

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Background Elevated plasma lipoprotein (a) (Lp(a)) levels are established as a risk factor of coronary heart disease (CHD) in populations mainly of European and African ancestry. The objective of this study was to examine the association of lipoprotein (a) (Lp(a)) levels with coronary heart disease (CHD) events in 4 racial/ethnic groups in the Multi-Ethnic Study of Atherosclerosis (MESA).

Methods The MESA consists of 6,814 individuals without clinical evidence of CHD (aged 45-84 years) at the initial recruitment. Individuals taking lipid-lowering medication at baseline or with unavailable samples were excluded from this analysis resulting in a remaining sample of 4,387 who were followed for 8.5 years. Incident CHD was defined as the first occurrence of myocardial infarction, resuscitated cardiac arrest, CHD death, or definite angina. Lp(a) mass concentration was measured with an isoform-insensitive turbidimetric immunoassay (Denka Seiken, Japan) at the Health Diagnostic Laboratory Inc. (Richmond, VA) with *inter-assay* coefficients of *variation* less than 5%. Statistical analyses were conducted using Stata (version 12.1, Stata Corp, College Station, TX) and R. Tukey-Kramer HSD was used to test differences between groups. Since residuals analyses suggested a non-linear relationship between CHD risk and Lp(a) as a continuous measure, we dichotomized Lp(a) into < and > median groups. Cox regression was used to test for association between Lp(a) and CHD events, adjusting for age, gender, race, diabetes, hypertension, smoking status, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and log-triglycerides.

Results The study population was composed of 28.7% African American (AA, n=1,257), 12.4% Chinese American (CA, n=546), 22.7% Hispanic (HS, n=996), and 36.2% Caucasian (CU, n=1,588) participants. The distributions of Lp(a) levels in all ethnic groups were left-skewed, and AA had a significantly higher level of Lp(a) compared to the 3 other ethnic groups ($p < 0.05$). The median levels of Lp(a) in the 4 ethnic groups were: AA 35.1 mg/dL, CA 12.9 mg/dL, HS 13.1 mg/dL, and CU 13.0 mg/dL. The numbers of CHD events were: AA 66, CA 18, HS 49, and CU 105. Using the Lp(a) median level of the entire cohort (17.8 mg/dL), Lp(a) > median was associated with a

significantly higher CHD event rate than Lp(a) < median (*hazard ratio* (HR) = 1.42, $p = 0.0095$) following adjustment for age, gender, race, diabetes, hypertension, smoking status, HDL-C, LDL-C and log-triglycerides. In analyses stratified by ethnic groups, Lp(a) level above the group-specific median was associated with a significantly higher incidence of CHD in CU (HR = 1.55, $p = 0.03$), but not in the 3 other ethnic groups ($p = 0.08$, 0.63 and 0.74 for AA, CA and HS, respectively).

Conclusion In MESA participants not on lipid-lowering medications at recruitment, we found that elevated Lp(a) levels were associated with increased risk of CHD independent of traditional CHD risk factors. When stratified by race and using race-specific Lp(a) median levels as the cutoff, the association was only significant in CU. However, the power of subgroup analysis may be limited by the number of events.

B-118

New Enzymatic Method for Sphingomyelin Measurement verified by Mass Spectrometry

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Background: Serum sphingomyelin (SM) can help to predict the development of coronary arterial diseases. However, no convenient and specific assay for measuring SM in serum is available for routine laboratory practice. We previously developed the new assay for SM using an enzymatic method in combination with a monoglycerolipase and two types of phospholipase D. In this assay, phosphatidylcholine (PC) and lysophosphatidylcholine are eliminated in the first step and the remaining SM is measured in the second step. To validate this assay, we correlated the measurement values with the data by mass spectrometric analysis.

Methods: We prepared 47 sera at Shinshu University Hospital and measured their SM content using an enzymatic method on a Hitachi-7170 autoanalyzer. We also analyzed the lipid extract of the same sera using a TripleTOF 4600 mass spectrometer (AB SCIEX). N-heptadecanoyl-sphingosylphosphorylcholine (sphingomyelin with C17:0 fatty acid; SM 17:0, Matreya) was used as an internal standard. Lipid extraction was performed according to Folch's method. The amount of each identified SM species was determined by the difference of their fatty acid forms. We correlated results of the enzymatic method with that determined by mass spectrometry.

Results: The total amount of SM in the 47 samples ranged from 0.249 to 0.945 mmol/L (mean : 0.497 mmol/L) in the enzymatic method and 0.241 to 0.870 mmol/L (mean : 0.417 mmol/L) in the mass spectrometric method. Identified SM species by the mass spectrometry were SM 16:0, 16:1, 18:0, 18:1, 20:0, 22:0, 22:1, 24:0, 24:1, 24:2. SM 16:0 was the most abundant (0.191±0.101 mmol/L, 46.2±8.3 %) and 24:0 was the second abundant (0.0632±0.0434 mmol/L, 15.1±4.0 %) among the SM species in the 47 samples. The other species each represented less than 10% of the total SM. Within-run coefficients of variation (CVs) at 0.422 and 0.756 mmol/L in pooled sera were 1.55% and 1.45% for the enzymatic method and 13.5% and 5.26% for the mass spectrometry, respectively. We found a high correlation between values of each SM species measured with the enzymatic method (X) and that determined by the mass spectrometry (Y). Correlation coefficients and regression equations were as follows; SM 16:0, r=0.897, Y=0.367X+0.649; SM 16:0+SM 24:1, r=0.912, Y=0.520X−0.249; all identified SM species, r=0.950, Y=0.894X−1.95. Conversely, the correlation coefficient for PC was low (PC 16:0/18:2 + PC 18:0/18:2, r=0.593). These results suggest that the proposed enzymatic method can measure most of SM species in serum with high specificity and accuracy.

Conclusion: Our new enzymatic method can measure SM in serum with high specificity and accuracy, and therefore is very useful in clinical practice.

B-119

Effects of myeloperoxidase-modified HDL on reverse cholesterol transport and monocytic migration

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Background: Myeloperoxidase (MPO) is one of the biomarkers for acute coronary syndromes. In the advanced atherosclerotic lesions, MPO, mainly secreted from macrophages, is known to induce the oxidized apolipoprotein AI (apoAI), such as 2-chloro- or 2-nitro-tyrosyl apoAI and apoAI-AII heterodimer. These products would be expected to give us the different informations from MPO activities in plasma, likely more specific to cardiac disease. We previously revealed that the plasma levels of apoAI-AII heterodimer in patients urgently hospitalized for the treatment of acute myocardial infarction were significantly higher than those of the healthy subjects. In the present study, we investigated the effect of MPO oxidation on the antiatherogenic properties of HDL, such as the cholesterol efflux capacity and the inhibition activity of monocytic migration.

Methods: 1) Oxidation of HDL by MPO; HDL (1.063<d<1.210 g/mL) was incubated with phosphate buffer (pH 7.4) containing hydrogen peroxide, diethylenetriamine pentaacid, L-tyrosine, and MPO for 24 h at 37 °C. 2) Evaluation of cholesterol efflux; THP-1 cells were differentiated into macrophages by addition of phorbol 12-myristrate 13-acetate. Then macrophages were loaded with acetylated LDL and [^{3}H]-cholesterol for 24 h. After 18 hours equilibration, cholesterol efflux was assessed in the media in the presence of HDL, MPO treated HDL, or no acceptor for 4 h. The radioactivity in the medium and the total cell-associated radioactivity were determined by scintillation counting. The cholesterol efflux was calculated as a percentage: [^{3}H]-cholesterol in medium/ ([^{3}H]-cholesterol in medium + [^{3}H]-cholesterol in the cells) x 100. 3) Evaluation of THP-1 cell migration; THP-1 cell migration assays were performed with 8 μm pore size inserts on the PET membranes. HUVEC (human umbilical vein endothelial cell) was stimulated by LPS at 37 °C for 16 h in the presence of HDL or MPO treated HDL. The lower compartments of chemotaxis chamber were filled with supernatant of HUVEC cultured medium. THP-1 cells were placed in upper chamber and incubated for more than 24 h at 37 °C. The THP-1 cells migrated in the lower chamber were counted using an inverted microscope. The THP-1 migration ability is defined by the percentage of migrated THP-1 number against the original number.

Results: ApoAI-AII heterodimer in HDL was apparently increased by the incubation with MPO, which was confirmed by SDS-PAGE under reducing condition followed by CBB R250 staining and immunoblotting using anti-apoAI and anti-apoAII antibodies. No difference in the cholesterol efflux capacity was observed between HDL and MPO treated HDL. In the THP-1 migration assay, the presence of HDL indicated the significant reductive effect (44%) against LPS stimulation of HUVEC. This effect was reduced to 34% by the treatment of HDL by MPO.

Conclusion: MPO oxidation did not largely affect the cholesterol efflux capacity of HDL. However, MPO oxidation partially impaired HDL property to inhibit the monocytic migration, suggesting that the oxidation of HDL by MPO would affect the progression of atherosclerotic plaque. It means that the products, such as apoAI-AII heterodimer, induced by MPO oxidation might be available as a biomarker to reflect a progression of atherosclerosis.

B-121

Increases in Large HDL Particles are associated with Improved Cardiopulmonary Fitness by Exercise-based Cardiac Rehabilitation in Patients with Acute Coronary

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Background: Exercised-based cardiac rehabilitation (CR) can increase HDL-cholesterol (HDL-C). However it remains unclear how elevated HDL-C and changes of HDL subfractions are correlated with the improvement of exercise tolerance in acute coronary syndrome (ACS) patients participated with CR.

Methods: Concentrations of cholesterol and apolipoproteins (Apo) in HDL subfractions separated by heparin-Mn precipitation method were measured at the onset of ACS and at the end of the 6-month CR program in patients (45 men and 6 women) aged of 64.3 ± 11.8 years. Cardiopulmonary exercise tests were performed at the beginning and the end of the CR program. All patients received successful percutaneous coronary intervention on admission, and then started to take statins.

Results: Serum levels of HDL-C and ApoA1, and concentrations of cholesterol and ApoA1 in large HDL fraction (HDL2) were significantly increased by CR (42.7 mg/dl ± 14.1 mg/dl to 47.4 mg/dl ± 14.2 mg/dl, 128.8 mg/dl ± 23.4 mg/dl to 139.2 mg/dl ± 26.0 mg/dl, 25.3 mg/dl ± 12.4 mg/dl to 30.3 mg/dl ± 13.6 mg/dl, 67.8 mg/dl ± 20.3 mg/dl to 79.4 mg/dl ± 24.5 mg/dl, respectively), while cholesterol and ApoA1 in small HDL fraction (HDL3) were not changed. Moreover HDL2-C / HDL-C ratio, and HDL2-ApoA1 / ApoA1ratio were significantly increased by CR. In addition, Spearman's rank correlation coefficient analysis revealed that only % increases of HDL2-C were significantly associated with % increased of peak oxygen consumption (VO2) and % increases of VO2 at anaerobic threshold ($\rho = 0.439$, $p = 0.007$, $\rho = 0.382$, $p = 0.020$), while neither HDL-C nor HDL3-C were associated with them.

Conclusion: CR can markedly increase the number of HDL2 particles, which is significantly associated with the improvement of cardiopulmonary fitness. These results suggest that CR is very useful therapy for the reverse cholesterol transport, and the secondary prevention.

B-122

A Novel Method Using Cation-exchange and Heparin Affinity Columns Arranged Tandemly to Determine ApoE-containing HDL-cholesterol in Un-pretreated Whole Serum

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Background: Measurement of HDL-related subclasses has been suggested to be more useful for evaluating coronary artery disease risk than total HDL. Clinical significance of apolipoprotein E-containing HDL (apoE-HDL) has not been clarified. Development of a reliable and rapid assay system to measure serum apoE-HDL levels is thus essential.

Aims: We developed a high-performance liquid chromatography (HPLC) equipped with cation-exchange and heparin affinity columns to measure apoE-HDL-cholesterol (apoE-HDLc) levels in untreated whole serum, and studied the analytical performance for serum apoE-HDLc determination. Separation characteristics of the system and their isolated lipoprotein fractions were also studied.

Methods: An un-pretreated whole serum sample was injected into two tandemly connected columns and eluted by a step-wise gradient manner, as shown in Fig.1. Non-HDL lipoproteins are bound to the cation-exchange column, and unbound HDLs next enter the heparin column. The heparin column retains apoE-HDL but no other HDLs (apoE-deficient HDL).

Results: Our developed HPLC system completed the assay within 16 mins and separated lipoproteins in un-pretreated serum into 3 peaks on the cholesterol pattern. Each peak corresponded specifically to apoE-deficient HDL (first peak), apoE-HDL (second peak), and non-HDL (third peak). The present HPLC system provided acceptable small within-day imprecision values; 1.3% CV (n=8) and good acceptable linearity with the serially diluted pooled serum, up to 18 mg/dL for apoE-HDLc. The system was not affected by triglycerides at concentrations up to 450 mg/dL in apoE-HDLc measurement. The apoE-HDLc levels of healthy volunteers determined by the present HPLC system were 5.3±1.6 mg/dL (n=26), which accounted for approximately 6-11% of total HDLc.

Conclusion: Our developed HPLC system with cation-exchange and heparin affinity columns showed a rapid and precise determination of apoE-HDLc levels in un-pretreated serum. The present system is thus useful in both clinical settings as well as for lipid research.

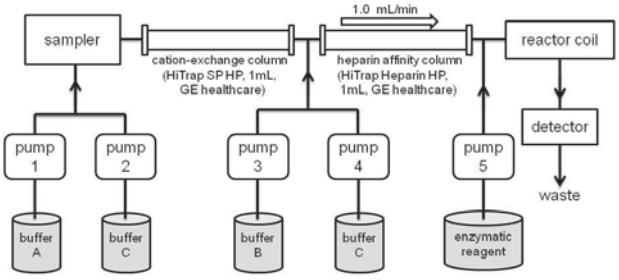

Fig.1 HPLC system
Buffers A (10 mM MOPS, 5 mM magnesium acetate, pH 7.2), B (10 mM MOPS, 0.1 M magnesium acetate, pH 7.2), and C (10 mM MOPS, 1.0 M sodium acetate, 0.01% nonionic detergent, pH 7.2) were used for eluting lipoproteins. The column effluent was mixed with an enzymatic reagent for cholesterol. Enzymatic reactions proceeded at 37°C in a reactor coil, and developed color was detected at 555 nm. Arrows indicate the flow direction.

B-123

Small Dense LDL Ratio Associates with the Metabolic Syndrome

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Background: Low-density lipoprotein (LDL) cholesterol is often not an effective predictor of cardiovascular risk because of the variability of the cholesterol content within lipid particles. We investigated the association of lipoprotein subclasses, classified with a polyacrylamide tube gel electrophoresis (PGE) method, with scoring for metabolic syndrome (MetS).

Methods: A total of 242 outpatients were scored into six groups, based on their number of MetS components (from 0 to 5 variables) defined by the NCEP ATP III criteria, modified for the Asian cutoff for waist circumference. Blood samples were analyzed for lipid profile, LDL subclass (Quantimetrix Lipoprint™, CA), and atherosclerosis-related markers: apolipoprotein A-I (apoA-I), apoB, glucose, hemoglobin A1c (HbA1C), high sensitive C-reactive protein (hsCRP), creatinine, cystatin C, and vitamin D. The PGE method separates the intermediate density lipoprotein (IDL) particles into three midbands (MID-A to C) and the LDL particles into larger-buoyant LDL (lbLDL; LDL1 and LDL2), small-dense LDL (sdLDL; LDL3 to LDL7), and HDL; sdLDL was calculated as the sum of LDL3 to LDL7.

Results: The mean levels of triglycerides, glucose, and HbA1C rose with increasing MetS score, whereas those of HDL-cholesterol decreased. However, the concentration of total cholesterol, LDL-cholesterol, non HDL-cholesterol, apoAI, hsCRP, and vitamin D did not trend with increasing MetS score (all P >0.170). Using PGE, the mean concentrations of VLDL, MIDC, MIDB, LDL2, and sdLDL positively correlated with increasing MetS score, but those of MIDA and LDL1 inversely correlated, similar to the pattern observed for HDL. Using backward stepwise logistic regression, MIDC, MIDB, MIDA, LDL1, LDL2, and sdLDL were considered the independent variables. LDL1 and sdLDL [regression coefficient = -0.033 and 0.054, odds ratio = 0.968 (95% CI, 0.943-0.994) and 1.055 (95% CI, 1.023-1.089), respectively] were identified as being significantly associated with MetS (P <0.02). In the logistic model, the sdLDL/LDL1 ratio showed the strongest association with MetS and demonstrated an odds ratio of 5.544 (2.030-14.542, 95% CI). For predicting MetS, the area under the ROC curve of the sdLDL/LDL1 ratio had the greatest diagnostic value (0.700), followed by VLDL (0.694), sdLDL (0.689), HDL (0.669), LDL1 (0.648), MIDC (0.605), and MIDB (0.596), which showed good discrimination power for MetS (P ≤0.010), whereas the value of MIDA (0.572) indicated a poor power (P = 0.055).

Conclusion: Respective subpopulations of IDL and LDL particles can vary in their ability to identify MetS. These variations may partially explain why a quantitative assessment using absolute LDL-cholesterol concentrations, as typically measured in conventional practice, is poorly associated with MetS. We show that the ratio of sdLDL/lbLDL is strongly associated with the metabolic syndrome (high odds ratio and highest area-under the curve). It may be a potentially important tool to maximize the effectiveness of risk assessment for cardiovascular disease.