Wednesday, July 29, 2015

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

Lipids/Lipoproteins

B-117

Quantitative Characterization of Protein Content from HDL and LDL Size Fractions

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Background:There is building evidence in recent years that measurement of LDL and HDL particle number concentration (LDL-P and HDL-P) in serum provides significant improvement in cardiovascular disease (CVD) risk assessment relative HDL and LDL cholesterol (HDL-C and LDL-C). The higher relative CVD risk associated with HDL-P and LDL-P is due to the particle size heterogeneity of both HDL and LDL. In spite of being potentially stronger risk factors, the more general use of clinical assays for quantification of particle number, particle size and other emerging lipoprotein associated protein/lipid biomarkers is impaired by lack of satisfactory inter-laboratory comparison and standardization. For this reason, we seek to develop a potentially gold standard method by preparatively separating lipoprotein sub-classes based on hydrodynamic size, coupled with quantitative protein/lipid measurements using isotope dilution mass spectrometry (IDMS).

Methods:A volume of 50 µL of human sera was separated by asymmetric field flow fractionation (AF4) into 20-40 fractions in a hydrodynamic diameter range of 5-45 nm in phosphate buffered saline. Size information was determined by dynamic light scattering measurements in each fraction after collection. An aliquot of each fraction was batch digested with trypsin. An additional aliquot was used for simultaneous basic hydrolysis of cholesteryl esters and triglycerides. Fast IDMS methods were developed for the high throughput quantitative analysis of all digested and hydrolyzed AF4 fractions and diluted serum aliquots, 3 min IDMS run for cholesterol and glycerol, and 8 min run for protein specific peptide cleavage products.

Results:Based on AF4 fractionation, size measurement, and IDMS analysis, highly selective concentration versus hydrodynamic size profiles were constructed for cholesterol, glycerol and apolipoproteins. Individual protein species profiles had a 2.5 nm half peak width on the hydrodynamic size scale allowing differentiation of 5 ApoA-I and >5 ApoB-100 containing subclasses using profile deconvolution, in the range 7-17 nm and in the 18-45 nm range respectively. The accuracy and precision of the concentration measurements of ApoA-I and ApoB-100 were evaluated based on currently used primary and secondary serum reference materials. Our IDMS method matched the assigned concentrations of ApoA-I and ApoB-100 with 95% accuracy and ~8% CV. This work has been expanded to the quantitative measurement of >10 proteins associated with ApoA-I and ApoB-100 containing sub-species based on size profile overlap in multiple serum samples with wide range of cholesterol and triglyceride levels. ApoA-II, ApoA-IV and ApoM were uniquely distributed in ApoA-I containing sub-fractions. ApoC-II/, ApoC-III and ApoE could be measured in both large size ApoA-I and small size ApoB-100 containing sub-fractions. A significant concentration of ApoE was in ~18 nm particle size fractions where it did not correlate with ApoA-I or ApoB-100 subclass concentration. The apolipoprotein profiles varied significantly between samples with low and high total cholesterol/triglyceride serum levels (low vs. high CVD risk samples).

Conclusions:We demonstrate an advanced lipoprotein particle sizing and sub-class particle number analysis approach which has the specificity, accuracy and precision to directly measure both size and composition of lipoproteins, in a manner that is traceable to universal calibrators.

B-118

A serum oxidized high-density lipoprotein marker and its association with metabolic syndrome in males

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Background: A high-density lipoprotein (HDL) particle, whose major protein is apolipoprotein A-I (apoA-I), plays relevant roles in cardioprotection with its antioxidant properties. The oxidative modification of apoA-I can be involved in dysfunctional HDL. Metabolic syndrome (MetS), a cardiovascular risk factor and an oxidative stress condition, is often accompanied by low HDL levels in the circulation. However, the cardiovascular burden of MetS is still not well understood with regard to dysfunctional HDL, and easy biomarkers for MetS-related HDL modifications are also needed. We developed a new assay to measure oxidized apoA-I, a suitable biomarker for oxidized HDL (oxHDL), since we found high oxHDL levels under several oxidative stress-related conditions. The aim of this study was to investigate the association between the oxHDL levels and the MetS status in males.

Methods: A total of 269 Japanese males (mean age, 61 years) were consecutively recruited from general health checkups. Any subjects who had been treated for cardiovascular diseases were excluded. Clinical data, including the waist circumference, blood pressure, and serum lipid and glucose levels, were obtained from the subjects in a fasting state. The serum oxHDL levels were quantified using a sandwich ELISA system, which utilizes monoclonal antibodies prepared by immunization with H2O2-oxidized human apoA-I. The presence of MetS was diagnosed by ≥ three of five metabolic criteria (including ≥ 85 cm of waist circumference for Japanese males).

Results: There were 77 subjects with MetS. The subjects with MetS showed a significantly lower level of mean HDL-cholesterol (1.27 mmol/L) than those without MetS (1.55 mmol/L, $p < 0.05$). The subjects with MetS showed a significantly higher level of the mean oxHDL/HDL-cholesterol ratio, an index of HDL oxidation, than those without (5.3 versus 4.3, respectively, $p < 0.05$). Additionally, a significant stepwise increase of the oxHDL/HDL-cholesterol ratio with an increase in the number of MetS criteria ($r = 0.2$, $p < 0.01$) was found.

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

B-119

Standardized NMR Spectroscopy for Metabolic Profiling in Clinical Diagnostics and Life Science Research

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Background

NMR spectroscopy is a powerful method for metabolomic profiling of human body fluids such as serum and urine. In lipoprotein profiling NMR has proven its unique potential for structural analysis of the complex lipoprotein particles, giving access to lipoprotein particle numbers and sizes as well as lipid concentrations.

Despite its potential and distinct advantages compared to other diagnostic systems, advanced lipoprotein testing by NMR spectroscopy has so far only partly found its way into routine diagnostics. The lack of accessibility and standardization, the complexity of the system, as well as long-term spectrometer drifts and insufficient comparability of different devices are reasons for this situation.

Here we describe a robust and highly standardized measurement process which overcomes individual spectrometer variability and other contributors to variation to achieve consistently reliable results even on different devices. According to a high level of automation and standardization, NMR spectroscopy now becomes available for routine diagnostics.

Methods

Our method provides particle concentrations and subfractions, mean particle sizes, cholesterol concentrations in fractions and subfractions, standard lipid parameter (total cholesterol, triglycerides, LDL-C, HDL-C) as well as metabolic parameters such as glucose, lactate, alanine and other amino acids.

Lipoprotein analysis was conducted via deconvolution of the broad methyl group signal at about 0.90.8 ppm utilizing fourteen base functions. In this process,

lipoprotein subclasses are reflected by this fixed number of predefined bell shaped base functions, each of which has a constant position and defined width. From the resulting fit parameters we compute the integrals attributable to each base function. NMR lipoprotein profiling is then carried out by finding an optimal linear combination of these base functions that deviates from the originally measured methyl NMR signal as little as possible. To assess the lipid concentrations, a conversion step is necessary to weight the achieved integrals of the base functions based on their contribution to the total signal fit.

Results

Based on our unique calibrator system, we end up with excellent precision, i.e. a mean total imprecision between 3 different systems of 3.7%, a repeatability of all within-runs < 4.5%, and a reproducibility between 3 different sites <5.1%. Thus, according to the high degree of standardization and automation, it is possible to acquire several hundred serum spectra per day on a single instrument in routine operation.

Conclusion

We have demonstrated that our automated NMR approach is capable of creating a standardized, stable and reproducible spectrum output, independent of spectrometer instabilities or differences, and thus is suitable for routine diagnostics. With respect to lipoprotein profiling our approach may be used for diagnostic tests to identify patients at risk for CVD, predict and monitor the course of the disease or monitor treatment efficacy. Furthermore, the approach can be used in Life Science Research for biomarker discovery, validation and development of new diagnostic tests

B-121

The LDLR variant c.1426C>T; p.P476S as a novel cause of Familial Hypercholesterolaemia

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Objective

The aim was to identify the cause of a raised serum cholesterol concentration of 8.8 mmol/L in a 26 year old male.

Methodology

On clinical examination there was no evidence of tendon xanthomas nor a family history of hypercholesterolaemia or premature death from vascular disease. The patient did not meet the Simon Broome criteria for Familial Hypercholesterolaemia (FH) and had a BMI of 31.5 kg/m^2. Lifestyle modification was suggested.

The following year a younger brother presented with a raised cholesterol concentration of 9.6 mmol/L. He was screened by fluorescent sequence analysis of the promoter and coding sequence (including intron-exon boundaries) of the LDLR gene, and for PCSK9 (exon 7) and ApoB (part of exon 26). MLPA analysis was also carried out for the LDLR gene. The variant c.1426C>T;p.P476S within exon 10 of the LDLR gene was identified. Although this mutation has been reported in one paper as a cause of FH, computer predictions are equivocal. The mutation was reported as possibly pathogenic and may be consistent with the diagnosis of FH.

Fluorescent DNA sequence analysis identified the same LDLR variant in the patient, their mother and a sister. The mother had a cholesterol concentration of 7.8 mmol/L and the sister had a cholesterol concentration of 9.1 mmol/L. The variant was absent in the father who had a cholesterol of 6.6 mmol/L. The mutation therefore does co-segregate with raised cholesterol concentrations within the family and the diagnosis of FH was given.

Outcome

LDL receptor mutations are the most common genetic defect and the prevalence of heterozygous FH in the UK population is estimated to be 1 in 500. The LDLR variant c.1426C>T;p.P476S is a novel mutation and the cause of the families raised cholesterol. Atorvastatin was prescribed to lower the cholesterol and due to the 50% risk of transmitting the variant to offspring the siblings have been referred for genetic counselling.

B-122

Large HDL-Cholesterol Concentrations Predict Long-term Outcomes in Patients with Acute Coronary Syndrome

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Background: HDL-cholesterol is an important independent predictor of atherosclerosis, however, it remains to be determined how HDL subfractions are associated with long-term cardiovascular events in patients with acute coronary syndrome (ACS).

Methods: Concentrations of cholesterol and apolipoproteins (Apo) in large (HDL2) and small (HDL3) HDL fractions separated by heparin-Mn and dextran sulfate precipitation methods were measured at the onset of ACS in 146 patients (119 men), aged 64.3 +/- 11.8 years. Cardiovascular events defined as death, ACS, stroke, heart failure requiring hospitalization and/or any revascularization were evaluated during 6-year follow-up periods.

Results: Not HDL-cholesterol, ApoA1, HDL3-cholesterol, or HDL3-apoA1 but lower levels of cholesterol and apoA1 in HDL2 were associated with higher incidence of cardiovascular events (p = 0.045 log-rank 4.037).

Conclusion: Measurement of HDL2 fractions is an important strategy to detect high-risk patients with ACS.

B-123

Carotid artery IMT is more closely related to small dense low-density lipoprotein cholesterol concentrations than other lipid parameters

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BACKGROUND: Small dense low-density lipoprotein cholesterol (sdLDL-C) concentrations correlate more strongly with coronary heart disease than total LDL-C and large LDL particle concentrations. We investigated the association between carotid artery intima-media thickness (IMT) and sdLDL-C concentrations in Japanese subjects.

METHODS: Carotid artery IMT, blood pressure (BP), fasting plasma sdLDL-C, glucose metabolism, lipid, and C-reactive protein concentrations were measured in 97 native Japanese subjects. Carotid artery IMT was assessed by ultrasonography, and sdLDL-C concentrations were measured by a homogenous assay (Denka Seiken Co., Ltd.). Pearson's correlation coefficient analyses and multiple regression analyses were used to examine the relationships between carotid artery IMT values, sdLDL-C values, and other clinical variables.

RESULTS: After multiple regression analysis, including age, sex, body mass index, systolic BP, diastolic BP, fasting plasma glucose, HbA1c, estimated glomerular filtration rate (eGFR), total-cholesterol, high-density lipoprotein (HDL)-C, triglyceride, LDL-C, non-HDL-C, large buoyant LDL-C, and sdLDL-C, carotid artery IMT remained significantly associated with age, systolic BP, diastolic BP, and sdLDL-C, whereas sdLDL-C remained significantly associated with age, total-cholesterol, HDL-C, triglycerides, and carotid artery IMT.

CONCLUSION: Carotid artery IMT has a closer relationship with sdLDL-C concentrations than other lipid parameters in Japanese subjects. sdLDL-C may be a potentially useful risk marker when assessing carotid artery IMT in Japanese subjects.

B-125

The AtherOx™ Assay detects oxLDL-β2GPI antigen complexes associated with atherosclerotic vascular disease

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Background: Low-density lipoprotein (LDL) is the principal form of cholesterol that accumulates in atherosclerotic lesions or plaques, but before accumulation occurs,

it must be modified into oxidized LDL (oxLDL) by oxidative stress. Only oxidized LDL binds to β2 glycoprotein 1 (β2GPI) to form oxLDL-β2GPI complexes; initially as unstable complexes followed by the formation of stable complexes. While highly pathogenic free oxLDL can be detected in serum, complexed oxidized LDL is also pathogenic and can be detected more reliably in serum. OxLDL-β2GPI complexes act as pro-inflammatory chemotactic factors for T lymphocytes and macrophage uptake of complexes leading to the development of foam cells within atherosclerotic lesions. Thus, circulating oxLDL-β2GPI complexes are implicated as pro-atherogenic antigens which may represent a serologic risk factor for the development of thrombosis and atherosclerosis. Here we describe the analytical and clinical performance of the AtherOx™ ELISA for the detection of oxLDL-β2GPI antigen complex in serum.

Methods: The AtherOx™ test is performed as an indirect ELISA. Diluted serum samples, calibrator, and controls are incubated in microwells coated with a monoclonal antibody directed towards only complexed oxLDL-β2GPI. After washing, biotin conjugated detection monoclonal antibodies specific for human apoB100 are added, followed by horseradish peroxidase conjugated to Streptavidin. Following substrate development, sample values are determined by comparison of optical densities to a calibrator curve on a 0-5 units/mL scale. Pre-defined assay performance characteristics were compared across two lots including control recovery, determination of LOB/LOD/LOQ, Linearity, Precision, Prozone, and Interference Testing. OxLDL-β2GPI and free oxLDL levels were compared in human subjects with and without vascular disease.

Results: The Limit of Blank (LOB) of the assay is 0.025 units/mL. The LOD is 0.075 units/mL and the LOQ is 0.19 units/mL. The linear range was determined to be 0.2 to 7.5 U/mL. Control recoveries were within labeled ranges with CVs between 9 and 12%. Within run precision of three levels spanning the linear range of the assay was 6-11%. Total precision was 12-23%. The assay exhibits no prozone effect during assays of synthetic oxLDL or very high titer human serum. Twenty four interfering substances, drugs, uncomplexed oxLDL and β2GPI were tested in the AtherOx™ assay. Only high levels of hemoglobin interfered with value recovery of serum samples. The assay exhibits strong specificity for only complexed oxLDL- β2GPI. In clinical studies, patients with vascular disease showed no difference in free oxLDL levels ($p=0.8$), but significantly higher values ($p<0.0001$) of serum oxLDL- β2GPI than controls assayed with the AtherOx™ assay.

Conclusion: Based on the performance data, the AtherOx™ test kit can be manufactured consistently across multiple lots and meets performance criteria. The assay has robust precision, linearity, interference and LOB/LOD/LOQ profiles. A significant increase in AtherOx™ levels was detected in patients with atherosclerotic vascular disease. These data point to the utility of detecting complexed oxLDL-β2GPI as a biomarker for the development of thrombosis and atherosclerosis.

B-126

Correlation between subfractional high-density lipoprotein cholesterol levels and obesity traits: an electrophoretic method and a homogeneous assay method

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Background:

Subfractional high-density lipoprotein (HDL) analyses can be useful for assessing the cardiovascular risk relative to a simple measurement of total HDL. A homogeneous assay for HDL2- and HDL3-cholesterol (HDL2-C and HDL3-C) was recently developed with a good correlation with a standard ultracentrifugation method, and its clinical applications are being studied. Obesity is known to modulate HDL metabolism and often induces low HDL-C levels. This study was performed to see the correlation between the subfractional HDL-C ratio, as measured by the homogeneous method, and the obesity traits.

Methods:

The serum samples of 54 subjects were analyzed by the direct assay for HDL-C, as well as the homogeneous method (Denka Seiken Co. Ltd., Japan) and the electrophoretic method for subfractional HDL levels. The data obtained by the electrophoretic method were expressed as the cholesterol levels of respective subfractions (with multiplication by total HDL-C). The HDL2-C/HDL3-C ratio and the large (L)+intermediate (IM) HDL-C/small (S) HDL-C ratio are possible indices for the obesity traits.

Results:

The subjects' mean levels were as follows: body mass index (BMI) 24 kg/m2, HDL-C 67 mg/dL, HDL2-C 40 mg/dL, HDL3-C 23 mg/dL, HDL2-C/HDL-3-C ratio 1.8, L+IM HDL-C 59 mg/dL, S HDL-C 8 mg/dL and L+IM HDL-C/S HDL-C ratio 13. The correlation coefficients were as follows: HDL2-C/HDL3-C ratio and BMI -0.33 ($p = 0.02$), and L+IM HDL-C/S HDL-C ratio and BMI 0.07 ($p > 0.05$).

Conclusion:

The HDL2-C/HDL-3-C ratio by the homogeneous method was significantly correlated with the BMI, suggesting the subfractional HDL-C ratio by the method might be helpful for observing the obesity-related pathophysiology. Subfractional HDL analyses by the electrophoretic method may also provide different information from those by the homogeneous method, and further studies using both methods are warranted.

B-127

Accurate Assessment of LDL Cholesterol Reduction at Levels below 70mg/dL has Implications in the Estimation of Efficacy for New Drugs in Development

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Objective: The objective of this study was to compare low density lipoprotein cholesterol (LDL-C) measurements obtained by two calculated methods, the Friedewald and Hopkins formulas, and a direct homogeneous enzymatic assay to preparative ultracentrifugation (PUC).

Relevance: Calculated LDL-C by the Friedewald formula has been the foundation for assessing both clinical response to lipid modifying therapies and determination of efficacy in clinical trials. As new drugs in development achieve very low LDL-C levels, the validity of the Friedewald formula is questioned as it significantly underestimates LDL-C < 70 mg/dL, with increasing inaccuracy when used to estimate levels < 50 mg/dL. Recently, the Hopkins method for calculating LDL-C has been introduced; which is based on the Friedewald formula but uses a variable triglyceride (TG):very low-density lipoprotein cholesterol ratio. PUC is widely considered to be the "gold standard", reference method to measure LDL-C, but is labor-intensive and requires specialized instrumentation. Direct, homogenous enzymatic methods have been proposed as an alternative.

Methodology: LDL-C measurements for PUC, a direct homogenous enzymatic assay, and calculations by both Friedewald and Hopkins formulas were obtained from 1299 patients. Samples were analyzed in a CDC-NHLBI Part 3 lipid standardized central laboratory. The four methods were compared for LDL-C across TG cutpoints.

Results: See table. Overall, both direct and calculated methodologies were significantly different from PUC at LDL-C < 70 mg/dL with increasing bias at LDL-C ≤50 and 25 mg/dL. However, the direct assay varied less from PUC than the calculated methods.

Conclusion: At LDL-C levels < 70 mg/dL, LDL-C calculated by both the Friedewald and Hopkins formulas, and 'direct' LDL-C measurement, show significant differences when compared to LDL-C by PUC. Underestimation of LDL-C levels when post treatment LDL-C is very low has implications for the accurate assessment of LDL-C lowering by new therapeutic agents in development.

Summary Statistics of Calculated LDL-C and PUC LDL-C by PUC Categories

		PUC	Direct LDL		Friedewald		Hopkins	
PUC LDL-C Category (mg/dL)	N	Mean	Mean	% Diff,a	Mean	% Diff,b	Mean	% Diff,c
≤25	322	18.1	18.9	8.8	12.3	-32.9	14.6	-19.7
26-50	538	36.0	34.3	-4.3	28.5	-20.9	32.8	-9.3
51-70	87	59.5	57.0	-4.1	50.9	-14.3	58.1	-2.3
71-100	76	86.2	83.6	-2.7	80.2	-6.9	84.2	-2.2
101-200	258	138.0	132.9	-3.7	133.4	-3.4	135.8	-1.4
>200	18	267.5	235.6	-3.5	261.9	-2.4	261.4	-2.4
≤50	860	29.3	28.6	0.6	22.4	-25.4	26.0	-13.2
≤70	947	32.1	31.2	0.1	25.1	-24.4	28.9	-12.2
≤100	1023	36.1	35.0	-0.1	29.2	-23.1	33.0	-11.4

a, Percent difference = 100*(Direct LDL – BQ LDL-C)/BQ LDL-C; b, Percent difference = 100*(Calculated LDL-C by Friedewald – BQ LDL-C)/BQ LDL-C; c, Percent difference = 100*(Calculated LDL-C by Hopkin's – BQ LDL-C)/BQ LDL-C P-values are from a one sample t-test performed on percent difference.

B-129

Incidence of risk factors in patients with acute myocardial infarction at young age

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Background: The incidence of acute myocardial infarction (AMI) in people under age 45 years is rare, less than 5% of all cases. It is emphasized that presentation of AMI in this population is peculiar, whose etiopathogenic, anatomic and prognostic characteristics are singular, being different from older patients. The purpose of this study was describe social-demographic-metabolic outline and incidence of cardiovascular risk factors in patients (age below 45 years) with AMI diagnosis. **Methods:** This was a cross-sectional study conducted from November 2010 to January 2015 which included 103 young patients under age 45 years, both sex, with AMI diagnosis, established based on clinical and coronary angiography criteria. The laboratorial evaluation includes analysis of glycosylated hemoglobin, fasting blood glucose, triglycerides, total cholesterol and fractions. The cardiovascular risk factors evaluated were smoking, alcoholism, familiar history, dyslipidemia, hypertension, diabetes mellitus, sedentary behavior and metabolic syndrome. **Results:** A total of 103 patients were included. The mean age of participants at the time of study was 39,6 ± 5,7 years. Fifty-eight percent of the participants were men. Smoking was found in 57 % of cases. Alcoholism and sedentary behavior were presented in 7% e 81% of persons respectively. Diagnosis of hypertension and dysglycemia were evidenced in 42% and 69%, respectively. Metabolic syndrome was confirmed in 58% of patients. Forty-one percent of persons had positive familiar history for coronary arterial disease. The ST-segment elevation was identified in 59% of cases. The most commonly compromising coronary artery was descendent anterior (72%). The subjects showed unfavorable lipid profile characterized for low levels of HDL-cholesterol (91% of men with HDL-C ≤ 40mg/dL and 95% of women with HDL-C ≤ 50mg/dL). **Conclusion:** Acute coronary syndrome in people younger than 45 years is rare and appears to be associated with dysglycemia and unhealthy lifestyle: smoking, hypertension, sedentary behavior and low HDL-cholesterol.

B-130

Validation of a new equation for LDL-c estimation in a German population-based study cohort

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Background: A recently established simple equation by Cordova & Cordova [LDL-COR = 3/4 (Total Cholesterol - HDL-cholesterol)], provided an improved estimation of LDL-cholesterol and correlated higher with directly measured LDL-c compared to the Friedewald's Formula (LDL-FW) in a sample of 10,664 Brazilian individuals. It showed a better performance compared to other previously published equations using or not triglycerides (TG) values. However, validation of this new equation in a distinct population is mandatory.

Methods: We have evaluated the performance of this new equation in a German general population cohort (SHIP), also considering age, gender, use of lipid lowering therapy, and associated co-morbidities such as diabetes, hepatic, kidney and thyroid disease. In total, we used data from 4,075 individuals from SHIP (SHIP-0: 1997-2001) aged 20 to 79 years. We excluded subjects with TG>400 mg/dL or with an incomplete lipid profile. Data (in quartiles when appropriated) were analyzed by linear regression models (with 95% Confidence Interval) and also by fractional polynomials to account for possible non-linear associations.

Results: LDL-COR showed a better performance, with a lower standard deviation (28.83%), compared to the previously published equations (31.42% for LDL-FW). The differences between LDL-COR and LDL-FW increase with higher Total Cholesterol [-6.6 (-27.60 to 15.49); -9.9 (33.80 to 13.99); -12.67 (-38.27 to 12.92); -21.21 (50.38 to 7.96)] and HDL-c [-6.38 (-35.63 to 22.86); -11.73 (-38.89 to 15.43); -14.43 (-39.34 to 10.48); -15.93 (-40.76 to 8.90)] quartiles, and decrease with higher TG quartiles [-20.03 (-38.03 to -2.02); -17.76 (-37.23 to 1.70); -13.22 (-34.68 to 8.24); 1.87 (-25.03 to 28.78)]. Considering the co-morbidities and the other evaluated factors, all except fibrates, being TSH borderline (P=0.06), were associated with the difference between LDL-COR and LDL-FW (P<0.01).

Conclusion: It is possible to hypothesize that, if there is a change in the absolute difference between the two formulae in these conditions, the LDL-FW values are being mostly affected, and not the ones obtained by LDL-COR, since these co-morbidities are well known conditions to affect the values obtained by LDL-FW. A study evaluating the performance of the new formula using a reference method (ultra-centrifugation and beta-quantification) in a well-defined population is still necessary to validate its use. Support: DAAD, CAPES, CNPq, FURB.

B-132

Is lipoprotein lipase a monomer or dimmer in pre- and post-heparin plasma?

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Background:

The structure of lipoprotein lipase (LPL) has been long controversial because of the difference of enzymatic activity between pre-heparin and post-heparin plasma. Active form of LPL found in post-heparin plasma has been recognized as dimmer LPL, while inactive form of LPL found in pre-heparin plasma has been believed as monomer LPL. However,

we recently found that dimmer form of LPL prevalently exists in pre-heparin plasma. Using LPL-ELISA and LPL activity assay, we have investigated the structure and function of LPL in pre-heparin plasma. Also the relationship between circulating LPL and remnant lipoproteins (RLP) in plasma was investigated.

Methods:

Monoclonal antibodies against LPL (57A5 and 88B8) were raised against human recombinant LPL and established two sandwich ELISA systems. LPL mass and activity were determined in healthy volunteers (n=40) of pre-heparin and post-heparin plasma with TG, HDL, RLP-C, RLP-TG, apoC1, apoC3, apoE and other plasma parameters.

Results:

Two LPL-ELISA systems were established. One assay used two different monoclonal antibodies against LPL (57A5 and 88B8) for the sandwich ELISA which can detect both monomer (inactive form) and dimer LPL (active form) (Assay 1). The other assay used the same monoclonal antibody (88B8) for the sandwich ELISA which can detect dimer

LPL only (Assay 2). The Assay 2 detected about 70 % of LPL concentration compared with Assay 1 in both pre- and post-heparin plasma using the same recombinant LPL as a calibrator. Both ELISAs were significantly correlated with activity assay in post-heparin plasma (r=0.6). Pre-heparin LPL mass was less than 20% of post-heparin LPL mass. In pre-heparin plasma, more than 80% of LPL mass was found in RLP but no activity was detected in it. LPL mass in RLP increased 2-folds with inactive form after heparin injection, while more than 5-fold increase of LPL mass in post-heparin plasma with significantly high LPL activity. Significant amount of apoC1 and apoC3 were detected in RLP, suggesting the inhibition of LPL activity.

Conclusion:

Sensitive and specific LPL-ELISA assays were developed to distinguish a monomer and dimmer form of LPL in RLP with and without heparin injection. More than 80% of LPL mass in pre-heparin plasma was found in remnant lipoproteins. LPL in RLP isolated from both pre-heparin and post-heparin plasma was found to be inactive, although those LPL was found to have mostly dimmer structure. ApoC1 or C3 in RLP may inhibit the LPL activity in spite of dimmer structure.

B-133

Does increased adiponectin influence lipid profile and inflammatory status in chronic kidney disease undergoing hemodialysis?

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Patients with chronic kidney disease present markedly elevated mortality from cardiovascular causes, even in patients undergoing hemodialysis (HD) substitutive treatment. Adiponectin (ADP) is an anti-inflammatory and insulin-sensitizing cytokine decreased in insulin resistance (IR) states. End-stage renal disease (ESRD) is associated with insulin resistance, nevertheless, ADP has been observed paradoxically elevated, probably due to an impairment in renal clearance rate of ADP. It is not clear if elevated ADP function in ESRD is conserved. Objective: To assess whether increased ADP in HD patients is associated with a more favorable metabolic and inflammatory profile.

We studied 120 HD patients. Fasting blood was drawn in the interdialysis day and serum total ADP was measured by ELISA method. According to the median of ADP (18 ug/ml) in the studied population, two groups were established (n=60 each): High ADP levels (ADP-HL) ≥18.0 ug/ml (range: 18.0 to 33.2) and low ADP levels (ADP-LL) <18.0 (4.3 to 17.9). Both groups did not differ in age (61±14 years-old vs 62±19; p=0.99), gender (F/M: 32/28vs 25/35; p=0.27), duration of HD treatment (12±3 years vs 14±5; p=0.86) and frequency of diabetes (diabetes/no-diabetes: 20/40 vs 24/36; p=0.18). Serum lipid profile, inflammatory and insulin resistance parameters were assessed.

The ADP-HL group showed lower triglyceride (TG) (1.32 ± 0.63 vs 1.81 ± 0.91 mmol/L, p <0.001) and higher HDL-cholesterol (1.14 ± 0.31 vs 1.01 ± 0.36 mmol/L, p = 0.03). Free fatty acids (p=0.87) and LDL-cholesterol (p=0.54) showed no differences between groups.

Insulin levels were lower in ADP-HL, median (range): 8.0 (2.0 to 32.9) IU/L vs 12.7 (2.0 to 44.9) p=0.008, without differences in fasting glucose concentration between groups (p=0.07). ADP-HL showed a HOMA-IR: 1.57 (0.30 to 10.70) and ADP-LL: 2.24 (0.31 to 9.65) p=0.003. As expected, ADP was positively associated with HDL-cholesterol (r=0.33, p<0.001) and negatively with TG (r=-0.27, p<0.001), insulin (r=-0.28, p=0.02) and HOMA-IR (r=-0.30, p=0.01).

Regarding the analysis of inflammatory markers, all patients with hs-CRP over 10 mg/L were excluded (n=8 in ADP-HL and n=10 in ADP-LL). No differences were observed between groups in hs-CRP (ADP-HL: 4.9±3.2 mg/L vs ADP-LL: 5.9±3.5 p=0.21) and interleukin-6 (ADP-HL: 11.5 (2.8-43.1) pg/mL vs ADP-LL: 10.0 (4.3-43.3) p=0.79. No significant correlations between ADP and inflammatory markers were found.

Conclusion: elevated ADP in HD patients would act favorably on lipid profile and insulin sensitization, but it may not exert its anti-inflammatory function possibly due to a deregulation of adiponectin signal pathways.

B-134

Elevated small dense LDL cholesterol in metabolic syndrome and diabetes patients with a fatty liver

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Background: Serum small dense LDL-cholesterol (sdLDL-C) levels were determined in healthy controls, type 2 diabetic patients (T2DM) and people suffering metabolic syndrome (MetS) with or without a fatty liver.

Methods: The plasma lipids and lipoproteins including sdLDL-C were determined in controls, MetS and T2DM patients (n = 5255). sdLDL-C levels were measured by a homogeneous assay (Denka Seiken Co., Ltd.). The cases with MetS and preliminary MetS (pre-MetS) as well as T2DM and preliminary T2DM (pre-DM) were selected based on the Japanese criteria. Fatty liver was diagnosed using the ultrasonography.

Results: The 75th percentile values for sdLDL-C were 27.5 mg/dl for men and 23.3 mg/dl for women and increased with age. Significantly increased sdLDL-C concentrations were found in the controls, pre-MetS, MetS and pre-T2DM and, T2DM cases with a fatty liver compared to the groups without a fatty liver

Conclusion: Fatty liver significantly increased serum sdLDL-C levels and the multiple regression analyses ascertained that fatty liver was an independent determinant for sdLDL-C levels in serum.

B-135

The usefulness of a new equation to estimate LDL cholesterol compared with the Friedewald formula and direct measurement for the assessment of cardiovascular risk according to current European guidelines

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Introduction: Cardiovascular risk (CVR) assessment is based on Total, HDL, LDL Cholesterol (TC, HDL, LDL) and Triglycerides (TG) concentrations. A new equation (NE) for estimating LDL has been proposed as an alternative to the Friedewald formula (FF). At our hospital we measure LDL (mLDL) with TG>250 mg/dL, due to possible inaccuracies using FF to estimate LDL.

Objective: Evaluate the usefulness of the new equation to estimate LDL cholesterol when compared to Friedewald formula for the assessment of cardiovascular risk in patients with moderate hypertriglyceridemia.

Patients and Methods: Results for TC, mLDL, HDL and TG (Advia 2400; Siemens HD) from patients with moderate hypertriglyceridemia (250-400 mg/dL) were gathered from laboratory information system over a ten months period, and NE and FF calculated. LDL intervals derived from 2012 European ESC/EAS Guideline were used. Concordance between mLDL and estimated LDL by both equations were calculated using the Cohen's Kappa index (k) with a 95% Confidence Interval (CI). Correct patient's classification according to CVR guidelines was studied.

Results: 7120 results from 5870 patients (60.9% males) were obtained; median age was 54 years (IQR 44-65). Medians and IQRs for concentrations in mg/dL are: TC 202 (174-230); mLDL 120 (95-147); HDL 39 (33-45); TG 289 (267-326).

Next table shows the concordance (Kappa index) and percentage of correct classification of both equations when compared to mLDL.

mLDL (mg/dL)	LDL NE	LDL FF
	Kappa index (CI 95%)	
	0.609 (0.595-0.623)	0.327 (0.313-0.340)
	% Correct clasification (CI 95%)	
<70 (n=667)	62.1 (58.3-65.8)	90.6 (88.3-92.8)
70-99 (n=1510)	72.8 (70.5-75.1)	42.0 (39.5-44.5)
100-154 (n=3558)	85.4 (84.2-86.6)	57.7 (56.0-59.4)
154-189 (n=998)	48.1 (44.9-51.3)	25.2 (22.4-27.9)
>189 (n=387)	57.4 (52.3-62.4)	41.1 (36.1-46.1)
Total (n=7120)	**73.9 (72.8-74.9)**	**52.0 (50.8-53.2)**

Conclusions: In patients with moderate hypertrygliceridemia there is a good concordance using the new equation for CVR assessment when compared to mLDL versus FF (fair agreement).

In this group, NE improves the correct classification of patient's CVR according to current European guidelines.

New equation can be an adequate alternative to assess the CVR in patients with moderate hypertriglyceridemia with an added advantage for saving of material resources.

B-136

Validation of a New Lipoprotein(a) Particle Number ELISA

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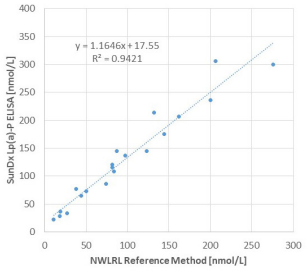
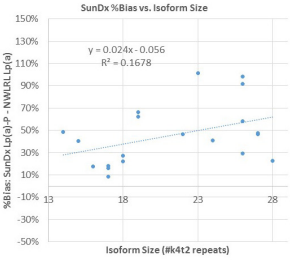
Background: Measurement of lipoprotein(a) particle number [Lp(a)-P] is important in CHD risk assessment. Unfortunately, turbidimetric assays for Lp(a) mass are biased due to apo(a) size isoforms. Therefore, we developed an ELISA for Lp(a)-P without isoform bias. Methods: Limit of the Blank (LOB) was determined using delipidated human serum assayed 50X over 5 days (LOB = mean ± 1.654 SD). Limit of Detection (LOD) was determined by measuring 2 serum samples with low Lp(a) ~50X over 5 days (LOD = LOB + 1.654 SD). LOQ was the lowest concentration with <20% CV. Precision was assessed by measuring 10 replicates of 5 serum pools over 5

plates. Linearity was assessed by intermixing samples with low and high Lp(a) pools. Accuracy and bias vs. size isoforms was assessed using 20 samples from Northwest Lipid and Diabetes Research Laboratory (Seattle, WA) with reference method-assigned concentrations and known kringle 4 type 2 repeats by linear regression analysis. Results: The LOB and LOD were 1 and 13 nmol/L, respectively. The LOQ was equal to the LOD. Imprecision at the extreme low and high ends of the analytical range were 12.1% and 11.4%, respectively. Imprecision in the midrange varied from 6.9% to 7.7%. Results were linear from ~2 to 500 nmol/L. Lp(a)-P was highly correlated to the reference method (r=0.971) with an average bias of 34 nmol/L, which was corrected by adjusting the master calibrator assigned values. There was no statistically significant association between bias and the number of kringle 4 type 2 repeats. Conclusion: The Sun Dx Lp(a)-P ELISA is sensitive, precise, and linear over a wider analytical range than most Lp(a) assays, and is strongly correlated to the reference method (r= 0.971). Importantly, the assay shows no bias due to apo(a) size isoforms.





B-137

Accuracy and Precision of Cholesterol, Triglycerides and HDL-Cholesterol Methods: A Retrospective Assessment of Quality

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Introduction: Accuracy-based proficiency testing programs utilize commutable samples and reference laboratory results to assess accuracy and precision of routine clinical methods. The Institute for Quality Management in Healthcare (IQMH) provides ISO 17043:2010 accredited Proficiency Testing (PT) programs for lipids. Ten years of data was evaluated to determine average bias and imprecision, and then compared to desirable performance targets based on biological variation.

Methods: Twenty-nine surveys distributed between June 2005 and December 2014 were included. PT samples consisted of single donor sera from healthy donors with no additives. Two challenges were distributed in each survey. Participants' results were assessed against the Centers for Disease Control and Prevention's certified methods. Robust statistics based on ISO 13528:2005 were used to calculate peer group means and standard deviations to eliminate outliers' effects.

Results: Cholesterol (CHOL) was assessed across all PT samples with 374 peer groups; average method bias: -0.23% (range: 19.4%-6.5%), average CV: 1.9% (range: 0.4%-11.3%). For triglycerides (TRIG), 362 peer groups; average method bias: -3.26% (range: -23.8%-12.2%); CV: 2.8% (range: 0.4%-14.8%). HDL-Cholesterol (HDL-C), 372 peer groups; average method bias: 0.9% (range: -34.6%-105.4 %); CV: 3.5% (range 0.7%-14.6%).

Ninety-five per cent of method bias estimates were within the desirable limits for CHOL; 91% and 69% for triglycerides and HDL-C. Ninety-five per cent of the peer group CVs were within the desirable CV limits for CHOL; 99% for TRIG and 66% for HDL-C.

Table 1. Average Method Bias and peer group CV values

	CHOL		TRIG		HDL-C	
	Bias (%)[b] (N)[c]	CV (%) (N)	Bias (%) (N)	CV (%) (N)	Bias (%) (N)	CV (%) (N)
Desirable Target[a]	4.1%	3.0%	9.6%	10%	5.6%	3.7%
Optimum Target[a]	2.1%	1.5%	4.8%	5.0%	2.8%	1.8%
Abbott	1.4 (40)	1.3 (40)	2.2 (38)	2.4 (38)	3.0 (38)	3.7 (38)
Beckman Coulter	-0.4 (80)	1.7 (80)	-6.3 (77)	2.8 (78)	-1.2 (80)	4.5 (80)
Ortho	-0.8 (80)	2.4 (80)	-2.0 (77)	2.5 (78)	-0.6 (80)	3.6 (80)
Roche	0.5 (34)	1.9 (34)	-2.1 (32)	2.8 (32)	1.3 (34)	2.7 (34)
Roche (BMC)	-0.7 (46)	1.7 (46)	-3.1 (45)	2.2 (46)	3.7 (46)	3.0 (46)
Siemens (Bayer)	-0.6 (36)	1.8 (36)	-3.5 (34)	1.8 (34)	0.0 (36)	2.5 (36)
Siemens (DB)	-0.1 (58)	2.3 (58)	-2.2 (56)	4.8 (56)	2.4 (58)	3.3 (58)

[a]Desirable and optimum targets refer to desirable and optimum specifications based on biological variation.
[b]Bias indicates the % difference between a peer group mean and reference laboratory result.
[c]N: number of peer groups. Analyte concentration ranges were cholesterol [117-364 mg/dL (3.0 -9.4mmol/L)], triglycerides [49 -1537 mg/dL (0.6 – 17.4 mmol/L)] and HDL-Cholesterol [22-115 mg/dL (0.6 -3.0 mmol/L)].

Conclusion: Overall biases for CHOL, TRIG and HDL-C and the imprecision for CHOL and TRIG were within the desirable limits. However there were peer groups bias and CV values exceeding these limits. This was more prominent in the HDL-C with only 69% of the HDL-C method bias and 66% of the CV estimates were within the desirable limits, indicating the lack of method standardization.

B-138

Hepatic triglyceride lipase is mainly distributed on apoE-rich HD in post-heparin plasma

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Background: We developed HPLC system with cation-exchange and heparin affinity columns to isolate and determine apoE-rich HDL in serum. As HTGL is known to metabolize HDL2 to HDL3, we have studied the relationship between HTGL activity and apoE-rich HDL, using our specific apoE-rich HDL isolation technique.Method: The HPLC system (Tosoh) consisted of AS-8020 sampler, five pumps (CCPS and CCPM-II) and UV-8020 detector and a SC-8020 system controller (Tosoh) was used for instrument regulation. Two columns were used tandemly to determine apoE-HDLc in plasma. First, a cation-exchange column (HiTrap SP HP, 1 mL, GE healthcare) was used for retaining non-HDL lipoproteins. Second, a heparin affinity column (HiTrap Heparin HP, 1 mL, GE healthcare) was used for retaining apoE-HDL. Three types of elution buffers for a step-wise gradient were mixed on line at a constant flow rate of 1.0 mL/min. To analyze HTG distributions in post-heparin plasma, 0.4-min fractions were collected and analyzed for HTGL activity by Imamura method (J Lipid Res 2008; 49: 1431). Remnant lipoprotein (RLP) fraction was isolated by immuno-separation method and used for the detection of HTGL and LPL activity and mass in RLP isolated by HPLC. Thirty units/kg of heparin was injected to healthy controls and post-heparin plasma was collected in 15 min. Tetrahydrolipstatin (lipase inhibitor) was added in test tube right after blood withdrawal. Results: Post-heparin plasma was applied to this HPLC system and fractionated into three groups, apoE-poor HDL, apoE-rich HDL and non-HDL, respectively. Also RLP fraction isolated from the post-heparin plasma by immunoaffinity gel was applied to this HPLC system. HTGL activity and mass was prevalently found in apoE-rich HDL. No LPL activity and mass was found in apoE-rich HDL. HTGL activity and mass was not found in apoE-poor HDL and non-HDL fraction. HTGL activity was completely inhibited by tetrahydrolipstatin in plasma and RLP.

Conclusion: This is the first report that apoE-rich HDL prevalently carries HTGL in post-heparin plasma. Although HTGL was found in RLP, HTGL did not bind remnant lipoproteins but bound apoE-rich HDL in RLP. Addition of tetrahydrolipstatin completely inhabited HTGL activity, similar with LPL activity.