

## Wednesday, August 2, 2017

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

Lipids/Lipoproteins

## B-130

**Apo B/A Ratio: A Predictor of Cardio Vascular Disease in Patients with or without Type II Diabetes Mellitus**

P. Rayamajhi<sup>1</sup>, S. Malla<sup>1</sup>, B. Poudel<sup>2</sup>, A. Mittal<sup>1</sup>. <sup>1</sup>Nepalese Army Institute of Health Sciences, Kathmandu, Nepal, <sup>2</sup>Manipal College of Medical Sciences, Pokhara, Nepal

**BACKGROUND:** Cardiovascular disease (CVD) is the growing issue of public health problem, and first major cause of mortality and morbidity throughout the globe. DM, Hypertension, Obesity, MetS and Dyslipidemia are the potential risk factors for the development of CVD. Apolipoprotein B (Apo B) transports all potentially atherogenic very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and low density lipoprotein (LDL) particles, and Apo A transports and acts as the major antiatherogenic protein in the HDL particles. Although conventional lipid parameters are known to predict CVD, limited data are available regarding Apo B/A ratio. The aim of this study was to find out the association of Apo B/A ratio to predict the risk of CVD with or without DM in patients visiting Shree Birendra Army Hospital, Kathmandu, Nepal.

**METHODS:** A cross-sectional study was conducted at Shree Birendra Army Hospital, Kathmandu from 2014/10/16 to 2016/7/30. We enrolled 180 participants including 45 healthy controls, 45 cases of DM and 90 cases of CVD. CVD was categorized into two groups into CVD with DM and CVD without DM. Demographic and anthropometric data of patient was collected. Fasting blood samples was collected from the patient. Fasting Glucose, Total Cholesterol, Triglyceride (TG), HDL-C, LDL-C, Apoprotein-A and Apoprotein-B were measured. One way ANOVA and Student-t test were applied for the comparison of mean between and among the group respectively. Chi square test was applied to see the categorical association between the group.

**RESULTS:** In this study, we have reported the difference in mean±SD of lipid parameters such as TCHO, HDL-C, LDL-C and TG among controls, DM and CVD were found to be statistically significant. Out of 180 subjects included in this study CVD cases had significantly lower levels of HDL and Apo A and significantly higher levels of TCHO, LDL, TG and Apo B regardless of DM. Apo B/A ratio was significantly elevated in CVD than the DM and control cases. Apo B/A ratio showed a strong positive correlation with CVD. The mean±SD of Apolipoproteins A in control, DM and CVD were found to be 173.9 ± 21.5, 119.5 ± 33.1 and 99.6 ± 28.3 respectively. The mean±SD of Apolipoproteins B in control, DM and CVD were found to be Apo B 104.7 ± 17.9, 126.1 ± 51.8 and 192.1 ± 79.5) and respectively. Similarly, the ratio of Apo B/A in control, DM and CVD were found to be 0.6 ± 0.1, 1.2 ± 0.8 and 2.2 ± 1.3 respectively. The mean±SD of Apo A and Apo B in CVD with DM and CVD without DM were found to be in significant. Similarly the ratio of Apo A/B were found to be significant.

**CONCLUSION:** This study reveals that Apolipoprotein measurement significantly predict CVD in patients suffering from type II DM and also concludes that the DM can be associated with recognized risk factors for the development of CVD. The predictive ability of Apo B/A ratio was found to be better than any of the routine clinical lipids measurements therefore, their inclusion in further clinical guidelines should not be discarded.

## B-131

**Development of new HDL-Cholesterol Generation 4 assay on Roche Clinical Chemistry Analyzers**

H. W. A. Klima<sup>1</sup>, C. Schühlmann<sup>1</sup>, G. Sobczak<sup>1</sup>, B. Löhr<sup>2</sup>. <sup>1</sup>Roche Diagnostics GmbH, Penzberg, Germany, <sup>2</sup>Roche Diagnostics GmbH, Mannheim, Germany

**Medical background**

Monitoring of HDL-cholesterol (HDL-C) in patients is of clinical relevance as the HDL-C concentration is important in the assessment of atherosclerotic risk. Elevated HDL-C concentrations protect against coronary heart disease (CHD), whereas reduced HDL-C concentrations, particularly in conjunction with elevated triglycerides, increase cardiovascular risk.

**Test principle**

NonHDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a watersoluble complex. In this complex the enzymatic reaction of cholesterol oxidase (CHER) and cholesterol oxidase (CHOD) towards nonHDL lipoproteins is blocked. The concentration of HDL-cholesterol is determined enzymatically by CHER and CHOD.

**Development Goals HDL-Cholesterol Gen.4:**

- Improved specificity for HDL-C in human samples due to reduced sensitivity towards denatured lipoprotein
- Extended measuring range of 0.083.88 mmol/L (3.09150 mg/dL)
- Less sensitivity to denatured lipoproteins and less matrix effects
- Increased reactivity towards apo E enriched HDL (Important for people with reduced CETP (cholesterol ester transport protein) activity)
- Reduced high recovery in samples from patients with liver disease and lipid disorder
- Improved stability over shelf life

**Results****Measuring range and lower limits of measurement**

The linear assay range of the HDLC4 assay is from 0.083.88 mmol/L (3.09150 mg/dL). The LoB, LoQ and LoD are 0.08 mmol/L (3.09 mg/dL).

**Traceability**

HDLC4 assay has been standardized against the ultracentrifugation method according to CDC (center of disease control) manufacture's protocol.

**Limitations and interferences**

No interference of bilirubin (conjugated and non-conjugated) up to 1026 µmol/L (60 mg/dL), I-Index of 60, no interference of lipaemia (Intralipid) up to a L-Index of 2000, no significant interference from native triglycerides up to 13.7 mmol/L (1200 mg/dL), no interference of hemoglobin up to 745 µmol/L (1200 mg/dL), H-Index of 1200. Statins (Simvastatin) and fibrates (Bezafibrate) tested at therapeutic concentration ranges do not interfere. Ascorbic acid up to 2.84 mmol/L (50 mg/dL) does not interfere.

**Precision - CLSI EP5 - 21 days**

Repeatability ≤ 1.8% and Intermediate precision ≤ 2.2% (concentration range 0.25 mmol/L (9.67 mg/dL) to 3.66 (141 mg/dL).

**Method comparison study:**

Human serum and plasma samples obtained on a Roche/Hitachi cobas c 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche cobas c 501 analyzer (x) (n = 59). Passing/Bablok regression:  $y = 1.006x + 0.032$  mmol/L. The sample concentrations were between 0.11 and 3.69 mmol/L (4.25 and 143 mg/dL).

**Conclusions**

All development goals for the new HDLC4 assay were met. The use of HDLC4 method in laboratory routine will improve quality of test results for HDL-C.

## B-132

**Development of an HPLC Assay for Measurement of Very Long-Chain Fatty Acids in the Plasma**

R. Shrestha<sup>1</sup>, S. Hui<sup>1</sup>, H. Chiba<sup>1</sup>, K. Hirano<sup>2</sup>. <sup>1</sup>Faculty of Health Sciences, Hokkaido University, Sapporo, Japan, <sup>2</sup>Department of Cardiovascular Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan

**Background:** Measurement of plasma very-chain fatty acids (VLCFA) provides important information to aid the diagnosis of various peroxisomal disorders, including Zellweger syndrome, Rhizomelic chondrodysplasia punctata (RCDP), and X-linked adrenoleukodystrophy (XALD). Furthermore, there are increasing evidence that VLCFA is associated with coronary heart diseases, therefore, can be used to refine the risk stratification of it. In this study, we aimed to develop a simple, rapid and sensitive method for the measurement of plasma VLCFA that can be easily adopted in the clinical laboratories.

**Method:** Fasting blood samples were collected from 200 healthy human volunteers (120 female and 80 male; mean age ±SD – 57.4 ±13.3 years) and 30 subjects with hypertriglyceridemia (10 female and 20 male; mean age ±SD – 59.8 ±10.2 years). 50 µL of the plasma was mixed with 4 nmol of tricosanoic acid (FA23:0) as internal standard (IS). After saponification of esterified fatty acids (FA) in the plasma using 0.3M KOH in ethanol, the liberated free VLCFA are labeled with 2-nitrophenylhydrazine (NPH) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and pyridine. The labeled VLCFA are then extracted in ether, reconstituted in methanol, and subjected to HPLC analysis with a UV/VIS detector. The chromatographic

separation was performed with  $C_4$  Mightysil reversed-phase column. Gradient elution was done with the solvent system composed of water (pH adjusted to 4.0 with 1% trifluoroacetic acid) and methanol. A series of experiments were performed to determine optimal condition for the labeling of VLCFA, extraction solvent, type of column and solvents, and its elution profiles for the distinct separation of each VLCFA including IS. Calibration curves of each FA were plotted by analyzing a series of standards with concentration ranging from 0.1 – 20  $\mu\text{mol/L}$ . The assay was validated by evaluating its accuracy, precision, and recovery.

**Results:** Optimal HPLC condition was established with distinct isolated peaks for each VLCFA free from interfering peaks eluting between 18 to 24 min. The lower limit of quantification was 4 pmol for each FA at S/N 5:1 per injection.

The mean concentration of total VLCFA among the healthy controls was found to be  $12.5 \pm 3.8$  for male and  $13.9 \pm 4.5$   $\mu\text{mol/L}$  for female. Hypertriglyceridemia is associated with significant increase in total VLCFA compared to the healthy controls ( $33.2 \pm 18.6$  vs  $13.3 \pm 4.3$   $\mu\text{mol/L}$ ,  $p < 0.01$ ). There is a significant difference in mean of arachidic acid (FA20:0), behenic acid (FA22:0), lignoceric acid (FA24:0) and cerotic acid (FA26:0) between healthy and hypertriglyceridemic subjects ( $7.1 \pm 2.8$  vs  $19.9 \pm 12.4$   $\mu\text{mol/L}$ ,  $p < 0.01$ ;  $3.7 \pm 1.2$  vs  $8.7 \pm 5.5$   $\mu\text{mol/L}$ ,  $p < 0.01$ ;  $2.1 \pm 0.9$  vs  $3.8 \pm 1.6$   $\mu\text{mol/L}$ ,  $p < 0.01$ ;  $0.5 \pm 0.6$  vs  $0.8 \pm 0.7$   $\mu\text{mol/L}$ ,  $p = 0.03$ , respectively). Significant correlation was observed between concentration of FA20:0, FA22:0, FA24:0, and FA26:0 ( $p < 0.01$ ).

**Conclusion:** We established a simple, accurate and reliable method for the quantification of VLCFA in the plasma. Application of this method may be valuable in diagnosis of peroxisomal disorders.

### B-133

#### Evaluation of different formulas for estimating LDL-c in Nepalese population

P. Padmavathi<sup>1</sup>, G. Brindha<sup>1</sup>, V. Sathiyapriya<sup>1</sup>, N. Gautam<sup>2</sup>. <sup>1</sup>A.C.S Medical College & Hospital, Chennai, India, <sup>2</sup>Universal College of Medical Sciences and Teaching Hospital, Bhairahawa, Nepal

**Introduction:** According to NCEP ATP III guideline the primary target of therapy in cardiovascular disease is based on Low Density Lipoprotein-cholesterol (LDL-c) concentration. Estimating LDL-c by direct homogenous assay is expensive especially in developing countries. Hence in clinical practice most of the clinical laboratory uses calculation method for estimating LDL-c concentration. There are different formulas been used to calculate LDL-c concentration, the most frequently used is Friedewald formula. In recent studies have shown that among them novel 180-c method showed the best agreement with direct LDL-c method when compared with other formulas in Asian population. The aim of the study was to (i) Evaluate different formulas for estimation of LDL-c in Nepalese population. (ii) And to identify which formula shows best agreement with novel 180-c method. (iii) And to determine whether it is really in the need to substitute the Friedewald formula. **Materials and methods:** This is prospective study conducted at Universal College of Medical Sciences teaching hospital (UCMSTH), Bhairahawa, Nepal. Lipid profile measured (n=1000) out patients samples which were sent to the clinical biochemistry laboratory, from January to December 2013. Total seven formulas used for estimating LDL-c in mg/dl. These are novel 180-cell method and equations suggested by Friedewald et al, Hattori et al, Anandaraja et al, Cordova et al, Ahmadi et al, & Rao et al. Two- statistical concepts were utilised for comparison of accuracy of different equations to estimate LDL-c by comparing with novel 180-cell method as reference value. The intra class correlation coefficient ICC was calculated to compare the degrees of agreement between the formulas. Good agreement ICC  $> 0.75$ , moderate agreement ICC  $< 0.5$  or  $< 0.75$ . Calculated weighted kappa (k) index to distinguish the most accurate equation for estimating LDL-c according to NCEP ATP III classification guideline. **Results:** The intraclass correlation coefficient for Friedewald equations (ICC: 0.991), Hattori et al. (ICC: 0.969), Anandaraja et al. (ICC: 0.979), Cordova et al. (ICC: 0.965), Ahmadi et al. (ICC: 0.623), Rao et al. (ICC: 0.989) ( $p < 0.001$ ). Among these Friedewald equation showed the highest agreement in terms of ICC and also highest weighted kappa index (k index: 97.49) which indicating the best agreement with novel 180 cell measurement. **Discussion/Conclusion:** Studies have highlighted Novel 180 cell method with high accuracy to use in clinical practice which uses adjustable factor for TG: VLDL-c ratio using stratification approach according to TG and non HDL-c levels (TC-HDL-c = non HDL-c) But in routine clinical practice calculating LDL-c by novel 180 cell method for each patient where there is manual entry of reports seems to be burden to the laboratory technologist. We used 180-c method as a reference value for LDL-c estimation with previously reported formulas as the first external validation in Nepalese population. The most commonly used Friedewald equation appeared to be more accurate than the newly derived formulas. This suggests that we still can use Friedewald formula in Nepalese population.

### B-134

#### Comparison of Friedewald's Formula with de Cordova's Formula for Calculating Low Density Lipoprotein Cholesterol in a Nepalese Population

S. Pradhan, K. Gautam, D. Pyakurel. *Samyak Diagnostic Pvt. Ltd, Lalitpur, Nepal*

**Background:** As serum Low Density Lipoprotein Cholesterol (LDL-C) concentration is positively related to the increase in incidence of coronary heart disease, its measurement plays vital role in the prevention and management of cardiovascular disease. Ultracentrifugation and beta quantification, the gold standard method, and direct measurement of LDL-C using homogenous assays are quite expensive and inconvenient in laboratory setups for countries like Nepal. To address this problem numbers of formulas have been established which calculate LDL-C level. Among these Friedewald's formula is being widely used in laboratories of Nepal even though it has limitations. Recently Cordova et al developed formula and claimed to outperform Friedewald's formula. This study aims to evaluate the performance of Cordova's formula for calculation of LDL-C in Nepalese population. **Methods:** A total of 270 participants were included in the study. Serum total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) were measured by direct automated method. LDL-C was also calculated using Friedewald's and Cordova's formula. SPSS ver. 22 was used to analyze the data. Pearson's correlation coefficient (r), Passing and Bablok regression, Bland-Altman plots were used to compare performance of different formula. **Results:** The mean values of directly measured LDL-C and LDL-C calculated by Cordova's formula and Friedewald's formula were  $120.87 \pm 41.55$  mg/dl,  $106.71 \pm 38.60$  mg/dl and  $104.95 \pm 33.88$  mg/dl respectively. The correlation coefficient of measured LDL-C for Cordova's formula was 0.857 ( $p < 0.01$ ) and for Friedewald's formula was 0.886 ( $p < 0.01$ ). Passing and Bablok regression yielded the equation  $y = 20.12 + 0.7x$  for Cordova's formula and  $y = 7.17 + 0.82x$  for Friedewald's formula. The bias of calculated LDL-C against measured LDL-C were 15.92% for Cordova's formula and 14.15% for Friedewald's formula. **Conclusion:**

This study showed better performance of the Friedewald's formula than Cordova's formula for approximate calculation of LDL-C. However further studies on a large population is required to strengthen this statement.

### B-135

#### Serum Bone Morphogenic Protein 4 levels are conversely correlated with high sensitive- C Reactive Protein in Obese Men

T. Yulianti. *Prodia Laboratory, Jakarta, Indonesia*

**Background:** The prevalence of obesity is increasing in many parts of the world, including Indonesia. The reframing of obesity as an inflammatory condition has had a wide impact on the conceptualization of obesity-associated disease. BMP4 is a growth factor of the transforming growth factor- $\beta$  superfamily. Initially, BMPs were identified as inducer of ectopic bone formation. Recent in vitro study on cultured human adipocytes have highlighted the important roles of BMP4 in the regulation of adipogenesis as an anti-inflammatory. However the correlation between serum BMP4 and inflammation in adult obese is unknown yet. The objective of this study was to evaluate the correlation between common inflammation marker, serum hs-CRP and BMP4 in obese men.

**Methods:** A total of 80 obese adult male subjects were included in the present study, out of which 33 were non metabolic syndrome (nonMetS) and the remaining 47 were metabolic syndrome, age range from 31 to 60 years old. Serum BMP4 and TNF $\alpha$  concentrations were quantified by ELISA principle. Serum hsCRP were quantified by immulite2000 (DPC cat L2KCRP-2). All assays were performed according to the manufacture instruction. Statistical analysis was performed with SPSS for windows ver 20. Significance value were define as alpha level  $< 0.05$  based on two-tailed tests.

**Results:** The mean levels of serum BMP4 in obese MetS is lower (470 pg/ml) as compared to obese nonMetS group (613 pg/ml). However, inverse results were seen for mean serum hsCRP levels which were higher in obese MetS group (2.69 mg/l) compared to obese nonMetS group (1.89 mg/l). Bivariate analysis (n=80) revealed that serum BMP4 was inversely correlated with hsCRP, TNF- $\alpha$ , triglyceride, blood fasting glucose. A significant inverse correlation were found between BMP4 and hsCRP ( $r = -0.277$ ;  $p = 0.013$ ).

**Conclusion:** In conclusion, this study provides evidence that in obese men serum BMP4 inversely correlated with hsCRP. This finding demonstrate the importance of BMP4 as an anti inflammatory protective factor of obesity-related diseases. Further study is needed to validate that BMP4 may serve as a therapeutic target for intervention in the control of metabolic disease.

**B-136****Evaluation of Free Fatty Acid Assay adapted on the Abbott Architect c8000 analyser**

J. W. S. Setoh, C. K. M. Ho, S. P. Peh. *KK Women's and Children's Hospital, Singapore, Singapore*

**Background:**

Fatty acids provide a source of energy alternative to glucose for some organs such as skeletal muscle. In liver, fatty acids can be converted to ketones, which in turn provide energy for the brain during starvation or when blood glucose levels are low. In blood, most fatty acids are esterified with either cholesterol or glycerol, and transported by lipoprotein particles, whereas non-esterified (free) fatty acids are bound to proteins in plasma.

In this study, we aimed to study the performance of the Randox non-esterified fatty acid, also known as free fatty acid (FFA) on the Abbott Architect c8000 analyser.

**Methods:**

We evaluated inter-day imprecision, linearity and limit of quantitation (LOQ) of the FFA assay adapted to the Abbott Architect c8000 analyser and its performance in two external quality assessment (EQA) schemes. Potential interference of the assay by hemolysis and bilirubin was also investigated using serum specimens spiked with known concentrations of hemoglobin or unconjugated bilirubin.

**Results:**

Inter-day imprecision, CV (%), over 10 days was 4.3% at 0.52 mmol/L and 2.5% at 1.59 mmol/L. The FFA assay demonstrated linearity for measurement between 0.10 and 2.97 mmol/L. Limit of quantitation (LOQ) was found to be 0.16 mmol/L with CV = 2.6%.

EQA results were comparable with peers participating in the Randox International Quality Assessment Scheme (RIQAS) and European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism (ERNDIM).

Hemoglobin concentration at 300 mg/dL and 600 mg/dL increased FFA results by 11.7% and 23.9%, respectively, at FFA concentration of 0.51 mmol/L ( $p < 0.001$ ). Bilirubin concentration at 100  $\mu$ mol/L, 200  $\mu$ mol/L and 400  $\mu$ mol/L decreased FFA results by 12.6%, 21.7% and 29.1%, respectively, at FFA concentration of 0.64 mmol/L ( $p < 0.001$ ).

**Conclusion:**

We have evaluated the performance (imprecision, linearity and LOQ) of the Randox free fatty acid assay on the Abbott Architect c8000 analyser. Assay results were found to be satisfactory when compared to all users' means in both RIQAS and ERNDIM external quality assessment programs. Hemoglobin at 300 mg/dL and above resulted in statistically higher FFA results compared with controls, whereas bilirubin at 100  $\mu$ mol/L and above resulted in statistically lower FFA results.

**B-137****An Improved Method for LDL-Cholesterol Beta-Quantification for Clinical Drug Trials**

H. L. Kincaid<sup>1</sup>, S. L. Mankin<sup>1</sup>, R. Duncan<sup>1</sup>, E. Wagner<sup>1</sup>, Y. Olivera<sup>2</sup>.  
<sup>1</sup>Covance Central Laboratory Services, Indianapolis, IN, <sup>2</sup>Covance Central Laboratory Services, Geneva, Switzerland

**Background:** Low density lipoproteins (LDL) are the major cholesterol-carrying lipoproteins in plasma. LDL is primarily cleared from the bloodstream via hepatic LDL receptors, and to a lesser extent, by extra-hepatic tissues. The CDC reference method for the measurement of LDL cholesterol (LDL-C) is beta quantification using ultracentrifugation. The original reference method for LDL-C by beta quantification requires 5 mL of serum; later adaptations apply 1 mL serum. This report describes an adaptation utilizing 0.2 mL of serum with a reduced centrifugation time, which satisfies IRB requirements for pharmaceutical companies investigating PCSK9 inhibitors and other drug development studies designed to improve LDL-C clearance.

**Methods:** Specimens were centrifuged for 3 hours 54 minutes, as opposed to 20 hours 15 minutes (existing method) in a Model Optima XPN-80 ultracentrifuge with titanium rotor Type 42.2 Ti (Beckman Coulter®, Brea, CA). A Beckman CentriTube™ Slicer and Beckman Coulter 7x20 mm cellulose propionate centrifuge tubes were used. Total cholesterol was measured on Modular Analytics or cobas® 8000 instrumentation (Roche Diagnostics, Indianapolis, IN) using Roche cholesterol reagent (Catalog # 05168538190). LDL-C by direct assay was performed using Roche reagents (Catalog #05171369190). An LDL-C determination by Friedewald

calculation was calculated from total cholesterol, high density lipoprotein-cholesterol (HDL-C), and triglycerides (glycerol-blanked) measurements. HDL-C was measured by dextran-magnesium sulfate precipitation and subsequent total cholesterol measurement. Triglycerides (glycerol-blanked) were measured using Roche reagents (Catalog #05976006190). Data reduction utilized EP Evaluator® (Data Innovations, South Burlington, VT). The new method was validated in the US and subsequently in Shanghai, PRC and Geneva, Switzerland. Global correlation studies were conducted according to Covance CLS established methods.

**Results:** The new process demonstrated an inter-assay imprecision of 2.6%, which satisfied imprecision requirements using an allowable total error of 10.5%. Accuracy, as determined by method comparisons to the Friedewald calculation and LDL-C by direct assay demonstrated concordance within 20% of the LDL-C ultracentrifugation results. The correlation between the 1 mL and 0.2 mL methods, as well as inter-laboratory global correlations, met acceptance criteria (95% CI for the slope includes 1.00, the 95% CI for the y-intercept includes 0.00, and correlation coefficient,  $R \geq 0.95$ ). Global correlations met acceptance criteria and proved commutability of the method.

**Conclusion:** The method enhancements for LDL-C by ultracentrifugation demonstrated excellent analytical performance, and provided significant value to pharmaceutical clients and laboratory operations both in turnaround time and test cancellation because of sample quantity not sufficient for testing. Test cancellations due to insufficient specimen volume were reduced from 4.3% to 0.2%. The time required for specimen processing was reduced by 48% leading to significant savings in time and labor expense.

**B-138****Comparison between cholesterol or low-density lipoprotein concentrations obtained by the direct method and those estimated through application of the Martin and Friedewald equations, in a Brazilian population sample: Hypertriglyceridemia limits the accuracy of the Martin equation.**

A. C. Dias, J. F. F. Oliveira, L. A. M. B. Gracindo, L. A. Silva, G. Barra, S. F. Fonseca, R. H. Jácomo, A. L. Barbosa, L. F. Abdalla. *Laboratório Sabin, Brasília, Brazil*

**Background:**

In 2013, Martin et al proposed a new equation for estimating cholesterol or low-density lipoprotein levels (LDL-C), [LDL-C(M)], which, by having an adjustable factor in its formula, would be more accurate than the more traditional Friedewald equation, [LDL-C(F)]. In December of 2016, the Brazilian Consensus for the Normalization of Laboratory Determination of Lipid Profiles was published, and this document recommended using LDL-C(M), regardless of the triglyceride (TG) levels. The objective of the current study was to evaluate the correlation and concordance between LDL-C(M) or LDL-C(F) and the direct dosage determination of LDL-C [LDL-C(D)], and to determine the triglyceride concentrations that may limit the accuracy of the LDL-C(M) values.

**Methods:**

LDL-C(D), TG, total cholesterol and HDL-C concentrations were determined for 680 serum samples, using the Advia 2400 analyser; LDL-C(M) and LDL-C(F) values were calculated by applying the respective equations of Martin or Friedewald. The clinical concordance was evaluated by using the total minimum error based on the biological variation components (17.84%). Statistical analyses were performed using the Pearson correlation, the Cohen's Kappa test, the Lin Concordance Correlation Coefficient, ROC curve analysis and the Bland-Altman graph.

**Results:**

The results are shown in Table. For TG values  $< 400$  mg/dL, the concordance between LDL-C(M) and LDL-C(D) was 97.4% ( $p = 0.238$ ), and between LDL-C(F) and LDL-C(D) was 84.7% ( $p < 0.0001$ ). For TG values  $\geq 400$  mg/dL, the concordance between LDL-C(M) and LDL-C(D) was 68.3% ( $p < 0.0001$ ), and between LDL-C(F) and LDL-C(D) was 43.3% ( $p < 0.0001$ ), considering a total maximum error of 17.84%. Analysis of the ROC curve showed that a TG concentration of 432 mg/dL limited the accuracy of the LDL-C(M) results.

**Conclusions:**

For the LDL-C calculation, the Martin equation is more accurate than that of Friedewald, despite presenting limitations with regard to samples with TG values greater than or equal to 432 mg/dL.

Concordance and correlation by Friedewald vs Martin Estimates of Low-Density Lipoprotein Cholesterol								
	TG < 400 mg/dL (n=620)				TG ≥ 400 mg/dL (n=60)			
	LDL-C(F)		LDL-C(M)		LDL-C(F)		LDL-C(M)	
	Value	95% CI	Value	95% CI	Value	95% CI	Value	95% CI
Percent Agreement based on Total Error	84.7	81.8 to 87.5	97.4	96.2 to 98.7	43.33	30.79 - 55.87	68.3	56.56 - 80.1
Pearson's correlation coefficient	0.95	0.92 to 0.97	0.98	0.97 to 0.98	0.95	0.92 - 0.97	0.93	0.89 - 0.96
Lin's Concordance Correlation Coefficient (Interpretation)	0.94 (Moderate)	0.92 to 0.94	0.92 to 0.94	0.97 to 0.979	0.78 (Poor)	0.70 - 0.84	0.81 (Poor)	0.74 - 0.87
Cohen's kappa coefficient (Interpretation)	0.65 (Good)	0.61 to 0.70	0.85 (Very good)	0.82 to 0.88	0.26 (Fair)	0.13 - 0.41	0.53 (Moderate)	0.38 - 0.69
Mean difference, mg/dL	-5.87	-6.74 to -5.00	-0.35	-0.94 to 0.23	-19.8	25.3 to -14.3	16.5	11.9 to 21.1

### B-139

#### Multicenter Evaluation of new HDL-Cholesterol Generation 4 assay (HDL-C4) on Roche Clinical Chemistry Analyzers

B. Loehr<sup>1</sup>, I. Paege<sup>2</sup>, Y. De Rijke<sup>3</sup>, D. Plonné<sup>4</sup>, H. Klima<sup>5</sup>. <sup>1</sup>Roche Diagnostics GmbH, Mannheim, Germany, <sup>2</sup>Otto-von-Guericke University, Institut für Clinical Chemistry and Pathological Biochemistry, Magdeburg, Germany, <sup>3</sup>Erasmus Medical Center, Department of Clinical Chemistry, Rotterdam, Netherlands, <sup>4</sup>Medical Center of human genetics Ulm, Department of Laboratory Medicine, Ulm, Germany, <sup>5</sup>Roche Diagnostics GmbH, Penzberg, Germany

#### Background:

Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. A new formulation of the Roche Diagnostics HDL-Cholesterol Gen.4 reagent (HDL-C4) was developed to increase specificity for HDL-C and to widen the measuring range up to 3.88 mmol/L (150 mg/dL). The analytical performance of the assay was tested in three laboratories.

#### Assay principle:

Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of cholesterol esterase (CHER) and cholesterol oxidase (CHOD) towards non-HDL lipoproteins is blocked. The concentration of HDL-cholesterol is determined enzymatically by CHER and CHOD.

#### Study Design:

The analytical performance of the new HDL-C4 assay was evaluated in three independent laboratories using **cobas c 702**, **cobas c 502** and **cobas c 501** instruments. Recovery of Roche PreciControl ClinChem Multi 1 and 2, recovery of Ring Trial samples from RfB and Instand e.V. and controls from Bio-Rad, within-run precision of human samples pools, precision according to CLSI EP5-A3, method comparisons HDL-C Gen.3 vs. HDL-C Gen.4, instrument-to-instrument (**cobas c 701** vs **cobas c 502**), method comparison to HDL-C assays from competitors.

#### Methods and Results:

Repeatability and intermediate precision were measured in the concentration range from 0.66 mmol/L (25.5 mg/dL) to 2.55 mmol/L (98.6 mg/dL) according to the CLSI EP5-A3 protocol using two Roche controls and five human serum pools. For the repeatability the coefficients of variation (CVs) were determined to be less than 1.9 % and for intermediate precision yielded CVs ranging between 1.1 and 2.7 % (two runs/day, 21 days). The recovery of two controls (Roche Diagnostics) was determined in three independent runs measuring 3 aliquots. The recovery of HDL-C target values ranged from 97.4 to 105.6 %. Method comparison experiments were designed in compliance with CLSI EP09-A3, using ≥ 117 serum samples. Passing-Bablok regression analysis of Roche HDL-C methods resulted in slopes in a range of 0.97 to 0.86, intercepts of 0.02 mmol/L (0.77 mg/dL) to 0.13 mmol/L (5.02 mg/dL), and Pearson correlation factors from 0.977 to 0.998. The HDL-C concentration of samples ranged from 0.08 mmol/L (3.09 mg/dL) up to 3.05 mmol/L (117.91 mg/dL). The measured biases at the medical decision points were acceptable (≤ 5 %).

#### Conclusions:

The results of the multicenter evaluation study prove a good analytical performance of the new HDL-C4 assay as well as an increased resistance to endogenous interferences and an increased specificity for HDL-C. The assay is well-suitable for routine use.

### B-140

#### Specific Sandwich-type Enzyme-Linked Immunosorbent Assay (ELISA) for Oxidized High-Density Lipoprotein and Its Clinical Application

M. Sumida<sup>1</sup>, T. Okada<sup>2</sup>, Y. Katayama<sup>1</sup>, T. Ohama<sup>2</sup>, K. Kanno<sup>2</sup>, H. Matsuda<sup>2</sup>, M. Sairyō<sup>2</sup>, Y. Zhu<sup>2</sup>, A. Saga<sup>2</sup>, T. Kobayashi<sup>2</sup>, D. Matsuda<sup>2</sup>, M. Koseki<sup>2</sup>, M. Nishida<sup>2</sup>, N. Kayahara<sup>3</sup>, Y. Sakata<sup>2</sup>, S. Yamashita<sup>1</sup>. <sup>1</sup>Research Laboratory, Kyowa Medex Co., Ltd., Shizuoka, Japan, <sup>2</sup>Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan, <sup>3</sup>Research and Development Division, Kyowa Medex Co., Ltd., Tokyo, Japan, <sup>4</sup>Department of Cardiology, Rinku General Medical Center, Osaka, Japan

**Background:** High-density lipoprotein (HDL) has anti-atherogenic functions such as cholesterol efflux. It is also well known that plasma HDL-C levels are negatively correlated with cardiovascular events. Consequently HDL has been considered as "good" cholesterol. However, recent studies show that HDL from coronary artery disease patients have poor cholesterol efflux, and such HDL is defined as "dysfunctional HDL". Oxidative stress is one of the factors which lead HDL to dysfunctional, so we consider oxidized HDL (Ox-HDL) is useful to assess the quality or functionality of HDL. The aim of this study is to develop a specific sandwich-type enzyme-linked immunosorbent assay (ELISA) for Ox-HDL and to analyze the serum Ox-HDL levels of both normolipidemic healthy subjects and dyslipidemic patients.

**Methods:** We have developed a sandwich ELISA for Ox-HDL with DLH3 antibody that can specifically recognize oxidized phospholipid. We assessed specificity of the assay using native HDL and LDL as well as Cu-oxidized HDL and LDL. Each lipoprotein was separated from human plasma by sodium bromide stepwise density gradient ultracentrifugation. Additionally we assessed the non-specific reaction of unoxidized phosphatidylcholine (PC) by inhibition assay. Next, we defined Ox-HDL obtained by forced-oxidation of 1 mg/L HDL phospholipids (HDL-PL) as 1 U/L Ox-HDL, and analyzed the sera of 118 healthy normolipidemic controls and 177 dyslipidemic outpatients, respectively.

**Results:** *Specificity analysis*-In this ELISA assay, Cu-oxidized HDL was specifically detected and neither Cu-oxidized LDL nor native LDL was detected. Native HDL was also recognized but the signal was low. Inhibition assay with various types of phospholipids revealed that lysophosphatidylcholine (lyso-PC) showed concentration-dependent inhibition. On the other hand, no inhibition by unoxidized PC was observed even in the condition of excess concentration. These results strongly suggested that oxidized phospholipid in HDL is specifically recognized in this ELISA assay. *Clinical evaluation*-The serum Ox-HDL levels in healthy controls were 28.3 ± 4.9 U/L (mean ± SD) and in dyslipidemic patients treated with drugs were 27.7 ± 9.3 U/L. Ox-HDL levels were moderately correlated with HDL-PL levels (r=0.59), therefore we also evaluated Ox-HDL/HDL-PL ratio, which represents oxidation degree of phospholipids in the HDL fraction. Patients treated with probucol, which is a potentially anti-oxidative and anti-hyperlipidemic drug, showed significantly lower Ox-HDL level (18.7 ± 6.6 U/L vs 30.0 ± 8.1 U/L, p<0.001) and Ox-HDL/HDL-PL ratio (0.206 ± 0.045 vs 0.224 ± 0.049, p=0.043) than those without probucol, suggesting that probucol may prevent oxidation of HDL. These probucol effects were also seen in Familial Hypercholesterolemia patients (Ox-HDL: 16.1 ± 5.8 U/L vs 30.2 ± 5.4 U/L, p<0.001; Ox-HDL/HDL-PL: 0.205 ± 0.037 vs 0.238 ± 0.043, p=0.015, respectively).

**Conclusion:** Our sandwich ELISA is an assay with a good specificity to Ox-HDL. In addition, clinical evaluation using our assay showed that probucol may prevent the formation of these Ox-HDLs.

### B-141

#### Validation of the need for 12-hour fasting and non-fasting for dosages of simple lipid profile parameters in our laboratory

M. C. Feres<sup>1</sup>, D. Ramadan<sup>2</sup>, R. Bini Jr.<sup>2</sup>, J. T. M. Torrecilhas<sup>2</sup>, B. B. Perez<sup>2</sup>, M. C. De Martino<sup>2</sup>, S. Tufik<sup>2</sup>. <sup>1</sup>Universidade Federal de Sao Paulo- Brazil, Sao Paulo, Brazil, <sup>2</sup>Associação Fundo de Incentivo a Pesquisa- AFIP, Sao Paulo, Brazil

12-hour fasting is a big problem for people who need to do exams, such as children and the elderly. A recent study in the journal, "European Heart Journal," shows

that assessment of lipid profile parameters at 12-hour fasting or after meal does not clinically impact patient outcomes. To improve patient adherence to lipid tests, many laboratories worldwide perform these tests without the need for 12-hour fasting at random times during the day. We know that exams processed immediately after the meal have the effect of postprandial lipemia, which in many laboratory methods undergo turbidity interference. In this context, before adopting non-fasting to perform dosages of the lipid profile in our laboratories, the authors found it prudent to do a study to determine the best time interval after the meal that we could recommend in our laboratory, considering the inter variability of absorption of Lipids of each individual. The authors of this study aimed to validate within our conditions, equipment and methodologies, so that we can introduce these changes in the pre-analytical phase of our laboratory routine. It was also part of our objectives to determine the mean concentration range of each parameter of the lipid profile studied at 2, 3 and 4 hours after meal. **Casuistic and Methods:** Our sample consisted initially of 62 volunteers from the community and venous blood was collected in a 12-hour fast and after a meal the following day the volunteer returns to the laboratory after having taken his usual breakfast to be collected blood 2, 3 and 4 hours after this meal. The following tests will be carried out: Cholesterol, Triglycerides, C-LDL, C-HDL and VLDL with the Enzymatic/Colorimetric method on Beckman-Coulter® AU5800 equipment and Beckman-Coulter reagent. In addition to dosing the C-LDL was calculated by the Friedewald equation. **Results:** In the comparison of the lipid profile at fasting versus 2, 3 and 4 h after meal, it was observed that there was no significant difference for the parameters of CT and HDL-C and for the calculated LDL-C, with mean indices for TC ( $p=0.237$ ), C-HDL ( $p=0.130$ ) and for C-LDL ( $p=0.089$ ). However, C-LDL dosed, TG and VLDL presented significant differences with their respective mean concentrations and standard deviation for each hour after 2h C-LDL ( $112.4 \pm 4.8\text{mg/dL}$ ,  $p=0.008$ ), 3h ( $111.9 \pm 5.0\text{mg/dL}$ ,  $p=0.019$ ) and 4h ( $116.0 \pm 4.9\text{mg/dL}$ ;  $p=0.017$ ) for TG 2h ( $156.1 \pm 12.5\text{mg/dL}$ ,  $p=0.000$ ), 3h ( $150.0 \pm 13.3\text{mg/dL}$ ,  $p=0.000$ ) and 4h ( $144.1 \pm 13.5\text{mg/dL}$ ,  $p=0.000$ ), and for VLDL calculated: 2, 3 and 4h after the indices were  $p=0.000$ . **Conclusion:** Our data confirmed that the meal did not influence the TC, C-HDL and calculated C-LDL data, but for TG, VLDL and C-LDL dosed, a significant difference was observed in the post-meal concentrations. Although disturbing the difference in C-LDL for methodological reasons, it did not impact on the clinic. Analyzing our data, we observed that the best blood collection time could be between 2 and 3 hours after the meal where the degree of lipemia would already have a lower influence in most individuals.

**B-142**

**ApoE-rich HDL in pre-heparin plasma may be HDL remnants which remain in the circulation**

S. Usui<sup>1</sup>, R. Shinohata<sup>1</sup>, K. Miyashita<sup>2</sup>, S. Hirohata<sup>1</sup>, M. Shibakura<sup>1</sup>, I. Fukamachi<sup>2</sup>, K. Nakajima<sup>3</sup>. <sup>1</sup>Okayama University Graduate School of Health Sciences, Okayama, Japan, <sup>2</sup>Immuno-Biological Laboratories, Fujioka, Japan, <sup>3</sup>Gunma University Graduate School of Medicine, Maebashi, Japan

**Background:** High-density lipoprotein (HDL) containing apolipoprotein E (apoE-rich HDL) represents only a small portion of plasma HDL. Hepatic triglyceride lipase (HTGL) is well known to play a major role in HDL metabolism, and promotes the selective uptake of cholesteryl esters from HDL via the scavenger receptor B1. We previously reported that HTGL may be associated with apoE-rich HDL in post-heparin plasma, but it has not been clear whether this observation is found in pre-heparin plasma. In this study, we propose the possibility of this lipoprotein fraction as HDL remnants based on the interaction with HTGL in pre-heparin plasma. **Methods:** A total HDL fraction was isolated from healthy serum samples (pre-heparin plasma) using 13% polyethylene glycol (PEG), and applied to a cation-exchange column (HiTrap SP HP, 1 mL, GE Healthcare) to obtain apoE-rich and apoE-poor HDL fractions. For the determination of HTGL distribution, the column effluent was collected in 0.4-mL fractions and analyzed by a newly developed HTGL-ELISA. Furthermore, the apoE-rich HDL fraction was applied into a gel-permeation column (Superose 6HR) to investigate the interaction between apoE-rich HDL and HTGL. **Results:** Approximately 90% of serum HTGL mass was recovered in the total HDL fraction (PEG supernatant), indicating apoB-containing lipoproteins were not associated with HTGL in pre-heparin plasma. Only 5% of total HDL-cholesterol was found in apoE-rich HDL fraction but 40% of HTGL in the total HDL was detected in apoE-rich HDL fraction. Therefore, the ratio of HTGL (ng/mL) to cholesterol (mg/dL) was much higher (approximately 7.0-fold) in apoE-rich HDL than apoE-poor HDL. Furthermore, the gel-permeation HPLC analysis revealed that HTGL and apoE-rich HDL were co-eluted at larger HDL position. These findings indicate that HTGL interacts preferably with apoE-rich HDL. **Conclusion:** This is the first report that apoE-rich HDL prevalently carries HTGL in pre-heparin plasma. Although lipoprotein

lipase was found in VLDL fraction as remnants, HTGL was found in apoE-rich HDL. Both fractions contain apoE-rich lipoproteins and carry the lipases as bound forms in pre-heparin plasma. Therefore, we propose that apoE-rich HDL is "HDL remnants" which were not rapidly cleared from the circulation.

**B-143**

**Comparability of commercial assays & commutability of evaluated materials for apolipoprotein A1 measurement**

J. Zeng<sup>1</sup>, T. Qi<sup>1</sup>, S. Wang<sup>2</sup>, T. Zhang<sup>1</sup>, W. Zhou<sup>1</sup>, H. Zhao<sup>1</sup>, R. Ma<sup>1</sup>, J. Zhang<sup>1</sup>, Y. Yan<sup>1</sup>, J. Dong<sup>2</sup>, C. Zhang<sup>1</sup>, W. Chen<sup>1</sup>. <sup>1</sup>National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing Engineering Research Center of Laboratory Medicine, Beijing, China, <sup>2</sup>The MOH Key Laboratory of Geriatrics, Beijing Hospital, National Center of Gerontology, Beijing, China

**Background:** Harmonization & comparability of apolipoprotein A1 (apo A1) measurement results are crucial to assess cardiovascular risk. Non-commutable materials used as calibrators & external quality assessment (EQA) materials can result in misdiagnosis or provide misleading information leading to limited EQA schemes. Potential reference materials should therefore be selected & evaluated for commutability.

**Methods:** In the present study, we determined apo A1 levels in commercial control materials used in the 2013 EQA Program, human serum pools prepared from leftover samples (LHSPs) & fresh donations (FHSPs), & a set of 50 individual samples using nine commercially available assays. Original or logarithm-transformed results were pairwise-analyzed to estimate the slopes & intercepts by Deming regression. The 95 % prediction interval (PI) & the minimum bias from biological variability were used to analyze the commutability of these materials. Matrix-related biases were also estimated.

**Results:** All commercially available assays in the present study showed acceptable within-run precision, compared with the minimum specification for imprecision derived from biological variability (4.9 %), & almost all combinations correlated well ( $r>0.975$ ). The slopes varied from 0.65 to 1.35, & the intercepts varied from -0.45 to 0.43. FHSPs were commutable in more combinations than LHSPs or EQA materials, & both 95 % PI & minimum bias (5.6 %) criteria for commutability yielded similar results for FHSPs. The matrix-related biases of EQA materials, LHSPs, & FHSPs varied from -42.28 % to 25.58 %, -7.29 % to 12.53 %, & -8.38 % to 11.22 %, respectively.

**Conclusion:** Human serum pools prepared from fresh donations are thus a more appropriate source of reference materials. The metrological traceability of calibrators is therefore essential for the harmonization & comparability of apo A1 measurement procedures.

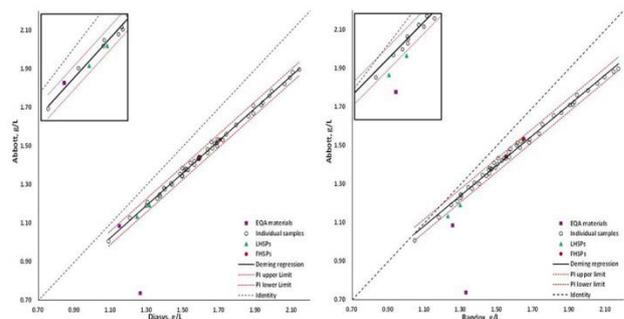


Fig. Examples for commutability assessment of apo A1 measurements in EQA materials and HSPs. The Deming fit with 95 % PI (red dotted lines) were calculated from individual sample results. Evaluated materials within 95% PI were considered commutable, otherwise they were non-commutable. The black dotted lines were identity lines (y = x).

**B-144****The laboratory's contribution in the clinical assessment of cardiovascular risk: evaluation of Imprecision and Reference Change Value of lipid profile.**

F. B. Ronchi, S. Caria, G. Demuro, G. B. Scrocco, G. Serra. *Clinical Pathology Service, Dept. of Services, P.O. N.S. Bonaria, ASSL Sanluri, ATS Sardegna, San Gavino Monreale, Italy*

**Background:** Variability in measurements of serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) has important repercussions on clinical decision-making. To test the impact of analytic variability ( $V_a$ ) of lipid measurements on the cardiovascular risk estimation we evaluated our  $V_a$  close to decision limits suggested by ACC/AHA 2013 and ESC/EAS 2016 guidelines. Since the state of art suggests the use of Reference Change Value (RCV) to evaluate significant changes in serial lipids results we also define our RCV. **Methods:** Analytical imprecision of lipid tests was evaluated throughout intra-(CVr) and inter-assay (CVd) performed in 10 days with Abbott Architect c8000. Samples: Bio-Rad Quality Control third-part (QC<sub>1</sub>, QC<sub>2</sub>) and 1 sample with values close to 200, 240, 290 mg/dL TC (S<sub>1</sub>-S<sub>2</sub>-S<sub>3</sub>); 115, 150 mg/dL LDL-C (S<sub>1</sub>-S<sub>2</sub>); 150, 180 mg/dL TG (S<sub>1</sub>-S<sub>2</sub>); 45 mg/dL HDL-C (S<sub>1</sub>). We further tested 10 patient samples (S<sub>var</sub>) close to decision limits (200 mg/dL TC; 115 mg/dL LDL-C; 150 mg/dL TG; 45 mg/dL HDL-C). To define RCV we used biological variation from Ricos's database, we monthly calculated our analytical imprecision (CV<sub>A</sub>) performing daily measurements of QC<sub>1</sub> and QC<sub>2</sub> during two years by Unity Real Time software, Bio-Rad Laboratories. Then we compared CV<sub>A</sub> with analytical goals (AG) for imprecision derived from biological variation and calculated by Fraser's formulas. **Results:** Table below shows all results expressed as Coefficient of Variation (CV%). RCVs of TC, LDL-C, HDL-C and TG were 17.3, 22.5, 22.1, and 55.5 % respectively ( $Z = 1.96$  for bidirectional changes;  $p < 0.05$ ). **Conclusion:** The accordance of CVr and CVd versus CV<sub>A</sub> obtained from QC highlight good analytical performances at different concentrations close to decision limits. The definition of CV<sub>A</sub> and RCV is a useful tool used by our cardiologists to assess cardiovascular risk and response to lipid-lowering therapy of patients.

		QC <sub>1</sub> CV%	QC <sub>2</sub> CV%	S <sub>1</sub> CV%	S <sub>2</sub> CV%	S <sub>3</sub> CV%	S <sub>var</sub> CV%	Average CV <sub>A</sub> %	AG Imprecision CV%
TC	CVr	0.76	0.47	0.51	0.37	0.68	<0.54	1.8	Desirable 3.0
TC	CVd	1.51	1.43	0.86	0.88	1.32	-	1.8	Desirable 3.0
LDL-C	CVr	0.79	0.64	0.50	0.68	-	<0.91	2.3	Desirable 3.9
LDL-C	CVd	2.24	2.34	1.52	1.55	-	-	2.3	Desirable 3.9
HDL-C	CVr	1.76	0.89	0.77	-	-	<0.71	3.2	Desirable 3.6
HDL-C	CVd	1.35	1.69	1.26	-	-	-	3.2	Desirable 3.6
TG	CVr	0.73	0.36	0.37	0.57	-	<0.58	2.4	Optimal 5.0
TG	CVd	1.75	1.88	1.71	1.74	-	-	2.4	Optimal 5.0

**B-145****Fasting Versus Nonfasting serum lipid profile in newly diagnosed Diabetes Mellitus**

R. Shrestha<sup>1</sup>, N. P. Bhatt<sup>2</sup>, S. Dahal<sup>3</sup>, N. Shrestha<sup>4</sup>, M. Khanal<sup>3</sup>. <sup>1</sup>Hokkaido University, Sapporo, Japan, <sup>2</sup>Department of Pharmacology, Kangwon National University, Chuncheon, Korea, Democratic People's Republic of, <sup>3</sup>Nepal Medical College and Teaching Hospital, Kathmandu, Nepal, <sup>4</sup>Chonbuk National University Hospital, Jeonju, Korea, Democratic People's Republic of

**Background:** Determination of serum lipids in the routine non-fasting state compared to the conventional measurement in the fasting provides several discrete advantages for both patients and the healthcare providers. Importantly, several lines of evidence supported that the non-fasting triglycerides serve as a better risk factor for the future coronary events. However, still, the majority of laboratory and clinician appear to have hesitation and uncertainty in the replacement of fasting lipids testing. This is particularly concerned in the patients with insulin resistance where metabolic handling of dietary lipids is remarkably disordered. Therefore, this study was designed to observe the variation of serum lipids between the fasting and non-fasting state in the newly diagnosed untreated diabetes.

**Methods:** A total of 96 (male:36/female:60) newly diagnosed untreated patients with type 2 diabetes mellitus were recruited (mean age:52.0±10.6) in Nepal Medical

College and Teaching Hospital. Equal numbers of age and sex matched healthy control were also included. Blood samples were collected twice from all the volunteers, one after 12-14 hr of fasting and other at 1-5 hr of a recent regular meal. Total cholesterol (TC), LDL-cholesterol, HDL-cholesterol and triglycerides were measured in an automated chemistry analyzer.

**Results:** Diabetic subjects have significantly higher level of TC (176.3±34.6 vs 165.8±27.4;  $P < 0.05$ ), triglycerides (176.2±77.4 vs 126.3±48.8;  $P < 0.05$ ) and LDL-cholesterol (101.9±31.4 vs 89.0±24.1;  $P < 0.05$ ) and significantly lower level HDL-cholesterol (38.7±12.0 vs 51.0±11.5;  $P < 0.05$ ) as compared to non-diabetic controls in the fasting state. There is a minimal increase in TC and LDL-cholesterol in both diabetes and control group in the non-fasting state. Mean TC increased by 5.1 mg/dL and 4.9 mg/dL in diabetes and control groups, respectively. However, we observed no statistical difference in the mean of HDL-cholesterol in both groups. Serum triglyceride shows remarkable difference in both diabetes (mean increased by 46.5 mg/dL) and control (mean increased by 32 mg/dL). Interestingly, when the cutoff limit was set at 150 mg/dL, 52% of diabetic subjects were shifted to hypertriglyceridemia with the non-fasting triglycerides measurement.

**Conclusion:** Despite the non-fasting sampling for lipid measurement appears to be convenient, lipids particularly triglycerides is significantly elevated in diabetes compared to fasting samples. Therefore, it is crucial to determine an optimal cutoff limit of non-fasting triglyceride to be flagged as abnormal.

**B-146****Establishment of an automated assay for cholesterol uptake capacity, a new concept of high-density lipoprotein functionality**

T. Iino<sup>1</sup>, K. Murakami<sup>1</sup>, M. Kiriyama<sup>1</sup>, T. Kubo<sup>1</sup>, K. Yoshikawa<sup>1</sup>, K. Miwa<sup>1</sup>, R. Toh<sup>2</sup>, K. Hirata<sup>2</sup>, A. Harada<sup>1</sup>. <sup>1</sup>Systemex Corporation, Kobe, Japan, <sup>2</sup>Kobe University Graduate School of Medicine, Kobe, Japan

**Background:** Recent studies have shown that the capacity of high density lipoprotein (HDL) to stimulate cholesterol efflux from lipid-laden macrophages is a better predictor of cardiovascular disease status than overall HDL cholesterol concentration. Recently, we established a cell-free plate assay system to evaluate cholesterol uptake capacity (CUC) of HDL using a fluorescence-labeled cholesterol and an apolipoprotein A1 (apoA1) specific antibody, and demonstrated feasibility of CUC for potential substitution of cholesterol efflux capacity and coronary risk stratification. To apply this concept in the clinical settings, this study aimed to establish a high-throughput method capable of measuring CUC.

**Methods:** We had developed a fluorescence-based manual plate assay as a pilot method for measuring CUC, which was presented at the AACC 2016 Annual Meeting. In this study, we converted it into automated magnetic bead-based chemiluminescence detection system. Briefly, HDL was incubated in reaction buffer containing dinitrophenyl (DNP)-labeled cholesterol, followed by its capture to an anti-apoA1 antibody coated onto the beads. Using alkaline phosphatase labeled anti-DNP antibody and specific substrate, chemi-luminescence signals were detected with our automated immunoassay system HISCL<sup>®</sup>. To investigate the throughput and analytical performance of our method, we quantified CUC of apoB-depleted serum samples and the recombinant apoA-1 as a standard.

**Results:** In this system, measurement of CUC was completed about 40 minutes per sample, notably rapid compared with that of conventional cholesterol efflux capacity assay, which requires 2-3 days. Each assay proceed sequentially every one minutes, so more than 10 samples could be analyzed within an hour. The assay system had high reproducibility (CV < 10%) and was linear from 5 to 200 ng/mL. The LoQ was validated at 5 ng/mL. CUC measured by the present automated method correlated well with that of our previous pilot method, which inversely associated with recurrence of coronary artery disease in patients with optimal control of low-density lipoprotein cholesterol.

**Conclusion:** Our novel automated method can measure CUC quickly and accurately. This method would expand the dysfunctional HDL research from the bench to the clinical arena.

**B-147****ELISA system for GPIHBP1 levels in human plasma**

K. Miyashita<sup>1</sup>, K. Nakajima<sup>2</sup>, I. Fukamachi<sup>1</sup>, A. P. Beigneux<sup>3</sup>, S. G. Young<sup>3</sup>, T. Machida<sup>2</sup>, K. Nakajima<sup>2</sup>, M. Murakami<sup>2</sup>. <sup>1</sup>*Immuno-Biological Laboratories, Fujioka, Japan*, <sup>2</sup>*Gunma University Graduate School of Medicine, Maebashi, Japan*, <sup>3</sup>*Department of Medicine, University of California, Los Angeles, CA*

**Background:** GPIHBP1, a glycosylphosphatidylinositol (GPI)-anchored protein of capillary endothelial cells, is crucial for the lipolytic processing of triglyceride-rich lipoproteins (TRLs). GPIHBP1 binds lipoprotein lipase (LPL) in the interstitial spaces and shuttles the enzyme to its site of action in the capillary lumen. GPIHBP1 is also required for the margination of TRLs along capillaries—so that lipolytic processing can proceed. A deficiency of GPIHBP1 in humans causes severe hypertriglyceridemia (chylomicronemia). Even though GPI-anchored proteins are tethered to the plasma membrane, it has been possible to detect some GPI-anchored proteins (e.g., uPAR) in the plasma. We hypothesized that it might be possible to detect GPIHBP1 in human plasma. To test this hypothesis, we created a sensitive sandwich immunoassay for human GPIHBP1 with two newly created human GPIHBP1-specific monoclonal antibodies.

**Methods:** We created a solid-phase sandwich ELISA for human GPIHBP1. The wells of 96-well plates were coated with Mab-CH79A4. After first incubating plasma samples at 37°C for 60 min and then washing the plates, the GPIHBP1 captured was detected with horseradish peroxidase-labeled Mab-HE20A6. Recombinant GPIHBP1 was used as a calibration standard.

**Results:** The ELISA detected GPIHBP1 in human plasma. The median concentration of GPIHBP1 in plasma samples from healthy subjects was range, 570-1523 pg/ml; the detection limit was 10 pg/ml. When plasma samples were “spiked” with recombinant GPIHBP1, the recovery of the spiked GPIHBP1 was excellent (ranging from 85 to 115% of the amount added). The assay was linear over a >4-fold dilution of plasma. The intra- and inter-assay coefficients of variation were <15%. The plasma levels of GPIHBP1 were not different in pre- and post-heparin plasma samples.

**Conclusion:** We developed a solid-phase ELISA for GPIHBP1 in plasma and used the assay to measure levels of GPIHBP1 in human plasma. In future studies, we will determine whether plasma levels of GPIHBP1 correlate with plasma lipid levels and whether plasma GPIHBP1 levels are a useful biomarker for metabolic or cardiovascular disease.

**B-148****A new enzyme-linked immunosorbent assay system for human serum hepatic triglyceride lipase**

K. Miyashita<sup>1</sup>, K. Nakajima<sup>2</sup>, I. Fukamachi<sup>1</sup>, Y. Muraba<sup>3</sup>, T. Koga<sup>3</sup>, Y. Shimomura<sup>3</sup>, T. Machida<sup>2</sup>, K. Nakajima<sup>2</sup>, M. Murakami<sup>2</sup>, J. Kobayashi<sup>4</sup>. <sup>1</sup>*Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan*, <sup>2</sup>*Gunma University Graduate School of Medicine, Maebashi, Japan*, <sup>3</sup>*Hidaka Hospital, Takasaki, Japan*, <sup>4</sup>*Department of General Medicine Kanazawa Medical University, Ishikawa, Japan*

**Background:** There is no previously established method for measuring human hepatic triglyceride lipase (HTGL) concentration in plasma obtained without heparin injection.

**Methods:** We developed new monoclonal antibodies (MoAb mouse 9A1, MoAb rat 141A1) that react with both HTGL in serum and in post-heparin plasma (PHP) and established a novel enzyme-linked immunosorbent assay (ELISA) system that was able to measure both serum HTGL concentration and PHP-HTGL concentration.

**Results:** To confirm the specificity of antibodies, we performed an immunoprecipitation-immunoblotting analysis. Both MoAb mouse 9A1 and MoAb rat 141 A1 were able to immune-precipitate not only recombinant HTGL and PHP-HTGL but also serum HTGL, demonstrating that HTGL exists in plasma obtained without heparin injection. This method yielded a coefficient of variation of less than 6 % in intra- and inter-assays and did not cross-react with lipoprotein lipase (LPL) or endothelial lipase (EL). In clinical analysis on 42 male subjects with coronary artery disease, there were strongly positive correlations of serum HTGL concentration to PHP-HTGL concentration ( $r=0.727$ ,  $p<0.01$ ). Serum HTGL concentrations had positive correlations to serum triglycerides ( $r=0.314$ ,  $p<0.05$ ) and ALT ( $r=0.406$ ,  $p<0.01$ ) and tendency toward positive correlations to LDL-C, small, dense LDL and  $\gamma$ GTP.

**Conclusion:** These results indicate that this new ELISA method for measuring pre-HTGL is applicable in daily clinical practice.

**B-149****Homogenous LDL Cholesterol assays - How does the Roche Generation 3 LDL-Cholesterol measure up?**

S. Y. Wong, C. J. Gea, B. X. S. Quek, N. Iu, C. P. Yeo. *Singapore General Hospital, Department of Clinical Pathology, Clinical Biochemistry Laboratory, Singapore, Singapore*

**Background:**

Low Density Lipoprotein Cholesterol (LDL-C) is an important risk assessment biomarker for cardiovascular disease and a primary target of cholesterol lowering therapy, hence accurate measurement is pertinent in routine clinical practice. Homogenous assays for direct measurement of LDL-C are gaining recognition due to its capability of being adapted to automated analysers and its merits in improved assay precision. However not all homogenous assays perform alike, due to differences in the ability of the assays to reliably detect LDL-C particularly in more complex specimen matrix. Our laboratory currently offers, in addition to the more routinely requested traditional Friedewald calculated LDL-C, testing for direct LDL-C measurements using the Wako LDL-C assay on the Beckman Coulter AU 5800 automated analyser. Lack of appropriate peers and sub-optimal performance in external quality assurance (EQA) programs for our current LDL-C assay have propelled our laboratory to proactively explore alternative assays as part of continuous improvement initiatives. Roche Diagnostics has recently launched a new improved formulated LDL-C Generation 3 (Gen 3) assay, which claims to offer improved specificity for LDL-C and better comparison with the beta-quantification reference method. Our study evaluated the analytical performance of the Roche LDL-C Gen 3 assay on the Roche Cobas c502 and compared it against our current existing LDL-C assay on the Beckman Coulter AU5800.

**Methods:**

Performance validation parameters of the Roche LDL-C Gen 3 assay included assay imprecision (within and total), lower limit of detection, linearity and carry-over. Imprecision was assessed using manufacturer's quality control materials measured in triplicates over 5 days. Limit of detection, linearity and carry-over studies were performed using appropriate low and high concentration patient samples. Method correlation studies using fresh patient serum samples (n=120) were performed on the AU5800 and Cobas c502 platforms. EQA performance of the Roche LDL-C Gen 3 assay was also assessed using leftover samples from accuracy-based surveys.

**Results:**

Within run and total imprecision were determined to be  $\leq 1.1\%$  on Roche c502. Roche LDL Gen 3 assay demonstrated linearity across the analytical measurement range of 0.1 - 14.2 mmol/L, with recoveries between 97-100%. The lower limit of detectable concentration was assessed to be in agreement with the manufacturer's claims. Results of carry-over studies were insignificant. Method correlation with Beckman AU revealed Passing Bablok regression slope of 1.16 and intercept of -0.7; mean absolute bias (Altman Bland) of -0.18 mmol/L (95% CI: -0.22 to -0.13) and Spearman's correlation coefficient of 0.98. Indirect assessment of Roche's LDL Gen 3 EQA performance using post-survey samples from accuracy-based surveys showed better agreement with reference method beta-quantification.

**Conclusions:**

Overall, the Roche LDL-C Gen 3 assay performed within manufacturer's specifications and showed good correlation with beta-quantification. A small but insignificant negative bias is detected in the Roche assay when compared with Beckman AU assay (Wako). Our study demonstrated that the Roche Gen 3 LDL-C fulfills the accuracy and imprecision assay goals in the National Cholesterol Education Program (NCEP) requirements and represents a good alternative choice of direct LDL-C testing for clinical laboratories.

**B-150****Small Dense LDL Cholesterol as Strong Predictors for Secondary Cardiovascular Events Compared with LDL Cholesterol in Elder Patients.**

Y. Ito<sup>1</sup>, S. Koba<sup>2</sup>, Y. Yokota<sup>2</sup>, K. Sakai<sup>2</sup>, Y. Nakamura<sup>2</sup>, F. Tsunoda<sup>2</sup>, M. Shoji<sup>2</sup>, Y. Kobayashi<sup>2</sup>. <sup>1</sup>*Denka Seiken Co., Ltd., Niigata, Japan*, <sup>2</sup>*Showa university school of medicine, Tokyo, Japan*

**Background:** Small dense (sd) LDL particles have been suggested to be highly atherogenic due to their higher penetration into arterial walls, their lower binding affinity for LDL receptors, their prolonged plasma half-life, and their lower resistance to oxidative stress compared to large buoyant (lb) LDL. Though several methods have been developed for the measurement of serum sd LDL levels, the methods are

laborious and time-consuming. We have developed a simple fully-automated direct homogeneous measurement method of sd LDL-C. With our assay kit, it has been revealed that the rise of sd LDL-C is a risk factor for coronary heart disease (CHD) independent from LDL-C. However, the significance as a risk factor in the secondary prevention of CHD has been unexplained yet. Method: Subjects were 356 male and 73 female patients aged of 65 years and older with stable coronary artery disease (CAD) who did or did not develop major cardiovascular events (MACEs) during 5-year follow-up period. MACEs were defined as all cause death, onset of acute coronary syndrome, need for coronary and peripheral arterial revascularization, hospitalization for heart failure, surgical procedure for any cardiovascular diseases, and/or requiring hospitalization for stroke. Besides sd LDL-C with our assay kit, the following biomarkers were measured or calculated, and then analyzed: LDL-C, lb LDL-C, non-HDL-C, RLP-C, apoA1, apoB, glucose, HbA1c, BNP, and eGFR. Result: First-time MACEs were observed in 141 patients. Male patients with MACEs had significantly higher levels of LDL-C, lb LDL-C, sd LDL-C, non HDL-C, apoB, RLP-C, fasting plasma glucose, HbA1c, BNP, and high-sensitive CRP, and significantly lower levels of HDL-C, apoA1, and eGFR were observed in the patients. Multivariate Cox regression analysis results showed that all of biomarkers except lb LDL-C and eGFR were significantly associated with MACEs. Among lipid biomarkers, only sd LDL-C had a significant association with MACEs in male patients treated with statins (Hazard ratio (HR) 1.022, 95% confidence interval (CI) 1.003-1.042) and diabetic male patients (HR 1.019, 95% CI 1.001-1.036). Kaplan-Meier MACE-free survival curve analysis was conducted for 2 groups dichotomized by sd LDL-C 25 mg/dL, which is the median of whole subject. Significantly decreased MACE-free Survival ratio was observed in high sd LDL-C group. Conclusion: These results confirm that sd LDL-C is the most important residual risk to predict future MACEs in stable CAD patients including patients treated with statins.

### B-151

#### Stability of fatty acids in serum stored at -20°C for up to 5 years

C. D. Powers<sup>1</sup>, M. R. Sternberg<sup>1</sup>, S. S. Momin<sup>1</sup>, E. C. Pendergrast<sup>2</sup>, D. C. Scully<sup>3</sup>, R. L. Schleicher<sup>1</sup>. <sup>1</sup>CDC, Atlanta, GA, <sup>2</sup>Battelle, Columbus, OH, <sup>3</sup>ORISE, Oak Ridge, TN

In a pilot study, we saw up to 45% degradation of polyunsaturated fatty acids (PUFA) in plasma stored for 6 months at -20°C. It is known that PUFA are highly sensitive to oxidative degradation. This study evaluates the stability of a panel of fatty acids in serum stored with and without butylated hydroxytoluene (BHT) at -20°C ≤ 5 years. The panel includes 11 saturated (SFA), 6 monounsaturated (MUFA), and 13 PUFA fatty acids. A modification of Lagerstedt *et al.* (2001) was used to quantitate unfractionated serum fatty acids. Briefly, total fatty acids were hydrolyzed, hexane-extracted, derivatized to pentafluorobenzyl bromide esters, and detected using electron capture negative-ion gas chromatography-mass spectrometry.

Serum pools were prepared (±)BHT (3.33 g/L methanol). The serum pools (±) BHT were stored at -70°C or -20°C for up to 5 years. All vials were kept frozen and analyzed at designated times, (3 months, 1.5 years, and 5 years). To assess assay performance, serum quality control pools (+)BHT were used. Serum was measured in duplicate at each time point except one storage condition, serum (-)BHT stored at -70°C for 5 years was measured in singlicate due to availability.

For each time period and fatty acid in the panel, concentrations were log transformed and a separate two-way analysis of variance was performed using pool and storage condition as the main effects. The reference condition was storage at -70°C (+)BHT for the specified length of time (3 months, 1.5 years, or 5 years). Relative percent changes for storage at -20°C (±)BHT compared to the reference condition for a given time period were calculated from a two-way ANOVA model with no interaction. However, interactions between the storage conditions and pools were noted if the pool-by-storage-condition interaction was both statistically significant and a > 5% difference existed between any of the pools.

The average percent difference for SFA in serum (±)BHT stored at -20°C ranged from -2% to 0.2% at 3 months and 1.5 years ( $p \leq 0.05$ ); MUFA were ≤ 4% different at 3 months ( $p \leq 0.05$ ). PUFA with ≥ 3 double bonds decreased from 0.4% to 9% (+)BHT and from 16% to 36% (-)BHT in serum stored at -20°C for up to 5 years ( $p \leq 0.05$ ). Eicosapentaenoic acid and docosahexaenoic acid were particularly unstable in the absence of BHT, losing 34% and 35%, respectively, after 5 years at -20°C ( $p \leq 0.05$ ). Serum fatty acids stored for 5 years at -70°C (±)BHT were on average < 1% different across all classes compared to the reference condition.

In conclusion, SFA and MUFA in unfractionated serum are relatively stable when stored at -20°C (±)BHT. However, PUFA degrade when stored without BHT at -20°C for longer than 3 months; generally, greater numbers of double bonds were associated with lower stability. To prevent substantial PUFA degradation, BHT should be added to the serum prior to storage at -20°C or preferably serum should be stored at ≤ -70°C.

### B-152

#### Non-HDL cholesterol secondary therapeutic goals based on LDL cholesterol population percentiles: Contribution of Longitudinal Study of Adult Health (ELSA-Brasil)

F. A. Brito<sup>1</sup>, W. Pedrosa<sup>2</sup>, C. B. Maluf<sup>1</sup>, P. G. Vidigal<sup>1</sup>. <sup>1</sup>Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, <sup>2</sup>Hermes Pardini Laboratory, Belo Horizonte, Brazil

**Background:** Current international guidelines consider non-high-density lipoprotein cholesterol (non-HDL-C) as a goal for the prevention and treatment of atherosclerotic cardiovascular disease. These guidelines established non-HDL-C targets 30 mg/dL higher than the respective low-density lipoprotein cholesterol (LDL-C) cut-off points, considering the very-low-density lipoprotein (VLDL-C), estimated by the Friedewald equation, when the triglyceride concentration is 150 mg/dL. These definitions do not consider the population distribution of the non-HDL-C values, and recent studies in different populations have shown that they were lower than the guidelines cut-off points. The aim of this study is to establish cut-off points for non-HDL cholesterol (non-HDL-C) at the same population percentiles of LDL-C. The frequency of reclassification of therapeutic category using current guideline-based non-HDL-C and population percentiles-based cut-off points were also assessed.

**Methods:** A total of 14,837 participants from the Longitudinal Study of Adult Health (ELSA-Brasil), aged from 35 to 74 years old and with triglycerides levels < 400 mg/dL were included. Total cholesterol, HDL-C, and triglycerides were measured in the ADVIA Chemistry system after 12 hours of fasting and LDL-C was calculated with the Friedewald equation. Initially, population percentiles were established for the LDL-C values corresponding to the therapeutic goals, and non-HDL-C values equivalent to the respective LDL-C percentiles were assigned. Next, we assessed the reclassification of therapeutic category based on non-HDL-C, defined as the frequency of subjects with LDL-C levels in the recommended treatment category, but non-HDL-C levels higher than the recommended treatment goal.

**Results:** The LDL-C values of 70, 100, 130 and 160 mg/dL corresponded to the population percentiles 3, 18, 52 and 82, respectively. The non-HDL-C cut-off points for the same percentiles were 92, 122, 156 and 191 mg/dL. When using the latter non-HDL-C cut-off points, 2305 (15.5%) participants were reclassified upwards and 2410 (16.2%) downward. Using current guidelines-based cut-off points, 2068 (14.0%) participants were reclassified upwards and 3957 (26.7%) downward. Among participants with triglycerides between 150 and 199 mg/dL and with concurrently LDL-C level < 70 mg/dL, 26.3% were reclassified upward according to the guideline-based non-HDL-C cut-off point (≥ 100 mg/dL), and 65.8% according to the population percentile-based cut-off point (non-HDL-C ≥ 92 mg/dL). Of participants with LDL-C level between 70 and 99 mg/dL, 14.3% were reclassified upward according to the guideline-based cut-off point (non-HDL-C ≥ 130 mg/dL), and 48.9% according to the population percentile-based cut-off point (non-HDL-C ≥ 122 mg/dL).

**Conclusion:** In this large cohort of free living Brazilians, our results indicates that the recommended target value for non-HDL-C should be reduced to match the same population percentile equivalent to LDL-C goals. This change resulted in a significant increase in the frequency of reclassification of patients to a higher therapeutic category, especially in the presence of low LDL-C level and hypertriglyceridemia.

### B-153

#### RNASE L regulates the expression of fatty acid synthase in the mouse liver

R. Wei, A. Zhou. Cleveland State University, Cleveland, OH

#### Background:

Interferons (IFNs) are cytokines that participate in the innate immunity against viruses and other microbial pathogens. In addition, they also display anti-tumor, anti-proliferative and immuno-regulatory activities. The function of IFNs is mediated through proteins encoded by IFN-stimulated genes (ISGs). Ribonuclease L (RNase L) is a type of ISGs. Studies have shown that RNase L contributes to anti-viral infection, apoptosis and anti-cell proliferation. RNase L knockout mice are significantly larger than wide type mice in terms of body weight, and have more oil droplets in the organ tissues such as liver and kidney, suggesting that RNase L may be involved in lipid metabolism.

#### Method and Results:

To determine if RNase L regulates the expression of any genes in the liver, liver tissue extracts from RNase L knockout and wild type mice were subjected to SDS-polyacrylamide electrophoresis and Coomassie blue staining. The differentially

expressed protein bands between the two tissue extracts were excised and digested by trypsin, and subsequently analyzed by LC/MS. Interestingly, the results indicated that fatty acid synthase (FAS) was significantly higher expressed in the liver deficient RNase L. The results were further confirmed with Western Blot analysis by using a mouse monoclonal antibody against fatty acid synthase. Further investigation revealed that RNase L regulates its expression at the transcriptional level and is age and gender dependent. This observation is consistent with our previous report that the lipid level is relatively higher in the plasma of RNase L deficient mice.

**Conclusion:**

Our study reveals that FAS is higher expressed in the liver of RNase L deficient mice, as a result, an increased level of lipid in the plasma of the animals was observed. RNase L transcriptionally regulates the expression of FAS, which could be a potential target of hyperlipidemia and a prognostic marker obesity.

**B-154**

**The Performance of Calculated and Directly-Measured Low Density Lipoprotein Cholesterol in a Pediatric Population**

S. M. Roper<sup>1</sup>, J. Cao<sup>1</sup>, E. Tam<sup>2</sup>, S. Devaraj<sup>1</sup>. <sup>1</sup>Baylor College of Medicine/Texas Children's Hospital, Houston, TX, <sup>2</sup>Texas Children's Hospital, Houston, TX

**Introduction:** Accurate measurement of low-density lipoprotein cholesterol (LDL-C) is essential for the identification and monitoring of dyslipidemia. Recent studies indicate that the Friedewald equation is biased when LDL-C levels fall below 100 mg/dL and triglycerides are elevated. The objective of this study was to evaluate the use of a novel equation and a direct homogenous assay to correct the bias of the Friedewald equation in a pediatric population. As a secondary goal, the utility of these methods was assessed by determining the proportion of cases which fall into the biased range of the Friedewald equation at Texas Children's Hospital.

**Study design:** LDL-C concentrations were determined by the Friedewald equation, a direct homogenous assay, the novel equation<sup>1</sup>, and the reference method in 127 pediatric samples with triglycerides between 150-399 mg/dL. Bias was measured by regression analysis at selected LDL-C cutoffs and after stratifying samples by triglyceride content. Statistical significance (p=0.001) was determined by the Wilcoxon Signed-Rank test at LDL-C cutoffs of ≤ 70, ≤ 100, and > 100 mg/dL. The concordance of each method, relative to the reference method, was calculated at LDL-C cut-points of <70, 70-99, and 100-129 mg/dL. Retrospective data analysis was carried out to determine the prevalence of low LDL-C (≤100 mg/dL) and elevated TG (150-399 mg/dL) over a 2.5-month period at Texas Children's Hospital in Houston, TX.

**Results:** The Friedewald equation significantly underestimated pediatric LDL-C concentrations below 100 mg/dL (-21.6%) and the direct-LDL assay was positively biased (10.8%); the performance of both methods worsened with decreasing LDL concentrations and increasing triglyceride content. The novel equation most-effectively reduced the bias (-1.2%) and increased the concordance of sample classification to the reference method. Approximately 19% (157 of 846) of samples submitted for LDL-C and TG measurement between October 2016 and December 2016 fell into the biased range of the Friedewald equation.

**Conclusions:** The novel equation should be used for accurate measurement of pediatric LDL-C when the concentration is below 100mg/dL and the triglycerides are between 150-399mg/dL. Granted the large percentage of samples having low LDL-C and elevated triglycerides at our institution, values from the novel equation should be included in lipid results.

1. Martin SS, Blaha MJ, Elshazly MB, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA*. 2013;310(19):2061-2068.

**B-155**

**Monogenic dyslipidemia in a child followed due disnutrition diagnosis: the importance of clinical suspicion and cascading screening**

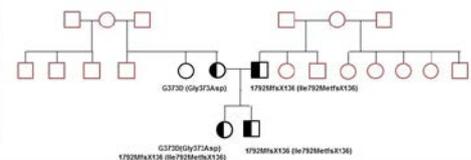
M. C. Gurgel Castelo<sup>1</sup>, L. A. A. Batista<sup>2</sup>, S. C. Lopes<sup>2</sup>, F. L. B. Furtado<sup>2</sup>, G. A. Campana<sup>3</sup>, R. M. Montenegro Jr<sup>2</sup>, R. D. Santos Filho<sup>4</sup>, A. C. Pereira<sup>4</sup>, C. E. Jannes<sup>5</sup>. <sup>1</sup>Universidade Federal do Ceará; DASA, Fortaleza, Brazil, <sup>2</sup>Universidade Federal do Ceará, Fortaleza, Brazil, <sup>3</sup>DASA, São Paulo, Brazil, <sup>4</sup>Incor- USP, São Paulo, Brazil, <sup>5</sup>Incor - USP, São Paulo, Brazil

**Background:** The screening to identify children with lipid disorders, and diagnosis of familial hypercholesterolemia (FH) is uncommon in clinical practice. In general, lipid profile measured in early childhood usually do so as a result of a metabolic condition arising from obesity and the family history of cardiovascular disease in the pediatric age group is not always obtained.

**Methods:** Case report

**Results:** We described an 11 year old child attended a pediatric referral center with complains of xanthomas (figure 1) in the knee, elbow and buttocks associated with LDL-c high levels (above 200 mg/dL), despite the rosuvastatin use, since the age 2. Additionally, the child had positive family history with her maternal grandmother surgically revascularized at 47 years old. As the child did not respond to the treatment, the pediatrician decided to refer her for clinical and genetic investigation to a primary dyslipidemia center. At the first visit, the lipid panel showed total cholesterol of 436mg/dL, LDL-c:383mg/dL, HDL-c:38mg/dL and triglyceride:75mg/dL. The calculate Dutch score was 8 with causality classified as "probable" for FH. The genetic sequencing was performed, using the Sanger method and revealed mutation on compound heterozygosis: G373D(Gly373Asp) on exon 8 and 1792MfsX136 (Ile792MetfsX136) on exon 16 of LDL receptor gene. After confirmation of this index-case (IC), a cascade screening was started on her asymptomatic first-degree relatives and identified the following mutations in heterozygosis at the LDL-c receptor gene: G373D(Gly373Asp) on exon 08 (mother- 27 yrs); 1792MfsX136 (Ile792MetfsX136) on exon 16(father- 45yrs and brother- 5 yrs).

**Conclusion:** Monogenic conditions due to a single gene defect, such as FH is a rare condition in children. Clinical suspicion in non-obese children is uncommon. The most frequent mutation is that of the LDL gene receptor. First-degree relatives should always be screened.



**B-156**

**Genetic and clinical-demographic profile of patients with familial hypercholesterolemia (FH) followed on an ambulatory of primary dyslipidemia in a university hospital**

M. C. Gurgel Castelo<sup>1</sup>, L. A. A. Batista<sup>2</sup>, S. C. Lopes<sup>2</sup>, F. L. B. Furtado<sup>2</sup>, R. M. Montenegro Jr<sup>2</sup>, G. A. Campana<sup>3</sup>, R. D. Santos Filho<sup>4</sup>, C. E. Jannes<sup>4</sup>, A. C. Pereira<sup>4</sup>. <sup>1</sup>Universidade Federal do Ceará; DASA, Fortaleza, Brazil, <sup>2</sup>Universidade Federal do Ceará, Fortaleza, Brazil, <sup>3</sup>DASA, São Paulo, Brazil, <sup>4</sup>Incor - USP, São Paulo, Brazil

**Background:** FH is a genetic disease caused by mutations in genes related to the metabolism of low-density lipoprotein (LDL-c), resulting in LDL-c high levels and cardiovascular risk (CVR) increase. The course of FH is silent and less than 10% FH patients are diagnosed. **Methods:** This was a cross-sectional study that evaluated patients from a FH screening program, selected through the LDL-c levels ≥210mg/dl (adults) and ≥170mg/dl (children). The patients were classified according to Dutch criteria and genetic data. The DNA sequencing was performed by Sanger's methodology. After the identification of a causal mutation, a cascade screening (CS) in first-degree relatives was pursued.

**Results:** For the 63 index cases (IC), the mean age was 51.44 ± 15.36 years and 72.73% (48) were female. On the 31 related cases, from CS, 22 ± 17.3 years and 27.27% (18) were female. From 63 IC, 79.36% (50) completed the genetic sequencing with 20.96% heterozygosis mutation confirmed on LDL-c receptor gene. The positivity for those mutation detection on SC patient was 25.8%. The majority of

mutations were found on exons 8,9,12,14 and 16. From the population (63 IC and 31 CS), 67.02% (63) patients met DUTCH criteria and 52.38% (11) had a positive family history for cardiovascular disease. Overweight or obesity was not identified in the study population. Less than half of the patients received at least one pharmacological agent for dyslipidemia (45.74%).

**Conclusion:** The diagnosis of FH is based on clinical criteria although the genetic diagnosis is very relevant. Most of the patients are heterozygote. The CS is crucial on the FH identification, which allows early intervention and cardiovascular mortality decrease.

### B-157

#### Markers of Inflammation in Acne Vulgaris

L. Aslan<sup>1</sup>, F. Ozcan<sup>2</sup>, T. Karaarslan<sup>1</sup>, E. Kıracı<sup>2</sup>, M. Aslan<sup>2</sup>. <sup>1</sup>*Antalya Training and Research Hospital, University of Health Sciences, Antalya, Turkey,* <sup>2</sup>*Akdeniz University Faculty of Medicine, Antalya, Turkey*

**Background:** Acne vulgaris (AV) is a inflammatory skin disease of unknown etiology. Eicosanoids derived from omega-6 (n-6) polyunsaturated fatty acids (PUFAs) have proinflammatory functions whereas eicosanoids derived from n-3 PUFAs have anti-inflammatory properties. Studies have documented that dietary supplementation with n-3 fatty acids ameliorates AV however serum PUFA levels have not been evaluated in patients with AV. Excess levels of secretory phospholipase A2 (sPLA2) also contributes to inflammatory diseases and studies indicate that lipoprotein lipase (LPL) has differential effects on several inflammatory pathways. This study aimed to determine circulating levels of n-6, n-3 PUFAs, assess serum activity of sPLA2, LPL and evaluate changes in circulating protein levels of angiotensin-like protein 3 (ANGPTL3), ANGPTL4, cyclooxygenase-2(COX-2) and prostaglandin E2 (PGE2) in patients with AV.

**Methods:** Serum from 31 AV patients and 21 age and gender matched control subjects were evaluated for levels of sPLA2, COX, PGE2, LPL, ANGPTL3 and ANGPTL4 via commercial assay kits. Serum PUFA levels were measured by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Lipid profile, routine biochemical and hormone parameters were assayed by standard kit methods using autoanalyzers.

**Results:** No significant differences were found for the lipid profile, routine biochemical and hormone parameters between AV patients and controls. Serum EPA levels were significantly decreased while AA/EPA and DGLA/EPA ratio were significantly increased in AV patients compared to controls. Serum levels of AA, DGLA and DHA showed no significant difference between patients and controls. Serum activity of sPLA2 and LPL were significantly increased in AV compared to controls. No significant differences were found for COX, PGE2, ANGPTL3 and ANGPTL4 levels.

**Conclusion:** The results of this study reveal the presence of a proinflammatory state in AV as shown by significantly decreased serum EPA levels and increased activity of sPLA2, AA/EPA and DGLA/EPA ratio. Increased LPL activity in the serum of AV patients can be protective through its anti-dyslipidemic actions. This is the first study reporting altered EPA levels and increased sPLA2 activity in AV and supports the use of omega-3 fatty acids as adjuvant treatment for acne patients. **Acknowledgement:** This study was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK; #115S940).

### B-158

#### Only fresh samples should be allowed for lipid profile evaluation

G. Lima-Oliveira, G. Lippi, G. C. Guidi. *University of Verona, Verona, Italy*

**Background:** This study was aimed to investigate whether the laboratory could assay lipid profile using either fresh or thawed samples.

**Methods:** Serum from 10 volunteers were immediately assayed for total cholesterol (CHOL), high density lipoprotein (HDL), and triglycerides (TG); then each volunteer's sample was divided in 2 identical aliquots. All aliquots were thawed after two-days freezing at -70°C. Immediately afterwards, the sera of the two paired aliquots were treated using two different techniques: (a) no mixing; (b) reference procedure, entailing 6 gentle inversions by overturning; The significance of the differences against the fresh (no frozen samples) was assessed with Wilcoxon signed rank test. The statistical significance was set at p<0.05.

**Results:** A significant variability was observed for CHOL, HDL, and TG when compared with thawed samples not mixed; whereas thawed samples mixed by inversion showed significant differences for CHOL, and HDL; but not for TG (Table 1).

**Table 1. Lipid profile assayed on fresh and thawed samples**

Test	Fresh samples	Thawed samples	
		Not mixed	Mixed
TG	1.32 [1.11 – 1.87]	0.82 [0.66 – 1.20] P=0.002	1.31 [1.10 – 1.86] P=0.588
CHOL	5.43 [5.18 – 5.68]	3.22 [2.90 – 3.42] P=0.002	5.33 [5.10 – 5.65] P=0.008
HDL	1.66 [1.38 – 1.75]	0.92 [0.80 – 1.02] P=0.002	1.65 [1.36 – 1.71] P=0.020

Data are presented as mean [interquartile range], in mmol/L.

**Conclusion:** Based on our results lipid profile should be assayed on fresh samples in order to guarantee both laboratory outcomes and reliable results, thus patient safety.