Tuesday, July 28, 2015

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

Nutrition/Trace Metals/Vitamins

A-307

Development of a Reference Material for the Determination of Vitamin B_{12} in Human Serum

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Background: The National Institute of Standards and Technology (NIST) provides a variety of Standard Reference Materials® (SRMs) for the analysis of nutrient levels in clinical matrices. One nutrient of interest is vitamin B_{12}, which is involved in DNA synthesis and red blood cell formation. Vitamin B_{12} deficiency can lead to anemia and permanent nerve damage. Vitamin B_{12} in serum is measured by a variety of analytical techniques, including microbiological, radioisotopic, and chemiluminescence assays. Concerns remain regarding the comparability and accuracy of these various methods, which supports the development of an accuracy control reference material. While the current SRM 1955 Homocysteine and Folate in Frozen Human Serum possesses reference values for vitamin B_{12} based on radioassay measurements at the Centers for Disease Control and Prevention (CDC), no material specifically designed for vitamin B_{12} determination in serum has been available to date. In addition, the SRM 1955 stock with be exhausted within a year and the replacement material is not slated for vitamin B_{12} value assignment. NIST, in collaboration with the National Institutes of Health-Office of Dietary Supplements (NIH-ODS), has developed a new material to fill this void.

Methods: Candidate SRM 3951 Vitamin B_{12} in Human Serum was designed to have three concentration levels of vitamin B_{12}: nominally, 100 pg/mL, 200 pg/mL, and 450 pg/mL. To produce candidate SRM 3951, a vendor pooled normal human serum to achieve the highest level. Since all donor serum screened for this project contained >200 pg/mL vitamin B_{12}, the remaining mid- and low-level materials were obtained by diluting higher-level serum with charcoal-stripped serum. All serum levels were packaged as 1-mL aliquots in glass vials and stored as fresh frozen serum.

Results: NIST intends to utilize measurement data from multiple methods in order to assign vitamin B_{12} values, as total cyanocobalamin, to all three levels of material. Duplicate analyses from six vials measured over three separate days by electrochemiluminescence immunoassay at the CDC resulted in mean values of 57.8 pg/mL (%CV=14 %), 155.1 pg/mL (%CV=5.3 %), and 383.7 pg/mL (%CV=2.6 %) for the low-, mid-, and high-level materials, respectively. In addition, NIST is developing an isotope-dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) method in order to provide a second, independent method for the determination of total vitamin B_{12}. The recent availability of ^{13}C-labeled cyanocobalamin has enabled the development of this method, which involves the spiking of serum samples at an approximately 1:1 mass ratio of labeled to unlabeled target analyte, followed by chemical conversion of various vitamin B_{12} metabolites to cyanocobalamin, affinity clean-up, and ID-LC-MS/MS analysis. The NIST method will also be utilized to establish homogeneity across the batch of candidate SRM 3951.

Conclusion: Candidate SRM 3951 will provide the first accuracy control reference material from NIST specifically designed with three unique levels of vitamin B_{12}, which will provide a valuable tool for *in vitro* diagnostics manufacturers, as well as other end-users, for validating their calibrators and methods. This, in turn, will help to ensure that clinical vitamin B_{12} measurements are accurate and that patients with deficiencies are treated appropriately.

A-308

The role of oxidized metabolites of linoleic acid in alcohol-mediated hepatic inflammation and injury in a mouse model of alcoholic liver disease

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Background/Aim: Dietary fat is an important determinant of alcoholic liver disease (ALD). It has been documented that experimental ALD is exacerbated by a high polyunsaturated fat diet rich in linoleic acid. Recent publications have shown that experimental and clinical alcohol-induced liver steatosis and injury were associated with elevated oxidized linoleic acid metabolites (OXLAMs), specifically 9- and 13-hydroxy-octadecadienoic acids (9- and 13-HODEs). OXLAMs are endogenous ligands for the Transient Receptor Potential Vanilloid 1 (TRPV1), a non-selective channel with high permeability for Ca2+. We postulate that bioactive OXLAMs play a critical role in the development and progression of alcohol-mediated hepatic inflammation and injury via OXLAM-TRPV1-mediated mechanism. The aim of the study was to evaluate the role of OXLAMs and OXLAM-TRPV1 signaling in an experimental animal model of ALD.

Methods: C57BL/6 wild type (WT) and Trpv1 knock out (Trpv1-/-) male mice were fed a Lieber-DeCarli diet containing 5% ethanol (v/v) for 10 days, followed by a single dose of ethanol (5 g/kg body weight) by gavage (chronic-binge model). Liver steatosis was evaluated by histopathology and hepatic triglyceride accumulation measurement. Liver injury was assessed by plasma ALT activity; hepatocyte cell death was determined by hepatic caspase-3 activity and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Expression of genes associated with liver inflammation was analyzed by qRT-PCR. Plasma OXLAM levels were determined by LC/ESI/MS/MS and stable isotope dilution GC-MS methods. In vitro studies using HepG2 cells were performed to evaluate OXLAM/TRPV1 signaling.

Results: Chronic-binge alcohol administration resulted in a marked increase in plasma OXLAM levels, specifically 9-HODE and 13-HODE, in parallel with up-regulation of hepatic Trpv1 in WT animals. These effects were associated with hepatic steatosis, inflammation and injury. Genetic depletion of Trpv1 did not blunt hepatic steatosis caused by EtOH, but ameliorated hepatic injury as assessed by ALT levels (354.7+54.0 U/L in WT vs 130.6+30.5 U/L in Trpv1-/-, $p<0.05$). Trpv1 deficiency protected from chronic-binge alcohol-induced hepatocyte death assessed by caspase-3 activity and TUNEL staining. Trpv1 deficiency also prevented the increase in pro-inflammatory cytokine and chemokine expression, including Tnf-α, IL-6, Mip-2, Mcp1. Moreover, Trpv1 depletion markedly blunted EtOH-mediated induction of plasminogen activator inhibitor-1 (Pai-1), an important mediator of alcohol-induced hepatic inflammation, via fibrin accumulation. EtOH-induced up-regulation of pro-inflammatory cytokines in WT but not in Trpv1-/- animals was in parallel with the activation of hepatic NF-kB and extracellular-signal-regulated kinase (ERK) pathways. One of the possible mechanism(s) underlying hepatic inflammation in our model might relate to the nature of TRPV1, as a channel with high permeability for Ca 2+. Thus, exposure of hepatocytes to 9-HODE and 13-HODE in vitro resulted in activation of TRPV1 signal transduction with increased intracellular Ca2+ levels, suggesting that OXLAM/TRPV1/Ca2+ signaling may be a potentially relevant pathway contributing to ALD.

Conclusions: This study indicates for the first time that the TRPV1 receptor pathway may be involved in the hepatic inflammatory response in an experimental animal model of ALD. TRPV1-OXLAM interactions appear to play a significant role in hepatic inflammation/injury, further supporting an important role for dietary lipids in ALD.

A-309

Assessment of excess cancer risk associated with exposure to some airborne trace elements

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Backgrounds: Airborne particulate matter (PM), especially fine particles, could be closely related to health problems in a typical urban environment. PM may consist of toxic trace elements that considered potential health risk factors due to their carcinogenic properties. Of these trace elements, cadmium (Cd), chromium (Cr), arsenic (As), beryllium (Be) and nickel (Ni) were classified as probable human carcinogens (group B1) in accordance to USEPA, 2011. Makkah is the holy city in Saudi Arabia with a total population of 1.7 million, although it has limited industrial activities, it has unique characteristics every year, over 2.3 million of pilgrims stay in Makkah during Hajj period which increase transportation pollution problems creating unspecified amount of trace elements pollution in air. Objective: This study aimed to determine the excess cancer risk (ECR) of population associated to the inhalation exposure of five heavy metals (Cd, Cr, As, Be, Ni) in ambient air of Makkah. Materials and Methods: The study was conducted in Arafat Area, east of Makkah (latitude 21° 21' 1 N, longitude 39° 58' 1 E), representing one of the holy places highly crowded in Hajj season. Meteorological data were recorded and air samples were collected using mini volume sampler (Airmetrics, USA) at 10m height on a 47mm Teflon filter at 16.6 l/min flow rate for 24 hrs once in a week for 6 months during summer and autumn 2014, in accordance to USEPA standards method (29/2000). Trace elements content of the collected PM10 samples were analysed using ICP-MS 7300 (Perkin Elmer, USA) in reference to a standard solution of trace elements using a protocol certified by (US-NIST). The recovery yields of trace elements were higher than 95% with

detection limits ≤3 ng/m3 for all trace metals. Since trace elements carcinogenicity risk were unknown in Makkah, determination of ECR due to inhalation exposure to each metal was calculated in accordance to unit risk factor (URF) presented in US-EPA-IRIS. Results: Mean atmospheric concentrations of Cd, Cr, As, Be and Ni were 0.098, 0.008, 0.016, 0.03 and 0.012 µg/m-3 respectively in summer months. While in autumn, the mean concentrations were 0.06, 0.006, 0.01, 0.002 and 0.01 µg/m-3, respectively. The ECRs for Arafat area, were found to be (1.08×10-4, 7.21×10-4, 4×10-6, 4.6×10-6 and 2.4×10-6) for Cd, Cr, As, Be and Ni respectively, exceeding USEPA's level of acceptable inhalation risk (1.0x10-6) for each element. Conclusions: Higher concentrations of atmospheric trace elements in summer are probably due to high temperature as well as high wind speed, a common phenomenon in Makkah, which increases atmospheric turbulence leading to a greater amount of re-suspension of dust from roadside and blowing sand particles. The findings of this study can be addressed as reference for authorities' regulations and to develop air quality management strategy for protecting the public health. However, larger prospective studies are warranted to explore the health effects of long-term exposure to ambient air trace elements. Acknowledgments: This work was supported by King Abdulaziz City for Science and Technology (KACST) under National Science, Technology and Innovation Plan (NSTIP), KSA.

A-311

Comparison Study of PAML's VDSP Certified LC/MS/MS Vitamin D Assay and the VDSP Certified Siemens Centaur Vitamin D Assay

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Objective:

The primary objective of this study was to compare two methods that are certified under the United States Centers for Disease Control (CDC) Vitamin D Standardization Certification Program (VDSCP). The methods are PAML's certified LC-MS/MS Vitamin D assay and the Siemens Healthcare Diagnostics ADVIA Centaur Vitamin D Total assay (Centaur).

Methods:

A total of 116 samples were analyzed by both methods in this study. The sample set included 39 samples obtained through the CDC, that are single donor serum materials independent of the VDSCP, with values ranging from 9.6-67.2 ng/mL. An additional 10 native serum samples were spiked with 25(OH)vitamin D_3 (D_3) and provided by Siemens. The remainder of the samples were PAML patient residuals, with total 25(OH)vitamin D values ranging from 9-84 ng/mL. All samples were stored frozen at -70°C. In preparation for analysis, the samples were thawed, vortexed, and aliquotted. The analysis by both assays was conducted by the respective clinical departments. The resulting data were compiled and analyzed using method comparison regression analysis and principle component analysis (PCA).

Results:

The study method comparison results are summarized in the table. The additional PCA results show a high influence of 25(OH)vitamin D_2 (D_2) in the second PC, and D_2 and the Centaur results are negatively correlated in the third PC.

Conclusions:

The data shows a negative bias of the Centaur compared to the LC-MS/MS. The PCA shows that the D_2 is influencing the data set, however the regression analysis does not show a marked difference between samples with D_3 only and samples that contain D_2. One source of potential bias is the D_3 epimer which co-elutes with D_3 in the LC-MS/MS method. The epimer does not cross-react in the Centaur assay. Additional studies are underway to determine if bias is due to individual measurements by the two assays.

Data Sets	Corr. Coeff.	Deming Slope	Deming Intercept	Average Bias
Full Study (N=116)	0.9555	0.882	-1.909	-6.137
CDC Sample Set (N=39)	0.8903	1.033	-3.706	-2.799
Samples with D_3 only (N=67)	0.9653	0.870	-0.199	-4.737
Samples with D_2 (N=42)	0.9158	0.923	-5.445	-8.123

A-312

The first decade of a global external quality assurance program for serum-based nutritional biomarkers

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*Background***:** The VITamin A Laboratory-External Quality Assurance program (VITAL-EQA) was established in 2003 to ensure the existence of laboratories around the globe that are proficient in quantitative analysis of serum retinol, particularly those labs taking part in national nutrition surveys. The program, administered by the U.S. Centers for Disease Control and Prevention, has since expanded to include vitamin D metabolites, inflammation and iron indicators, and B vitamins.

Methods: Twice each year, an average of 19 labs test serum samples in duplicate over the course of 3 days and estimate concentrations of nutritional biomarkers. Results are submitted to CDC, reports are issued, and labs are given guidance as requested or needed.

Results: The method used to determine the performance of each lab was updated in the 5th year to place an emphasis on imprecision and bias based on biological variation rather than scoring against acceptable and target ranges generated from consensus and CDC data, respectively. Using vitamin A as an example, based on biological variation, labs need a CV <2.4%, <4.8%, and <7.1% to achieve an optimum, desirable, or minimum imprecision performance rating, respectively. For bias, deviation from the target value needs to be <±4%, <±8%, and <±12% for an optimum, desirable, or minimum bias rating, respectively. Of the labs that participated in at least 2 rounds during the last 5 years of the program, 24% had an acceptable bias (minimum or better) for all 3 levels of vitamin A tested in both their first and last rounds. In their first round of participation, more than 1/3 of labs (38%) had an unacceptable bias for all 3 levels. Of this subgroup of labs, nearly 2/3 moved to having an acceptable bias in their last round (27% for all 3 levels, 27% for 2 of the 3 levels, and 9% for 1 level), while the remainder (36%) still had an unacceptable bias for all 3 levels. The program has grown from 11 to 21 participating labs, and from 2 to 7 nutritional indicators. As of the last round, the number of labs reporting biomarker data were as follows: 18 retinol, 5 25-hydroxyvitamin D, 7 C-reactive protein, 9 ferritin, 6 soluble transferrin receptor, 7 vitamin B12, and 7 folate.

Conclusions: The number of labs benefitting from this program has nearly doubled since its inception. More labs are added each year, and the performance rating method continues to evolve to better determine the quality of the data and aid the regions serviced by each lab.

A-313

Rapid and Simultaneous Quantitative Determination of Vitamin A, D and E by UPLC-UV

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Objective: To validate and implement a rapid, simple, and sensitive Ultra Performance Liquid Chromatography (UPLC) method for the simultaneous determination of Vitamin A (retinol), 25-OH-vitamin D2, 25-OH-vitamin D3 and Vitamin E-α (α-tocopherol) and Vitamin E-γ (γ- tocopherol) in human serum/plasma for routine clinical use at the Children's Hospital Los Angeles.

Method: Tocopherol Acetate (100 µL) as the internal standard was added to 500 µL of serum/plasma. After a 5 minute incubation at room temperature, 800 µL of M/I mixture (Methonol: 2-propanol, 9:1 v:v) was added and vortexed for 15 seconds, followed by 4 ml of hexane, vortexed for 4 minutes and then centrifuged at 1500 rpm for 2 minutes. The top phase was transferred to a 12 X 75 mm glass tube, and dried under N2. The pellet was then reconstituted with 150 µL of methanol, and filtered out with a 0.2 µm syringe filter. 4 µl of filtrate was injected into Waters UPLC Acquity system.

Retinol, 25-OH-vitamin D2, 25-OH-vitamin D3, α-tocopherol and γ-tocopherol and Tocopherol Acetate were analyzed simultaneously using the UPLC with UV detector at 265 mm. Separation was achieved with Water's UPLC BEH C18 1.7 µm, 150 x 2.1 mm column at 32 °C. The method incorporates gradient elution with mobile phase A (0.1% formic acid in 85% acetonitrile and 15% water) and mobile phase B (0.1% formic acid in 96% acetonitrile and 4% methanol).

Results: The linearity was up to 6.66 µg/ml for retinol, 200 mg/L for 25-OH-vitamin D3, 200 mg/L for 25-OH-vitamin D2, 190 mg/L for α-tocopherol and 42 mg/L for γ-tocopherol, respectively. The limit of quantitation for retinol, 25-OH-vitamin D3,

25-OH-vitamin D2, α-tocopherol and γ-tocopherol were as follows: 0.025 µg/ml, 4.2 mg/L, 4.2 mg/L, 0.28 mg/L and 0.7 mg/L respectively. The recoveries were 102-110% for retinol, 114-119% for 25-OH-vitamin D2, 109-117% for 25-OH-vitamin D3, 98-101% for α-tocopherol and 99-117% for γ-tocopherol. The inter-assay precision CV was 8% for retinol, 9.9% for 25-OH-vitamin D2, 6.9% for 25-OH-vitamin D3, 11% for α-tocopherol and 9.5% for γ-tocopherol. The intra-assay precision CV was 2.2% for retinol, 6.7% for 25-OH-vitamin-D2, 3.7% for 25-OH-vitamin-D3, 0.8% for α-tocopherol and 1.3% for γ-tocopherol. This UPLC method was compared with a LC/MS-MS method. The correlation coefficients (R) were 0.97 for retinol, 0.98 for total 25-OH-vitamin D, 0.97 and 0.96 for α-tocopherol and γ-tocopherol. Reference intervals were established for retinol and α-tocopherol and γ-tocopherol based on the healthy pediatric population seen at Children's Hospital Los Angeles. For retinol, 17y: 0.22-0.96 µg/ml. For α-tocopherol, 17y: 5.38-19.08 mg/L. For γ-tocopherol, 17y 0.33-4.19 mg/L.

Conclusion: Because of its low cost, short analysis time (8 min) and excellent chromatographic reproducibility, this UPLC method is suitable for simultaneous analysis of these three vitamins in human serum/plasma. This new method will allow laboratories to meet the increasing demands for monitoring of fat-soluble micronutrients, and can be easily adopted and scaled for high-throughput clinical practice if more expensive LC/MS-MS is not available.

A-314

Amalgam fillings, fish consumption and urbanization are associated with high urinary excretion of mercury in healthy individuals.

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Background: Mercury (Hg) heavy metal pollution is a serious environmental hazard all over the world. Bioaccumulation of Hg is associated with neurotoxicity and damaging effects on several cellular components such as membranes, proteins, and DNA. This study was performed to assess concentration of Hg in urine samples of otherwise healthy individuals and exposure to commonly known predisposing factors.

Methods: This study was performed in clinical chemistry department at King Khalid University, Riyadh between Jan. 2013 and March 2014. A total of 246 otherwise healthy individuals comprising of 153 (62.2%) males and 93 (37.8%) females with a mean age of 37±11 years were included in the study. Along with the demographic details information regarding consumption of fish, having amalgam tooth fillings, residence in urban or rural areas and smoking habit was also recorded at time of collection of random urine samples. Hg levels in urine were determined by inductively coupled plasma mass spectrometry.

Results: The median concentration of Hg [7.8 µg/g creatinine Interquartile range; (IQR) 4.55] in urine samples of 95/246 individuals with one or more amalgam fillings was significantly higher (p=0.00001) than 151/246 individuals with no amalgam filling (1.8 µg/g creatinine; IQR: 1.96). Participants consuming fish meals more than once a week (108/246) had higher urinary levels of Hg (median 4.04 µg/g creatinine; IQR: 3.44; p=0.0001) compared to those (138/246) consuming less than one fish meal per week (median 1.92 µg/g creatinine; IRQ: 2.97). Similarly individuals (174/246) residing in urban areas had higher urine concentration of Hg (median 3.64 µg/g creatinine; IQR: 3.3) compared to individuals (72/246) residing in rural areas (median 2.65 µg/g creatinine; IQR: 2.98; p=0.006). No difference in urinary Hg concentration was detected between smokers (114/246) consuming more than ten cigarettes a day (median 2.4 µg/g creatinine; IQR: 3.29) or less than ten cigarettes (132/246) a day (median 2.6 µg/g creatinine IQR: 3.1).

Conclusion: Amalgam fillings, frequent consumption of fish and residing in urban areas are significant predisposition to bioaccumulation of Hg.

A-315

Introducing Candidate Standard Reference Material (SRM) 2378 Fatty Acids in Human Serum

B. A. Benner, M. M. Schantz, K. E. Sharpless. *NIST, Gaithersburg, MD*

Objective: To characterize the fatty acid composition of a new SRM that consists of serum acquired from donors who were or were not taking dietary supplements that contained fish oil or flaxseed oil.

Relevance: Dietary fatty acids can be both beneficial and detrimental depending on the degree and type of saturation. Healthcare providers, the Centers for Disease Control and Prevention (CDC), and other organizations monitor fatty acid content of human plasma and serum as an indicator of health status and diet. Both the CDC and the National Institutes of Health - Office of Dietary Supplements are interested in serum fatty acids because they may be predictive of coronary heart disease or cancer. Candidate SRM 2378 is for use as a quality assurance sample in the analysis of serum for fatty acids, and consists of three different pools of serum acquired by a contracted laboratory from healthy donors who had been taking fish oil dietary supplements (at least 1000 mg per day) for at least one month (level 1 material), from healthy donors who had been taking flaxseed oil dietary supplements (at least 1000 mg per day) for at least one month (level 2 material), and from healthy donors eating "normal" diets without taking dietary supplements containing oils (level 3 material).

Methodology: Mass fractions of fatty acids in SRM 2378 were determined using four different extraction procedures, including saponification in methanolic KOH, treatment with sodium methoxide in methanol, hydrolysis using sequential addition of acetonitrile:hydrochloric acid and methanol:sodium hydroxide in the presence of heat, and microwave-assisted digestions using acetonitrile:hydrochloric acid. The fatty acids were esterified using sulfuric acid in methanol and pentafluorobenzyl bromide for subsequent measurement by GC with flame ionization detection (FID) and GC with electron capture ionization (negative chemical ionization) detection, respectively. SRM 1950 Metabolites in Human Plasma was employed for quality control of the methods used in this study.

Results: Significant differences in mass fractions of specific fatty acids were observed among the different levels representing different supplementations (flaxseed oil and fish oil) or no supplementation. For example, the concentration of α-linolenic acid, a major component of flaxseed oil, was observed at two times the concentration of the level 3 material in both the level 2 and level 1 materials [(32.5 ± 4.1) µg/g and (31.5 ± 1.3) µg/g, respectively versus 17.0 ± 0.1) µg/g]. In addition, the level 1 material (fish oil supplemented) was enriched with four times the levels of eicosapentaenoic acid [(84 ± 11) µg/g versus (20.7 ± 0.8) µg/g and (18.9 ± 2.2) µg/g] and two times the concentrations of docosahexaenoic acid [(104 ± 5) µg/g versus (55.4 ± 2.3) µg/g and (54.9 ± 2.4) µg/g] observed in the level 2 and 3 materials.

Conclusions: The enrichments in specific fatty acids observed in the level 2 and level 1 materials of SRM 2378 with respect to the level 3 material (no supplementation) are consistent with donors supplementing with flaxseed oil and fish oil, respectively.

A-316

Trace element levels in Korean pregnant women and their associations with pregnancy outcomes

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Background:

Maternal trace element levels can have multiple impacts on health outcomes in mothers and their offspring. The aim of this study was to investigate the trace element status [cobalt (Co), copper (Cu), zinc (Zn), and selenium (Se)] in Korean pregnant women to assess their effects on pregnancy related outcomes: gestational diabetes, preeclampsia, gestational age at delivery, delivery with emergent Caesarean section, baby weight at birth (including assessments of low birth weight infants and those small for gestational age), children born with congenital abnormalities, prelabor rupture of the fetal membranes (PROM), preterm birth, and preterm PROM.

Methods:

This prospective study is based on 243 Korean pregnant women recruited at a referral hospital from April 2012 to January 2013. We investigated serum micronutrient status according to demographics, seasons, and obstetric characteristics together with the assessment of pregnancy and infant outcomes.

Results:

The median (interquartile range) serum trace element concentrations of all participants were as follows: 0.39 µg/L (0.29-0.53 µg/L) for Co, 165.0 µg/dL (144.0-187.0 µg/dL) for Cu, 57.0 µg/dL (50.0-64.0 µg/dL) for Zn, and 94.0 µg/L (87.0-101.0 µg/L) for Se. The serum concentrations of all four trace elements were significantly different over all three trimesters ($P < 0.05$). The overall prevalence of an excess or deficiency of the trace elements were as follows: 3.7% (n = 9) for Co excess, 70.0% (n = 170) for Cu excess, 76.1% (n = 185) for Zn deficiency, and 2.1% (n = 5) for Se deficiency.

There was no significant association between maternal serum trace element status and pregnancy outcomes, except for Zn deficiency and the rate of emergent Caesarean sections (adjusted odds ratio 4.59, P = 0.04) and the risk of preeclampsia with Cu excess (adjusted odds ratio 12.37, P = 0.03).

Conclusion:

Our results suggest that maternal Zn and Cu status during pregnancy may influence the risk of emergent Caesarean sections and preeclampsia. Further research about the long-term consequences of trace element status during pregnancy is warranted.

A-318

Clinical utility of serum folate measurement in tertiary care patients Argument for revising reference range for serum folate from 3.0ng/mL to 13ng/mL

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Clinical utility of Serum folate:

Importance: The reference range for serum folate levels needs to be established and clinical utility of folate measurement assessed.

Objective: Asses the need for folate testing and determine reference level for serum folate.

Design: Serum folate levels in 5313 samples from 4448 patients, at two tertiary care medical centers were examined. Clinical data reviewed for patient characteristics in general and for evidence of corrective action in patients with serum folate values <5.5ng/mL. Clinical parameters in 128 patients with serum folate levels <5.5ng/mL and 128 patients with levels >25.7ng/mL at one of the medical centers were analyzed.

Setting: Medical School affiliated, tertiary care, safety net hospitals in Georgia and Missouri.

Participants: All samples and patients tested in July 2013 through June 2014 were included.

Results: The prevalence of serum folate levels, in patients, <3.0, <4.0, <5.5 and <13ng/mL was 0.58, 1.55, 4.9, and 43.21% respectively. Patients with serum folate levels <5.5ng/mL had lower serum albumin and hemoglobin as compared to those with serum folate >25.7ng/mL. In 82 of the 128 (64%) patients with serum folate >25.7ng/mL the sample was collected after supplementation with folic acid or multivitamins. Of the 20 patients with serum folate <3.4ng/mL there was evidence of supplementation in half. Of the 128 patients with serum <5.5ng/mL documentation of supplementation was present in 28.9%.

Conclusions: Serum folate levels are below the current "normal" level of 3.0ng/mL in a larger proportion of patients seen at tertiary care hospitals than that reported for ambulatory patients. In patients with subnormal serum folate levels, corrective action by supplementation is lacking in at least half of the patients. Since serum folate levels ≥13.0ng/mL are needed for optimal prevention of neural tube defects in the embryo/fetus and we propose that normal serum folate level should be designated to be ≥13.0ng/mL.

Table

	Georgia		Missouri		Total	
	Samples	Patients	Samples	Patients	Samples	Patients
Serum folate	2696	2075	2617	2373	5313	4448
<3.0ng/mL	15	14	12	12	0.51%	0.58%
<3.4ng/mL	21	20	18	18	0.73%	0.85%
<4.0ng/mL	41	36	34	33	1.41%	1.55%
<5.5ng/mL	153	128	100	90	4.76%	4.90%
<7.0ng/mL	304	260	202	184	9.52%	9.98%
<13ng/mL	1202	1014	967	908	40.82%	43.21%

A-319

Antioxidant Status of Subjects with Metabolic Syndrome in Port Harcourt, Nigeria

E. P. Odum, C. G. Orluwene, A. A. Ejilemele, V. C. Wakwe. *University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria*

Abstract

Background

Increased free radical production and thus oxidative stress have been implicated in the pathogenesis of metabolic syndrome. These cause depletion of defences against free radical damage which comprise antioxidant enzymes and vitamins like vitamins C and E, resulting in low plasma levels. This may aggravate impaired insulin action and endothelial dysfunction and predispose to diabetes and cardiovascular disease.

Aim/Objective

This study was designed to determine if the plasma total antioxidant status and vitamins C and E levels are lower in metabolic syndrome subjects living in Port Harcourt compared to healthy controls.

Subjects and Methods

Blood pressure, waist circumference, concentrations of plasma glucose (mmol/L), lipid profile (mmol/L), total antioxidant status (TAS, mmol/L), vitamin C (µmol/L) and vitamin E (µmol/L) were determined in 100 subjects who fulfilled the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III criteria for metabolic syndrome and 100 age- and sex-matched controls.

Statistical Analysis Used: Statistical Package for Social Sciences (SPSS) version 11.0

Results

The mean plasma TAS (1.23 ± 0.28mmol/L), vitamin C (27.5 ± 7.4µmol/L) and vitamin E (16.9 ± 4.9µmol/L) of metabolic syndrome subjects were significantly lower (P=0.0001 for all) than that of controls (1.58 ± 0.28mmol/L, 44.3 ± 7.3µmol/L, 30.8 ± 6.1µmol/L respectively).

Conclusion

The reduced TAS, vitamins C and E in metabolic syndrome subjects compared to controls may be due to increased oxidative stress resulting from an imbalance between antioxidant defences and increased free radical production. Increased intake of adequate dietary antioxidants and supplementation could be beneficial in preventing or delaying the consequences of metabolic syndrome.

Key Words: metabolic syndrome, total antioxidant status, vitamin E, Vitamin C.

Table 1: Antioxidant Levels of Subjects and Controls

Antioxidant	Subjects(N=100) Mean ± SD	Controls(N=100) Mean ± SD	P-value
Vitamin C (µmol/L)	27.5 ± 7.36	44.3 ± 7.30	0.0001*
Vitamin E (µmol/L)	16.9 ± 4.86	30.8 ± 6.12	0.0001*
Total Antioxidant Status (mmol/L)	1.2 ± 0.28	1.6 ± 0.28	0.0001*

* Statistically significant (P<0.05)

A-320

Vitamin D status in Korean children and adolescents: continuously increasing prevalence of vitamin D deficiency with advancing age

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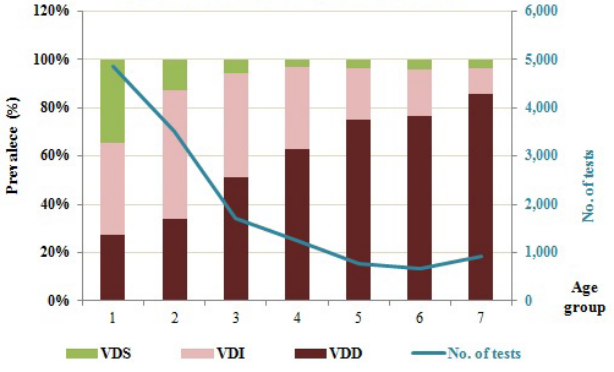
Background: The objective of this study was to assess the prevalence of vitamin D deficiency (VDD) and vitamin D insufficiency (VDI) across the age range of 0 to 20 in Korean children and adolescents using recent nationwide data.

Methods: The authors retrospectively analyzed the 25-hydroxyvitamin D (25(OH)D) results of 13,728 children and adolescents (6,755 boys, 6,973 girls) aged from 0 to 20 years. The tests were requested to Seoul Medical Science Institute from 332 medical institutions nationwide in South Korea between January 2014 and December 2014. Serum 25(OH)D was measured by chemiluminescence immunoassay (LIAISON system, DiaSorin, Italy). Participants were divided into 7 age groups (1, age <3 years; 2, 3-5 years; 3, 6-8 years; 4, 9-11 years; 5, 12-14 years; 6, 15-17 years; 7, 18-20 years). Prevalence of VDD and VDI according to gender and age groups were analyzed. VDD and VDI were defined by serum 25(OH)D <20 ng/mL and 20.0-29.9 ng/mL respectively.

Results: When the concentrations of 25(OH)D were categorized according to the above criteria, overall 44.3% and 38.5% of subjects were defined as VDD and VDI respectively. The mean concentration of 25(OH)D in girls was significantly lower than that of boys (20.9 ng/mL vs 22.7 ng/mL, p <0.0001). Concentrations of 25(OH)D were negatively correlated with age groups (r=-0.4185, p<0.0001). The prevalence of VDD according to age group was continuously increased with advancing age groups, reaching the highest VDD rate (85.8%,799/931) in the age group 7.

Conclusion: Our result shows that the prevalence of VDD is continuously increasing with advancing age in South Korean children and adolescents. In spite of very high prevalence of VDD in older children, 25(OH)D assays are requested much less than younger children. Therefore, more attention regarding vitamin D status is needed, and efforts for improving vitamin D status are required especially for older children and adolescents in South Korea.

Fig. 1. Prevalence of 25-hydroxyvitamin D (25(OH)D) deficiency and insufficiency in different age groups



A-321

Ramadan Fasting Reduces Serum Fetuin A And High Sensitive C Reactive Protein Levels

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Fasting during Ramadan is a religious act practiced by many Muslims worldwide. The aim of this study was to evaluate the effects of Ramadan fasting on body composition, Fetuin A, high sensitive C- reactive protein and tumor necrosis factor alpha in some Egyptian volunteers. Subjects and methods: This study was conducted on 40 Egyptian volunteers (20 females and 20 males) divided into 2 age groups: below or equal to 35 years and above 35 years. Data was collected twice: one day before Ramadan and on the 28thday of Ramadan fasting. The following parameters were measured :a) body composition was determined by bioelectric impedance using InBody-220; the fat mass, skeletal muscle mass, total body water and minerals. b) Body Mass Index (BMI). c) The waist circumference. d) blood samples were taken for determination of; serum Fetuin A by (ELISA technique), serum TNF-α by (ELISA technique) and High sensitive C reactive protein by (ELISA technique) Results: After Ramadan fasting, there was a significant decrease in the body weight of males > 35 years, the skeletal muscle mass of females ≤ 35 years and the body mass index of females >35 years. Furthermore, in most of the studied groups, there was a significant decrease in the level of fetuin A and high sensitive C reactive protein. However, no significant changes occurred in the level of tumor necrosis factor alpha after Ramadan in any group. Conclusion: Ramadan fasting has a potential anti-inflammatory effect manifested by lowering the levels of both fetuin A and Hs-CRP, irrespective to body fat percentage which did not significantly change in any of the studied groups.

Correlations between the different studied parameters before and after Ramadan fasting:			
	Before Ramadan	After Ramadan	Mean difference
	r p	r p	r p
Fetuin A versus Hs-CRP	0.718* <0.001	0.395* 0.012	0.535* <0.001
Fetuin A versus TNF-α	0.196 0.225	0.080 0.625	0.048 0.770
TNF-α versus Hs-CRP	-0.043 0.793	-0.119 0.466	0.213 0.187

A-322

Simultaneous Determination of Retinol and α-Tocopherol in Serum by Ultra Performance Liquid Chromatography

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Clinical interest in the evaluation of vitamin A and vitamin E nutriture has increased in recent years, mainly owing to the possible roles of retinol β-carotene, and α-tocopherol in decreasing the risk of cardiovascular disease and cancer.

The aim of this study was validate a simple, rapid, sensitive and cheap method for the simultaneous determination of retinol and α-tocopherol in serum using a Ultra Performance Liquid Chromatography with UV-VIS detection by comparison with a reference method.

The reference method was developed by ChromSystems®. The method was previously validated in the laboratory to confirm that the analytical procedure employed was suitable for the intended use.

The extraction procedure is based on protein precipitation and liquid-liquid extraction, in which 100 µL of serum were mixed with 450 µL of a solution of ethanol/n-butanol(50:50, v/v) (precipitant solution) and submitted a vortex for 30 seconds, followed by centrifugation at 14.000 rpm for 10 minutes. 400 µL of the clear supernatants were transferred to vials and 10 µL were injected into the UPLC equipment.

Retinol and α-tocopherol were separated by isocratic mobile phase containing a mixture of methanol:water (95:5 v/v). The flow rate was 400µL/min, using an Acquity UPLC BEH C18 1.7µm 2.1x50 mm column kept under thermostatization at 30 °C. The eluate absorbance was monitored at 325 nm until 0.9 min then turn to 295 nm until the end of the chromatographic running to quantify Retinol and α-tocopherol, respectively. The chromatographic running time was approximately 2.8 minutes.

A comparison test was carried out with 87samples, two levels of commercial controls and a commercial calibrator standard extracted and analyzed usint the two methods in order to verify the performance of the new test.

The correlation coefficient was 0.990 and 0,976 for retinol and α-tocopherol, respectively. The regression line (Passing-Bablok) showed In house = 1.14ChromSystem-0.0157 for retinol and In house = 1.21 ChromSystem-2.0628 for α-tocopherol. The recovery was calculated taking as the true value the value found in the reference method.We obtained an average recovery of 110.6% and 101.69% and the coefficient of variation was 7.1% and 4.0% for to retinol and α-tocopherol, respectively. The samples tested by both methods, showed an absolute agreement of 93.3% for retinol and 97.8% for α-tocopherol to clinical classification of patients.

With the data obtained and the statistical analysis, we can conclude that the methods tested are equivalent and produce concordant results.

A-323

Redox Modulatory Factors In Human Breast Milk

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Background:

In this study, we aimed to investigate the difference in total oxidant status (TOS), total antioxidant status (TAS) and nitric oxide (NO) concentrations in human breast milk, according to the gestational age (preterm, late preterm, term) and lactation days after delivery (3rd, 7th, and 28th day)

Methods:

26 mothers were included in this study (9 preterm, 8 late preterm and 9 term). Breast milk was collected from participants on 3rd, 7th and 28th days. Milk samples were stored at -80°C. NO analysis was performed by a spectrophotometric method using the Griess Reagent.TOS and TAS analysis were performed by a spectrophotometric method using commercial kits provided by the Rel Assay Company.

Results:

There was a significant difference between the TAS, TOS and NO levels of the groups of lactation days (p=0.480, 0.290, 0.426 respectively), without considering the gestational age. According the posthoc Tukey comparisons of NO levels between 3rd and 7th days, 3rd and 28th days; significant difference was seen (p values <0.001 and 0.028 respectively). There was also a significant difference between 3rd and 28th, 7th and 28th days considering the TAS results (p=0.007, 0.012 respectively). And TOS

result revealed a significant difference between 3rd and 7th days (P=0.022). TAS, TOS and NO levels of milk samples of the three groups, according to the gestational age, showed no significant difference. TAS and NO levels were found to be decreased during lactation.

Conclusion:

This study showed that the redox modulatory factors of human breast milk changed during lactation. However, gestational age on delivery did not affect the human breast milk with this aspect.

Table 1: TAS, TOS and NO levels in human breast milk samples on 3rd, 7th and 28th days of lactation

	3rd day(Mean ± SD)	7th day(Mean ± SD)	28th day(Mean ± SD)
TAS (mmol Trolox Equiv./L)	0.944 ± 0.254	0.928 ± 0.272	0.810 ± 0.221
TOS (μmol H2O2 Equiv./L)	0.673 ± 0.588	1.196 ± 1.121	1.096 ± 0.744
NO (mM)	0.382 ± 0.098	0.322 ± 0.092	0.282 ± 0.089

A-324

Performance and Certification of the ADVIA Centaur Vitamin D Total Assay

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Background: Vitamin D helps regulate calcium in the development and maintenance of healthy bones. The National Institutes of Health Office of Dietary Supplements created the Vitamin D Standardization Program (VDSP) to establish a standard for accurate and comparable results for the detection of 25(OH)D across laboratories. The Centers for Disease Control (CDC) provides assay manufacturers and laboratories with the Vitamin D Standardization-Certification Program (VDSCP) to help assess assay calibration.

Methods: Between January and December 2013, the Centers for Disease Controls (CDC) provided 40 blinded 25(OH)D samples in the Vitamin D Standardization-Certification Program (VDSCP), in which a set of 10 samples was evaluated each quarter against the CDC and University of Ghent Vitamin D_2 and D_3 Reference Measurement Procedure (RMP). Samples were tested blindly in replicates of four over 2 days, two replicates per day. Additional supplemental samples were also evaluated, including the four standards from the National Institute of Standards and Technology (NIST).

Results: The ADVIA Centaur® Vitamin D Total assay met the criteria for VDSCP certification. The mean bias to the reference method was 0.3%, within the acceptable bias of ±5.0%. The assay's imprecision of 5.5% was also within the acceptable range of ≤10.0%. A linear regression of the blinded samples demonstrates a slope of 1.01 and an intercept of -1.89nmol/L. The ADVIA Centaur Vitamin D Total assay also shows an acceptable bias with the NIST Standard Reference Material (SRM) 972a vitamin D metabolite samples.

Conclusion: The VDSCP certification for the ADVIA Centaur Vitamin D Total assay establishes an acceptable alignment to a harmonized testing standard for 25(OH)D. The ADVIA Centaur Vitamin D Total assay provides laboratories with a standardized and automated means for quickly and efficiently testing patients' 25(OH)D levels.

A-325

The impact plastic tube EDTA K2 metal free in tha analysis of trace metals

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Introduction:

The trace metal determinations are performed either to evaluate the deficiency or excess of these elements in the body. Thus, pre-analytical losses and elevated values due to contamination should be evaluated. Contamination sources include: clothing and skin of the patient; collection materials such as needles, anticoagulants, covers, gel separator and tubes; material was particulated in the laboratory. The loss may be attributed to the surface adsorption of metals from the sample collection tubes or due to the presence of anticoagulants and a complex formation. There are on the market a limited number of free metal tube suppliers. The most common material was glass, but due to biosecurity issues its use was discontinued.

Purpose:

This study aimed to evaluate the performance of plastic tube metal-free with anticoagulant EDTA K2, in the determination of trace metals - Al, Cu, Zn and Se compared to glass tubes.

Materials and methods:

Samples from volunteer donors were collected in tubes BD Vacutainer® glass dry. The material was centrifuged and aliquoted into identical tube. The same procedure was carried out using BD Vacutainer ™ plastic tubes K2 EDTA. All tubes are metal-free.

The equipment used for the analysis of Al and Se was an atomic absorption spectrometer AAnalyst 800, Perkin Elmer with graphite oven with correction by Zeeman Effect. For Cu and Zn, we used the same equipment in the flame module. The samples were diluted only with Triton X® in 0.1% HNO3. Calibration curves were prepared from primary standard ICP Multi Element Standard Solution VI Certipur®.

Results

The data for all elements were evaluated for correlation of results using EP Evaluator software. The possible loss or contamination generated by the use of Tubes BD Vacutainer® EDTA K2, metal-free was investigated. The number of analysis was 53 for Al; 42 for Se; 36 for Cu and 36 for Zn.

The correlation coefficients were: Al = 0.9903; Se = 0.9551; Cu = 0.9772 and Zn = 0.8265. The slopes and intercepts were: Al 0.859 / 0.995; Se 0.897 / 9.58; Cu 1,082 / -9.5 and Zn 1.183 / -0.077. The bias was found -2.07 to Al; -0.52 to Se; -0.1 to Cu and 0.042 for Zn.

Discussion and Conclusion:

Among the metals analyzed, Al is what attracts more attention, by the contamination of sample facility. Factor noticed in a lesser extent in Zn. But the performance of the test tube was satisfactory in this regard. We did not observe losses by adsorption.

Found proper correlation in all metals, noting that the plastic tube with EDTA anticoagulant use K2 metal free proved to be compatible with the standard required by our Quality System.

A-327

Can the folate microbiologic assay be harmonized across laboratories?

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Background: There is lack of agreement among methods to measure serum and whole blood folate. The fully automated protein binding assays performed on clinical analyzers offer high sample throughput and generally good precision, but they have questionable accuracy and may suffer from lot-to-lot variation. Chromatography-based methods have high specificity, sensitivity, and precision, but they are expensive and require complex sample preparation. The microbiologic assay is considered a gold standard method, because it fairly equally measures all biologically active forms of folate, needs only a small sample volume, and can be performed in a low-resource laboratory. However, the microbiologic assay has not yet been harmonized and use of different calibrators or microorganisms can lead to different results. We will investigate whether providing different laboratories the same "microbiologic assay kit" leads to comparable serum and whole blood folate results.

Methods: We plan to recruit laboratories that are proficient in carrying out the folate microbiologic assay and are interested in participating in a Round Robin study. We will provide them with a "microbiologic assay kit" consisting of calibrator, microorganism inoculum, and unknown serum and whole blood lysate samples to be analyzed over 2 days each, first using the laboratory's reagents, and then using the CDC kit. The laboratories will use their own protocol, instrumentation, consumables, and basic reagents throughout the Round Robin study. We will compare results among laboratories and assess whether the harmonized results using the CDC kit are more comparable than the non-harmonized results.

Results: We created sufficient amounts of 5-methyltetrahydrofolate calibrator, *Lactobacillus rhamnosus* microorganism inoculum, and 20 pools each of serum and whole blood hemolysate. The folate concentrations in these pools span the range of results found in a population exposed to folic acid fortified foods: 9-90 nmol/L for serum folate and 170-600 nmol/L for whole blood folate. This will allow the laboratories to use higher sample dilutions compared to when samples are from a folate-deficient population, minimizing any potential of interferences or inhibition effects. We tested the homogeneity of these pools and found it to be adequate (≤10% variability for serum and whole blood folate). We invited 15 laboratories to participate in the Round Robin study. Eight laboratories have agreed to participate. The Round

Robin study will be carried out in the spring of 2015 and results will be available by the summer.

Conclusions: The microbiologic assay is a reliable and practical method that could be used in the low-resource setting to assess the folate status of the population. We expect that comparable folate results can be generated as long as laboratories use the same calibrator and microorganism inoculum. If that is the case, a global network of resource laboratories could be established to facilitate the implementation of nutrition surveys that assess whether an optimal blood folate concentration is achieved in women of reproductive age for prevention of neural tube defects.

A-328

Associations between Vitamin D Status and Serum Cardiovascular Risk Markers in an Adult Brazilian Population.

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Background: Vitamin D is essential for bone mineralization, but a growing body of evidence points at a broader role; vitamin D deficiency has been found to be associated with mortality and several diseases ranging from cardiovascular disease to autoimmune diseases and liver diseases. Low serum 25-hydroxyvitamin D (25OHD) levels have been associated with increased prevalence of cardiovascular diseases. A possible relation between serum cardiovascular risk markers and 25OHD might explain this association. The objective of this study was to evaluate the association between the serum 25OHD levels and cardiovascular risk markers in a population from Rio de Janeiro State in Brazil. **Methods:** This was a cross-sectional study in a sample of patients who performed the dosage of 25OHD in a private reference laboratory in Brazil over a period of one year (January to December 2014). Data on laboratory tests and demographic variables were available from a database of the local Laboratory Information System. We analyzed samples of both genders aged ≥ 20 years who tested for 25OHD (Chemiluminescence, Abbott) and also for total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TG) (Enzymatic Colorimetric, Roche), apolipoprotein ApoA-1 and ApoB (Nephelometry, Siemens) and homocysteine (Chemiluminescence, Abbott). Vitamin D deficiency was defined as serum concentration below 20.0 ng/mL, values between 20.0 and 30.0 ng/mL were considered insufficiency and those above 30.0 up to 100.0 ng/mL corresponded to the optimal levels. **Results:** Laboratory tests were equally distributed throughout the different seasons of the year (332,565 samples). 77 percent of the studied population was female and the mean age was 53 ± 18 years. Serum 25OHD ranged between 3.2 and 99.8 ng/mL, with a mean of 29.4 ± 10.4 ng/mL. Vitamin D deficiency, insufficiency and normal were observed in 16.6%, 42.0%, 41.4% in female versus 14.8%, 42.0% and 43.2% in male, respectively ($p<0.01$). There was also a difference in frequency of vitamin D deficiency/insufficiency in the second half of the year, corresponding to winter and spring months in south hemisphere (48.2% first half vs 66.7% in second half; $p<0.01$). In our data, multiple linear regression showed a correlation of 25OHD levels with lipids profile values ($p<0.01$) as well as for apolipoprotein A-1 levels ($p<0.01$). Results were not able to show correlation with apolipoprotein B and homocysteine. **Conclusions:** Our data support the literature where other tropical regions also have a high level of patients with deficiency / insufficiency of 25OHD, which was more frequent in winter and spring months. This study also supports an association between low 25OHD levels and lipids profile and ApoA-1, markers that contribute to cardiovascular diseases, suggesting that Vitamin D may be important in maintaining cardiovascular health.

A-329

Comparability of whole blood folate results from the microbiologic assay with a high-throughput LC-MS/MS method: a pilot study

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Background: The microbiologic assay (MA) responds nearly equally to

biologically active folate forms and is therefore regarded as a gold standard for the measurement of serum and whole blood folate. The advantage of LC-MS/MS is that it provides information on different folate forms and is less prone to interferences such as antibiotics. While we showed earlier good agreement (within ±10%) between the MA and LC-MS/MS for serum folate, we observed larger differences (~25%) for whole blood folate, with the LC-MS/MS method measuring on average lower. We were interested in an up-to-date comparison of whole blood folate data between these two assays using our newest high-throughput LC-MS/MS method.

Methods: We used conventionally prepared hemolysates (whole blood diluted 1:11 with 1% ascorbic acid; $n = 289$) to measure total folate by MA ($tFOL_{MA}$) and five folate forms (5-methyltetrahydrofolate [5-methylTHF], folic acid, THF, 5-formylTHF, and 5, 10-methenylTHF) and one oxidation product of 5-methylTHF (MeFox; pyrizino-s-triazine derivative of 4α-hydroxy-5-methylTHF) by LC-MS/MS. The sample vials were shared and processed by each assay at the same time. For the LC-MS/MS method, we added $^{13}C_5$-labeled folate analogs to serve as internal standards and subjected the hemolysates to 4-h incubation at 37ºC to deconjugate polyglutamates to monoglutamates. For the MA, we processed thehemolysates directly without further incubation. We calculated $tFOL_{LC\text{-}MS/MS}$ as the sum of folate forms measured by LC-MS/MS without including the biologically inactive MeFox form, because the MA does not respond to MeFox. We evaluated the comparability of the two methods by assessing weighted Deming regression and relative Bland-Altman bias to account for an increase in variance with increasing concentration.

Results: We found excellent correlation (R^2= 0.95) and goodcorrespondence between $tFOL_{LC\text{-}MS/MS}$ (mean [SD]: 432 [158] nmol/L) and $tFOL_{MA}$ (459 [178] nmol/L). While both the Deming slope (estimate [95% CI]: 0.89 [0.85 to 0.92]) and intercept (24.6 [10.3 to 38.9] nmol/L) were significant, they almost cancelled each other out, leaving a small but significant negative relative bias (-5.5 [-6.7 to 4.2] %). Most of the $tFOL_{LC\text{-}MS/MS}$ was composed of 5-methylTHF (mean [SD]: 404 [161] nmol/L), with the other folate vitamers contributing on average only 7%. The mean [SD] MeFox concentration was 66.7 [25.3] nmol/L, which corresponded to 17% of the 5-methylTHF concentration. This was a larger proportion of MeFox than what we typically observed in serum samples (~5%) and may be a result of 5-methylTHF oxidation during the 4-h incubation at 37ºC. The strong correlation (Spearman r = 0.94) between MeFox and 5-methylTHF in these whole blood samples, compared to a weak correlation (Spearman r = 0.25) observed earlier in serum samples, seems to support this hypothesis.

Conclusions: In this pilot study, we found good agreement for whole blood tFOL between the two test methods, with our new high-throughput LC-MS/MS method measuring on average only ~5% lower than the MA. However, based on the higher than expected proportion of MeFox, we need to explore whether we can shorten the incubation period and whether that would better maintain 5-methylTHF and other folate vitamers.

A-330

Determination of B-Carotene in Serum by Ultra Performance Liquid Chromatography

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Clinical interest in evaluation of vitamin A and vitamin E nutriture has increased in recent years, mainly owing to thepossible roles of retinol (vitamin A), β-carotene, and α-tocopherol (vitamin E) in decreasing the risk of cardiovascular disease and cancer.

The aim of this study was to validate by comparison a simple, rapid, sensitive and cheap method for the determination of β-carotene in serum by Ultra Performance Liquid Chromatography with UV-VIS detection. The instrument used was a chromatograph Acquity UPLC Waters® with a sample manager FTN and TUV detector.

The reference method was developed by ChromSystems® and contains commercial reagents kit for the HPLC analysis of β-carotene in serum/plasma. The method was previously validated in the laboratory to confirm that the analytical procedure employed is suitable for the intended use.

In clinical measurement comparison of a new measurement technique with an established one is often needed to see whether they agree sufficiently for the new to replace the old.

The extraction procedure is based on protein precipitation and liquid-liquid extraction, in which 100 µL of serum were mixed with 450 µL of a solution of ethanol/n-butanol (50:50, v/v) (precipitant solution). Then vortex mixed for 30 seconds, followed by centrifugation at 14.000 rpm for 10 minutes. 400 µL of the clear supernatants were transferred to vials. In UPLC equipment are injected 10 µL.

B-carotene was separated by isocratic elution with a mixture of acetonitrile:ethanol (90:10 v/v) as mobile phase. The flow rate was set to 400 µL/min, using an Acquity UPLC BEH C18 1.7µm 2.1x50 mm column kept under thermostatization at 30 °C. The eluate absorbance was monitored at 453 nm to quantify β-carotene. The chromatographic running time is approximately 3.0 minutes.

There was a comparison test on 60 samples, two levels of commercial control and a commercial standard extracted and analyzed with the two methods in order to verify the performance between the method developed by the laboratory method used.

It was made a direct comparative analysis in which a method is confronted with each other. The correlation coefficient was 0.9960. The regression line (Passing-Bablok) shows In house = 0.9157 ChromSystem- 11.567. The recovery was calculated taking as the true value the value found in the reference method. There was obtained an average recovery of 95.8%, with acceptable values for each sample are 80 to 110%. The mean coefficient of variation was 6.2%. Of the 60 samples and 2 commercial control tested by both methods obtained an absolute agreement of 93.6% in clinical classification of patients.

With the data obtained and the statistical analysis, we can conclude that the tested methods are equivalent and produce results with the same agreement, without causing any difference in clinical interpretation.The performance after the migration to the new methodology was significantly better, and observed the reduction of time analyses, cost, reduction troubles with the instruments and also higher rate of approval of controls.

A-331

Hair trace elements concentrations in obese females and their relation to type 2 diabetes in Saudi Arabia

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Backgrounds: Trace elements excess or deficiency could induce metabolic disorders and cellular growth disturbance; their regulatory, immunologic and antioxidant functions resulting from their action as essential components or cofactors throughout metabolism. Obesity is a worldwide disease affecting population of all age groups. In Saudi Arabia, the population is going through a nutrition transition where customary and traditional food are being replaced by fast food high in fat, sugar, salt as well as changes in lifestyle and reduced physical activity leading to increase the overall obesity prevalence above 35% and type 2 diabetes mellitus (DM) which is projected to rise from 890,000 in 2000 to 2,523,000 in 2030.

Objective: This study aimed to explore the correlations of hair trace elements as long-term biomarkers with obesity and type 2 DM in adult female Saudi patients.

Patients and Methods: This study included 169 women grouped as 64 diabetic obese (BMI > 30 kg/m2), 45 non-diabetic obese and 60 healthy non-obese (BMI 18-25). All subjects were randomly selected among the volunteers of matched age and similar socio-economic status. Renal or liver diseases patients, smokers and individuals who were taking trace elements supplements for the past three months, were excluded from the study. Full history, clinical data and anthropometric measurements were taken for all subjects. Hair samples collected from the nape section and hair trace elements Se, Zn, Cu, Mn and Fe concentrations were analysed by ICP-MS (Perkin- Elmer 7300, USA). Serum levels of total cholesterol, triglyceride, high and low density lipoprotein cholesterol (HDL and LDL) were analysed. DM confirmed by both fasting blood glucose (FBS) and glycated haemoglobin (HbA1c) levels. Odd Ratio of hair trace elements concentrations were adjusted for family history.

Results: FBS, HbA1c, cholesterol, LDL and triglycerides levels of the diabetic obese females were significantly higher than non-diabetic obese and healthy women ($P<0.005$). Although serum HDL levels had no statistical difference between diabetic and non-diabetic obese women, they were significantly lower than healthy group ($P<0.05$). Hair Cu concentrations of the diabetic and non-diabetic obese women were significantly higher than healthy group ($P<0.05$). On the other hand, hair Zn, Mn and Fe concentrations were significantly lower than those of the healthy women ($P<0.05$) as well as hair Zn concentrations was negatively correlated with FBS and HbA1c levels in the diabetic obese women ($P<0.05$). Hair Cu/Zn ratio was significantly higher in the diabetic obese than healthy women ($P<0.05$). Hair Se concentrations had no statistical differences among studied groups. However, they were positively correlated with FBS and HbA1c levels in the diabetic obese women.

Conclusion: Impaired trace-element metabolism may have a role in the pathogenesis and progression of both obesity and type-2 DM. Obesity is a clinically manifested metabolic disorder, including mineral imbalances, our findings showed significant association between obesity and Zn and Fe deficiencies. Hair trace elements can be a useful diagnostic tool as long-term biomarkers for metabolic disorders; however, larger prospective studies are warranted to validate their diagnostic value in obesity and type 2 DM.