400 Analyzer.

Results. The average CAR concentration (values in µmol/l, mean ± SD) in healthy persons was 40.1 ± 8.9, in HD patients 30.5 ± 10.3 before HD ($p < 0.001$). Values after HD decreased to 9.4 ± 4.6 ($p < 0.001$ vs. predialysis values). Both subgroups of HD patients had comparable levels of CAR in serum at the beginning of the study. While this value markedly increased after 3 and 6 months of supplementation (29.5 ± 8.7 vs. 135.3 ± 39.9 vs. 111.7 ± 35.7; $p < 0.001$), there was a tendency to a decrease in non-supplemented subgroup (30.4 ± 11.5 vs. 30.2 ± 12.5 vs. 25.7 ± 10.0; non-significant). The levels of CAR were still higher 2 months after the supplementation had ended (65.0 ± 16.6; $p < 0.01$ vs. initial values).

Conclusions. About 60 % of the patients before HD had a significant deficit of free CAR in serum. The others had comparable values with the healthy population. All observed patients had lower values of CAR after HD (approximately 30 % of the value before HD). There was a high increase after CAR supplementation, so that the values after HD were within reference ranges for healthy population. These higher levels still remained 2 months after the end of supplementation.

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D-117

Selenium replacement, HLA-DR expression and outcome in septic ICU patients

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Objective: This study was undertaken to determine the effect of selenium (Se) supplementation on outcome markers in septic patients in an adult ICU.

Methods: A prospective double-blind intervention study. Patients (APACHE II >15) were randomly stratified to receive either a high dose of Se intravenously [6, 4 & 2 µmol consecutive dosages, each for a 3 day period, group Se +, n=18] or a standard dose [0.4 µmol of Se group Se -, n=22]. Blood samples were taken for measurements of HLA-DR expression on monocytes, plasma Se and glutathione peroxidase (GPx), RBC GPx on day 0, 3, 7, 14. Clinical outcome markers included SOFA score, 28 day mortality, duration of ICU stay, infection rate and renal replacement therapy. One way ANOVA was used for statistical analysis.

Results:

TABLE 1: Outcome markers (mean ±SD) in Se+ & Se-

Analytes	Day 0		Day 3		Day 7		Day 14	
	Se+	Se-	Se+	Se-	Se+	Se-	Se+	Se-
SOFA score	10 (4.1)	8.7 (3.2)	8.5 (4.2)	8.4 (3.6)	5.7 (2.9)	6.7 (3.8)	4.5 (3.8)	6.3 (4.5)
HLA-DR (%)	21 (22.3)	49 (33.7)	38 (30.6)	39.2 (27.6)	32 (18.8)	42 (34.3)	48 (25.6)	58 (26.1)
Plasma Se (µmol/L)	0.6 (0.27)	0.7 (0.22)	1.6 (0.42) *	0.8 (0.28) *	1.6 (0.34) *	0.9 (0.26) *	1.4 (0.35)	1.1 (0.37)
Plasma GPx (U/L)	410 (163) **		564 (277) **	870 (253) **	606 (326) **	994 (334) **	657 (321) **	918 (349)	699 (305)
RBC GPx (U/gHb)	39.4 (8.2)		43.1 (8.0)	37. 1(9.4)	42.6 (8.9)	40.6 (9.7)	39 (8.4)	3.1 (6.2)	42.1 (11.3)

* p<0.0001, ** p=0.05(day 0), p=0.01(day 3),p=0.007(day 7)

Conclusions: There was a significant increase in plasma Se and GPx in Se+ by day 3 and 7 as compared to Se- group. However, there was no significant difference in mortality, infection rate, renal replacement therapy or duration of stay in ICU between two groups. Our study was unable to confirm that an increased dose of Se improves outcome in critically ill patients.

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Evaluation of Vitamin B12, Folate and rbcFolate Assays on the ADVIA IMS® 800i System

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Automated anemia assays are developed on the ADVIA IMS® 800i System for *in vitro* measurement of vitamin B12 and folate in human serum and whole blood samples. The modular system includes Immunochemistry, Chemistry and ISE modules on a single platform with a capability of continuous operation and stats.

All three assays are based on competitive protein binding. For rbcFolate, the red blood cells need to be manually lysed first. Folate (either from serum or lysed red blood cells) and B12 are released from endogenous binding proteins by treating with the Anemia Pretreatment Reagents (DTT/NaOH) at 42°C. The released B12 or folate reacts with the fluoresceinated (FITC) B12 intrinsic factor or folate binding protein reagent (R1) at 37°C. The B12 or folate-ALP conjugate reagent (R2) is then added to compete with B12 or folate in the sample for the R1 binding sites. Magnetic particles coated with the anti-FITC antibodies are subsequently added. After incubation, the complexes are washed and the dioxetane phosphate substrate is added, which produces a chemiluminescence signal upon hydrolysis.

Precision and accuracy were assessed at external clinical trial sites, by testing control samples over multiple runs and days, and by comparing patient sample results to the corresponding assays on the Bayer ADVIA Centaur® immunoassay system. The minimum detectable concentration (MDC) was determined by calculating the average dose at the 95% confidence limit of 20 replicates of the zero calibrators. Performance data are summarized in the following table:

	Vitamin B12, pg/mL	Serum Folate, ng/mL	rbcFolate, ng/mL
Minimal Detectable Concentration			
	26	0.3	7.5
Control Recovery and Precision Recovery(Within Run / Total % CV)			
Level 1	280 (2.1 / 6.5)	1.69 (5.6 / 11.3)	78.5 (3.0 / 8.6)
Level 2	516 (1.2 / 3.6)	6.15 (2.6 / 6.7)	207.3 (3.0 / 8.0)
Level 3	1051 (1.4 / 2.5)	10.13 (2.7 / 6.9)	N/A
Correlation (versus Bayer ADVIA Centaur)			
Slope	1.04	0.92	1.06
Intercept	-11	0.13	-13.9
r	0.982	*N/A	0.870
N	132	127	124

* Passing-Bablok analysis

In conclusion, the Bayer ADVIA IMS 800i anemia assays demonstrated good precision and correlated well with Bayer ADVIA Centaur anemia assays.

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Trace element determinations in blood serum samples from elderly population in the city of São Paulo, Brazil

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Trace element determinations in human blood serum samples are becoming of great interest. They provide an important basis for the diagnosis of clinical disorders and intoxication, as well as, for the investigation of their vital role in metabolism. Besides, data about element determinations in serum from an elderly Brazilian population are practically non-existent. Therefore, this study could contribute to the future monitoring of aging pathologies and to detect micronutrient deficiencies. The objective of this study was to establish adequate procedures for trace element determinations in serum samples from an elderly population living in the city of São Paulo. The selection of donors was carried out following the SENIEUR protocol. Blood sera were collected after a 12 h fast from 25 subjects (7 male and 18 female) aged 60 to 84 years, using sterile metallic needles. The sera were then placed into heparin free Becton Dickinson vacuum tubes. Sample contamination problems were examined and considered negligible. The blood was centrifuged to separate the serum that was placed in clean polyethylene vials and freeze-dried for neutron activation analysis (NAA). The NAA was performed using serum (about 200 mg) weighed in clean polyethylene bags, and irradiated at the IEA-R1 nuclear research reactor along with elemental standards. Irradiations of 30s under a thermal neutron flux of 1.4 x 10^{12} n cm^{-2} s^{-1} were carried out for Cl and Na determinations. Longer irradiations of 16h under a flux of about 5 x 10^{12} n cm^{-2} s^{-1} were performed for Br, Ca, Fe, Rb, Se and Zn determinations. Samples and standards were measured using an HGe detector coupled to a gamma-ray spectrometer. Radioisotopes were identified according to their half-lives and gamma ray energies and, element concentrations were calculated by comparative method. For quality control of the results NIST 1566b Oyster Tissue certified reference material was analyzed. Colorimetric methods for Ca, Fe and Mg and ion-selective electrode (ISE) method for Na and K were also applied in these analyses using Roche/Hitachi MODULAR *ANALYTICS* PP (Roche Diagnostics GmbH, Mannheim, Germany). Results obtained were: Br = 3.26±0.02 mg/L; Ca= 9.7 ± 0.5 mg/dL; Cl=88.6 ± 2.2 Meq/L; Fe = 129.8± 6.9µg/dL; Na = 128±2 Meq/L;

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Rb=341.3±5.2 µg/L; Se = 79.6±2.0 µg/L and Zn = 110.6±1.2µg/dL by NAA and Ca = 9.7±0.4 mg/dL; Fe = 116±459µg/dL, Mg = 2.0±0.4 Meq/L; Na = 139±2 Meq/L and K = 4.7±0.3 Meq/L by colorimetric and ISE analyses. Our findings, though in elderly population, agree with literature values for several elements. In conclusion, this study revealed that the experimental procedures used could be successfully applied in serum element determination. These preliminary data encourage further study for a larger population to obtain reference values. Acknowledgements: To FAPESP for financial support.

D-120

Soy Protein Reduces Lipid Acumulation and SREBP1 Gene Expression in Liver of Rats Fed a High-fat Diet: Implications for Lipotoxicity.

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Lipid overaccumulation in nonadipose tissues due to hyperinsulinemia and leptin resistance causes the lipotoxicity observed in obesity related diseases, and is associated with an increase in hepatic expression of the lipogenic transcription factor SREBP1. In the other hand, a soy protein (SP) diet reduces steatosis and expression of lipogenic genes in liver of diabetic (fa/fa) rats. The **objective** of the present study was evaluate the expression of SREBP1 and its association with lipid accumulation in liver of rats fed a SP High-Fat (HF) diet. After 180d, rats fed SP-HF diet had 21% lower body weight than those fed casein-HF diet, despite similar energy consumption. SP-HF diet reduced serum leptin, cholesterol and triglycerides concentration by 58%, 45%, 70% respectively, and the insulin/glucagon ratio by 78%. SP-HF diet also reduced liver cholesterol and triglycerides concentration by 57% and 64% compared with rats fed casein-HF. Real time PCR analysis revealed a reduction in the mRNA of SREBP1. These results indicate that SP prevents hyperleptinemia and reduces steatosis in liver of rats fed a HF diet by reducing SREBP1 expression related to a lower insulin/glucagon ratio.

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Soy protein effect on insulin resistance and insulin secretion in a rat model of diet induced obesity.

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Complications of obesity are hipertension, dislipidemia and diabetes. These are due to increase of lipid content in periferic tissues, insulin resistance and hyperinsulinemia. On the other hand, soy protein intake has a hipolipidemic effect and maintain serum insulin concentration in the normal range. Therefore the purpose of the present work was to assess if soy protein intake reduces insulin resistance or insulin secretion in a model of obesity induced by diet. Rats fed soy protein-high fat diet showed lower weight gain and 55%, 70% and 58% reduction in serum cholesterol, triglyceride and leptin concentration respectively than rats fed casein-high fat diet. Euglicemic-hyperinsulinemic clamps did not show significant difference in the rates of glucose utilization in rats fed casein or soy protein diet. However, studies with isolated pancreatic islets showed 70% lower insulin secretion and a significant decrease in SREBP-1c expression in rats fed soy protein than those fed casein diet. These results suggest that soy protein has an hipocolesterolemic, hipotrigliceridemic and hipoleptinemic effect in a model of diet induced obesity due to decrease in insulin secretion rather than changes of insulin sensitivity.

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Development of a Serum-Based Standard Reference Material with Certified Values for Homocysteine and Folate

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Hyperhomocysteinemia and folate deficiency are clinically measurable risk factors for cardiovascular disease in the general population. Additionally, hyperhomocysteinemia is associated with increased risk of renal failure and psychiatric disorders and folate deficiency is an established risk factor for the development of neural tube defects in newborns and for the development of certain types of cancers and anemias in adults. There exist many different analytical methods for the measurement of both homocysteine (measured as free homocysteine - Hcy or as total homocysteine - tHcy) and folate (measured as individual folate forms or as total folate - tFol); however, most inter-method and inter-laboratory comparison studies yield discordant results for both types of analytes. To rectify this situation, the National Institute of Standards and Technology (NIST), in collaboration with the Centers for Disease Control and Prevention (CDC), have developed a serum-based Standard Reference Material (SRM 1955 - Homocysteine and Folate in Frozen Human Serum) with certified values for tHcy and the predominant circulating blood folate (5-methyltetrahydrofolic acid - 5MT). SRM 1955 was prepared with three different levels of Hcy and 5MT based upon input from the clinical laboratory community. Certified values for the analytes were assigned based upon the use of higher-order reference methodology (gas chromatography/mass spectrometry - GC/MS, liquid chromatography/mass spectrometry - LC/MS and liquid chromatography/tandem mass spectrometry - LC/MS/MS). Reference values for folic acid (FA), along with information-only values for 5-formyltetrahydrofolic acid (5FT), tFol, tHcy (non-MS methods) and vitamin B12 were assigned to each SRM level. Reference values for FA were assigned on the basis of limited measurements via LC/MS/MS. Information-only values for 5FT were assigned on the basis of limited measurements via LC/MS/MS. The individual method-dependent values for tFol were assigned via LC/MS/MS folate form summation, microbiologic assay and radioassay measurements. The information-only values for tHcy were assigned via fluorescence polarization immunoassay (FPIA) and liquid chromatography with fluorescence detection (LC/FD) measurements, while the values for vitamin B12 were assigned via radioassay measurements. A Bayesian statistical model was utilized to obtain the certified and reference values, concurrent with assignment of a NIST defined 2-sigma (k = 2) coverage interval (approximate 95% confidence range) for all values. Information-only values were defined as the mean analyte value ± 2 times the standard deviation of the mean of the measurement. SRM 1955 should provide improved inter-method and inter-laboratory assessment and traceability for homocysteine and folate (in various forms) serum levels. Additionally, SRM 1955 should satisfy the recent EU *In Vitro* Diagnostic Devices Directive which requires traceability of values assigned to calibrators and control materials for *in vitro* diagnostic devices.

D-123

Reevaluation of the Traditional Microbiologic Assay for Serum Folate Measurement by Comparison to LC/MS/MS

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Objective: The microbiologic assay (MA) has been used for many years to measure total folate (TFOL) concentrations in serum and whole blood (WB). Although it is a low-cost, easy to perform assay, it has relatively high day-to-day variability. We tested calibration of the assay with different forms of folate, freeze-thaw stability of serum, recovery of folates added to serum, and the effect of various anticoagulants. We also compared the MA to a novel LC/MS/MS method using stable-isotopically labeled internal standards for each folate form measured.
Relevance: Folates reduce serum homocysteine levels, a suspected risk factor for vascular diseases, and reduce the occurrence of neural-tube defects.
Methodology: We used a 96-well microplate assay with *Lactobacillus casei* (*L. casei* NCIB 10463) as organism and 5-methyltetrahydrofolic acid ($5CH_3THF$) as calibrator (11 calibration points, range 0-0.5ng/mL). Serum was used for quality control (QC) at three concentrations. After incubating the inoculated medium at 37°C for 42 h, the turbidity was read at 590 nm. Eight replicates per sample were used to calculate the final TFOL concentration. We tested four different forms of folate from Eprova Merck: $5CH_3THF$, 5-formyltetrahydrofolic acid (5CHOTHF), folic acid (FA), and tetrahydrofolic acid (THF). FA from Sigma was also tested.
Results: *L. casei* produced the highest growth response to $5CH_3THF$ and 5CHOTHF; we obtained lower responses for other folate forms: FA (Eprova Merck) 96%, FA (Sigma) 87%, and THF 65%. The day-to-day variability over 60 days was ~10%. Serum sample with very high TFOL concentration (~470 ng/mL) showed excellent linearity when diluted up to 1:12000. We obtained recoveries for different folates added to serum at two concentrations: 102.5% ± 5.4% for $5CH_3THF$, 89.4% ± 6.7% for Eprova FA, 107.0% ± 2.4% for 5CHOTHF and 37.7% ± 7.8% for THF. After three freeze-thaw cycles, the serum QC samples did not show any significant degradation. Compared with conventional serum, serum collected into a serum separator tube and heparin plasma did not show different TFOL concentrations. Acidic citrate (ACD) plasma gave 15% lower results due mostly to the volume of the ACD solution in the tube that inevitably diluted the samples by 12%. EDTA plasma gave 13% lower results. The microbiologic assay correlated well with LC/MS/MS ($r = 0.98$) and was biased 12% higher. It is unclear at this point whether this small difference is due to nonspecific response of the microbiologic assay or whether a folate form exists that we don't capture with the LC/MS/MS assay.