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Nutrition, Vitamins and Trace Elements

Cod: W183

PREVALENCE OF ESSENTIAL TRACE ELEMENT DEFICIENCY IN PATIENTS AFTER BARIATRIC SURGERY

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INTRODUCTION
Due to malabsorptive surgical techniques (MabsT), patients undergoing bariatric surgery may develop severe nutritional deficiencies. Essential trace element (ETE) deficiency is common in the first months after surgery.
Our objective was to assess ETE deficiency (copper, zinc, selenium) in patients with morbid obesity who have undergone bariatric surgery, in relation with the surgical technique.

METHODS
This is an observational study performed in a tertiary care hospital. Data since year 2000 were retrieved after malabsorptive bariatric surgery (Scopinaro, gastric by-pass and Y-en-Roux gastrojejunostomy) and restrictive (tubular or longitudinal gastrectomy). Only patients having normal kidney and liver functions, and no anemia or leukocytosis were included.
Serum Cu, Zn and Se levels were measured by ICP-MS (NexION 300X, Perkin-Elmer). Trace element concentrations were compared with epidemiological variables (age, gender, BMI reduction and time since surgery) and with surgical technique, using the Mann-Whitney's U-test. P-values below 0.05 were considered significant.

RESULTS
Up to 55 patients were included (27% male), with a mean age of 52 years (95%CI: 49-55). No difference was seen between genders. Mean follow-up time was 75 months (95%CI: 64-88) in MabsT, and 38 months (95%CI: 29-44) in restrictive.
While 92% of patients underwent MabsT (of these, 80% Scopinaro, 15% gastric by-pass and 5% Y-en-Roux gastrojejunostomy), the other were operated by a restrictive technique. The mean reduction in BMI was 15 kg/m2, without differences among surgery types.
Selenium deficiency was seen in 70% of patients undergoing MabsT (61% Scopinaro, 50% by-pass, 100% Roux-en-Y) and 25% for restrictive techniques (p=0.009). For copper, 67% of malabsorptively operated patients was deficient and 75% for restrictive techniques (p=0.007). Zn deficiency was seen in 75% undergoing MabsT and 50% for restrictive techniques (p=0.002).

CONCLUSIONS
Patients under malabsorptive surgical techniques show different degrees of ETE deficiency. Copper deficiency is the most severe (lowest concentrations) and low selenium is common in all of them.
Restrictive techniques have a better trace element profile after surgery, while achieving a similar BMI reduction than malabsorptive techniques.

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Cod: W184

EFFECTS OF MORUS NIGRA EXTRACT AND ITS PHYTOCHEMICALS ON COLORECTAL CANCER

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Background: Several phytochemicals like chalcone derivatives and plant lectins isolated from medicinal plants have been shown to decrease cell proliferation, induce apoptosis, and enhance drug binding to cancer cells. Morus nigra is one of the medicinal plants that have been reported to have anticancer effect and it's lectin Morniga G (MorG) have been demonstrated to show high affinity to T/Tn antigens and enhance efficiency of therapy. Also different chalcone derivatives were shown to have anticancer properties and Chalcone 4 hydrate potently and selectively inhibits chemokine CXCL12 but hasn't been studied on cancer cells before. In this study, we investigated effects of M. nigra extract, MorG and Chalcone 4 hydrate on colorectal adenocarcinoma cell line HT-29 and that if these natural compounds change effectiveness of targeted therapy.
Methods: Effects of compounds on cell proliferation were determined with xCELLigence SP system. Wound healing model was used for migration assay. To measure effects on targeted therapy, cells were treated with compounds and Cetuximab together.
Results: While MorG and M. nigra extract concentrations reduced cell viability, the effect of Chalcone 4 hydrate on cell viability varied in a dose-dependent manner. M. nigra extract reduced cell migration and Chalcone 4 hydrate increased cell migration. While MorG and M. nigra extract concentrations enhanced effect of targeted therapy, Chalcone 4 hydrate decreased effects of drug.
Conclusions: We showed effects of Chalcone 4 hydrate, MorG and M. nigra extract on HT-29 cell proliferation and targeted therapy for the first time. We can speculate that CXCL12 inhibitor Chalcone 4 hydrate alters effect of Cetuximab due to absence of EGFR activation. However, futher studies are required to fully understand it's mechanisms of action.

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Cod: W186

ABETALIPOPROTEINEMIA AND CHYLOMICRON RENTENTION DISEASE: DIFFERENT RESPONSES FOR A SAME TREATMENT WITH VITAMIN E

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Objective: Abetalipoproteinemia (ABL) and chylomicron retention disease (CMRD) are rare recessive forms of genetic hypobetalipoproteinemia characterized by an intestinal lipid malabsorption and a severe vitamin E deficiency leading to disabling neuro-ophtalmologic sequelae. Oral alpha-tocopherol supplementation with high doses has to be initiated as early as possible to prevent or halt progression of complications. However, there is no real consensus regarding the treatment by vitamin E and its monitoring in genetic hypocholesterolemias. Vitamin E is often supplemented in the form of vitamin E acetate but fat malabsorption considerably limits the correction of deficiency. Tocofersolan, a water-soluble derivative of RRR-α-tocopherol, is a commercially available vitamin E supplement which has proven its efficiency in chronic cholestasis but which has never been evaluated in hypobetalipoproteinemias. Therefore, the purpose of this study was to compare the responses to the same daily dose (50 UI/kg for 4 months) of tocofersolan and α-tocopherol acetate in patients with ABL and CMRD.
Methods: 3 ABL (1 girl, 2 boys; age = 18.3 ± 2.5 y) and 4 CMRD (3 girls, 1 boy ; age = 19.4 ± 2.8 y) were included in this study. The efficiency of these 2 molecules to restore vitamin E storage were evaluated by the concentrations of α-tocopherol in plasma, red blood cells (RBC) and adipose tissue (AT) after a 4 months period treatment in a randomized cross-over clinical study.
Results : With the same dose of α-tocopherol, CMRD patients had higher concentrations of α-tocopherol in plasma ($p < 0.05$), RBC ($p = ns$) and AT ($p < 0.05$) at baseline and were better corrected after 4 months of treatment than ABL ($p < 0.05$).
Conclusion: This study provides new insights about the vitamin E status in ABL and CMRD diseases. It appears that RBC and AT α-tocopherol are relevant and complementary biomarker of vitamin E status. This study allows to confirm that 50 UI/kg/d is sufficient to treat CMRD wheareas higher dose for supplementation are required in ABL. Efficacy of tocofersolan and α-tocopherol acetate are equivalent in patients with hypocholesterolemia in the conditions of the study, but tocofersolan may be of great interest to young children due to its water-soluble and therefore liquid form for easier administration.

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Cod: W187

MAGNESIUM SUPPLEMENTATION ALTERS SALIVARY T/C RATIO IN ATHLETES AND SEDENTARY INDIVIDUALS

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Background: It is well known that magnesium ion plays an important role in stress response and diminishes the excitability of nervous system. Testosterone to cortisol ratio (T/C) can be used as a marker of disrupted anabolic/catabolic balance caused by the physical and/or psychological stress. Salivary levels of these two hormones correlate well with their biologically active forms. The purpose of this study was to investigate: a) if magnesium supplementation might have any influence on salivary cortisol and testosterone levels as well on salivary T/C ratio; b) if this effect is different in physically active and sedentary individuals.
Methods: Thirteen male rugby players and 15 male students between 18 and 25 years of age participated in the study. Saliva samples were taken in the morning, at 09:00-10:00 a.m., after an overnight fast and rest. Appropriate samples were centrifuged 2 minutes at 1000g and hormones were determined in cleared supernatant with ECLIA method (Elecsys 2010, Roche Diagnostics) both before and after 28 days of magnesium supplementation (500 mg MgO/day). Data were analyzed using the Student's t-test and $p<0.05$ considered to be statistically significant.
Results: There were no statistically significant differences in salivary cortisol and testosterone levels between groups, neither before nor after magnesium supplementation. The decrease in salivary cortisol was seen in both groups after magnesium administration. Salivary T/C ratio showed statistically significant increase ($p<0.01$) in rugby players following magnesium supplementation, while in sedentary individuals this rise was of no significance. Although before magnesium administration, the salivary T/C ratio was statistically lower in rugby players ($p<0.05$), following supplementation in both groups of participants T/C ratio achieved almost the same level.
Conclusion: The results of our investigation suggest that magnesium supplementation has a greater influence on athletes' ability to cope with stress situations, which can be due to the fact that physically active persons are more prone to develop magnesium deficiency because of intensive energy expenditure. Further investigations are needed in order to explain the exact mechanism by which magnesium modulate the balance between testosterone and cortisol.

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Cod: W188

THE "VITAMINS" SFVB–ASQUALAB EXTERNAL QUALITY ASSESSMENT SCHEMES (EQAS)

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BACKGROUND :
The SFVB is a non-profit organization involving searchers, physicians and manufacturers developing studies related to the role and metabolism of the vitamins. Since 2002, a national survey has been organized by SFVB, with currently 56 participant laboratories (40 French and 16 European or non European laboratories). To meet ISO 9001 and 17043 requirements, SFVB associated to ASQUALAB, a French EEQ organizer, in order to improve organisation of EQAS surveys. This exercise is intended to increase the level of reliability of the results provided by the laboratories through improvement of analytical methods used and practices. Furthermore this work could facilitate the development of standard methods.
METHOD :
Human serum from French Blood Bank Donors was pooled and spiked with vitamin A (retinol), vitamin E (alpha tocopherol), vitamin C (ascorbic acid), vitamin D (25 OH-vitamin D3), vitamin B6 (pyridoxal phosphate (PLP)), vitamin B9 (methyl tetrahydrofolic acid), vitamin B12 (cyanocobalamine), homocysteine, beta-caroten then lyophilised. 16 samples were shipped to 56 laboratories for the 8 surveys of the year; each of them included 2 different samples. Samples are sent once in the beginning of the year and must be kept at -20° C .
RESULTS AND DISCUSSION :
- The number of participants was depending on the molecule under investigation from 14 to 46 laboratories.
-Tow group of method are used, immunological (vitamin B9, B12, D, homocysteine) or liquid chromatography (vita min A, E, C, B6, homocysteine) with different kind of detection
- Inter-laboratory variations expressed as coefficients of variation (RSD), for molecules with at least 20 participants, range from 8 to 19%,. Vitamin A, E, B9, B12, homocysteine are less than 10% but the dispersion is more important for vitamin C, beta-caroten and vitamin B6
-During 2016 (10 samples) inter-laboratories CV ranged from 5 to 11 % for Vitamin A (1.60 to 3,30 µmol/l), vitamin E (21.9 to 44.3 µmol/l) and homocystein (12.3 to 40.0 µmol/l).
For vitamin C (9.3 to 31.8 µmol/l) ranged from 11.0 to 18.7 %, for PLP (29 to 112 nmol/l) 7.5 to 13 % and beta-caroten (0.6 to 1.34 µmol/l) 11 to 25%.
CONCLUSION :
The spiked concentrations of the samples were chosen to mimic physiological or pathological levels as observed in patient samples. Those surveys are a useful tool for increasing inter-laboratory consistency of the results and consequently to improve the evaluation of the vitaminic state of the patients.

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Cod: W189

THE INVISIBLE PART OF THE ICEBERG IN TURKEY: METABOLIC IMBALANCE IN A NEWBORN WITH VITAMIN B12 DEFICIENCY

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BACKGROUND: Vitamin B12 which is a cofactor only in two reactions (the methylation of homocysteine to methionine and the reversible rearrangement of methylmalonyl-CoA to succinyl-CoA) in human body can cause a reversible cause of bone marrow failure and demyelinating nervous system disease when it is deficient. Especially neonatal or infant vitamin B12 deficiency which is most commonly caused by maternal vitamin B12 deficiency, can result in anemia, developmental regression, hypotonia, lethargy, tremors, hyperirritability, and coma. Because of the longer the period of deficiency, the more likely that there will be permanent disabilities, early diagnosis and treatment can prevent irreversible brain injury. METHODS: An eight days old, full termed male newborn with a birth weight of 2760 gr was presented with breastfeeding difficulty, lethargy, hypotonia. RESULTS:In arterial blood gas analysis lactate level was slightly increased and pH and HCO3 levels indicated metabolic asidosis. Urine organic acid (OA) analysis showed elevated methylmalonic acid (MMA) and methylcitric acid level (MMA :87.8 mmol/mol crea (N: <5) and Methylcitric acid: 3.6 mmol/mol crea (N:0)). Serum homocysteine (Hyc) level was mildly elevated (Hyc:23 nmol/l (N:5-15). Serum vitamin B12 level was found 168 pg/ml (N: 211-946). In the expanded Newborn Screening (NBS), methionine, methylmalonylcarnitine (C4-DC) and propionylcarnitine (C3) levels were normal. At this time, maternal vitamin B12 and Hyc levels were measured and results were 163.2 pg/ml and 17.3 nmol/l respectively. The infant's elevated urine MMA level was resulted from vitamin B12 deficiency secondary to maternal deficiency. Intramuscular injection of 1000 µgr hydroxycobalamine corrected the infant's vitamin B12 deficiency and homocysteinemia. The urine organic acid profile normalized. CONCLUSIONS: Biochemically, vitamin B12 deficiency leads to an accumulation of Hcy, MMA and propionylcarnitine. Levels of MMA and Hcy are more sensitive markers of vitamin B12 status than serum B12 levels. Although vitamin B12 deficiency is not a primary target of NBS programs, measurement of MMA may incidentally identify vitamin B12–deficient newborns.

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Nutrition, Vitamins and Trace Elements

Cod: W190

MEDITERRANEAN DIET AND SERUM CYSTATIN C LEVELS

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Background: The aim of the present study was to examine serum cystatin C levels in association with the Mediterranean diet in a healthy Greek population.
Methods: Cystatin C together with basic clinical chemistry tests was measured in a total of 490 adults (46 ± 16 yrs, 40% males), who underwent an annual health check. For the above mentioned tests, within run and between day coefficients of variation (CV) were less than 7%, respectively. Accuracy of results was further supported by participation in suitable external quality assurance programs (ESEAP). All measurements were performed on a Roche/Modular Analytics analyzer. Reagents, calibrators, controls and consumables were purchased from the same supplier (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, Germany). Demographic, anthropometric, and lifestyle characteristics were recorded, while adherence to the Mediterranean Diet was evaluated through the MedDietScore (0-55).
Results: The mean level of serum cystatin C was 0.84 mg/L, while men had increased serum cystatin C levels as compared to women (0.86 mg/L vs. 0.83 mg/L respectively, 0.017). After adjusting for age, gender, Body Mass Index and smoking status, each unit increase in MedDietScore lead to 0.003 mg/dL reduction to cystatin C levels. After further adjustment for the presence of hypertension, diabetes and hypercholesterolemia, each unit increase in MedDietScore lead to a 0.003 mg/dL decrease of cystatin C levels. In the final model, three more confounding factors were taken into account (i.e., GFR, albumin and ferritin levels). Even in this model, each unit increase in MedDietScore lead to 0.002 mg/dL drop off in cystatin C serum levels.
Conclusions: We have demonstrated an inverse relationship regarding MedDietScore and serum cystatin C levels. Our finding that increases in MedDietScore are associated with decreases in serum cystatin C levels could imply that adherence to the Mediterranean Diet may reduce the cardiovascular risk, as assessed by cystatin C, a prognostic marker of the cardiometabolic risk. This notion could have a great impact on public health.

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Nutrition, Vitamins and Trace Elements

Cod: W191

THE EFFECTS OF VITAMIN D STATUS ON SERUM LIPIDS LEVELS

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Background:Vitamin D deficiency has been associated with mortality and several diseases including cardiovascular disease, obesity, diabetes mellitus and cancer. Although dyslipidemia is a major risk factor for cardiovascular diseases data about the relationships between vitamin D and lipids are conflicting. The aim of this study was to explore the effects of vitamin D status on serum lipids level.
Methods: Lipids, HbA1c and 25(OH)D3 levels of 6234 patients (1700 male and 4534 female) between 18 and 65 years old were scanned retrospectively. Patients were divided into the groups based on their vitamin D status: insufficient (25(OH)D3<20 ng/mL) and sufficient (25(OH)D3≥20 ng/mL). Patients whose 25(OH)D3>50 ng/mL weren't included in the study because of the possibility of supplementation.
Results: In females, vitamin D insufficient group's HbA1c and triglyceride levels were significantly higher ($p<0.001$ and $p<0.001$, respectively), whereas high density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) levels were significantly lower ($p<0.001$ and $p=0.003$, respectively) than the vitamin D sufficient group. There was no difference in low density lipoprotein cholesterol (LDL-C) levels between groups ($p=0.06$). In males, there were no differences in all these parameters between the vitamin D insufficient and sufficient groups. In females, 25(OH)D3 levels were negatively correlated with HbA1c ($r=-0.13$, $p<0.001$) and triglyceride ($r=-0.07$, $p<0.001$) levels, whereas 25(OH)D3 levels were positively correlated with HDL-C ($r=0.12$, $p<0.001$) and TC ($r=0.03$, $p=0.02$) levels.
Conclusion: Vitamin D deficiency may be associated with the increased risk of dyslipidemias, especially in female. Our study supports that vitamin D can affect lipid status and was inversely associated with cardiovascular risk. Vitamin D may be protective in cardiovascular diseases and supplementation will need to be elucidate in larger clinical trials.

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Cod: W192

STUDY ON CERULOPLASMIN ACTIVITY IN HEALTHY BULGARIANS

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Background: Ceruloplasmin (Cp) is the major carrier of copper (Cu) in the body. Typically, it is measured for diagnosis of rare diseases such as Wilson disease (WD), Menke's syndrome and aceruloplasminemia. Cp could be determined both as protein concentration (iCp) and enzymatic activity (eCp). Routine immunological testing is loaded with some methodological limitations, so evaluation of enzymatic Cp activity might be useful approach.
Methods: The study comprised 41 healthy Bulgarians (male:female = 16:25) with average age of 43±13 years old. All sera were examined for iCp, eCp and serum Cu. The following methods were used: iCp - immunoturbidimetric method (Sigma-Aldrich, Pentra ABX); eCp - enzymatic method with chromogenic substrate o-dianisidine (Sigma-Aldrich, Pentra ABX); serum Cu – flame atomic absorption (Perkin Elmer, AAnalyst 400 Plus).
Results: Results are presented as mean±SD and range: iCp – 36,9±5,4 (27,9 to 50,99) mg/dL; eCp– 106,7±20,4 (34,32 to 156,13) IU/L and serum Cu – 15,4±1,8 (12,4 to 20,16) μmol/L. Slight significant difference between male and female eCp (p=0,0458) was found, with higher values in females (111.9±18,2 IU/L) vs. males (98,6±20,5 IU/L). The correlation between iCp and eCp was high – r=0,838. Mild correlation was established between Cu and iCp (r=0,520), and also between Cu and eCp (r=0,43).
Conclusion: This is the first study on eCp in healthy Bulgarians. We found mean values similar to those reported in the literature for other populations. In 5% (n=2) of individuals eCp was out of the reference range for the method (60 - 140 IU/L). Good correlation was established between iCp and eCp, and also for Cp (concentration and enzymatic activity) and Cu. Recently, increased clinical implication of eCp is observed. Copper status seems to be important not only for rare disorders but in social significant diseases also. Deeper knowledge on eCp (reference ranges, factors of variations, clinical significance) could be necessary in disordered copper balance.

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Cod: W193

MONITORING THE LEVELS OF B VITAMINS IN DIFFERENT HUMAN POPULATIONS USING HPLC METHOD

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BACKGROUND
The B vitamin family is made up of eight B vitamins. Although they are commonly recognized as a group and often work together in the body, each of the B vitamins performs unique and important functions. At present, increase the requirements on measurement particularly vitamins B1, B2 and B6 in different population groups and also in search of the possible range specifying certain diseases. The main objective of this work was to monitor the levels of vitamin B in population with a lower standard of living and its determination as a marker of early diagnosis.
METHODS
The level of vitamin B1, B2, B6 was studied in 257 men and women in a poor living conditions. The pilot study was focused on the detection of vitamin B1 in the group of 100 patients after intoxication with methanol and vitamin B6 in a group of 20 patients with autosomal dominant disease. Vitamin B1 and B2 was extracted from whole blood, vitamin B6 from plasma using two type of appropriate separation kits (RECIPE, CHROMSYSTEMS). Subsequent analysis was performed by high-performance liquid chromatography (HPLC, Agilent Technologies 1260).
RESULTS
We found out, that 50.5 % of men and 38.5 % of women with a poor living conditions had decreased levels of vitamin B2. On the other hand, people after methanol intoxication had values of vitamin B1 in an appropriate reference range. Vitamin B6 was significantly increased in patients with autosomal dominant disease.
CONCLUSIONS
The results have interesting predictive value among individual social population groups. The correctness of the analysis was confirmed by two different separation kits.
Work is supported by IGA MZ CR NR/8049-3, RVO-VFN64165

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Cod: W194

DIAGNOSTIC VALUE OF HOLOTRANSCOBALAMIN IN ASSESSMENT OF VITAMIN B12 DEFICIENCY

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Background
Different neurological manifestations could be a consequence of vitamin B12 deficit, even when vitamin B12 concentrations are within reference intervals. In theory, measurement of amount of vitamin B12 available for the cells, known as holotranscobalamin, could be a more sensitive indicator of B12 deficiency in such cases. The aim of the study was to examine the potential usefulness of holotranscobalamin in assessment of vitamin B12 deficiency.
Methods
The study included 54 subjects who were divided into two groups according to the homocysteine values: 31 patients with elevated homocysteine values (>15 μmol/L) and with neurological symptoms and 23 patients with normal homocysteine values and different diagnoses which represented controls. All subjects in both groups had vitamin B12 concentrations between 138 and 300 pmol/L. Serum samples were collected according to a standard operating procedure using a blood activator tubes (Becton-Dickinson, Eysins, Switzerland). Vitamin B12, holotranscobalamin and homocysteine were measured with chemiluminescent microparticle immunoassay on the Architect i1000SR analyzer (Abbott Diagnostics, Lake Forest, IL, USA). The results were processed using MedCalc Software version 12.4.0 (MedCalc Software, Mariakerke, Belgium). ROC curve was used for sensitivity and specificity testing, while Mann-Whitney test was used for groups comparison. For testing of the association between variables, Spearman's rank correlation test was used.
Results
Patients with neurological simptoms and hiperhomocysteinemia had only a bit lower median values of holotranscobalamin (50.4 pmol/L vs 54.6 pmol/L; P=0.896). Using homocysteine as a discriminating variable between the groups we obtained similar AUC values for vitamin B12 (AUC=0.596) and holotrancobalamin (AUC=0.511). We also found moderate correlation between holotranscobalamin and vitamin B12 (r=0.440; P<0,001).
Conclusion
Our study shows that holotranscobalamin is not a more sensitive indicator of vitamin B12 deficiency than vitamin B12. Holotranscobalamin and vitamin B12, separately or in combination, have almost equal diagnostic efficiency as early indicators of the vitamin B12 deficiency.

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Nutrition, Vitamins and Trace Elements

Cod: W195

EFFECT OF STEVIOL, STEVIOL GLYCOSIDES AND STEVIA EXTRACT ON GLUCOCORTICOID RECEPTOR SIGNALING IN NORMAL AND CANCER BLOOD CELLS.

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BACKGROUND. Due to the growing incidence of type 2 diabetes mellitus, obesity and metabolic disorders, steviol glycosides are widely used as non-caloric sweetener because of their low glycemic index. It has been suggested that they exert various beneficial biological activities however, the whole spectrum of their effects has not been completely investigated. Since glucocorticoid receptor mediates harmful effects in metabolic processes, in this study we investigate the possible glucocorticoid receptor (GR) modulated activity of steviol glycosides, steviol and Greek-derived stevia extract in normal human peripheral blood mononuclear cells (PBMCs) and human leukemic T-cells (Jurkat).
METHODS. Isolated PBMCs from healthy donors were exposed to steviol and its glycosides (0.1-10 μM) as well as to stevia extract (10-500 μg/ml) for 12 and 24 h. In these cells, we evaluated the expression of a primary GR-target gene, the Glucocorticoid-induced leucine zipper (GILZ) (using real time PCR), GR expression levels and GR subcellular localization (using western blot). Jurkat cells (untransfected and transfected cells) were also cultured as above for 24 h. In untransfected cells, the GILZ expression and GR subcellular localization was assessed (using immunoflorescence), while GRE-mediated luciferase reporter gene assay was performed in transfected Jurkat cells. GILZ mRNA levels were also measured in whole blood of normal human individuals before, 2 and 4 hours after oral administration of a mixture of steviol glycosides (60 mg steviol equivalents).
RESULTS. Our study demonstrates, in vitro and in vivo, that steviol glycosides, steviol and stevia extract do not alter GILZ mRNA levels, GR expression levels or GR subcellular localization in normal human PBMCs. In Jurkat cells, stevia compounds increase GILZ expression levels and luciferase activity in a concentration-dependent manner and also induce GR nuclear translocation.
CONCLUSIONS. Steviol glycosides, steviol and Greek-derived stevia do not exert any glucocorticoid receptor – mediated effects in normal human blood cells, thus implicating these compounds may show no adverse effects in metabolism. The GR modulated activity shown by the aforementioned compounds in Jurkat cells implicates their possible cell-type specific action.

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Nutrition, Vitamins and Trace Elements

Cod: W196

25-HYDROXYVITAMIN D: MONTHLY MINIMUM REFERENCE VALUES TO ASSURE LEVELS NON INFERIOR THAN 30 NG/ML EVERY MONTH IN BUENOS AIRES POPULATION (LATITUDE 34°36'S)

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Background. The main source of 25-hydroxyvitamin D (25(OH)D) is the ultraviolet radiation dose (UVR). All-cause mortality has been associated with 25(OH)D serum concentrations below 30 ng/mL. As a result of monthly variation in UVR we have observed marked reduction during the winter and the spring that could affect a proper clinical decision.
Methods. We analyzed 23920 serum samples of out patients (21387 females, mean age 61.2 years; and 2533 male, mean age 62.1 years). We partitioned the population by sex and age group: females until 60 years (FU60: n=9888), females older than 60 years (FO60: n=11499); males until 60 years (MU60: n=1032) and males older than 60 years (MO60: n=1501). For each group we registered on a monthly basis from January to December the percentage variation of 25(OH)D mean levels with respect to the previous month. Then, we calculated a correction factor, taking into account those months with negative variation. We took January and a minimum value of 30 ng/mL of 25(OH)D as a baseline. A correction factor of annual lost was applied to this value. Then we calculated, using a factor considering the variation respect to the previous month, the minimum values of 25(OH)D from February to December. With these minimum values and the applied factor, we checked that no month presented a 25(OH)D value less than 30 ng/mL.
Results. The minimum values required in January for FU60 and FO60 are 42.5 and 38.9 ng/mL respectively; and for MU60 and MO60, 48.4 and 44.5 ng/mL respectively. The annual minimum values required are: for FU60 and FO60, 31.6 ng/mL in September and 32.0 ng/mL in October respectively; for MU60 and MO60: 31.6 ng/mL and 33.3 ng/mL in September respectively; and the maximum values required are: for FU60 and FO60 46.1 ng/mL and 42.1 ng/mL in March respectively, and 51.4 ng/mL and 45.6 ng/mL in February for MU60 and MO60 respectively.
Conclusions. For Buenos Aires population (latitude 34°36'S) the minimum reference values required for protection against a decrease in 25(OH)D serum concentrations below 30 ng/mL are lower at the beginning of the spring and higher in summer and at the beginning of fall.

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Nutrition, Vitamins and Trace Elements

Cod: W197

EVALUATION OF SERUM VITAMIN B12 LEVEL IN TYPE 1 DIABETES MELLITUS

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Background:
Type 1 Diabetes Mellitus an autoimmune condition is known to be associated with multiple co-morbidities. Vitamin B12 deficiency is a potential co- morbidity that is often overlooked in these patients, despite the fact that many diabetic patients are at risk for this specific disorder. Studies done on the other population have demonstrated the presence of vitamin B12 deficiency or low vitamin B12 level in Type 1 diabetes. Defining the prevalence of low or deficient serum vitamin B12 levels in the diabetic population in this part of world may aid physicians to consider screening for vitamin B12 levels in Type 1 diabetic patients and carry out further evaluations.
Methods:
The cross sectional study was done by selecting 40 Type 1 Diabetes Mellitus patients from outpatient department (OPD) visiting endocrinology unit in Kanti children's Hospital. 30 healthy control groups were also selected based on inclusion/ exclusion criteria. Serum C-peptide, vitamin B12, creatinine, blood sugar level were assessed along with glycosylated hemoglobin in both groups. SPSS ver. 22 was used to analyze the data; t-test and one way ANOVA were used to find mean differences and Pearson's correlation was used to establish the correlation.
Results:
The mean age of Type 1 Diabetic patients was 10.44 ± 3.68 years. A total of 30 controls were also included in the study with the mean age of 4.87 ± 3.53 years.The case and the control group did not differ in biochemical and demographic characteristics except in their age; the difference in age was statistically significant. The mean serum vitamin B12 level of the case was 280.37 ± 111.34 pg/ml. Among the population 40.0% i.e. 16/40 were found to be deficient and 37.5 % i.e. 15/40 were sub clinically deficient. Whereas the mean serum vitamin B12 level of the control group was 462.67 ± 184.32 pg/ml. 2 out of 30(6.7%) were deficient, 8 out of 30 (26.75%) were found to be sub clinically deficient. Significant difference was noticed in the mean serum level of vitaminB12 between two groups.
Conclusion:
This study demonstrated the presence of low serum vitamin B12 levels in Type 1 Diabetics. The routine screening for this condition along with confirmatory test and detail clinical examination could benefit the Type 1 diabetic patients. However, further studies on a larger population using additional markers to investigate the actual cause of deficiency are must to strengthen this statement.

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Nutrition, Vitamins and Trace Elements

Cod: W198

FERRITIN ASSAY FOR THERMO SCIENTIFIC INDIKO AND INDIKO PLUS CLINICAL CHEMISTRY ANALYZERS

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Background: Ferritin is an iron storage protein found in nearly all cells of the body. The amount of storage iron in men is approximately 800 mg, mostly as ferritin; in healthy women, it ranges up to 200 mg. Minimum quantities of ferritin are also present in serum in concentrations roughly proportional to total body-iron stores.
The determination of ferritin is important in iron metabolism diagnosis, monitoring iron therapy, ascertaining the iron reserves in groups at risk and in differential diagnosis of anemia. While very low serum ferritin values are always indicative of iron deficiency, very high serum ferritin values have many implications. Increased serum ferritin can be suggestive of iron overload but is also seen in conjunction with other diseases.
Thermo Scientific™ Indiko™ and Indiko™ Plus used in this study are bench top clinical chemistry analyzers, especially suitable for small and medium sized laboratories or as a back-up analyzer for bigger ones. Colorimetric, turbidimetric and ISE methods are well applied and CE marked. The Indiko analyzers are complete easy-to-use systems including the instrument, system reagents, calibrators and controls.
Method: Ferritin (981949) used on Indiko applies immunoturbidimetric method. The absorbance is measured at 700nm when the reaction has reached the end-point. The change in absorbance is proportional to the amount of antigen (ferritin) in solution.
Results: The repeatability (within-run precision) is 1.5–4.6 % (CV; n=80) and the within device (total) precision is 2.7–9.4 % (CV; n=80).
A comparison study was performed using Konelab PRIME 30i Ferritin (981944) as a reference. Linear regression was $y = 0.930x – 0.34$ and $r = 0.985$ (N=74).
Conclusion: With this ready-to-use system reagent, ferritin analysis on Indiko analyzers is quick and accurate. Together with our other Indiko system test kits, Iron, sTfR and Transferrin, Ferritin delivers a comprehensive offering for anemia diagnosis.

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Nutrition, Vitamins and Trace Elements

Cod: W199

IMPACT OF A SINGLE ORAL DOSE OF 100,000 IU VITAMIN D3 ON VITAMIN D METABOLITE PROFILES IN HEALTHY ADULTS WITH VITAMIN D INSUFFICIENCY

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Purpose: To investigate the effect of a high dose of vitamin D3 on circulating concentrations of 25(OH)D3 and its metabolites 24,25(OH)2D3, 3-epi25(OH) D3, and 1,25(OH)2D3 in healthy individuals with vitamin D insufficiency (25(OH)D3 <50 nmol/L).
Methods: 107 healthy adults (age 20–50 years) with vitamin D insufficiency were randomized to receive a single 100,000 IU dose of vitamin D3 (n= 52) or placebo (n= 55), and vitamin D metabolite concentrations in serum were measured before, and 4 weeks after, supplementation.
Results: Overall, 52% of participants receiving vitamin D3 attained a serum 25(OH)D3 level >75 nmol/L. Among patients who received vitamin D3, there were significant increases in serum concentrations of 25(OH)D3 and its metabolites 24,25(OH)2D3, 3-epi25(OH) D3, and 1,25(OH)2D3 at 4 weeks; however, inter-individual variability in these changes was substantial. Positive correlations between serum 25(OH)D3 and 24,25(OH)2D3 and 3-epi-25(OH)D3, and a significant negative correlation between serum 1,25(OH)2D3 and 3-epi-25(OH)D3, were found 4 weeks after supplementation. The 24,25(OH)2D3/25(OH)D3 and 24,25(OH)2D3/1,25(OH)2D3 ratios were significantly increased, compared with baseline, in patients receiving vitamin D3. Baseline 25(OH)D3 concentration was the only factor predictive of the change in 25(OH)D3 after supplementation.
Conclusions: Administration of a single high dose of vitamin D3 leads to a significant increase in concentrations of 25(OH)D3, 24,25(OH)2D3, 3-epi (OH)D3 and 1,25(OH)2D3; induction of the catabolic pathway predominates over the production of 25(OH)D3. Due to the high inter-individual variation in the 25(OH)D3 response to supplementation, any given dose of vitamin D is unlikely to achieve optimal vitamin D status in all treated individuals.

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Nutrition, Vitamins and Trace Elements

Cod: W200

DETERMINATION OF 1ALPHA, 25 DIHYDROXYVITAMIN D IN HUMAN SERUM – AN EVALUATION OF COMMERCIALLY AVAILABLE AUTOMATED ASSAYS

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Background. 1,25 $(OH)_2$ Vitamin D (1,25$(OH)_2$ VitD) is the bioactive form of vitamin D. Due to the very low concentrations of 1,25$(OH)_2$ VitD in the blood and its lipophilic character, measurement of this parameter is analytically challenging. Radioimmunassays reach sufficient sensitivity but are labor- and time consuming. The development of automated assays facilitates and accelerates the measurement of 1,25$(OH)_2$ VitD in an increasing number of patient serum samples.

Methods. Remaining material of 93 serum samples sent for determination of 1,25$(OH)_2$ VitD to our laboratory was used for measurements with automated immunoassays from either IDS on the IDS iSYS immunoanalyzer or DiaSorin on the immunoassay analyzer LIAISON XL. Assay imprecision and linearity was estimated according to CLSI EP15-A3 guidelines. For testing of accuracy, 1,25$(OH)_2$ VitD results of the immunoassays were compared to 1,25$(OH)_2$ VitD concentrations obtained by LC-MS/MS as a reference method.

Results. Assay imprecision was 5.2% or less for the DiaSorin test, but reached 20.1% for the IDS iSYS test. 1,25$(OH)_2$ VitD concentrations measured with the Diasorin assay showed a strong correlation with the values obtained from measurements by LC-MS/MS (r= 0.967). By contrast, the IDS iSYS test overestimated 1,25$(OH)_2$ VitD concentrations in human serum, particularly at higher concentrations.

Conclusions. The use of automated assays enables the measurement of 1,25$(OH)_2$ VitD in the increasing number of serum samples within a shorter time frame with good reliability. Due to its high sensitivity, low imprecision, the broad measurement range and the good agreement with 1,25$(OH)_2$ VitD concentrations measured by LC-MS/MS, particularly the Diasorin test is a valuable analytical option for the determination of 1,25$(OH)_2$ VitD.

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Nutrition, Vitamins and Trace Elements

Cod: W201

ANTIATHEROGENIC AND ANTICANCER POTENTIAL OF STEVIOSIDE, REBAUDIANA A, STEVIOL AND STEVIA EXTRACT

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Stevia leaves and their extracted steviol glycosides (stevioside and rebaudiana A being the most abundant constituents) are used as natural non-caloric sweeteners in many countries. Stevioside has been reported to possess anti-inflammatory and anti-cancer properties in various cells. In this study we investigated, for the first time, the anti-inflammatory/antiatherogenic properties of Greek-derived stevia extract, stevioside, rebaudiana A as well as steviol (the major metabolite of steviol glycosides) in human aortic endothelial cells (HAEC) and their cytotoxic effect on prostate cancer cells (PC-3, LNCaP) and breast cancer (MCF-7) cells. In particular, we examined the potential of the above extract and the individual steviol glycosides 1) to inhibit the tumor necrosis factor-α (TNF-α) induced expression of the intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in HAEC, a widely used in vitro model assessing the anti-inflammatory/antiatherogenic effect of examined compounds, 2) to inhibit TNF-a-induced monocyte adhesion to HAEC and 3) to reduce the viability of PC-3, LNCaP and MCF-7 cells. Endothelial cells, in the presence and absence of TNF-α, were incubated with various concentrations of stevia extract (10-500 μg/ml) and steviol glycosides (10-5-10-7 M). The surface expression of adhesion molecules (ICAM-1 and VCAM-1) was assessed by cell ELISA and the adhesion of U937 monocytes to activated HAEC using a cell adhesion assay. MCF-7 cells as well as PC-3 and LNCaP cells were incubated with above compounds at the same concentrations for 48 hours. The cell viability was determined using the colorimetric MTT metabolic activity assay. Our data demonstrate that neither stevia extract nor steviol and steviol glycosides altered the viability of PC-3, LNCaP and MCF-7 cells. Stevia extract and steviol glycosides inhibited the ICAM-1 and VCAM-1 expression in TNF-α activated endothelial cells as well as the adhesion of U937 to endothelial cells. Our results demonstrate that Greek-derived Stevia extract and Stevioside exhibit strong anti-inflammatory/antiatherogenic potential but not anti-cancer properties.

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Nutrition, Vitamins and Trace Elements

Cod: W202

SUBOPTIMAL VITAMIN D STATUS IN CHRONIC PROSTATE DISEASES

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Background. A mounting evidence for the "nonmineral" effects of vitamin D (VD) and the connection of its deficiency with different chronic diseases was accumulated. Chronic prostate diseases (CPD) such as prostate cancer (PCa) and benign prostate hyperplasia (BPH) are leading cause of morbidity and mortality among men. Normal and malignant prostate cells contain VD receptors and 1-alpha-hydroxylase responsible for the formation of the active form of VD. Therefore, the antiproliferative and immunomodulatory effects of VD could play a beneficial role in CPD. We aimed to determine the VD status in patients with CPD and to examine its relationship with clinical and laboratory parameters related to disease severity and progression.

Methods. The study encompassed 85 PCa patients (mean age 66.76±6.25years) and 37 BPH patients (mean age 67.14±7.77years) who consented to participate. Diagnosis was confirmed histologically, PCa patients were graded by Gleason grading system, and risk for biochemical recurrence of localized and locally advanced PCa was assessed according to EAU guidelines. Prostate specific antigen (PSA) was measured immunochemically, and VD status was assessed by measuring 25-hydroxy-VD (25OHVD) with a validated LC-MS/MS method. Variation and correlation analysis were used for data handling, level of significance $p<0.05$.

Results. The mean 25OHVD (54.98±21.93nmol/L vs 42.85±18.88 nmol/L for BPH and PCa patients respectively, $p<0.05$) was below the optimal cut-off of 75-80 nmol/L, responsible for its noncalcemic effects. BPH patients with PSA<4ng/ml had higher 25OHVD (59.21±17.21nmol/L) compared to those with PSA>4ng/ml (46.12±23.76 nmol/L), $p=0.063$. PCa patients always remained VD deficient when stratified by their PSA, $p>0.05$. No associations between 25OHVD and PSA were found for both groups. Stratification by the risk of biochemical recurrence and by tumor grade revealed worse VD status for the highest risk group and in patients with Gleason score>7 (36.64±18.16nmol/L and 37.84±19.71nmol/L, respectively). A strong negative correlations between 25OHVD versus Gleason score and versus risk were found only during the warm season (Spearman $r=-0.44$, $p<0.01$; $r=-0.60$, $p<0.001$, respectively).

Conclusion. Improving VD status may have beneficial role for CPD patients.

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Nutrition, Vitamins and Trace Elements

Cod: W203

IMPACT OF VITAMIN D BINDING PROTEIN ON CALCULATED FREE 25OH VITAMIN D

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25OH vitamin D is used to diagnose vitamin D deficiency. Vitamin D and its metabolites are bound with high affinity to vitamin D binding protein (DBP), a specific proteic carrier.Two major SNPs in the DBP gene give rise to 3 principal isoforms that have various affinity for 25OHD (GC-1F>GC-1S>GC2). GC-1F, the wild-type allele, is predominantly found in African/East Asian populations while GC-1S is found among the European/South Asian populations. The GC2 variant is worldwide found at lower frequencies. A small fraction of 25OHD, called free 25OHD, is not bound to DBP and can be calculated from total 25OHD, DBP and albumin concentrations, with/without factor accounting for DBP genotype-specific binding affinities.

The cohort enrolled 765 belgian participants and from various ethnicities (442 Caucasians, 98 Africans, 225 Maghrebians). 25OHD concentrations were measured with a LCMS/MS method. Polyclonal (Immundiagnostik°) and monoclonal (R&D systems°) ELISA were used to measure DBP concentrations.

DBP concentrations measured by monoclonal assay were different from polyclonal results in each ethnic group ($p<0,0001$). Consistantly different mean concentration of DBP were found using monoclonal assay between the different ethnicities ($p<0,0001$). Mean monoclonal DBP in Africans were lower than Caucasians and Maghrebians ($p<0,0001$). On the contrary, there were no differences between ethnicities using polyclonal assay. Mean DBP concentration measured by polyclonal assay was 105,7% higher than with monoclonal assay. More specifically, mean polyclonal result was 75,3% higher in Caucasians, 254,2% in Africans and 106,9% in Maghrebians. 25OHD mean concentration was higher in Caucasians (26,5±13,1 ng/mL) than in Africans (22±12 ng/mL)($p=0,0015$) and Maghrebians (19,6±13 ng/mL)($p<0,0001$).

DBP isoform strongly influences DBP concentrations when using a monoclonal assay. Monoclonal assay underestimates the DBP GC-1F concentration, predominantly found in Africans while polyclonal assay recognizes all isoforms in a similar way and shows equivalent results between ethnicities. Africans have lower mean DBP concentration using monoclonal assay resulting in unvalid higher calculated free 25OHD. Calculated free 25OHD is impacted by use of polyclonal DBP assay. We found a higher prevalence of insufficiency in Africans as based on both their total and calculated free 25OHD.

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Nutrition, Vitamins and Trace Elements

Cod: W204

COMPARISON OF SIX AUTOMATED ASSAYS FOR THE THE DETERMINATION OF COBALAMIN AND HOLOTRANSCOBALAMIN IN SERUM. IS THERE ANY BETTER ASSAY?

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Cobalamin measurement is routinely performed for the screening of vitamin B12 deficiency. Unfortunately, there is no standardization between assays. Hence, there is no consensus on the cut-off values used for deficiency and a great variability exists between the reference values proposed by the manufacturers leading to potentially different clinical interpretation of the result according to the assay and/or chosen cut-off. Cobalamin circulates mostly bound to haptocorrin (±80%) and is metabolically inert. The remaining 20% is bound to transcobalamin and forms a biologically active complex named holotrancobalamin (holoTC). In this study, we decided to compare 6 automated assays for cobalamin and holoTC determination.

A total of 55 samples were tested for B12. The test were performed on five different automated immunoassay analyzers (cut-off pg/mL): Abbott Architect i1000sr (187), Siemens Centaur ADVIA (211), Ortho Clinical Diagnostics Vitros 5600 (239), Beckman Coulter Access (180), Roche Modular (191). HoloTC was performed on Abbott Architect i1000sr. Serum-based international reference material do exist for the standardization of serum cobalamin assays but Roche is the only manufacter to use it (WHO03/178 standard). Abbott use the USP standard, Vitros is calibrated against BioRad RIA and the two others use internal standards.

Close correlation was found between the 5 tests for cobalamin results, with Pearson's values ranging from 0,974 to 0,993. Wilcoxon test showed that all methods were different ($p<0,0001$) except Access- Centaur ($p=0,09$). Concordance correlation coefficient ranged from 0,676 (Centaur-Modular) to 0,982 (Vitros-Modular). Access is highly correlated to Centaur and Vitros is highly correlated to Modular. Abbott cobalamin is moderatly correlated with the 4 other tests. The five methods showed different standard calibration excepted Access-Centaur. HoloTC was poorly correlated to the 5 cobalamin assays. Applying manufacturer's cut-off for deficiency, many discrepancies are observed leading to lack of consistency in the number of subnormal results ranging from 1,8% for Roche to 21,8% for Siemens.

Our results show a clear lack of standardization between methods. Carefull care should be made on cut-off values since laboratory results are the first step to diagnose deficiency and patients could be classified differently according to the test, leading to different clinical care. Laboratory experts and clinicians should be aware of this disagreement.

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Nutrition, Vitamins and Trace Elements

Cod: W205

COMPARISON OF MEASURED AND CALCULATED FREE 25-HYDROXYVITAMIN D : IMPACT OF VITAMIN D BINDING PROTEIN ASSAY AND RACE

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Total 25OH vitamin D (t25OHD) is used to diagnose vitamin D deficiency. Vitamin D and its metabolites are bound with high affinity to vitamin D binding protein (DBP), a specific proteic carrier. Two major SNPs in the DBP gene give rise to 3 principal isoforms that have various affinity for 25OHD (GC1F>GC1S>GC2). GC1F, the wildtype allele, is predominantly found in African populations while GC1S is found among the European/South Asian populations. The GC2 variant is worldwide found at lower frequencies. A small fraction of 25OHD, called free 25OHD (f25OHD), is not bound to DBP and can be calculated from t25OHD, DBP and albumin concentrations, with/without factor accounting for DBP genotype-specific binding affinities. In this study, we aimed to compare the measured and calculated f25OHD concentrations in a population of first trimester pregnant women from various ethnicities.

The cohort enrolled 237 belgian women (90 Caucasians, 46 Africans, 102 Maghrebians). 25OHD concentrations were measured with a LCMS/MS method. Polyclonal (Immundiagnostik) and monoclonal (R&D systems) ELISA were used to measure DBP concentrations. F25OHD concentrations were directly measured by ELISA (DIASource).

DBP concentrations measured by monoclonal assay were different from polyclonal results ($p<0,0001$). There were no differences between ethnicities using polyclonal assay unlike using monoclonal assay ($p<0,0001$). T25OHD mean concentration was higher in Caucasians (26,3±8,7 ng/mL) than in Africans (19,6±9,5 ng/mL) and Maghrebians (16,9±11,8 ng/mL)($p<0,05$). Measured f25OHD were different from calculated f25OHD derived from polyclonal and monoclonal DBP assay ($p<0,0001$). Measured f25OHD showed poor correlation with both calculated f25OHD and t25OHD (spearman ranging from r=0,62 to 0,66). Calculated f25OHD derived from polyclonal measures of DBP were highly correlated with t25OHD (r=0,9). ANOVA showed that measured f25OHD was not influenced by race unlike t25OHD and calculated f25OHD derived from monoclonal and polyclonal DBP assays.

DBP assay strongly influences calculated f25OHD concentrations since only polyclonal assay recognizes all DBP isoforms in a similar way and shows equivalent results between ethnicities. Measured f25OHD is not influenced by race, which is not the case of t25OHD. Calculated f25OHD is clearly impacted by the DBP assay used and race. Measured f25OHD showed independent results from t25OHD, DBP assay and race that invalid calculated f25OHD equation.

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Nutrition, Vitamins and Trace Elements

Cod: W206

VITAMIN D LEVELS IN CHRONIC SPONTANEOUS URTICARIA AND DEPRESSION

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Background: There are various roles of vitamin D in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation. We aimed to find out whether vitamin D plays a role in the pathogenesis of chronic spontaneous urticaria and depression.
Methods: 30 patients diagnosed with chronic spontaneous urticaria whose Beck-Depression inventory score<17, 30 patients diagnosed with depression, 30 patients diagnosed with both chronic spontaneous urticaria and depression whose Beck-Depression inventory score≥17 and 30 healthy volunteers as control group were involved in the study. 25-hydroxy vitamin D_3 ($25(OH)D_3$) levels of these four groups were measured with LC/MS-MS.
Results: Healthy controls' $25(OH)D_3$ levels (17.2 ± 8.8 ng/mL) were significantly higher than urticaria (9.1 ± 5.1 ng/mL), depression (8.9 ± 6.1 ng/mL) and both urticaria and depression (7.7 ± 4.7 ng/mL) groups ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). In pairwise comparisons there were no differences between urticaria and depression groups, between urticaria and both urticaria and depression groups, between depression and both urticaria and depression groups ($p=0.92$, $p=0.43$ and $p=0.82$, respectively).
Conclusions: The low $25(OH)D_3$ levels in all three patient groups suggest that vitamin D may have a role in the pathogenesis of chronic spontaneous urticaria and depression. Evaluation of the vitamin D levels of chronic spontaneous urticaria and/or depression patients by clinicians has importance. Vitamin D may be protective in these diseases and supplementation will need to be assessed in well-designed clinical trials.

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Nutrition, Vitamins and Trace Elements

Cod: W207

DETERMINING THE 25-HYDROXY VITAMIN D DEFICIENCY LEVEL BY THE RESPONSE OF PARATHYROID HORMONE

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Background: As the information on the physiological effects of vitamin D and its roles in other metabolic pathways have increased it has become more important to determine and evaluate vitamin D levels. We aimed to contribute to the literature by determining deflection point of intact parathyroid hormone (iPTH) level compared to 25-hydroxy vitamin D_3 ($25(OH)D_3$) level and by determining the cut-off value of vitamin D deficiency in Turkey.
Methods: The results of 1684 patients between 18 and 65 years of age who had calcium (Ca), inorganic phosphate (P), creatinine, iPTH and $25(OH)D_3$ tests between the dates of 01/03/2015 and 01/03/2016 which were requested simultaneously were scanned retrospectively via laboratory software system. $25(OH)D_3$ levels were first classified as 80-50, 50-30, 30-25, 25-20, 20-15, 15-10, 10-5, 5-0 ng/mL and iPTH levels among those groups were compared by one-way ANOVA test.
Results: According to $25(OH)D_3$ levels groupings as 80-50, 50-30, 30-25, 25-20, 20-15, 15-10, 10-5, 5-0 ng/mL, significant difference in iPTH levels among groups was determined ($p<0.001$). In Student-t tests which were performed post-hoc in order to determine significant differences in iPTH levels; first significant difference was determined between $25(OH)D_3$ groups of 80-50 and 50-30 ng/mL ($p=0.007$). No significant difference between groups of 50-30 vs 30-25, 30-25 vs 25-20, 25-20 vs 20-15 and 20-15 vs 15-10 was determined ($p=0.75$, $p=0.74$, $p=0.40$ and $p=0.67$, respectively). Second and third significant differences has been determined between 15-10 and 10-5 groups and between 10-5 and 5-0 groups, respectively ($p=0.006$ and $p=0.033$, respectively).
Conclusions: We believe that cut-off value for vitamin D deficiency in Turkish society depending on PTH response should be used as 10 ng/mL. We think that for the diagnosis of vitamin D insufficiency, it is not sufficient to use PTH values alone. For the cut-off value required to be used in the diagnosis of vitamin D insufficiency, the results of clinical studies in which risk factors are evaluated should be taken into consideration.

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Nutrition, Vitamins and Trace Elements

Cod: W208

PREVALENCE OF MICRONUTRIENT DEFICIENCIES IN CHILDREN

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Micronutrient deficiencies and anemia among children continue to be major public health challenges in most developing countries. Studies have documented folate, ferritin, and cobalamin deficiencies to be the major causes of nutritional anemia. However, limited data is available on the prevalence of folate, ferritin, and cobalamin deficiencies in children. The present study was carried out to find out the magnitude of folate, ferritin, and cobalamin deficiencies in children of 1-18 years of age.

We retrospectively analyzed serum folate, vitamin B12, ferritin levels of 1–18 years aged outpatients attending Tepecik Training and Research Hospital during the period July 2008- June 2016. Micronutrients cut off levels used were: folate deficiency < 4 ng/ml; vitamin B12 deficiency < 200 pg/ml and ferritin deficiency < 12 ng/ml.

A total of 6722 children were included in the study. 1372 (20.4 %) subjects had ferritin deficiency, 1108 (16.5 %) had vitamin B12 deficiency and 493 (7.3 %) had folate deficiency. The mean levels of serum cobalamin, folate and ferritin were found to be 376 ± 210 pg/ml, 9.2 ± 4.8 ng/ml and 33 ± 51 ng/ml, respectively.

A high prevalence of anemia existed along with deficiency of ferritin, cobalamin, and folate in children. The strategies for prevention of anemia in children should also include cobalamin, iron and folate supplementation for prevention and control of nutritional anemia. Primary care physicians should suspect all the three causes for anemia.

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Nutrition, Vitamins and Trace Elements

Cod: W209

EVALUATION OF VITAMIN D STATUS IN HEALTHY ADULTS FROM URBAN POPULATION OF ZAGREB, CROATIA USING REFERENCE LC-MS/MS METHOD

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Background: Numerous studies have been conducted regarding vitamin D status and its importance for human health. Still, there is an on-going debate about reference intervals (RI) and deficiency defining cut-off levels for serum 25-OH vitamin D (25(OH)D) - commonly considered as the best biological marker for vitamin D status monitoring. The aim of this study was to evaluate 25(OH)D status using reference LC-MS/MS method among healthy adults from Zagreb, Croatia in order to verify the RI recommended by contemporary literature using CLSI C28-A3 guidelines.

Methods: 69 healthy adults (14 female) with a median age of 34 (21-56) years were evaluated in this study during the fall of 2016. Inclusion criteria were: patients that haven't been taking any drugs or supplements, had clinical and laboratory status without any abnormalities. 25(OH)D concentration was measured using the reference LC-MS/MS method, controlled by an external quality assessment scheme, organized by RfB, Germany. According to CLSI C28-A3 guidelines, verification criteria are met only if no more than 2 out of 20 tested subjects' values fall outside the RI. Differences between groups were tested by Kruskal-Wallis and Mann-Whitney statistical tests.

Results: 25(OH)D concentration median for all samples was 64,67 nmol/L (min = 23,11 nmol/L; max = 123,25 nmol/L) and there was no statistically significant difference between age or sex groups (P=0,085, P=0,024, respectively). In regards to various literature RIs and deficiency defining cut-off values, 19 (0,28) observed patients would be vitamin D deficient at cut-off <50 nmol/L, 2 (0,03) at cut-off <30 nmol/L and only 19 (0,28) had a concentration greater than 75 nmol/L - generally considered as the sufficient level of serum 25(OH)D.

Conclusion: Study results have shown that 50 out of 69 healthy individuals haven't met verification criteria, suggesting that own RI should be developed. Great variety of the available RI could be a possible cause of such discrepancy, considering they are universal regardless of the analytical methods used and that the methods are incomparable themselves. On the other hand, the best approach would be development of methods traceable to reference LC-MS/MS - currently the most specific method, prone to majority of the interferences.