Biological variation

Cod: 0117

THE EFFECT OF SOME SPORT BRANCHES ON URINARY CYCLIC NUCLEOTIDE LEVELS

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BACKGROUND: The effect of some sport branches on hormonal secretions in general was tried to explain on the basis of urinary cyclic nucleotide (cAMP and cGMP) excretion, which are only two of the second messengers, since the total, ultimate effects of secreted (agonistic and antagonistic) hormones under the effect of stress and/or exercise may be estimated by means of the changes in the second messenger concentrations.

METHODS: A total of sixty subjects from different sport branches were included in the study and pre- and post-training urinary cyclic nucleotide levels of them were determined. The urinary cyclic nucleotide, cAMP and cGMP, concentrations were determined by EIA method.

RESULTS: Mean age of subjects was 25.6 ± 6.79 years and mean Body Mass Index (BMI) was 24.43 ± 2.78. Pre-training and post-training urinary cAMP levels were measured as 8.95±3.53 and 21.56±1.92 mg/g creatinine, respectively. Pre-training and post-training urinary cGMP levels were measured as 4.50±3.85 and 7.5±4.8 mg/g creatinine, respectively. The statistical analyses of the results obtained showed that the difference in cAMP and cGMP between pre-training and post-training periods were significant at the level of $p< 0.05$ for both parameters, with the post-training levels being higher.

CONCLUSIONS: Our findings related to the increased post-training cyclic nucleotide concentrations in urine showed that a complex hormonal response occurs as a result of both metabolic state and the stress/exercise in sporters. The conclusion is that the complexed hormonal status of the sporting person may be explained only by determining the second messenger concentrations. Since the present study looking at this perspective is a preliminary one in this area, one needs the determination of all second messenger changes in any way for the evaluation of the total hormonal status of sporting person.

Biological variation

Cod: 0118

DEFINITION OF A MINIMUM DATA SET TO ACCOMPANY INDICES OF BIOLOGICAL VARIATION

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BACKGROUND: Biological variation data are used by laboratory professionals globally to enable interpretation of clinical laboratory test results and to set quality standards. The data are derived from varying populations with studies utilising a variety of experimental models and approaches. The data are of varying quality and sometimes poorly characterised. These data are effectively reference data and users of them need to be aware of the attributes of the data that impact upon the transferability of data across populations and time. There is a further need for users to understand the uncertainty applying to the estimates of published biological variation. The Biological Variation Working Group (BVWG), set up by the EFLM, have undertaken work to identify a minimum data set (MDS) to accompany published indices of within and between subject biological variations to enable critical appraisal of their utility to prospective users.

METHODS: The BVWG, as part of their remit to establish a critical appraisal checklist for publication of biological variation data, has studied existing literature and databases and undertaken discussions to identify the MDS required by users to enable transferability of biological variation data safely, accurately and effectively.

RESULTS: Six main data domains were identified with sub categories. The domains with example sub categories are:
Target - analyte and measurand, sample matrix, method characteristics.
Population characteristics- demographics, state of well being, physical/physiological characteristics, medication.
Study Characteristics- study duration and design, power of study to detect BV indices, model assumptions, statistical approach.
Data Characteristics- indices of biological variability, confidence intervals, tests for model assumptions
Publication Details- links to the original publication.
Data rating- new concept to be developed to indicate the quality of the BV data against a set of key criteria.
The group has identified that wherever possible international coding systems (e.g. LOINC, SNOMED) should be used to facilitate the accurate transmission of the relevant data.

CONCLUSIONS: An MDS has been identified for further development to enable safe, accurate and effective transmission of biological variation data.

Biological variation

Cod: 0119

BIOLOGICAL VARIATIONS OF OXIDATIVE STRESS BIOMARKERS IN URINES OF PATIENTS WITH TYPE 2 DIABETES MELLITUS AND DIABETIC NEPHROPATHY AS WELL AS HEALTHY INDIVIDUALS

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BACKGROUND: Quantitative data on the components of biological variation were estimated for oxidative stress biomarkers such as superoxide dismutase (CuZnSOD), catalase (CAT) and malondialdehyde (MDA) in the urines of 20 patients with type 2 diabetes mellitus (T2DM) and 20 patients with diabetic nephropathy (DN), and results were compared with those of 15 apparently healthy individuals.

METHODS: Timed first morning urine samples were taken from each individual on the zero, 1st, 3rd, 5th, 7th, 15th, 30th days. The within-subject and between-subject variations were calculated by nested analysis of variance (ANOVA) and used to derive the reference change value (RCV) required to be 95% certain to prove that a change has occured. Linear regression analysis was used to look for significant trends in values for CAT, CuZnSOD, MDA and to investigate the time dependence of the within-subject variations.

RESULTS: Within-subject variation was significantly higher in patients with DN and T2DM compared to healthy individuals. Analytical variability was the smallest component of variance for all variables. MDA showed low individuality, and within-subject variances of MDA were larger than between-subject variances in all groups. However, CAT and CuZnSOD showed strong individuality, but within-subject variances of them were smaller than between-subject variances in all groups. RCVs of all analytes in both diabetic patients were relatively higher, because of high within-subject variation, resulting in a higher RCV.

CONCLUSIONS: Within-subject biological variation contributes to the variation in serial results and should therefore be included in the criteria for oxidative stress biomarkers' assessment in urines of both healthy and diabetic patients.

Biological variation

Cod: 0120

CALCULATION OF REFERENCE CHANGE VALUES OF CLINICAL CHEMISTRY PARAMETERS

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BACKGROUND: It has been recommended that using Reference Change Values (RCV) is the most appropriate method to follow-up of individuals. Most of them are used to follow-up of individuals in acute situations and following the improvement or deterioration of chronic disease. The aim of this study was to calculate of RCV of 25 analytes of clinical chemistry parameters.

METHODS: Twenty -five serum analytes (glucose, urea, alkaline phosphatase (ALP), creatine kinase (CK), CKMB, amylase, protein total, albumin, bilurubin total, bilurubin conjugated, calcium, creatinine, urate, cholesterol total, trigyliceride, HDL-cholesterol, aspartate aminotransferase (AST), alanine amino transferase (ALT), gamma-glutamyl transferase (GGT), lactate dehiydrogenase (LDH), phosphate, sodium, potassium, chloride, magnesium) were analyzed with Abbott kits (Abbott Laboratories, IL, USA), which were manufactured to use Architect c8000® (AbbottLaboratories,IL, USA) auto-analyzer. We used analytic coefficient of variation (CVA) from internal quality control programmed. Intra-individual biological variation (CVW) were obtained from Ricos current updated published in Westgards website. This database was updated in 2014. We calculated RCV using this formula RCV= 21/2*Z* (CVA2 + CVW2) ½. Z=1.96 for a significant change, that is, a change with 95% propability, $p<0.05$.

RESULTS: RCVs of these analytes were calculated as 17.38 % for glucose, 35.49 % for urea, 21.09 % for ALP, 63.43% for CK, 55.80% for CKMB, 24.94% amylase, 9.39% for protein total, 10.34% for albumin, 60,68% for bilurubin total, 102.56% for bilurubin conjugated, 7.60% for calcium, 17.85% for creatinine, 25.50% for urate, 16.85% for cholesterol total, 55,38% for trigyliceride, 24.36% for HDL-cholesterol, 35.14% for AST, 55.12% for ALT, 37.46% for GGT, 24.85% for LDH, 23.46% for phosphate, 5.94% for sodium, 37.92% for potassium, 5.27% for chloride, 16.23% for magnesium.

CONCLUSIONS: We suggest to use RCV as well as to use population-based reference interval in clinical laboratories. RCV could be a valuable tool for clinical decision in especially monitoring indivuduals.

Biological variation

Cod: 0121

EVALUATION OF IMPRECISION AND REFERENCE CHANGE VALUE OF TUMOR MARKERS ON ABBOTT ARCHITECT I2000SR SYSTEM USING BIO-RAD QUALITY CONTROL

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BACKGROUND: Variations in serial tumor markers results are due not only to clinical conditions patients but also to analytical imprecision (CV(A)) and intra-individual biological variation (CV(i)). Whereas the index of individuality (II) for the tumor markers is <0.6, the state of art recommends the use of the Reference Change Value (RCV) to evaluate the significant changes in serial results instead of conventional cutoff limits. The aim of this study was to define our RCV in order to improve the clinical use of these biomarkers.

METHODS: To define the RCV we used biological variation from C. Ricos's database and we calculated our CV(A). We performed daily measurement of human serum based control Bio-Rad with tumor markers concentrations close to the cut-offs by ARCHITECT i2000SR instrument (Abbott Diagnostic Division). During two years we collected, calculated monthly CV(A) and monitored our results with an interlaboratory quality control program (Unity Real Time Bio-Rad). We compared CV(A) with corresponding analytical goals derived from biological variation and calculated by Fraser's formulas.

RESULTS: The averages CV(A) and analytical goals (AG), consistently maintained during the two years period, were as follows: AFP 3.01% (3.05% optimal AG); CA15-3 4.46% (4.58% minimal AG); CA19-9 6.40% (8.0% desirable AG); CA125 3.87% (6.10% optimal AG); CEA 4.68 % (6.35% desirable AG); total PSA 4.97% (4.53% optimal AG). The RCVs of AFP, CA 15-3, CA 19-9, CA 125, CEA, total PSA were 34.81%, 20.94 %, 47.73 %, 69.25 %, 37.49 % and 51.99 % respectively (Z = 1.96 for bidirectional changes; $p<0.05$).

CONCLUSIONS: The measurement and continuous monitoring of CV(A) are essential to ensure the reliability of an analytical result. Since our CV(A) remained constant during two years and in accordance with analytical goals we could define our RCV. The RCV represents an optimization of laboratory reporting and could be an additional tool for clinical decision, instead of the only use of conventional reference intervals in follow-up of cancer. The next step of our study will be the application of RCV to clinical cases to evaluate its performance.

Biological variation

Cod: 0122

USEFULNESS OF BIOLOGICAL VARIATION ESTABLISHED AS ANALYTICAL SPECIFICATIONS FOR TUMOR MARKERS. A COMPARISON OF TWO DIFFERENT CA 19.9 METHODOLOGIES

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BACKGROUND: Biological variation (BV) is widely used by laboratory professionals to set analytical quality specifications. The use of BV as quality standards for tumor markers (TM) is controversial due to their BV is generally high. Although there is not a definitive consensus, an increase of 30-35% is commonly considered as a significant change in patient's follow-up.The aim is to assess if BV is an adequate quality standard to be applied as a method comparison goal and to evaluate the possible impact on patients' monitoring

METHODS: Before a change of laboratory analytical system, following CLSI EP9-A2IR protocol, CA 19.9 from 40 randomly selected patient samples (analytical range: 2.6 to 803 IU/mL) were performed in two analyzers, Architect i2000 (Abbott) and Advia Centauro XP (Siemens) by duplicate. Analytical imprecision of all methods were within desirable limits based on BV. Desirable and optimum BV bias was set as analytical goal for comparability of patients' results. Confidence interval at 95% (CI) of the predicted difference (pD) was compared to laboratory allowable bias (LBa) at medical decision levels (MDL)

RESULTS: Linear regression equation: y= 0.78x + 7.14 (x: Architect, y:Centauro) r=0.989.Difference between methods was evaluated for desirable BV bias claim (25.8%) and optimum (12.9%) at all MDL. At 37 IU/mL, pD:-0.96, CI:-8.4 to 6.5; desirable LBa was 9.6 and optimum 4.8 .At 300 IU/mL, pD:-58.6, CI:-69.1 to -48.1, while LBa was 77.4 and 38.7 for desirable and optimum BV respectively.At 1000 IU/mL, pD:-212, CI:-248 to -176, being desirable BV LBa 258 and optimum LBa 129.The difference between methods with desirable BV was verified at all MDL. However, it was not verified when optimum BV claim was used

CONCLUSIONS: Desirable biological variation bias set as method comparison goal does not ensure that a modification in methodology for CA 19.9 fulfils clinical needs. Due to that, optimum Biological Variation should be preferred or, even to ascend to the upper step of Stockholm hierarchy.Moreover, when a method change exist, it is always necessary to perform a comparison protocol with an appropriate goal for comparability depending upon tumor marker and pathology. Clinicians should be notified of the change and a consensus should be reached

Biological variation

Cod: 0123

THE CORRELATION BETWEEN HBAS-GENE AND CONSANGUINITY AMONG FORTY FAMILIES WITHIN TWO GENERATIONS IN MARZOUK REGION - SOUTHERN LIBYA

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BACKGROUND: Sickle cell anaemia is a hereditary disease most commonly seen in African population due to point mutation in beta globin gene. Studies in sickle cell anaemia are very scarce in the Southern part of Libya. The objectives of the study were to detect the pattern of sickle cell gene in these families as well as to study the relation of consanguinity and the occurrence of such genes among these families.

METHODS: In this study, 210 native Libyans from different families from Marzouk Region were tested for the presence of HbS either in heterozygous or homozygous form. The families were selected by tracing blood transfusion dependent sickle cell patients attending the Marzouk hospital Haematological test including CBC, PBS as well as sickling tests by both slides and tube methods were carried out. Positive cases for HbS were confirmed by Hb electrophoresis.

RESULTS: Results of this study revealed 10% of cases with HbSS, 53.34% with HbAS and 35.71% with HbAA. A case of HbSC (0.95%) was also detected. Sickled cells were seen in all PBS of homozygous and in most of heterozygous patients. Heterozygous individuals, who are usually asymptomatic presented with some clinical manifestations such as joint pain, jaundice and eye problems. Consanguinity was found in 72% of cases and could play a major role in the spread of this disease in this region.

CONCLUSIONS: The incidence of sickle cell trait (HbAS) was high with 53.34% in Marzouk region compared to other parts of the country. The rate of frequency of consanguinity in this study showed a relevant high result of 72% and gave a clear noticed that interrelation marriages in this region is well practiced and that could be a major factor of high rate of sickle cell trait.

Biological variation

Cod: 0124

ACUTE EFFECTS OF TRAINING IN BOXING SPORTSMEN

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BACKGROUND: Boxing sport is characterized by short duration, high intensity bursts of activity. The aim of this study was to analyze some biochemical parameters of boxing sportsmen before and after training.

METHODS: Twenty professional boxing men (age range: from 16 to 30 years and BMI range: from 19.0 to 26.8) were included in the study. Venous blood samples were taken from the subjects, and some biochemical parameters were measured before and 6 minutes after fighting. This study was approved by local ethics committee, and in-formed consent was obtained from each participant. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), and al-kaline phosphatase (ALP) activities were measured using Beckman Coulter AU5800 analyzer by enzymatic methods; total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides assays were carried out in the same analyzer by respective methods. Adrenocorticotropic hormone (ACTH), growth hormone (GH), and insulin-like growth factor-1 (IGF-1) assays were performed using Immulite 2000 analyzer with chemiluminescence method.

RESULTS: Serum levels of ACTH, AST, GGT, ALT, and GH were significantly in-creased and serum levels of IGF was decreased by fighting exercise ($p<0.05$). There were no significant changes in serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides and ALP levels after fighting exercise ($p>0.05$).

CONCLUSIONS: Increases in serum ALT and AST activities reflect mechanical damage to the muscle cells. Additionally, the increases in serum enzyme activities may indi-cate minor hepatocellular damage. The increased levels of serum GH is associated with the physical and exercise stress. Considering that aerobic exercise positively affects plasma lipid profile, we can conclude that the sportsmen were exposed to anaerobic training at a higher rate more than aerobic training. Serum IGF-1 level is generally reported to be increased, mostly in aerobic exercises. The serum IGF-1 levels significantly decreased in our study, which supports our conclusion that the exercising man were exposed to anaerobic training at a higher rate more than aerobic one. The results suggest that the exercise affects some substantial biochemical parameters in the selected subjects.

Biological variation

Cod: 0125

CIRCULATING CARDIAC TROPONIN T EXHIBITS A DIURNAL RHYTHM

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BACKGROUND: Cardiac troponin (cTn) is the preferred biomarker for the diagnosis of acute myocardial infarction (AMI). However, acute and chronic conditions distinct from AMI frequently lead to elevated cTn levels. Therefore, the diagnosis of AMI strongly relies on the interpretation of cTn kinetics with serial testing. Essential in this regard is a profound understanding of the biological cTn variation. Aim of this study was to investigate the unverified assumption that cTn levels fluctuate randomly around a homeostatic setpoint.

METHODS: To optimize comparability to the typical patient that requires evaluation for chest pain, 23 type 2 diabetes patients (age 63±7 yrs, BMI 30±4 kg/m^2, mean±SD) with a high cardiovascular risk profile were included. In study 1, three extended biological variation experiments were conducted to assess within-day (8:30-19:30h, 9 samples) and between-week (3 samples collected at 8:30h at a one-week interval) biological variation of cardiac troponin T (cTnT). In study 2, the presence of a diurnal rhythm was investigated by sampling 7 subjects every hour over a time span of 25h.

RESULTS: Circulating cTnT exhibited a diurnal rhythm, with peak concentrations during morning hours, gradually decreasing throughout daytime and rising during nighttime (17±3 ng/L at 8:30h, 12±2 ng/L at 20:30h, 16±3 ng/L at 8:30h day 2, mean±SEM). In all participants, the diurnal cTnT oscillation was significantly described by a cosine curve (R2 0.55-0.91, all p=0.001). As a consequence of the rhythm, the biological variation between 8:30 and 12:30h was twice the biological variation assessed between 16:30 and 20:30h (CVi 9% (95%CI 7-13) vs. CVi 4% (3-6)). In addition, the within-day biological variation was significantly larger than between-week biological variation (CVi 14% (95%CI 13-16) vs. CVi 10% (8-12)). The cTnT kinetics was not related to changes in hydration status, posture, kidney function or exercise.

CONCLUSIONS: The presence of a diurnal cTnT rhythm substantiates the recommendation that all dynamic changes of cTn should be interpreted in relation to the clinical presentation. Epidemiological studies and risk stratification protocols using cTnT may benefit from standardized sampling times.

Biological variation

Cod: 0126

EFFECTS OF INTENSE EXERCISE ON BIOCHEMICAL PARAMETERS IN KICK BOXING SPORTSMEN

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BACKGROUND: Kick boxing, boxing and other fighting sports popularity is increasing because of such benefits as personal protection and keeping the body fit. Exercise has various effects on body weight, serum lipids levels, psychosocial stress, blood pressure, and insulin resistance and diabetes mellitus. Depending on the duration and intensity of exercise, physiological and laboratory changes may occur. Objective of the study was to determine the effects of a kick-boxing match on some biochemical parameters.

METHODS: The local ethics committee approved this study. At the beginning of the study, all kick-boxing sportsmen (n = 23) were informed about the aim of the study, and the written informed consents of them were taken. Serum alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferases (GGT) activities were analyzed in Beckman Coulter AU5800 analyzer by the respective spectrophotometric enzymatic methods. Cortisol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH) and free thyroid hormones were analyzed in Beckman Coulter DXI800 analyzer by chemiluminescence method. Growth hormone (GH), adrenocorticotropic hormone (ACTH) and insulin-like growth factor- 1 (IGF-1) were analyzed at Immulite 2000 analyzer by the chemiluminescence method. Somatic and some biochemistry parameters were measured in all kick boxing sportsmen, before and after matching which following one-hour training. Age of all studied sportsmen ranged from 15 to 46 years.

RESULTS: The comparative analysis of some biochemical and somatic parameters measured before and after the training program and kicks boxing match showed that the exercise causes significant increase in AST, ALT, GGT, and ALP activities and total cholesterol, HDL-cholesterol, and LDL-cholesterol levels, and significant decrease in IGF-1 concentration ($P<0.05$). Exercise did not significantly affect the serum GH and triglyceride concentrations ($p>0.05$). After exercise, weight and body mass index were significantly decreased ($p<0.05$).

CONCLUSIONS: Our study demonstrated that significant changes may appear in some biochemical parameters after training program and kicks boxing match.

Biological variation

Cod: 0127

DETERMINATION OF BIOLOGICAL VARIATION DATA AND ANALYTICAL QUALITY SPECIFICATIONS IN TROPONIN, MYOGLOBIN AND CK-MB

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BACKGROUND: Biological variation is an important factor for the interpretion of laboratory test results. Biological variation (BV) has two separate components which are represented by the coefficients of variation (CV) of within-subject (CVI) and between-subject biological variation (CVG). The ratio between CVI and CVG is known as the index of individuality (II). Reference change values (RCVs) provide the information whether the difference between replicate test results of a patient are clinically significant. BV data can be used to establish quality specifications, such as imprecision (I), bias (B) and total error (TE). We aimed to establish analytical quality specifications on the basis of BV for common analytes used for cardiac ischemia.

METHODS: To perform BV data, 20 healthy and fasting volunteers (10 females, 10 males) were chosen from the reference group and blood samples were taken at 0,7,14 and 21 days into gel containing tubes. Serum samples were stored at -80°C until the analysis and were collectively analysed in Uludag University, Bursa using Abbott reagents and analyzer. CVI , CVG, RCV and II were estimated for both genders and separately. The Fraser and Harris methods were used to calculate the components of BV and RCV.

RESULTS: For the whole group, CVI (%) values for troponin I, myoglobin and CK-MB were 61,15,21 and CVG (%) were 71,40,47 respectively. For myoglobin, the CVI, CVG, RCV and II values were determined as 11,10,31,1.1 for females and 19,17,53,1.1 for males. For CK-MB, CVI,CVG,RCV and II values were 26, 23,71,1.1 for females and 16,15,43,1.0 for males. In the total data evaluations of I,B and TE values for troponin were determined as 31,24,74 respectively, for myoglobin as 8,11,23 and for CK-MB as 11,13,30.

CONCLUSIONS: The results of this study have shown that in addition to the calculations of total biological data and RCV, the separate gender calculations will be of benefit in the laboratory information system and therefore will affect clinical outcomes. The BV components, RCV and analytical specifications determined in the present study may be used to evaluate the results of cardiac markers.