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Recent doctoral theses (biochemistry, biology and biophysics) in Lithuania

Prepared by Indrė LIPATOVA

ANALYSIS OF MATURATION OF MEASLES VIRUS HEMAGLUTININ IN YEAST *S. CEREVISIAE* AND *P. PASTORIS* SECRETORY PATHWAY AND HUMANIZATION OF YEAST CELLS

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The dissertation defended:
16 December 2011

The aims of the study were to determine the reasons for unsuccessful expression of measles virus hemaglutinin (MeH) in the yeast cells and to generate stable yeast strains with integrated genes of protein secretory pathway of human cells and to examine influence of coded human proteins on MeH maturation. For the firs time overexpression of MeH in yeast *S. cerevisiae* and *P. pastoris* was described. It was demonstrated that mechanisms of cotranslational translocation into the endoplasmic reticulum (ER) and protein maturation in the ER of yeast cells are not adapted to deal with for such complex virus glycoproteins. Proteomic analysis revealed that overexpression of human virus surface protein precursors induces cytosolic unfolded protein response (UPR-cyto) in the yeast *S. cerevisiae*. A key feature of this response is the formation of extremely large aggregates involving macromolecular structures of eEF1A. Efficient mammalian like cotranslational translocation pathway was attempted to reconstitute in yeast cells by transferring human SRP, Sec61 complexes and TRAM1 protein. Human chaperones BiP, clanexin, calreticulin, ERp57 and PDI were transferred to the yeast cells to create suitable environment for maturation of MeH in the ER. Even though yeast strains able to produce biologically active MeH protein were not generated during this study, the results show that humanization of yeast secretory pathway designed for producing active virus glycoproteins is possible.

ON THE PROCESSES OF CELL DEATH INDUCED BY PHOTODYNAMIC TREATMENT *IN VITRO*: IMPACT OF THE PHOTOSENSITISER LOCALISATION

Aušra Sasnauskienė

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The dissertation defended:
21 October 2011

Photodynamic treatment (PDT) is used to treat cancer and other diseases caused by cellular overgrowth. The initial damage of PDT is restricted to the site of photosensitiser accumulation. Clinically used photosensitisers show affinity to multiple cellular organelles. We have studied cell response to PDT (apoptosis, autophagy, cell cycle, expression of several genes) mediated by photosensitisers localised to: 1) mitochondria – 2-(6-amino-3-imino-3H-xanthen-9-yl) benzoic acid methyl ester (Rh123) and 3,7-diamino-2,8-dimethyl-5-phenylphenazinium chloride (Safr); 2) lysosomes – aluminium (III) phthalocyanine tetrasulfonate (AlPcS4); 3) multiple cellular organelles – meso-tetra(3-hydroxyphenyl)chlorin (mTHPC).

It was determined that PDT mediated by Safr, AlPcS4 and mTHPC induced apoptosis at high cytotoxic dose (CD80), reducing cellular viability for 80%. Medium cytotoxic dose of PDT mediated by Rh123 and Safr did not induce cell death, but after Safr-FDP the cell cycle arrest was registered. Medium (CD50) and high (CD80) cytotoxic doses of PDT mediated by Safr and mTHPC induced autophagy. The amount of autophagosomes was increased after small, medium and high dose of PDT mediated by AlPcS4, but the flux through autophagy pathway proceeded only after the small dose (CD20). PDT mediated by Safr, AlPcS4 or mTHPC increased the expression of cytokines VEGF-A, IL-1 alpha and transcription factor HIF-1 alpha subunit at the RNA level.

ABSORPTION AND TISSUE DISTRIBUTION OF DRUG-LIKE COMPOUNDS: QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS

Kiril Lanevskij

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VšĮ „Aukštieji algoritmai“

The dissertation defended:
30 September 2011

The objective of this work was to develop mechanistic quantitative structure activity relationship models that would facilitate the assessment of drug properties related to their absorption and distribution in the body. The analysis involved several parameters reflecting the rate of passive diffusion across brain endothelium and intestinal epithelium, and thermodynamic constants related to drug distribution between plasma and tissues.

Permeation through cellular transport barriers was modeled by nonlinear equations relating the passive diffusion rate to physicochemical properties of drugs: lipophilicity, ionization, hydrogen bonding potential and molecular size. It was demonstrated that brain endothelium and intestinal epithelium exhibit a quantitatively similar pattern of permeability-ionization dependence – ionized species permeate 2–3 orders of magnitude slower than neutral molecules.

Analysis of tissue to plasma partitioning data revealed the necessity to split original experimental values into separate terms reflecting plasma and tissue binding strength. Drugs' affinity to tissues could then be described by their lipophilicity, whereas detrimental effect of ionization was only observed for acidic drugs. Finally, it was shown that a linear combination of quantitative blood-brain barrier transport parameters allows classifying drugs according to their access to central nervous system with 94% overall accuracy.

COMPUTATIONAL MODELING OF CYTOCHROME P450-MEDIATED DRUG METABOLISM

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The main objective of this study was the development of QSAR models for drug metabolism-related properties. Novel GALAS (Global, Adjusted Locally According to Similarity) modeling method was used, which is a combination of baseline global QSAR model and local similarity based corrections. GALAS modeling method allows forecasting the reliability of prediction thus defining the model applicability domain. Models predicting CYP3A4 inhibition and regioselectivity of metabolism in human liver microsomes were developed and validated using external test sets. In all cases the baseline models already showed acceptable results, and the overall accuracy of predictions increased after the similarity based corrections. Moreover, the numbers of mispredictions reduced significantly when only results of higher reliability were taken into account. However, the original models are applicable only for less than a half of external datasets. Since the similarity correction procedure of GALAS modeling method allows simple model training, the possibility to expand the applicability domain has been tested. The CYP3A4 inhibition model was successfully adapted to PubChem data and compounds with a novel chemical scaffold. After training the regioselectivity model new metabolism sites could be identified in compounds of new chemical class. Moreover, this model was adapted for human cytochrome P450 isoform profiling.

GENERATION OF ANTICANCER VACCINE BASED ON VIRUS-LIKE PARTICLES

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The dissertation defended:
17 June 2011

In this dissertation the investigation of potential applications of hamster polyomavirus (HaPyV) major capsid protein VP1 based chimeric virus-like particles (VLPs) harboring CTL epitopes for anticancer vaccine development is presented.

The objective of this study was to investigate the potential of recombinant HaPyV, VP1 based VLPs for anticancer vaccine generation in model systems, including investigation of VP1 applicability for heterologous CTL epitopes insertions, VLPs assembly and ability to induce insert specific immune response *in vivo*.

HaPyV, VP1, VLPs carrying CLT epitopes derived from different proteins were generated, most suitable positions for insertion into VP1 protein were selected, the ways to improve assembly and yield of the chimeric VLPs were determined and a new VLPs purification procedure was created allowing to purify VLPs cheaper, faster and more efficiently. HaPyV, VP1 based VLPs ability to induce CTL immune response in vivo was evaluated for the first time. It was demonstrated that model chimeric VLPs were able to stimulate antigen specific CTL cells *in vitro* and *in vivo*, induced insert specific humoral and CTL immune response *in vivo* and protected mice from insert specific virus infection and antigen-specific tumor growth. The presented data confirmed that HaPyV protein VP1 is a universal carrier for CTL epitopes, capable to tolerate insertions, to form VLPs and to induce effective, long lasting immune response against inserted antigens *in vivo*.

PHOTOSENSITIZED DAMAGE TO *ESCHERICHIA COLI* MEDIATED BY NEW TETRACATIONIC PORPHYRIN

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The dissertation defended:
9 September 2011

The development of multidrug resistance and its spreading among the pathogens increased the demand for alternative treatment methods of bacterial infections. One of the most promising techniques is the photodynamic antibacterial chemotherapy. The main idea of it lays in combining three initially harmless components (the oxygen, photosensitizer and appropriate light) which has fatal consequences to bacteria.

The study was designed to investigate spectroscopic features of a new tetra-cationic meso-substituted photosensitizer tetrakis(N-ethylpyridinium-4-yl)porphyrin tetratosylate (TN–Et–PyP), its interaction with different cellular components and to analyse the photodynamic action of the porphyrin on the integrity and selected functions of *E. coli* KMY1 cell membranes. It was shown that tetracationic TN–Et–PyP symmetry changes strongly depend on the polarity of the solvent. The dye forms complexes with nucleic acids and lipopolysaccharides in buffer solution, the interaction being of electrostatic nature. The interaction with surface structures of *E. coli* may facilitate the targeting of the bacterial envelope. The study presents evidence for outer membrane permeabilization, inactivation of periplasmic marker enzyme alkaline phosphatase and impairment of the inner membrane functions expressed in drop in respiration efficiency and decrease of membrane voltage upon irradiation with blue light. The inner components of the cells are consequently damaged as has been shown with decreased cytoplasmic marker enzyme β – galactosidase activity. The cell death is prompted by blow of the big vesicular structure formed in one of the cell poles and full of inner cellular material which is consequently leaking into the media.

SYNTHESIS OF PARAMYXOVIRIDAE NUCLEOPROTEINS IN YEAST *SACCHAROMYCES CEREVISIAE* AND THEIR APPLICATION IN VIRAL DIAGNOSTICS

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The dissertation defended:
20 June 2011

The aim of this study was to investigate synthesis of SeV, hPIV1, hPIV3, hRSV, NiV, HeV and MenV nucleocapside (N) proteins in yeast *Saccharomyces cerevisiae*, to determine properties of recombinant N proteins and evaluate the feasibility to use them in diagnostics. In this study it was demonstrated that yeast *S. cerevisiae* is an excellent host for a high-level production of proteins SeV, hPIV1, hPIV3, hRSV, NiV, HeV and MenV N proteins as virus nukleokapsid-like particles (vNLP). The yeast-expressed hPIV1, hPIV3, hRSV, NiV, HeV and MenV, vNLPs represent useful tools for the development of new virus detection systems and demonstrate the effectiveness of yeast as a host for generation of recombinant proteins organized in complex structures like human virus NLPs.

INVESTIGATION OF AMYLOID FIBRILS FORMING PROTEINS

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The dissertation defended:
27 May 2011

Self-assembly of biomolecules into beta-sheet structures can be applied in the creation of nano-materials with novel electrical, optical, catalytical, or / and mechanical characteristics. This work was directed towards the construction of nano-derivatives based on amyloid fibrils forming proteins (Abeta40 peptide, a-Synuclein (a-Syn), equine lysozyme (EL)). Such nanostructures can be used to produce nanoscale functional systems. Herein, different mutant and hybrid proteins, which were able to form fibrillar structures, were constructed and the properties of fibrils were investigated. Designed cysteine mutants of Abeta40 and a-Syn can be modified through thiol group of cysteine. Herein, for the first time it was demonstrated that a-Syncys141 fibrils could be modified with biotin and gold nanoparticles with neutravidin molecules. Hybrid proteins of Abeta40 or a-Syn and other non-amyloid proteins were designed on purpose to obtain fibrils with active functional non-amyloid proteins. Under appropriate conditions these proteins aggregated into beta-sheet structures. Hybrid protein of streptavidin and Abeta40 formed a net-like fibrillar structure, and streptavidin was active. For the first time the production of recombinant EL in *E. coli* was described. Moreover, active EL can form fibrils which are similar to those formed by native EL. The constructed novel hybrids and mutants that are able to form amyloid fibrils can be applied for the creation of functionalized nanodevices.

A DIRECTED EVOLUTION DESIGN OF TARGET SPECIFICITY AND KINETIC ANALYSIS OF CONFORMATIONAL TRANSITIONS IN THE HHAI METHYLTRANSFERASE

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The dissertation defended:
2 May 2011

DNA cytosine-5 methyltransferases (MTases) recognize short DNA sequences and catalyze the transfer of the methyl group from the cofactor AdoMet to the C5-position of a target cytosine. In this work both these aspects of the MTase mechanism have been addressed. First, using rational protein design and directed evolution approaches the specificity of the HhaI MTase, GCGC, has been changed to GCG by functional elimination of one of the two target recognition elements. In addition, the introduced structural changes endowed the MTase with the ability to transfer extended groups from synthetic cofactor analogs, providing the first example of a dual specificity change in a DNA MTase.

Second, the kinetics of fast pre-catalytic conformational transitions in the MTase and DNA has been investigated. A new method to follow the target cytosine flipping and its subsequent covalent activation has been proposed which allows a direct real-time observation of these processes by monitoring associated UV absorbance changes in a chemically unperturbed DNA. For the first time these studies demonstrate that the flipping of the target cytosine and the closure of the catalytic loop in the enzyme occur simultaneously, whereas the covalent activation of the target cytosine and the transfer of the methyl group are temporally distinct steps in the catalytic cycle of M. HhaI. Since the new method is based on the general phenomenon of hyperchromicity, it is thus applicable for studies of other systems involving base-flipping.

TOXICITY OF ENVIRONMENTAL POLLUTANTS AROCLOR 1248, OLEIC ACID AND SULFUR TO *VIBRIO FISCHERI* AND BIOCHEMICAL MECHANISMS

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The dissertation defended:
1 April 2011

Vibrio fischeri bacteria are used as a bioobject of the standard bioluminescence quenching test (EN ISO 11348-3:1998), which is designed for the determination of the toxicity of single chemicals, their mixtures and environmental samples. The evaluation of the target sites of chemicals in redox enzyme systems (*in vivo*) and individual enzymes (*in vitro*) of *V. fischeri* bacteria is a valuable tool for understanding the mechanisms of chemical action, for the prediction of mixture toxicity and explanation of the toxicodynamic effects.

The use of multiple regression method revealed the different toxicodynamic effects of binary mixtures of Aroclor 1248 (A), oleic acid (OA) and elemental sulfur (S80) on bioluminescence of *V. fischeri* and NADH : FMN oxidoreductase – luciferase *in vitro*. A and S80 acted additively with synergistic interaction *in vivo* and additively without significant interaction *in vitro*; A and OA acted antagonistically in both systems. OA and S80 caused an additive effect with antagonistic component of interaction *in vivo* and had additive effect with synergistic interaction *in vitro*. Toxicity of the individual pollutants (A, OA and S80) and their binary mixtures to bioluminescence diminished with increasing exposure time (30, 60 min) in the *in vivo*, but not in the *in vitro* system. It was indicated that A and OA, but not S80, enhanced ROS generation in *V. fischeri* cells. Contrarily, S80 (greater than A and OA) enhanced reducing ability of cells. It is concluded that the principle target site of OA and S80 is the active center of the luciferase enzyme, and the main site of A action is a NADH : FMN oxidoreductase in the bioluminescence complex.

REPEATED BOUT EFFECT ON PEOPLE OF DIFFERENT AGE AND GENDER

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The dissertation defended:
23 September 2011

The aim of the research was to study repeated bout effect on people of different age and gender after muscle disturbance intensity. Our results showed that the maximal intensity of eccentric concentric bout effect on people of different age and gender causes muscle disturbance, but repeated bout effect reduces disturbance symptoms.

INVESTIGATION OF BONE MARROW HEMATOPOIETIC STEM CELL MIGRATION DURING INFLAMMATION IN BALB/C MICE

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The dissertation defended:
28 October 2011

The aim of dissertation work was to investigate the anti-inflammatory effect of murine bone marrow hematopoietic stem cells and their migration in the BALB/c mouse contact hypersensitivity model *in vivo*. It was found that isolated hematopoietic cell populations had a significant anti-inflammatory effect and inhibited the edema. The studies showed that the most efficient (up to 66%) inhibition of foot edema was obtained using the HSC population. For the first time the HSC population migration kinetics in the BALB/c mouse contact hypersensitivity model in vivo was investigated. We determined that cells of this population can be found in mice paw edema and liver after just one hour. Slightly later they are detected in the spleen. We did the HSC population quantitative migration kinetic studies and found that in case of foot inflammation there is a secondary migration of the transplanted HSC migrating from the bone marrow to the spleen hematopoietic niche. We have shown that these cells selectively migrate into the inflammation areas of the foot edema. The quantity of transplanted cells in the samples of foot edema, as compared with the untreated foot, was more than 1 000 times higher. A transplanted hematopoietic stem cell migration research during inflammation carried out in this work contributes to clarification of stem cell migration patterns in case of pathological processes. This is particularly important in ensuring a safe and effective stem cell application in practice.

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL PATTERNS OF THE INTRINSIC GANGLIONATED NERVE PLEXUS IN THE MOUSE HEART

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The dissertation defended:
30 August 2011

Intrinsic neural plexus of the mouse heart has not been adequately investigated despite the extensive use of this species in experimental cardiology. Both normal and genetically modified mice are excellent models for investigations of molecular mechanisms of cardiac arrhythmia associated with disbalance between sympathetic and parasympathetic neural inputs to the heart. The purpose of this study was to determine the topographical and structural organization of the mouse cardiac neural plexus, also to determine the distribution of cholinergic (parasyphatetic), adrenergic (symphatetic), and sensory (peptidergic) neural components in the whole-mount mouse heart preparations using double immunohistochemical labeling. The results showed that despite substantial anatomic differences in the number and distribution of epicardiac ganglia, the structural organization of intrinsic ganglionated plexus in the heart of mouse in general corresponds to that of other mammalian species, including the human. The majority of nerves and neural bundles in the heart of mouse are mixed, but a lot of them express either adrenergic or cholinergic phenotype. Therefore, the selective stimulation and / or ablation of the functionally distinct intrinsic neural pathways appear fairly available in the mouse heart model and this should allow further investigations on specific effects of distinct intrinsic nerves and ganglia on cardiac function.

INFLUENCE OF EXTRACTS OF GINKGO BILOBA LEAVES ON MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION SYSTEM

Giedrė Baliutytė

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The dissertation defended:
26 August 2011

Ginkgo biloba-derived preparations have become widely used in medical practice. Although extracts of Ginkgo biloba leaves have a wide pharmacological application, little is known about the effects of these extracts on mitochondria. Therefore the aim of this study was to investigate the influence of extracts of Ginkgo biloba leaves on mitochondrial oxidative phosphorylation system. The tasks of the study were as follows: 1. To investigate the effects of extract of Ginkgo biloba leaves on the respiration of isolated heart and liver mitochondria and permeabilized heart fibers. 2. To analyze the mechanism(s) of extract of Ginkgo biloba leaves on mitochondrial oxidative phosphorylation system. 3. To determine the effect of extract of Ginkgo biloba leaves on perfused rat heart electromechanical activity and to analyze how GBE given to isolated perfused rat hearts can readily penetrate into the heart cells and mitochondria. 4. To test whether perfusion with Ginkgo biloba extract protects heart mitochondria against ischemia / reperfusion damage. 5. To investigate the effect of extract of Ginkgo biloba leaves on mitochondrial oxidative phosphorylation system *in vivo*.

A STUDY OF MATRIX METALLOPROTEINASES AND ANGIOTENSIN-CONVERTING ENZYMES EXPRESSION IN THE MORPHOGENESIS OF DILATATIVE PATHOLOGY OF THE ASCENDING THORACIC AORTA

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The dissertation defended:
30 June 2011

The aim of the study was to investigate factors involved in the expression regulation of the matrix metalloproteinases (MMP-2, MMP-3, MMP-9) and angiotensin-converting enzymes and their impact on the morphogenesis of dilatative pathology of the ascending thoracic aorta.

This study provides for a more detailed understanding of the morphogenesis mechanisms of the molecular dilatative pathology of the thoracic aorta aneurysm. It is the first work where molecular genetic and tissue histology methods are applied in a comprehensive manner to analyze the impact of the renin-angiotensin and MMP systems on the development of dilatative pathology of the ascending thoracic aorta.

THE INFLUENCE OF MORPHOLOGICAL AND BIOMECHANICAL PROPERTIES OF THE ACHILLES TENDON AND CALF MUSCLES ON BALANCE STABILITY

Laimutis Škikas

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The dissertation defended:
30 June 2011

The aim of the study was to detect the influence of morphological and biomechanical properties of the Achilles tendon and calf muscles on balance stability. Four experiments were done using different methods – echoscope, miotonometry, posturograpgy.

INFLUENCE OF THE MEMBERS OF RAS / PKA SIGNAL TRANSDUCTION PATHWAY ON THE YEAST *SACCHAROMYCES CEREVISIAE* CELL DEATH INDUCED BY NATURAL ACIDIFICATION OF THE MEDIUM

Eglė Lastauskienė

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The dissertation defended:
11 May 2011

Constantly changing environment is the major factor controlling the growth and development of microorganisms. For a rapid generation of the cell response, information about changes in the cell environment is rapidly transmitted to the inner molecules of the cell. During the course of evolution the cells have developed signaling systems that are able to combine extracellular signals with the inner processes, as transcription, translation, etc. One of the universal signaling systems is Ras / PKA signal transduction pathway. This system helps cells recognize the nutrient sources present in the growth medium. Environmental pH is one of the main factors influencing the growth, physiology and differentiation of yeast. In *S. cerevisiae*, the response to pH is determined by RIM101 pathway. Recently it was shown that Ras / PKA signal transduction pathway regulates cell aging as response to environmental pH. Many aging and apoptosis features are conserved between yeast and multicellular microorganisms, and this makes them perfect model organisms. Yeasts are also suitable for acidosis related disease studies. Evaluation of the cell growth and medium acidification of isogenic strains containing mutation in the members of Ras / PKA signal transduction pathway was performed. Mutations in these genes cause changes in metabolic activity of the cell. Members of Ras / PKA signal transduction pathway participate in regulation of cell viability and lifespan during the natural gradual acidification of the medium and under acidic stress conditions. In this process RAS genes are acting as negative regulators. PDE1 gene is also a negative regulator of the cell viability in these conditions. It was determined that buffering of the medium significantly increases the lifespan. Analysis of the cell death type showed that hyperactivation of the Ras / PKA signal transduction pathway caused by Ras2Val19 mutation induces apoptosis in yeast cells. Contarily, downregulation of the pathway by deletion of RAS1 and RAS2 genes act as necrosis inducer. Activity of the phosphodiesterase 2 is related to the termination of the apoptosis in yeast cells and inactivation of both phosphodiesterase genes induces necrosis in yeast cells. Buffering of the medium causes decreased cell mortality with apoptosis related markers. It was proved that members of Ras / PKA signal transduction pathway regulate cell metabolism, viability, aging and death type during natural acidification of the environment and under acidic stress conditions.

THE RESIDUAL EFFECT OF ECCENTRIC CONCENTRIC PRIOR EXERCISE ON AEROBIC CAPACITY AND MUSCLE ELECTRICAL ACTIVITY DURING RUNNING OF DIFFERENT INTENSITY

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The dissertation defended:
23 June 2011

The aim of the research was to study residual effect of eccentric concentric prior exercise on aerobic capacity and muscle electrical activity during running of different intensity.

Research hypothesis. Eccentric concentric stepping and jumping exercise can induce calf muscle damage and decrease strength, thus it might affect the indices of aerobic capacity, and especially its efficiency, and leg muscle electrical activity during increasing and constant running on a treadmill. This would confirm the hypothesis that after increasing muscle fatigue or inducing muscle damage, motor unit recruitment changes not only during static, but also during dynamic exercise, and this affects VO2 and running economy or other aerobic capacity indices.

Research originality. For the first time we have studied the effect of eccentric concentric (stepping and jumping) prior load on gas metabolism, aerobic capacity and EMG indices during increasing, moderate and heavy intensity constant speed running 1 and 24 hours after such prior load. We have established that the greatest changes in the studied indices appeared 1 hour after such prior load: VO2 max and VT2 decreased, VO2 and ratings of perceived exertion increased, EMG amplitude of calf muscles for young women increased.

DETERMINATION OF GENOMIC VARIANTS OF THE COMPLEX AETIOLOGY CLEFT LIP AND (OR) PALATE IN LITHUANIAN PATIENT GROUP

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The dissertation defended:
22 June 2011

The incidence of cleft lip and (or) palate (CL/P) varies from 0.4 to 2.0 in 1 000 live births across populations. More and more CL/P candidate loci are being confirmed using novel genome-wide methods of molecular genetics and tools of statistical analysis. The aim of this study was to identify the alleles of the candidate genes for cleft lip with or without cleft palate and isolated cleft palate in the Lithuanian patient group, applying the molecular genotyping of the genomic markers in 42 CL/P candidate genes and association analysis using transmission disequilibrium test approach.

Using families' studies and case-control studies approach CL/P candidate genes TIMP2, BMP2, FN1 variants were confirmed for cleft lip with or without cleft palate and COL11A1, COL11A2, COL2A1 for cleft palate only in the population of Lithuania. Estimating the association analysis results of the CL/P patients of Lithuania, Latvia and Estonia, FGF1, TIMP2 were identified as candidate genes for cleft lip with or without cleft palate and COL2A1, COL11A2 for isolated cleft palate in the North East European populations. Different genomic risk variants have influence for cleft lip with or without cleft palate and for cleft palate only, which confirms the hypothesis of different mechanisms for these two phenotypes.

THE EXPRESSION OF *SACCHAROMYCES CEREVISIAE* K2 PREPROTOXIN GENE IN PLANT *NICOTIANA TABACUM* L. AND THE SEARCH OF TOXINS PRODUCING MICROORGANISMS AND ANALYSIS OF THEIR USE

Brigita Čapukoitienė

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The dissertation defended:
25 May 2011

Approximately 230 spontaneous fermentations were analyzed and prepared out of fruits and berries. 17 strains were extracted, characterised with activated biocidic, immune and fungicidal properties were evaluated comparing it with yeast *S. cerevisiae* sensitive and killer strains. The temperature and pH for the best killer toxin secretion of killer yeast strains and bacterial isolates were defined. It shows that toxins of the isolates can be used against plants, animals and humans pathogenic microorganisms: *Candida* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Verticillium* spp., *Venturia* spp. 1M yeast strain was found which had features necessary for wine yeast and fermentated apple juice quality parameters and was used for natural apple wine semimanufactures production in cooperative company „Vaisių sultys". During this research it was for the first time that K2 killer preprotoxin gene was cloned successfully to plant *Nicotiana tabacum* L. and its expression analysed.

VARIABILITY AND ACCURACY IN THE FORCE OF MUSCLE ISOMETRIC CONTRACTIONS OF PLANTARFLEXORS AND DORSIFLEXORS AND BALANCE CONTROL CHANGES FOR WOMEN OF DIFFERENT AGE

Vida Janina Česnaitienė

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The dissertation defended:
1 April 2011

The aim of the research was to study variability and accuracy of the force of isometric muscle contractions of plantarflexors and dorsiflexors and balance control changes.

Objectives: 1. To establish the dependence of variability and accuracy of the force of continuous isometric muscle contractions of plantarflexors and dorsiflexors on age, dominating side, visual feedback and the size of muscle torque. 2. To establish the dependence of variability and accuracy of the force of repeated isometric muscle contractions of plantarflexors and dorsiflexors on age, dominating side, visual feedback and the size of muscle torque. 3. To establish the dependence of the amount of energy consumed for balance control and the complexity of keeping balance on age and visual feedback.

Originality of the research. The results of the research showed that due to ageing of the motor system variability of the force of continuous and repeated isometric muscle contractions of plantarflexors and dorsiflexors increases and the accuracy decreases. Moreover, it was established that gross plantarflexors were controlled better than fine dorsiflexors when performing repeated and continuous isometric contractions, and this does not depend on age and bilateralism. We established that due to the aging of motor system the dependence of accuracy of repeated isometric contractions force on visual feedback increases. Changes in the variability and accuracy of the contraction force of plantarflexors and dorsiflexors due to aging of the motor system appear more in the performance of complicated tasks, i. e. repeated accurate isometric contractions compared to continuous isometric contractions. Balance control becomes not so complex with aging (i. e. the behaviour of the system is easier predictable and repeated), more energy is used to maintain it because the power of fluctuations in the centre of pressure increases. Furthemore, due to aging the dependence of the energy consumed for balance control on visual feedback increases. We suggest that all those facts make the cognition of motor control phenomenon of elderly people easier.

DYNAMICS OF INTERACTIONS OF CARDIOVASCULAR INDICES IN EVALUATION OF SPORTSMEN'S BODY FUNCTIONAL STATE

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The dissertation defended:
23 May 2011

The dynamic of the human body as a complex system of processes is reflected in the registration process of physiological signals which are characterized by varying degrees of oscillations, for example, electrocardiogram (ECG) signals. Typically, these signals are selected for the statistical analysis methods that are more applicable to global processes of the body and requiring large amounts of information. Accordingly, the mathematical analysis methods are used to examine the local processes of the body and thus do not require such a large amount of information (in our case, enough of three deductions). Physiological signal analysis matrix helps to reveal the complex human organism as a complex adaptive system and fatigue phenomena, which reflect the functional state of homeostasis and adaptation processes in fractal and chaotic nature of features.

In this context, the aim of the study is to reveal the dynamical peculiarities of interactions of cardiovascular system indices in evaluation of sportsmen's body functional state.

GENETIC CHARACTERISTICS OF LITHUANIAN AND LATVIAN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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The dissertation defended:
15 April 2011

The aim of the thesis was to investigate the role of the inflammatory bowel disease associated genetic variants in a subset of Crohn's disease and ulcerative colitis patients from Lithuania and Latvia and to test the relation of genetic markers to disease phenotype. The following objectives were accomplished: the associations of the inflammatory bowel disease associated single nucleotide polymorphisms in the subset of Crohn's disease and ulcerative colitis patients were determined; the associations of the single nucleotide polymorphisms with the phenotype of the inflammatory bowel disease were evaluated; the interactions of single nucleotide polymorphisms (SNP-SNP) and their association with inflammatory bowel disease were determined; the significance of the combinations of disease associated single nucleotide polymorphisms for diagnosis of inflammatory bowel disease was evaluated. TaqMan and SNPlex genotyping methods were used in this work. The statistical data analysis consisted of: statistical study power and data quality evaluations; single marker case-control association analysis using χ^2 or Fisher's exact tests, Breslow-Day test, Cochran-Mantel-Haenzsel test and post-hoc Bonferroni correction; genotype–phenotype association analysis using χ^2 test and post-hoc Bonferroni correction; SNP-SNP epistasis for case-control sample using logistic regression test and post-hoc Bonferroni correction; In sicilo prediction of gene interactive network; genetic risk profile construction using logistic regression analysis.

STUDY ON GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIPS IN GENUS *LONICERA* L. USING DNA MARKER METHODS

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The dissertation defended:
4 March 2011

The aim of this study was to examine genetic diversity of the *Lonicera* L. collection of the Botanical Garden of Vilnius University, to carry out intra-specific and inter-specific phylogenetic analysis through methods of molecular markers, and to compare sequencing results with homologous cpDNA sequences registered in the databases. For the first time investigations on genetic diversity and intra-specific taxonomy of the blue-berried honeysuckle (*L. caerulea* L.) were conducted using DNA marker methods (RAPD and sequencing of cpDNA non-coding regions). Representatives of *L. caerulea* were found to have a high level of DNA polymorphism. A species-specific RAPD marker was identified and sequenced for this polymorphic complex species. Genetic lines of the blue-berried honeysuckle derived in Vilnius University distinguish themselves by unique genetic properties and can be used in breeding to increase genetic diversity of new cultivars. The sequencing of six cpDNA non-coding regions of twelve taxa samples of genus *Lonicera* L. was performed. Phylogenetic trees based on RAPD markers and cpDNA sequencing data were similar. The phylogenetic analysis showed that subspecies of the blue-berried honeysuckle described in scientific literature do not form one claster, but group with other taxa of disputable status and form with them a monophyletic group.

ASSESSMENT OF THE MODULATION OF PHOTODYNAMIC EFFECT BY B-GLUCAN AND CHARACTERISTICS OF ANTI-CD7 MONOCLONAL ANTIBODY DURING TUMOR PROCESS

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Activation of the immune system during photodynamic therapy (PDT) and improvement of the effector functions of mAbs – these are the ways to use and enhance the potential of the immune system to fight cancer. Tumor cells lack β-glucan as a surface component and cannot trigger complement receptor 3-dependent cellular cytotoxicity and initiate tumor-killing activity during PDT. Thus it gave rise to a hypothesis that β-glucan in combination with PDT will produce more effective killing of by iC3b fragment opsonized tumor cells. The human Fc portion is essential for the recruiting of human effector immune cells to produce antitumor effect. Therefore, connection of Fv portion of murine anti-CD7 antibody with Fc portion of human IgG1 can be helpful for such protein to obtain ideal features. However, each modification of the monoclonal antibody can cause a loss or decrease in the rate of protein expression and antigen-binding properties. Monoclonal antibody products are unique in their molecules. Due to post-translational modifications that often occur during the fermentation process, the final product is heterogeneous. Therefore, careful characterization of monoclonal antibodies is required in order to assess their identity, purity, potency and safety. The response of Lewis lung carcinoma tumor to PDT modulated by β-glucan was assessed in mice and functional characteristics of novel purified chimeric anti-CD7 antibody was tested in this study.

COGNITIVE EFFECTS OF HORMONE BASED CONTRACEPTION IN WOMEN

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Hormonal contraception (HC) – one of the most popular methods of contraception acts centrally and peripherally to inhibit follicular growth, reduce overall endogenous sex steroids level and prevent monthly fluctuations. A lot of structures in CNS, including the areas related to cognition, are affected by sex steroid hormones. In the present study we investigated the influence of HC and composition of HC on cognitive functions. In one group of our study there were young, healthy women, having natural regular menstrual cycle. The second group consisted of women who used hormonal contraception. Half of hormonal contraception users used HC with androgenic, and the other half – with anti-androgenic properties. The results of the present study showed that women who used HC with androgenic properties performed worse on verbal (verbal fluency) and slower on spatial (mental rotation) tasks. In addition, the increasing difficulty of spatial task significantly aggravated the performance in HC users group and had little effect in nonusers group. There were no memory and mood differences between HC users and nonusers. However, the comparison of personality traits showed that neuroticism is less and extraversion is more expressed in HC users as compared to nonusers.

STUDY OF PSYCHIATRIC DISORDERS AND EVALUATION OF THEIR TREATMENT USING METHOD OF AUDITORY EVOKED POTENTIAL P300

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Recording and analysis of event-related potentials is a safe and harmless method of evaluation of cognition and is suitable to follow the changes of cognitive processes induced by psychoactive drugs or other therapeutic procedures. The main aim of the work was to evaluate the influence of atypical antipsychotics risperidone and quetiapine and such nonpharmacological methods as electroconvulsive therapy and metaglossotherapy on the changes of information processing in the auditory system using event-related potential P300 recording and analysis method. Auditory P300 potential was elicited applying "odd-ball" paradigm and recorded at 3 electrode sites (Fz, Cz, Pz). 4 parameters of P300 potential were measured: N2 latency, P300 latency, P300 amplitude and recognition time of target stimulus. A total number of 85 patients with schizophrenia spectrum disorders and mood disorders were studied. The results of this work showed that the parameters of P300 potential are sensitive indicators of abnormalities of information processing in auditory system in case of schizophrenia spectrum disorders. More considerable positive influence on the event-related potential P300 had atypical antipsychotic quetiapine and that nonpharmacological methods of treatment of psychiatric disorders are as effective as drug therapy with atypical antipsychotics in remediation of cognitive functions.

REGULATION OF L-TYPE CALCIUM CURRENT BY PROTEIN KINASES AND OTHER SIGNALING MOLECULES IN CARDIAC MYOCYTES

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The dissertation defended:
30 August 2011

The objective of the study was to investigate the role of protein kinase A, protein kinase C, Src family nonreceptor protein tyrosine kinases and other signaling molecules involved in pathways regulating the L-type calcium current (ICa, L) in enzymatically isolated cardiac myocytes. This objective was realized by resolving four tasks: 1) Examination of the basal activity of β-adrenergic receptor (β-AR) signaling cascade involving β-ARs, adenylyl cyclases, phosphodiesterases, protein kinase A, protein phosphatases (protein phosphatase 1 and protein phosphatase 2A) and L-type voltage-dependent calcium channels in frog and rat ventricular myocytes and human atrial myocytes; 2) Investigation of the role of β3-ARs in regulation of ICa, L and force of contraction in human atrium; 3) Exploration of the role of Src family nonreceptor tyrosine kinases in regulation of ICa, L, determining the route of their activation and site of action in β-AR signaling cascade of human atriual myocytes; 4) Probing of the impact of protein kinase C on basal and β-AR stimulated ICa, L in human atrial myocytes. The experiments were performed using whole-cell configuration of the pach-clamp technique.

PHYSIOLOGICAL MODULATION OF AUDITORY STEADY-STATE RESPONSES: AROUSAL, ACTIVATION AND ATTENTION

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The auditory steady-state response (ASSR) is used to monitor the ability of the brain to generate synchronous response to the external stimulation. However it is not known how registration conditions influence ASSR. The aim of the study was to investigate the effects of varying arousal, activation and attention levels on the 20 Hz and 40 Hz auditory steady-state responses. Additionally, transient auditory responses were analyzed to monitor the effect of experimental modulation on P1–N1–P2 auditory complex that is similar to the initial part of auditory steady-state responses. Higher phase precision and larger evoked amplitudes were obtained for gamma range activity of 20 Hz and 40 Hz ASSRs in low arousal / activation closed eyes condition. The effect of arousal was obtained only for the total intensity of 40 Hz ASSR – it was larger in low arousal condition, and for P1 potential amplitude that was diminished in low arousal condition. The effect of arousal / activation and attention was prominent for phase precision and evoked amplitude of 40 Hz ASSR: measures were diminished in high arousal / activation low attentional demands – "distraction" conditions and were largest during low arousal / activation and unfocused attention conditions. The largest N1 amplitudes were also obtained in low arousal / activation unfocused attention condition. The current results suggest important improvements of the practical use of ASSRs: 1) a careful monitoring for arousal fluctuations should be performed and 2) in cases where ASSRs are applied to investigate the ability to generate high frequency cortical activity a "distraction" task is not favorable.

ANALYSIS OF IN VITRO FUNCTIONS OF MESENCHYMAL STEM CELLS ISOLATED FROM DIFFERENT HUMAN TISSUES

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Human mesenchymal stem cells (MSC) have attracted a great deal of interest for their potential use in regenerative medicine and suppression of inflammation. Nevertheless, all known therapy protocols require large amounts of MSCs, which can be obtained only by in vitro expansion. One of the most important methodological problems is associated with the use of animal-derived components in the cell culture medium. The main aim of the current research was to elucidate the impact of different serum substitutes on the proliferation, differentiation, expression of cell surface markers, and total protein expression of mesenchymal stem cells derived from human adipose tissue. In addition we were aiming to determine the features of mesenchymal stem cell populations from an exfoliated deciduous tooth (SHED) and their response to the multifunctional proinflammatory protein alpha1-antitrypsin. Our results indicate that adipose tissue derived MSCs cultivated in the presence of fetal calf serum and allogeneic human serum display similar properties, while a synthetic serum substitute induces increase in growth and differentiation potential of MSCs. Moreover, our results indicate that synthetic serum substitute also activates transcription of genes related to adipogenic and osteogenic differentiation and diminishes expression of cell surface marker CD146. In the present study we used a proteomic approach that allowed us to compare protein expression signatures between primary cell culture and its daughter clones. As a result, for the first time we established a map of abundantly expressed proteins in MSC-like cells derived from the dental pulp of human exfoliated deciduous teeth. We also demonstrated that physiological and inflammatory concentrations of human alpha1-antitrypsin increase the proliferation and motility of mesenchymal stem cells derived from exfoliated deciduous tooth.

The results of the present study extend our understanding of processes in MSCs during cultivation in vitro and explain some mechanisms responsible for the functionality of these cells.