Rudolf Buchheim 200:
New Essays on the Doctrine of Drugs

September 9-11, 2021

Abstract book & Programme

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The programme for the Rudolf Buchheim 200: New Essays on the Doctrine of Drugs

Thursday, 9 September

The White Hall of the University of Tartu Museum

18.30-22.00 **Welcome reception**
Opening speeches: Prof Jaanus Harro, Prof Lembit Allikmets

Plenary lecture*
History of Chemistry in University of Tartu
Prof Enn Lust, University of Tartu
Chair: Prof Jaanus Harro

Plenary lecture*
Rudolf Buchheim - materia medica becomes modern pharmacology
Prof Lars Oreland, Uppsala University, Sweden
Chair: Prof Jaanus Harro

** at the Old Anatomical Theatre*

Friday, 10 September

University of Tartu Main Building

8.15-8.20 **Assembly Hall**
Opening
Prof Toomas Asser, Rector of the University of Tartu
Chair: Prof Lembit Rägo

8.20-9.05 **Assembly Hall**
Plenary lecture
Pharmacogenatics in psychopharmacology: approaches of individualization
Prof Ingolf Cascorbi, Kiel University, President of International Union of Basic and Clinical Pharmacology (IUPHAR), Germany
Chair: Prof Lembit Rägo

9.05-10.00 **Room 139**
Poster session and coffee break

10.00-12.00 **Parallel symposia**

Neuropsychopharmacology 1 Chair: Prof Klaus-Peter Lesch **Assembly Hall**	**Recent Advancements in Drug Design and Delivery** Chair: Dr Karin Kogermann **Room 128**
The legacy of Arvid Carlsson: the past, present and future of psychopharmacology Prof Elias Eriksson, University of Gothenburg, Sweden	**Nanocrystal based formulations in drug delivery** Prof Leena Peltonen, University of Helsinki, Finland

	MANF in cortex development and therapeutic implications for stroke Prof Mikko Airavaara, University of Helsinki, Finland	**Peptide-based transfection: applications in biotechnology for the production of therapeutic proteins** Dr Kaido Kurrikoff, University of Tartu
	Personalized psychopharmacology of psychostimulants: reward sensitivity in animals and humans Prof Jaanus Harro, University of Tartu, North Estonia Medical Centre	**Identification and testing of new treatment targets in neuropsychiatric disorders** Prof Jan Haavik, University of Bergen, Norway
	Novel RNA methylation regulating compounds protect and regenerate dopamine neurons Prof Mart Saarma, University of Helsinki, Finland	**Preclinical and clinical development of vascular homing peptides for precision medicine** Prof Tambet Teesalu, University of Tartu
		Safety and biocompatibility of electrospun nanofiber mats as wound matrices Kaisa Põhako, MSc, University of Tartu
12.00-12.45	**Assembly Hall** **Oswald Schmiedeberg lecture** **The brain speaks many languages: focus on talking via neuropeptides** Prof Tomas Hökfelt, Karolinska Institutet, Sweden Chair: Prof Jaanus Harro	
12.45-13.45	**Lunch at the Ülikooli Kohvik**	

Parallel symposia

13.45-17.00	**13.45-15.45**	**15.00-18.30**
Oncology Chair: Dr Kersti Oselin **Assembly Hall**	**Network for European Clinical Trials for Children** Chair: Prof Tuuli Metsvaht **Room 128**	**Emerging drugs in Endocrinology and Diabetes** Chairs: Prof Ugis Gruntmanis, Prof Vallo Volke **Former Chemistry Building, room 226**
Pharmacogenomics of tyrosine kinase inhibitor resistance Prof Ingolf Cascorbi, Kiel University, President of International Union of Basic and Clinical Pharmacology (IUPHAR), Germany	**From microsampling to miniature mass spoctromoters - advaneos in bioanalysis with limited sample volume** Dr Karin Kipper, University of Tartu; Epilepsy Society, UK	**Development of cagrilintide, a long-acting amylin analogue** Dr Kirsten Raun, NovoNordisk, Denmark
Precision medicine in oncology - where we are? Dr Kersti Oselin, North Estonia Medical Centre	**The effects of augmented renal clearance on the pharmacokinetic profile of antibiotics** Dr Lenne-Triin Kõrgvee, Tartu University Hospital, University of Tartu	**Acute and Chronic Metabolic effects of Melatonin** Prof Ulla Kampmann Opstrup, Steno Diabetes Center Aarhus, Aarhus University, Denmark

The role of neurotrophic factors in nerve and cancer crosstalk Dr Anu Planken, North Estonia Medical Centre, University of Tartu	**Measurement of glomerular filtration rate using iohexol** Dr Hiie Soeorg, University of Tartu	**Citrulline - more than just an amino acid** Prof Alastair Forbes, Norwich Medical School, University of East Anglia, UK; University of Tartu
Current state of art - cancer immunotherapy in clinical practice Dr Ann Valter, North Estonia Medical Centre	**Vancomycin and DosOpt** Dr Riste Kalamees, Tartu University Hospital, University of Tartu	**16.25-16.40 Coffee break**
Future of cancer immunotherapy in experimental research Prof Pärt Peterson, University of Tartu	**Pharmacokinetics of spironolactone in children under 2 years** Dr Jana Lass, Tartu University Hospital, University of Tartu	**New drugs for osteoporosis** Prof Ugis Gruntmanis, Geisel School of Medicine at Dartmouth, USA
Cancer immunotherapy in early clinical development Dr Aidi Adamson-Raieste, North Estonia Medical Centre; The Christie Hospital, UK	**Pharmacokinetics and concentration related effects of dobutamine in neonates** Dr Maarja Hallik, East Tallinn Central Hospital, University of Tartu	**17.10 Short communications**
Endocrine side effects of new oncological drugs Dr Ingrid Reppo, University of Tartu		**Development of tolerance toward effects of GLP-1 receptor agonists** Dr Tuuli Sedman, University of Tartu
		GLP-1 receptor agonists affect other hormonal systems Dr Keiu Heinla, University of Tartu
		New drugs for the treatment of hypercortisolism Dr Kristina Isand, University of Tartu

18.15-19.45 Excursion in the city with a small coffee break

20.00-23.00 Gala Dinner at the Athena Centre

Saturday, 11 September

University of Tartu main building

8.20-8.50 **Assembly Hall**

Planary lecture

Pharmacovigilance: from reactive to proactive and predictive

Prof Lembit Rägo, Secretary-General of Council for International Organizations of Medical Sciences (CIOMS), Switzerland

Chair: Prof Vallo Volke

8.50-9.20	**Plenary lecture** **Can dysregulated myelination be linked to ADHD pathogenesis and does this generate new treatment targets?** Prof Klaus-Peter Lesch, University Hospital of Würzburg, Germany Chair: Prof Vallo Volke	
9.20-11.00	**Naw Challenges in Medicine** Chair: Prof Irja Lutsar **Changing patterns of the COVID-19 pandemic** Prof Irja Lutsar, University of Tartu **Lessons learned during the pandemic season 2020/21 and implications for the future** Prof Uga Dumpis, Pauls Stradins Clinical University Hospital, Latvia **Spread of COVID-19 from aerosol physics point of view** Prof Heikki Junninen, University of Tartu **Advanced Therapy Medicinal Products (ATMP)** Prof Toivo Maimets, University of Tartu	
11.00-11.20	Coffee break	
11.20-13.20	**Parallel symposia**	
	Neuropsychopharmacology 2 Chair: Prof Eero Vasar Assembly Hall	**New drugs in Cardiology** Chair: Dr Alar Irs Room 128
	The potential of nauropeptides in psychopharmacology - insights from animal models Prof David A. Slattery, Johann Wolfgang Goethe Universität Frankfurt am Main, Germany	**How to combine antiplatelet and anticoagulant drugs?** Dr Alar Irs, Tartu University Hospital, the Estonian State Agency of Medicines
	Epigenetics and Psychostimulant Addiction Prof Anti Kalda, University of Tartu	**How to minimize the risk of arrhythmia with QT-prolonging** Dr Jessica Schubert, Uppsala University Hospital, Sweden
	Extended release naltrexone for opioid use disorders: Advantages and problems Prof Evgeny Krupitsky, V.M. Bekhterev National Medical Center for Psychiatry and Neurology and First Pavlov Saint Petersburg State Medical University, Russia	**New drugs for treating dyslipidemia** Dr Martin Serg, North Estonia Medical Centre
	Neuronal plasticity and neurotrophin signaling in the antidepressant effect Prof Eero Castrén, University of Helsinki, Finland	
13.20-13.25	Assembly Hall **Closing remarks** Prof Jaanus Harro	
14.00-16.30	Excursion to the Estonian National Museum	

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OSWALD SCHMIEDEBERG LECTURE

Tomas Hökfelt, *Department of Neuroscience, Karolinska Institutet, Sweden*

The brain speaks many languages: focus on talking via neuropeptides

Neuropeptides are small, 3-45 amino acids long molecules that serve as extracellular messenger molecules in the nervous, endocrine and immune systems. They are thus different from classic transmitters in that they are ribosomally produced, previously thought only to occur in cell bodies. Peptides have turned out to be the most diverse family of signaling molecules (>100 members) in the nervous system acting via a correspondingly large number of G protein-coupled receptors. Early on we observed that neuropeptides coexist with classic transmitters, like monoamines. In fact, this type of coexistence seems to be a rule. We have used histochemical methods, especially immunohistochemistry and in situ hybridization to study levels and transcripts in the microscope, complemented with electrophysiology and behavioral experiments. We have focused on some peptides associated with Sweden: substance P (Ulf von Euler), cholecystokinin (CCK), galanin and neuropeptide Y (NPY) (the latter three Viktor Mutt). In early experiments rodents were studied, recently also human postmortem tissue. We have been interested in pain and depression-like behavior/depression and a possible involvement of the 27-aminoacid peptide galanin.

Regarding pain we have defined a new antinociceptive system against *neuropathic pain* intrinsic to a population of DRG neurons. This system is activated by nerve injury and involves upregulation of i.a. inhibitory galanin (and *downregulation* of the excitatory peptides substance P and CGRP in the same neuron subpopulation). If this protective mechanism is fatigued, neuropathic/chronic pain may arise. More detailed studies have shown that it is the GalR1 receptor, on glutamatergic spinal interneurons, that mediates antinociception. Pain alleviation could thus be achieved by a GalR1 agonist. Another endogenous antinociceptive system counteracting *inflammatory* pain is associated with dorsal horn interneurons expressing i.a. the enkephalins (opioid peptides) (their receptors also being targets for morphine). Inflammatory pain in addition *upregulates* expression of substance P and CGRP in DRG neurons.

Regarding depression, based on animal and human studies, we have the following view on involvement of galanin in mood regulation: Galanin synthesized in LC neurons is present in noradrenergic nerve terminals in cortex but also locally in their dendrites. Stress induces increased firing of the LC and upregulation of galanin synthesis. If firing becomes excessive, galanin will be released from dendrites and activate inhibitory GalR3/GalR1 autoreceptors to prevent overexcitation, a protective mechanism. We and others have proposed that this is part of the resilience machinery. When this protective mechanism is exhausted, depression may develop.

Taken together, we believe that the neuropeptide systems offer opportunities to develop novel medicines. In fact, there are some successes: CGRP antibodies/antagonists for treatment of migraine, orexin/hypocretin antagonists for insomnia and substance P antagonists for chemotherapy-induced nausea.

PLENARY LECTURES

Enn Lust, *University of Tartu*

Chemistry and Pharmacy at the University of Dorpat in the 19th century

Chemistry and pharmacy have been very well developed at the University of Tartu/ Dorpat /Yurjev since the 19th century. There have been numerous outstanding scientists, who worked at the University of Dorpat (Yurjev), just to mention Hermann Hess, a developer of the thermochemistry, Wilhelm Ostwald, a Nobel Prize winner in chemistry, the father of physical chemistry and green energetics, including the theory of fuel cells thermodynamics and electrochemistry, the world famous researcher of metallic alloys Gustav Tammann, the discoverer of the chemical element ruthenium Carl Claus, the first producer of synthetic caoutchouc Ivan Kondakov, etc. However, among the world leading scientists a special mention should be made of Carl Schmidt and Georg Dragendorff who worked with remarkable success at the Dorpat University for 30-40 years, shaping them into the outstanding research and teaching centres, which became well-known all over European countries.

It is very interesting that the first professor in chemistry E.H.G.Arzt was appointed in December 1800. Since 1803 lectures on pharmacy were read by a professor of chemistry and since 1820, the University had established a joint professorship for theoretical and practical chemistry and pharmacy. Two substantially close specialities remained well connected throughout nearly 50 years.

An independent professorship of pharmacy and, along with it, the institute of Pharmacy, had been established at the University of Dorpat by the ukase of Emperor as of October, 19 1842. Chemistry began to be taught separately from 1850. March 25, 1850 is the date which is considered as the time of establishment of the Department of Chemistry.

Lars Oreland, *Uppsala University, Sweden*

Rudolf Buchheim - materia medica becomes modern pharmacology

Rudolf Buchheim is rightly described as "the father of modern pharmacology". He was born in Germany in 1820 and by 2020 it could have been possible to celebrate the 200th anniversary if the corona pandemic had not got in the way. What lay be behind this epithet attributed to Buchheim? There are mainly five factors which will be discussed:

1. Buchheim was the first to arrange a laboratory specifically for experimental pharmacology as professor in Dorpat (1847)
2. Buchheim the first in the field of medicine to do research on pharmacology – identifying active components not being a physiologist, medicinal chemist or pharmacist – to be a pharmacologist.
3. Buchheim put emphasis on the importance of mechanism of action, absorption, distribution and elimination of drugs. He also underlined the importance of statistical data instead of reports of single experiments.
4. Before Buchheim drugs were organized in different and most often not very rational ways. Buchheim in his textbook from 1853 organized drugs according to a "natural system", based on a combination of chemistry and mode of action.
5. Buchheim realized the necessity to eliminate a number of traditional/obsolete drugs.

The private life of Buchheim will be outlined as well as an attempt to describe his strive to modernize research and use of drugs. Some history of materia medica before Buchheim will be given, as well as his activities resulting in a chair in Dorpat at the age of 27 – e.g. translation to German of Pereira's Encyclopedia on drugs (London 1839-40).

Buchheim's own research will be summarized, as well as his work strategies, which together with the Dorpat colleagues C Schmidt and F Bidder, was of a great importance for discoveries of later generations of researchers, as exemplified by Emil Heubel, Carl Gaehtgens and Oswald Schmiedeberg.

Ingolf Cascorbi, *Institute of Experimental and Clinical Pharmacology, University Hospital Schleswig-Holstein, Kiel, Germany*

Pharmacogenetics in psychopharmacology: approaches of individualization

Major depression and psychotic diseases are a major global health burden. Although the development of antidepressants of antipsychotics helped tremendously to overcome or at least mitigate the diseases, a substantial proportion of patients still suffer from non-response or side-effects. The wide variation in the pharmacokinetics of various drugs can be explained partly by genetic variation contributing to interindividual differences of the activity of metabolic enzymes i.e. cytochrome P450s. Further, drug-drug interactions may contribute to varying plasma concentrations. Although guidelines have been developed for dose-adaptation and selection of antidepressants, there is still a need to launch large trials to gain further evidence of the clinical benefit.

This talk will give an overview on major genetic factors contributing to interindividual variation of pharmacokinetics and drug response, the set-up of clinical trials and also approaches to explain side effects such as clozapine-associated agranulocytosis.

Lembit Rägo, *Council for International Organizations of Medical Sciences (CIOMS), Geneva, Switzerland*

Pharmacovigilance: from reactive to proactive and predictive

In 1848 Hannah Greener, near Newcastle in the UK, died after receiving chloroform for anesthesia. It took more than hundred years and Thalidomide catastrophy (first launch in 1957) to start systematic reporting of suspected adverse reactions, implementing specific safety regulations and setting up WHO International Programme for Drug Monitoring (1968). Setting up systems for reporting individual case safety reports (ICSRs) in 1960s and 70s enabled starting systematic signal detection. Due to signals detected and verified actions were taken, including Dear Doctor letters, label modifications and medicines withdrawals. Over the years ICSRs reporting, databases and signal detection improved but pharmacovigilance (PV) of medicines remained mostly reactive benefitting from improved signal detection. It took several more decades to add more proactive (PA) measures. Today the risk management plans (RMP) prepared for new medicines describe the activities of the marketing authorisation holder to further characterise the safety profile during post-marketing phase, and explain the measures that are taken in order to prevent or minimise the medicine's known risks in patients. Nowadays PV is PA in continuously evaluating the benefit-risk profile of a product based on new information gathered from various sources, and starting to elaborate predictive (PD) models leading to PD pharmacovigilance. The contribution of ICSRs, while still substantial and important, may in future be diminishing due to its limitations. Other more robust sources of real-world data (RWD), and new emerging methods become available. In addition, the future of PV is linked to increasing use of basic, process and cognitive automation combined with artificial intelligence.

Prof. Klaus-Peter Lesch, *University Hospital of Würzburg, Germany*

Can dysregulated myelination be linked to ADHD pathogenesis and does this generate new treatment targets?

Recent epidemiologic, genetic, epigenetic, neuroimaging, and experimental studies have provided further support to the concept that attention-deficit/hyperactivity disorder (ADHD) is causally related to dysregulated myelination and downstream alterations in neuronal plasticity. These alterations underlie suboptimal brain maturation that is shaped by gene-environment interactions, leading to the emergence and persistence of cognitive and emotional impairments across the life span. In humans, myelination begins early in the third trimester, progresses extensively in infancy and continues through the adolescent stage of life. The developmental delay in ADHD may be underpinned by dysregulated myelination – the facilitation of neurite outgrowth and axonal extension by the oligodendrocyte-dependent production of the myelin sheath. Several molecular culprit mechanisms have been identified by converging lines of evidence: For example, in the GWAS meta-analysis of ADHD (Demontis et al., Nature Genetics 2019; 51, 63-75) the statistically most significant locus (SNP rs1142027, p = 2.14 × 10–13) revealed a gene encoding the beta-galactoside-alpha-2,3-sialyltransferase-III membrane protein (ST3GAL3), while St3gal3-deficient mice display impaired motor coordination, disturbed gait, and profound cognitive dysfunction, this behavioural phenotype being caused by dysmyelination marked by a reduction in major myelin proteins, fewer myelinated axons, a decrease in myelin thickness, and molecular disruption at nodes of Ranvier (Yoo et al., 2015; FASEB Journal 29, 3040-3053). Several other candidate genes identified by GWAS in patients have the potential to interfere with the process of myelination. This mechanistic pathophysiologic concept should be amenable to therapeutic intervention.

SYMPOSIUM LECTURES

Neuropsychopharmacology 1

Elias Eriksson, *University of Gothenburg, Sweden*

The legacy of Arvid Carlsson: the past, present and future of psychopharmacology

A notion underlying modern neuropsychopharmacology ever since the birth of this relatively young field of science has been, *first*, that the different brain neurotransmitters have different roles in terms of the regulation of different aspects of behaviour, and with respect to their involvement in different brain disorders, and, *second*, that one by modulating the activity of a certain transmitter with drugs hence may produce symptom relief. A breakthrough in this regard was the observation in 1957 by Arvid Carlsson (1923-2018), Swedish pharmacologist (and Nobel laureate in 2000), that the levels of dopamine in rabbits administered reserpine are depleted, and that one may counter the profound impact on locomotion displayed by these animals by administration of the dopamine precursor levodopa. By paving the way for the introduction of levodopa for the treatment of Parkinson´s disease, Carlsson's discovery was of immense importance for neurology, but it was equally significant for psychiatry, since reserpine was known to produce antipsychotic effects. The possibility that schizophrenia may indeed be associated with elevated release of dopamine gained shortly (1963) support from another pivotal observation by Carlsson, i.e., that the antipsychotic drugs chlorpromazine and haloperidol act as antagonists at dopaminergic receptors. In this presentation, these and other contributions by Carlsson, such as the development of the first SSRI antidepressant, will be discussed with respect to their conceptual relevance for more recent developments in neuropsychopharmacology, as well as for the future of this field.

Mikko Airavaara, *University of Helsinki, Finland*

MANF in cortex development and therapeutic implications for stroke

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an endoplasmic reticulum (ER) luminal protein that has protective activities in a wide range disease models. Together with its homologue cerebral dopamine neurotrophic factor (CDNF) they form highly interesting protein family with therapeutic effects. Despite named as neurotrophic factor, the protein is drastically different from classical neurotrophic factors. MANF act as unfolded protein response (UPR) gene that modulate the UPR and inflammatory processes. We have found that MANF exerts protective effects in stroke animal models and when given after stroke it hastens recovery. Our recent work also show that MANF is important for cortex development and MANF deletion causes slower formation of cerebral cortex, and the work is among the first to show that UPR has critical role in cortex development. After stroke MANF increases the number of developing neurons in the damaged adult brain and in vitro studies showed that MANF increases the migration of developing neurons. We have also found that MANF increase the number of phagocytic cells in the brain after stroke, indicating that MANF enhances the endogenous repair processes after stroke.

Jaanus Harro, *University of Tartu*

Personalized psychopharmacology of psychostimulants: reward sensitivity in animals and humans

Co-authors: **Kadri Kõiv**, *University of Tartu*; **Katre Sakala**, *National Institute for Health Development*; **Marten Vares**, *University of Tartu*; *Margus Kanarik University of Tartu*; **Evelyn Kiive**, *University of Tartu*; **Aleksander Pulver**, *Tallinn University*; **Ruth Shimmo**, *Tallinn University*; **Toomas Veidebaum**, *National Institute for Health Development*

Inter-individual differences in illicit drug use and sensitivity to their effects and addiction have been attributed to sensitivity to rewards in general. What exactly constitutes reward sensitivity in neural terms is not clear, possibly because 'reward' is an imprecise construct that often is extended to the experience of positive affect. We have shown that rats with high sucrose intake are more sensitive to amphetamine and more vulnerable to amphetamine sensitization if previously chronically stressed (Kõiv et al., J. Psychopharmacol. 2019; 33, 1512-1512) but the effect of amphetamine is mitigated by stress in animals with low inherent positive affect as expressed in 50-kHz ultrasonic vocalization (Kõiv et al., Eur. Neuropsychopharmacol. 2016; 26, 631-643). Within a single experiment, chronic stress again reduced the rewarding effect of amphetamine to the highest degree in animals with high sucrose intake and low positive affect. In humans, reward sensitivity can be parsed into striving towards multiple rewards (Openess to Rewards) and fixation to a specific reward (Insatiability by Reward) that are differently associated with positive affect and ADHD symptoms (Pulver et al., Acta Neuropsychiatrica 2020; 32, 247-256). Analysis of the data of the longitudinal Estonian Children Personality Behaviour and Health Study (www.ecpbhs.ee) revealed that while sedative psychoactive drug use was associated selectively with Insatiability to Reward, psychostimulant use had an additional association with Openness to Rewards. However, positive affect was not related to use of either type of psychoactive substances. Conclusively, psychostimulant use and effect have separate associations with distinct aspects of reward sensitivity, and positive affectivity has a limited direct role.

Mart Saarma, *University of Helsinki, Finland*

Novel RNA methylation regulating compounds protect and regenerate dopamine neurons

Co-authors: **Ly-Ying Yu**, *University of Helsinki* and **Mati Karelson**, *University of Tartu*

N6-Methyladenosine (m6A) is the most common cellular modification that occurs in the mRNA of eukaryotes. This modification plays crucial role in mRNA post-transcriptional regulation and recent studies highlight that m6A mRNA methylation dysregulation occurs in Parkinson's diseases (PD). Effects of m6A modification of mRNAs on the survival and regeneration of dopamine neurons and in Parkinson's disease are very poorly known. The m6A methylation regulates the transport, stability and translation of mRNA. Three main classes of proteins regulate m6A methylation. The formation of m6A is catalysed by a methyltransferase complex that contains methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), and Wilm's-tumor-1-associated protein (WTAP). m6A demethylation that is the opposite reaction is catalysed by two enzymes: the fat-mass- and obesity-associated protein also known as α-ketoglutarate-dependent dioxygenase (FTO) and the RNA demethylase ALKBH5. YTH domain family proteins bind to and regulate the fate of m6A modified mRNAs. We have used in silico-based discovery to identify small-molecule ligands that bind to METTL3/METTL14/WTAP, FTO and ALKBH5 and determined experimentally their binding affinity, kinetics, and their effect on enzymatic functions. We found five ligands that serve as activators of the METTL3/METTL14/WTAP complex at nanomolar concentrations. We also discovered several very potent inhibitors of FTO and ALKBH5. No cytotoxicity was observed at up to 100 μM concentrations of the compounds when tested on HEK293 cells. Two METTL3/METTL14/WTAP activators and two FTP inhibitors even at 10 nM concentration protected and supported the survival of growth factor deprived or 6-OHDA treated dopamine neurons. Effects of METTL3-METTL14-WTAP activator C4 at two different does was compared with GDNF in the rat 6-OHDA neurorestoration model of PD. C4 more rapidly and more efficiently than GDNF restored animal motor behaviour and significantly more efficiently than GDNF protected and restored striatal fibres of DA neurons. Notably C4 has long lasting effects detectable even 6 weeks after delivery. This is the first demonstration that m6A mRNA methylation regulates the survival and regeneration of dopamine neurons.

Recent Advancements in Drug Design and Delivery

Leena Peltonen, *Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Finland*

Nanocrystal Based Formulations in Drug Delivery

The number of drug candidates having solubility issues is increasing, and numerous different techniques to improve the solubility properties are studied a lot. One very efficient way for improved solubility is nanosizing (formation of nanosized drug particles, also called drug nanocrystals). Drug nanocrystals are solid nanosized drug particles, which are covered by a stabilizer layer on top of the particles. Although improved solubility is the most important application of these nanosystems, they can be used for controlled release purposes, too.

Nanonization of drug particles is just a way to change the physical properties, the particle size, of the drug. For drug delivery purposes, further formulation is still needed. The first nanocrystal products were tablets, where dry nanocrystal powder - excipient mixture was just compressed to tablets. Later on, nanocrystals have been studied for many other drug delivery routes, like ocular, parenteral, dermal, pulmonary, buccal etc.

The biggest challenge with nanocrystal formulations is their inherent instability (aggregation tendency). Another challenge with nanoparticle based formulations is the precipitation tendency after the dissolution. Dissolution of nanosized particles forms supersaturated state. This may induce spontaneous and uncontrolled precipitation in vivo, followed by lower drug absorption. Both above mentioned challenges need to be taken care of when formulating nanocrystal based formulations.

In the presentation, case studies with different kind of nanocrystal formulations from 3D printed drug products to more conventional formulations are shown. The challenges faced with and how they are solved successfully are described. Solubilization capacity of nanocrystals are demonstrated by comparing them with other type of high solubility systems.

Kaido Kurrikoff, *University of Tartu*

Peptide-based transfection: applications in biotechnology for the production of therapeutic proteins

Therapeutic proteins (TP) represent a huge field in pharmaceutical medicine. Half of the global top 10 revenue drugs sold in 2020 were either humanized antibodies (mAb) or other TP. Therapeutic intervention using mAb has been very successful, especially in cancer and inflammatory diseases. Despite their many advantages over the classical, low-MW drugs, the disadvantage is that industrial production of TP is considerably more challenging.

Cell penetrating peptides (CPP) are a promising approach to facilitate the delivery of macromolecules through biological barriers. One of the applications of CPP is transient transfection of mammalian cells and implementation in TP production process.

We have compared various transfection methods and unexpectedly discovered that the classical methods of measuring transfection efficacy in small scale laboratory settings fail to predict their performance in industrial use. Unsurprisingly, the top performers in the lab scale were the most widely used industrial methods (i.e. PEI and liposome). However, these methods performed poorly in 7-day protein production settings and could not compete with the CPP approaches. We carefully and comprehensively analyzed various intracellular processes following transfection procedure and concluded that the main differentiator between good and poor performers were subtle adverse effects on normal cellular functioning that amplify over a longer time period, resulting in lower number of protein-producing cells.

Our results point to the methodological shortcomings in assessing transfection efficacies and acknowledge the need to develop better transfection methods of mammalian cells. We also found that some of our own CPP demonstrate superior transfection efficacy compared to the widely used alternatives.

Jan Haavik, *University of Bergen, Norway*

Identification and testing of new treatment targets in neuropsychiatric disorders

Neuropsychiatric disorders (NPDs) are major causes of human suffering, loss of lives and productivity all over the world. Currently used pharmacotherapies against NPDs have low efficacy and specificity and were introduced 50-100 years ago based on accidental findings. However, recent molecular genetic studies have revealed strongly associated genetic loci, enriched in brain pathways and proteins, and possible new therapeutic targets for NPDs. Moreover, revolutionary new technological breakthroughs have been reported in computational and experimental molecular life sciences. The application and combination of such technologies may accelerate drugs discovery. I will present a brief overview of some developing technologies and some recent results from our own work on "classical" monoamine transmitters, such as serotonin and dopamine, as well as new and emerging findings from genome wide studies.

I will also discuss the possibilities for repurposing old drugs for new indications.

Tambet Teesalu, *University of Tartu*

Preclinical and clinical development of vascular homing peptides for precision medicine

Precision delivery of diagnostic and therapeutic payloads remains a challenge. Each organ and pathology has a unique vascular ZIP code that can be targeted with affinity ligands. Our laboratory uses in vivo peptide phage display for unbiased mapping of the vascular diversity and for identification of short (typically <10 amino acids) homing peptides. These peptides are coupled on therapeutic and imaging compounds, or nanoparticles, to improve their target selectivity and activity.

I will discuss our work on identification and preclinical evaluation of the homing peptides selective for solid tumors and for normal blood-brain-barrier. I will also provide an update on the clinical development of tumor penetrating peptide iRGD that we discovered a decade ago and that is currently undergoing phase 2 clinical testing for precision targeting and treatment of pancreatic ductal adenocarcinoma.

Kaisa Põhako, *University of Tartu*

Safety and biocompatibility of electrospun nanofiber mats as wound matrices

Co-authors: **Tanel Tenson**, *University of Tartu*; **Külli Kingo**, *Dermatology Clinic of Tartu University Hospital*; **Karin Kogermann**, *University of Tartu*

The number of patients with chronic wounds increases because of population aging and non-efficient wound treatment methods. Most chronic wounds are infected with bacterial biofilms which in combination with the lack of effective topical antimicrobial treatment options make the wound care even more difficult. Therefore, new strategies for wound management are needed. During recent years antimicrobial electrospun nanofiber mats as novel wound matrices have been developed. These nanofiber mats enable to incorporate different drugs into their structure and deliver these successfully into the wound area. With new potential treatment, it is crucial to test the safety and biocompatibility of electrospun nanofibers before their administration to patients.

The aim of this study was to test the safety and biocompatibility of electrospun nanofiber mats with and without antimicrobial agents on two different eukaryotic cell lines (baby hamster kidney cells (BHK-21) and primary skin fibroblasts obtained from patient). The study had all relevant ethical committee permissions. Experiment setup was based on modified MTS cell viability assay, morphology of fibers and biocompatibility were evaluated with microscopy. The results showed that all tested electrospun fiber mats were safe and biocompatible. However, there were some differences in the viability and behaviour of cells when pristine polymeric mats and mats with antimicrobial agents were compared. Further studies will test the cell migration, proliferation and differentiation on electrospun wound matrices in more depth in order to understand better the cell-fiber interactions.

Oncology

Ingolf Cascorbi, *Institute of Experimental and Clinical Pharmacology, University Hospital Schleswig-Holstein, Kiel, Germany*

Pharmacogenomics of tyrosine kinase inhibitor resistance

Tyrosine kinase inhibitors (TKI) are widely used in the modern treatment of malignancies. Major success was achieved e.g. in the treatment of chronic myeloid leukemia (CML) through imatinib, inhibiting the catalytic domain of the BCR-ABL fusion gene product. However, resistances may occur mutants of the BCR-ABL gene, but also through changes of gene expression and (epi)genetics, some leading to overexpression of the efflux transporter ABCG2.

This talk will cover mechanisms of drug resistance observed in an CML in-vitro model of imatinib resistance focusing on changes of gene expression profiles, efflux transporters and their potential regulation by microRNAs.

Ann Valter, *North Estonia Medical Centre*

Current state of art - cancer immunotherapy in clinical practice

The beginning of cancer immunotherapy might be tracked back to 19th century when the 'Coley's toxin' was first described. However, the introduction of immune checkpoint inhibitors (CPIs) more than a decade ago has revolutionized the treatment of cancer. Firstly, used on malignant melanoma patients, the CPIs are now standard of care for many different cancer types making the quality of life of patients better and survival longer.

Aidi Adamson-Raieste, *North Estonia Medical Centre*

Cancer immunotherapy in early clinical development

Early phase studies are the starting position in the chain of clinical trials leading to the approval of new drugs.The immunotherapy field continues to grow, with more drugs and trials for almost all drug classes compared with previous years. Competition grows for validated targets such as PD1 and CD19 and the field is exploring immune cells and novel targets beyond T cells.

Network for European Clinical Trials for Children

Karin Kipper, *University of Tartu, Estonia; Epilepsy Society, Head of Therapeutic Drug Monitoring Unit, United Kingdom*

From microsampling to miniature mass spectrometers - advances in bioanalysis with limited sample volume

Covid-19 pandemic has highlighted the importance of diagnostic testing in healthcare. Through the surge in new Covid-19 positive cases, the healthcare sector was under tremendous pressure all over the world, and many routine appointments for chronic conditions got cancelled. The long-term impact of that is yet to be seen. However, the pandemic has clearly demonstrated that access to quick, reliable measurement; near-patient sample collection, and testing solutions have never before been as important as now.

The development of microsampling techniques for limited sample volume for most vulnerable patients, such as paediatric patients and neonates, has widened the toolkit for clinicians - allowing healthcare professionals to take biological samples with reduced volume. Available techniques vary from the required blood volume and resulting sample matrix to facilitate analytical needs for diagnostic testing. Moreover, available solutions offer less invasive alternatives for diagnostic testing. The downscaling and simplification of mass spectrometric measurement systems has advanced over recent years, allowing highly specific and accurate results through ready-made, customised, CE-marked assays and reduced instrumentation footprint that could potentially bring the lab to the patients' bedside.

Maarja Hallik, *University of Tartu, East Tallinn Central Hospital*

Pharmacokinetics and concentration related effects of dobutamine in neonates

Co-authors: **Mari-Liis Ilmoja**, *Tallinn Children's Hospital*; **Joseph F. Standing**, *University College London*; **Hiie Soeorg**, *University of Tartu*; **Kalev Takkis**, *University of Tartu*; **Rūta Veigure**, *University of Tartu*; **Karin Kipper**, *St George's University of London, University of Tartu*; **Joel Starkopf**, *University of Tartu*; **Tuuli Metsvaht**, *University of Tartu*

In critically ill neonates, dobutamine is often used to support the transitional circulation, but the pharmacokinetics (PK) and pharmacodynamics (PD) have not been very well described.

The aim of our study was to describe the dose-concentration-related effects of dobutamine in term and preterm neonates in the first days of life.

Neonates hospitalised in NICU within the first 72 h of life were included. Dobutamine was administered on clinical indication in escalating doses of 5-20 µg/kg/min. Left ventricular ejection fraction (LVEF), cardiac output of right and left ventricle (RVO, LVO) was measured on echocardiography; heart rate (HR), mean arterial pressure (MAP), peripheral arterial oxygen saturation and cerebral regional oxygen saturation were recorded from patient monitors. Simultaneous population PKPD modelling with nonlinear mixed-effects modelling soft-ware (NONMEM Version 7.3) was used.

Twenty-eight neonates with median (range) gestational age of 30.4(22.7 – 41.0) w, birth weight (BW) of 1618(465 – 4380) g and postnatal age of 6(2-28) h were included. PK of dobutamine was described by one-compartmental linear model with clearance (CL) allometrically scaled to BW and maturing with postmenstrual age (PMA). The final population PK model parameter mean typical value (SE) estimates, were 41.2(44.5) l/h/1618 g for CL and 5.29(0.821) l/1681 g for volume of distribution, with between subject variability of 29% (17.2%). The relationship between dobutamine plasma concentration and RVO/LVEF was described by linear model, between plasma concentration and LVO/HR/MAP/cerebral fractional tissue oxygen extraction by sigmoidal Emax model.

In neonates dobutamine elimination is related to BW and PMA. Highly variable PD response suggests the need for individual dose titration.

Emerging drugs in Endocrinology and Diabetes

Alastair Forbes, *University of Tartu*

Citrulline. More than just an amino acid

Citrulline is formed in the small intestine and, although it is not incorporated into protein, it is important, via the urea cycle, in nitrogen metabolism in virtually all cells. Its net clearance occurs only in the kidney. Citrulline is important in the production of arginine and as a marker of intestinal integrity. Plasma levels are stable in most contexts and are increased only transiently by consumption of watermelon which is its richest dietary source.

Low circulating levels are indicative of diminished functional intestinal mass and predict the need for parenteral nutrition in the short- and long-term. More general predictive value may exist with respect to intestinal function in unselected patients in critical care.

Citrulline may be a safer and more effective NO donor than arginine for which it is a precursor. There is parallel animal evidence in favour of a role against acute oxidative damage, and suggestions that it may protect against some elements of sarcopenia.

Inverse correlations with mucosal damage indicate a potential therapeutic role alongside forms of cancer chemotherapy that are commonly associated with therapy-limiting mucositis. Falling levels are also clinically valuable in identifying early rejection in patients with intestinal transplantation. Its exploration in sports medicine is incompletely validated but provides important safety data. There appears to be unrealised potential.

Kristina Isand, *University of Tartu*

New drugs for the treatment of Cushing's syndrome

The treatment and diagnosis of Cushing's syndrome (CS) is clinically challenging. Management of CS must include the treatment of hypercortisolism but not less importantly treating co-morbidities caused by high levels of cortisol.

The fist-line treatment for Cushing's disease is transsphenoidal surgery (TSS), but it does not always guarantee remission. Recurrence rates after primary and revision TSS around 18% and 28%. Second line therapy includes repeat surgery, medical therapy, bilateral adrenalectomy or radiation therapy.

Medical therapy for hypercortisolism includes pituitary-directed agents (Pasireotide and Cabergoline), steroidogenesis inhibitors (Osilodrostat, Ketoconazole, Metyrapone, Mitotane) and glucocorticoid receptor antagonist (Mifepristone). Cabergoline and Mifepristone are used off-label for the treatment of CS but are in-line with the current treatment guidelines.

Osilodrostat and Metyrapone have just showed new and promising data to be effective and safe treatments for the treatment of CS.

Medical treatment of hypercortisolism has to be individualised and there are various treatment options that can be used individually or combined.

New Challenges in Medicine

Irja Lutsar, *University of Tartu*

Changing patterns of COVID-19

COVID-19 pandemic that started in January 2020 in China and was characterised by relatively low transmission rate, limited availability of preventive measures and treatment, is not the same in autumn 2021. We have seen constant changes of viral variants, each of them being more transmissible and less susceptible to vaccines than previous one. We have seen new vaccines coming into the market with super speed but have also experienced breakthrough infections. We have experienced total lockdowns and are now moving towards easing restrictions. Although our knowledge about SARS-CoV-2 has increased enormously there are still more unknowns that knowns in this pandemic. Almost all countries have given up hopes of complete eradication of virus and we all still are learning how to live with SARS-CoV-2.

Uga Dumpis, *Pauls Stradins Clinical University Hospital, University of Latvia, Latvia*

Lessons learned during the pandemic season 2020/21 and implications for the future

The pandemic is yet to be overcome, but an end is conceivable if we act smart and learn from previous experiences. Three critical factors shape the development of the epidemic: population immunity and vaccination, variants of concern (VOCs) and public responses to public health policy.

With the spread of more contagious VOCs the airborne route of transmission will become even more significant. Prevention of superspreading events during mass gatherings will still be needed even in populations with high vaccination uptake. Rapid identification of cases and wide testing will have to be introduced in the light of new antiviral treatments. Novel screening approaches, eg. wastewater sampling, pool testing should be actively explored. One health approach should also be implemented since animal reservoirs most likely play role in SARS-Cov2 and other viral infections.

Two opposing public health strategies can be considered: either continue to rapidly lift restrictions, assuming the combination of past natural exposure and vaccination coverage would allow a high incidence to continue without overburdening health-care systems, or lift restrictions at the pace of vaccination progress with the aim to keep incidence low with actively introducing test-trace-isolate strategies.

Any strategy should not simply developed by politicians and imposed on the public. They should be largely based on societal consensus and accepted by the majority of the population. Unwillingness of the general public to follow the recommendations based on science has already become a major challenge.

Maintaining and communicating a clear science based strategy is key and pan European coordination and common goals across countries are more important than ever.

Prof. Toivo Maimets, *University of Tartu, European Medicines Agency Committee for Advanced Therapies*

Advanced therapy medicinal products (ATMPs)

Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells. ATMPs can be classified into three main types: gene therapy medicines, somatic-cell therapy medicines and tissue-engineered medicines. In addition, some ATMPs may contain one or more medical devices as an integral part of the medicine, which are referred to as combined ATMPs.

ATMPs differ from more "traditional" medicines in that their composition is chemically not well defined, they often require substantial manipulation of living material, they present special requirements for delivery, microbiological safety and monitoring of long-term clinical effects. Therefore, their quality control and marketing authorisation procedures are organized separately from other drugs. In the European Medicines Agency the authorisation starts from the EMA Committee for Advanced Therapies (CAT), a committee responsible for assessing the quality, safety and efficacy of ATMPs and following scientific developments in the field.

Neuropsychopharmacology 2

Prof David A. Slattery, *Johann Wolfgang Goethe-Universität Frankfurt am Main, Germany*

The potential of neuropeptides in psychopharmacology - insights from animal models

Neuropeptides and their receptors are established modulators of neuronal activity, shaping multiple behavioral and physiological responses to environmental stimuli. Neuropeptide signalling is, therefore, an important target for the development of pharmacological treatments of psychopathologies, such as autism- spectrum and substance- use disorders, schizophrenia, major depression and anxiety disorders, among others associated with social and emotional dysfunctions. In this context, the neuropeptide oxytocin has received considerable attention over the last decades due to its profound prosocial, fear-reducing and anxiolytic effects demonstrated both in human and animal studies. In most human studies, oxytocin has been applied acutely so far, and, even at relatively high doses. However, we and others have found adverse effects on anxiety, fear, and social behaviours after chronic OXT application, or otherwise artificially enhanced oxytocin signalling, in rodents. In my presentation, I will discuss the potential and pitfalls of oxytocin covering over a decade of research into this neuropeptide on its behavioural and molecular properties.

Evgeny Krupitsky, *V.M. Bekhterev National Medical Research Center for Psychiatry and Neurology, Russia*

Extended release naltrexone for opioid use disorders: Advantages and problems

Opioid addiction is one of the most severe drug problems due to its high level of morbidity, mortality, and psychosocial consequences. Naltrexone completely blocks the effects of opioids and, when administered to detoxified patients with opioid use disorders and taken as directed, prevents relapse and reduces the chances for a wide range of adverse effects associated with untreated addiction. It has been available as a 50 mg tablet since the mid 1970's and early studies showed very limited efficacy due to lack of adherence to oral naltrexone formulation, high dropout rates, and a preference for methadone maintenance. In addition, patients must be detoxified and free of physiologic opioid dependence prior to starting naltrexone because it will precipitate withdrawal if given to a person who is currently dependent, and such services are not always available or prohibitively expensive. In Russia, the law prohibits use of methadone, buprenorphine and other opioid agonist therapies and naltrexone is the only effective specific pharmacotherapy available for treating opioid addiction. Inpatient detoxification followed by 1-3 months of residential treatment is the standard treatment for opioid addiction in Russia. A number of randomized, placebo-controlled trials have been conducted in Russia that have demonstrated efficacy of the oral formulation with younger patients who are living with their families who improve adherence; that combining oral naltrexone with an antidepressant (fluoxetine) or an α-adrenergic agonist (guanfacine) does not increase significantly the proportion of patients who remain in treatment without relapsing; and that both long acting sustained-release formulations (injectable and implantable) are more effective compared to oral formulations of naltrexone and placebo. The implant that has been studied (Prodetoxone®) prevents relapse for 2-3 months and the injectable formulation (Vivitrol ®) prevents relapse for a month. Long acting extended release formulations of naltrexone reduce HIV drug risk behavior and improve adherence to antiretroviral therapy in HIV positive subjects with opioid use disorders. This talk will summarize the results of these studies, all conducted in Russia during the past 20 years

New drugs in Cardiology

Martin Serg, *North Estonia Medical Centre*

New drugs for treating dyslipidemia

Recent advances in dyslipidemia pharmacotherapy have led to improved outcomes in patients who are at very high cardiovascular risk. The current presentation gives an overview of the available evidence regarding the novel cholesterol-lowering drugs and critically reviews the efficacy and safety data.

Jessica Schubert, *MD, Clinical Pharmacology, Uppsala University Hospital, Sweden*

Minimizing the risk of arrythmia with QT-prolonging drugs – a written recommendation for the clinicians in the county of Uppsala, Sweden

Background. Many drugs inhibit potassium channels in myocardial cells, which can lead to a prolongation of the QT interval. This can in rare cases lead to syncope or death by the ventricular arrhythmia torsades de pointes (TdP). The probability of drug induced TdP is very low, and the condition is difficult to diagnose due to the brief duration of the arrhythmia. This makes it very difficult to estimate the incidence and the propensity of individual drugs to cause arrhythmia. It is established that two or more QT-prolonging drugs increase the risk of QT prolongation, and the general recommendation is to avoid such situations. In practice, however, this is not always possible or advisable.

Methods. Clinical pharmacologists together with adult and pediatric cardiologists have written a recommendation on how these situations can be handled. The document has been agreed upon by representatives from eight medical specialties in the county. The information is intended for prescribers who treat patients with drugs that may cause QT prolongation. The aim of the document is to support the prescriber in making risk assessments for each individual patient.

Summary of recommendations. A comprehensive risk assessment should be carried out on a case-by-case basis. The risk assessment consists of evaluating the QT prolonging drugs, the patient's risk factors and other concomitant medications. The risk of TdP is increased when, after initiation of a QT-prolonging drug, QTc is >500 ms or has increased by >60 ms. In these cases, a different drug should be considered.

If the QTc interval after initiation of the drug is >500 ms, has increased by >60 ms, or assistance is needed in interpreting the ECG, a cardiology consultation is recommended. For in-depth information about a drug's risks or discussion about alternative treatments, contact with the local Drug Information Center is recommended. The whole translated document can be found here: https://svelic.se/utredning/?id=14-500

POSTER PRESENTATIONS

Aneth Lvovs, *Tallinn University, University of Tartu*

Higher methylation in the TPH2 promoter region is associated with a greater extent of suicidal thoughts, adaptive impulsivity and a less playful personality

Co-authors: **Margus Kanarik**, *University of Tartu*, **Gabriela Ortega**, *University of Würzburg*; **Arunima Roy**, *University of Würzburg*; **Toomas Veidebaum**, *National Institute for Health Development*; **Klaus-Peter Lesch**, *University of Würzburg*; **Jaanus Harro**, *Tallinn University and University of Tartu*

Dysfunction in the 5-HT system is a known risk factor for affective disorders. Brain 5-HT synthesis depends on the enzyme tryptophan hydroxylase 2 (TPH2), thus TPH2 gene expression and enzyme activity has a direct influence on central 5-HT availability. Gene expression can be epigenetically regulated by DNA methylation (DNAm). Generally, DNAm inhibits transcription and high DNAm for TPH2 has been associated with a risk for ADHD (Heinrich et al., Scientific Reports 2017; 7:3823), suicide (Zhang et al., Molecular Medicine Reports 2015, 12:3184-3190) and lower social cognition (Reuter et al., Physiology & Behavior 2020; 227:113143). We have explored the association of TPH2 DNAm with depressiveness, anxiety, impulsivity and personality.

TPH2 promoter region DNAm was measured by pyrosequencing at 6 CpG sites in 195 males from the Estonian Children Personality Behaviour and Health Study. Subjects completed the Montgomery-Åsberg Depression Rating Scale, Adaptive and Maladaptive Impulsivity Scale, Affective Neuroscience Personality Scales (ANPS) and the Big Five Inventory.

DNAm in one CpG site was positively correlated with a higher extent of suicidal thoughts. Three CpG-s were positively correlated with excitement seeking and two with overall adaptive impulsivity. DNAm in four CpG-s correlated negatively to PLAY from the ANPS. One CpG was associated with all these measures, so that higher DNAm predicted higher impulsivity and lower positive emotion. Additional correlations were observed with several subscales of Extraversion (Gregariousness, Activity, Excitement-Seeking), Openness (Fantasy, Aesthetics, Actions) and Conscientiousness (Achievement Striving, Self-Discipline), altogether suggesting that TPH2 DNAm could lead to higher action-orientation and lower gregariousness.

Arle Kõrkjas, *University of Tartu*

Application of automated ultrasound signal generator in the development of electrospun multi-layered polymeric nanofibrous structures

Co-authors: **Ivo Laidmäe**, *University of Tartu*; **Karin Kogermann**, *University of Tartu*; **Ari Salmi**, *University of Helsinki*; **Heikki. J. Nieminen**, *Aalto University*; **Edward Hæggström**, *University of Helsinki* and **Jyrki Heinämäki**, *University of Tartu*

Multi-layered polymeric nanofibrous structures have found uses in tissue engineering and drug delivery applications. Ultrasound-enhanced electrospinning (USES) is a new nozzle-free continuous manufacturing technology to fabricate polymeric nanofibers. The aims of the present work were (1) to further develop the USES process in fabricating nanofibrous mats, and (2) to develop the multi-layered polymeric nanofibrous structures of a water-soluble polymer using an automated ultrasound signal generator.

An in-house USES method was used for fabricating the polymeric nanofibrous structures. A focusing ultrasonic transducer generates an ultrasonic fountain on top of a bath of polyethylene oxide aqueous solution. This fountain acts as Taylor cone, from where the nanofiber is spun. The humidity was kept at 4-5% with dehumidifier. A waveform generator was programmed by using NI Labview NXG 4.0 software. SEM was used for investigating the fiber size.

We were able to continuously adjust the US settings in a signal generator by using an automated program, and consequently, to generate nanofibrous mats with a nanofiber thickness gradient in a continuous manner. The SEM micrographs on the nanofibrous layers showed the difference of ~100 nm on average size of the fibers on the course of an USES process. To our best knowledge, this the first time ever to successfully generate a gradient structure of the nanofibers in a nozzle-free ES process.

In this study, we further developed the USES system for generating novel polymeric nanofibrous structures. Automation of the continuous change of US signal parameter in an USES process is possible with software. Automated programming assisted USES allows us to fabricate advanced nanofibrous structures (i.e., gradient structures).

Diva Eensoo, *National Institute for Health Development*

Systolic blood pressure: The role of body composition, diet, and the serotonin transporter promoter polymorphism

Co-authors: **Kadi Luht**, *Estonian Academy of Security Sciences*; **Inga Villa**, *University of Tartu*; **Anu Aaspõllu**, *National Institute for Health Development*; **Jaanus Harro**, *University of Tartu*

Introduction. Hypertension is one of the risk factors for lifestyle-related cardiovascular diseases. Lifestyle-related risks, including alcohol consumption and unhealthy eating, derive from daily decision-making that could be influenced by impulsivity and the serotonin system. Aim: Clarify whether associations between blood pressure and lifestyle-related risk factors are associated with impulsivity and the serotonin transporter promoter polymorphism (5-HTTPLR).

Materials and Methods. Data collected in the 4th study waves of both birth cohorts of the longitudinal Estonian Children Personality Behaviour and Health Study (www.ecpbhs.ee) were used (29.4±4.4 years). Subjects filled in a lifestyle questionnaire and the Adaptive and Maladaptive Impulsivity Scale. NaCl intake was calculated from 72 h diet diary followed by interview. Resting blood pressure was obtained as the average of 5 measurements. Triallelic 5-HTTLPR was genotyped (n=1234, l'/l' 32.8%, l'/s' 47.9%, s'/s' 19.3%).

Results. High blood pressure (≥140mmHg systolic and/or ≥90mmHg diastolic blood pressure) was revealed in 6.3% (n=59) subjects, whereas 5.6% (n=52) had high systolic pressure. Systolic blood pressure was positively correlated with sodium intake ($r=0.25$, $p<0.0001$) and also with beer consumption ($r=0.33$, $p<0.0001$). 5-HTTLPR was significantly associated with systolic blood pressure ($F_{2,927}=3.28$, $p=0.038$; l'/l' 116.7±15.0, l'/s' 114.1±13.3, and s'/s' 115.9±14.2mmHg. In a good fit model (RMSEA < 0.0001, NFI = 1.0, CFI = 1.0, and TLI = 1.0) higher systolic blood pressure was predicted by higher waist-hip ratio (WHR), NaCl intake, beer consumption and 5-HTTLPR l'-allele homozygocity.

Conclusion. Systolic blood pressure is mediated or moderated by body composition and dietary habits and also the 5-HTTLPR.

Evelyn Kiive, *University of Tartu*

Neuropeptide Y gene variants and agreeableness in young people: interaction effect with the birth cohort

Co-authors: **Toomas Veidebaum**, *National Institute for Health Development*; **Jaanus Harro**, *University of Tartu*

Neuropeptide Y (NPY) regulates essential basic and complex biological functions including social anxiety (Kask et al., NeuroReport 1998; 9, 2713-2716). Associations of common gene variants with behaviour have recently been described as subject to birth cohort effects if the behaviour in question is socially motivated (e.g., Vaht et al., Psychopharmacology 2014; 231, 2587-2594).

In the ECPBHS sample (www.ecpbhs.ee), the rs16147 and rs5574 polymorphisms of the NPY gene were genotyped (n= 1234). These variants were previously found to have a similar association with obesity, dietary intake, blood pressure, lipid and glucose metabolism in this sample (Katus et al., Peptides 2021; 139, 170524). Personality traits of the five-factor model were self-reported at age 25 (n = 856) by using EE.PIP-NEO (Mõttus et al., Eur. J. Psychol. Assess. 2006; 22, 149-157).

We found no significant main effect of NPY genotype on five-factor model personality domains in the total sample of ECPBHS. Nevertheless, there was a significant interaction of the NPY rs16147 and rs5574 variants and birth cohort on Agreeableness: $F(2,823)=5.27$ and $F(2,823)=4.45$, respectively, $p= 0.005$. The low expressing T/T genotype of NPY rs16147 resulted in high Agreeableness in the younger cohort (born 1989) and in low Agreeableness in the older cohort (born 1983). Similarly, the low expressing C/C genotype of NPY rs5574 was associated with higher Agreeableness in the younger cohort but not in the older.

In conclusion, the association between the NPY gene variants and a personality domain reflecting social desirability depends in times of rapid societal changes on the birth cohort, serving as an example of the relationship between the plasticity genes (Belsky et al., Mol Psychiatry 2010; 14, 746-754) and environment.

Farzaneh Zareei, *University of Tartu*

Family relationships and alcohol consumption: Interaction with the serotonin transporter gene linked polymorphic region (5-HTTLPR)

Co-authors: **Jaanus Harro**, *University of Tartu*; **Toomas Veidebaum**, *National Institute for Health Development*

The interaction of psychological, environmental, and inherited factors determines how a person gets involved in problematic behaviors such as drinking alcohol. The current study investigated the effects of the polymorphism in the serotonin transporter gene promoter region (5-HTTLPR) and family relationships on alcohol consumption. This longitudinal study was initiated in 1998, and the study participants (original n=1238) represent two birth cohorts of schoolchildren from Tartu County in Estonia. 1238 cases were included to the study. Family relationships of at age 15 years was significantly related to frequency of drinking alcohol. Specifically, association of Warmth (closeness plus support within family) with consuming alcohol was negative. On the other hand, Maltreatment (misprize and abuse) was in a positive relationship with alcohol consumption. At age 18, the effects of family relationships on consuming alcohol were lower and no longer statistically significant (p-values>0.10). The associations between family relations and alcohol use at age 15 varied by the 5-HTTLPR genotype. The impact of the family relations on the frequency of drinking alcohol was statistically significant among participants with the S/L genotype and similar results were obtained in the S/S genotype, but no relations were present between family relations and consuming alcohol in subjects with the L/L genotype. In conclusion, our findings reveal that family relations are related to alcohol consumption in S-allele carriers that is compatible with the hypothesis that S-allele carriers are more malleable by the environment.

Georg-Marten Lanno, *University of Tartu*

Incorporation of chloramphenicol loaded mesoporous silica nanoparticles (MSN) into electrospun polymeric nanofibrous scaffolds

Co-authors: **Sen Didem Karaman**, *İzmir Katip Çelebi University, Biomedical Engineering*; **Georg-Marten Lanno**, *University of Tartu*; **Liis Preem**, *University of Tartu*; **Gouani Zahara**, *Åbo Akademi University*; **Andres Meos**, *University of Tartu*; **Tanel Tenson**, *University of Tartu*; **Jessica Rosenholm**, *Åbo Akademi University*; **Karin Kogermann**, *University of Tartu*

The need for effective treatment of chronic wounds is increasing and one possible solution is electrospun nanofibrous scaffolds due to their unique structure and characteristics. (1)

Even more, the fibers can be incorporated with drug-loaded nanocarriers such as surface modifiable mesoporous silica nanoparticles (MSN) offering enhanced therapeutic effect. The aim of the study was to understand the effect of MSN incorporation to the drug release and antibacterial, antibiofilm and mechanical properties of electrospun polymeric scaffolds. Polycaprolactone (PCL) with acetic acid (AA)/formic acid (FA) solvent system was used for the electrospinning of scaffolds. Chloramphenicol (CAM) was used as a model antibacterial agent loaded into MSN. Modified dissolution tests and antibiofilm tests were used for analysis. Mechanical properties were investigated using texture analyzer.

The release profiles of CAM from MSN-loaded scaffolds showed initial burst release hence more than 60% of CAM was released already after 3 min. Antibacterial and antibiofilm activity of the MSN-loaded scaffolds correlated well with the drug release results. The planktonic and biofilm bacterial concentrations were reduced with CAM-loaded scaffolds, however no changes were seen compared to the PCL-CAM loaded scaffolds. The mechanical properties of the scaffolds were enhanced by the presence of MSN. MSN-CAM loaded fiber scaffolds had the highest Young's modulus and tensile strength.

In conclusion, MSN-loaded fiber scaffolds with improved mechanical properties were successfully prepared. Desired prolonged drug release and antibacterial and antibiofilm activity were not achieved with a developed system, however further bioactivity tests will be performed.

1. Preem L et al. Mol. Pharm. 2017, 14 (12), 4417–4430

Hanna Keidong, *University of Tartu*

Introduction of a smart medication dispenser in Estonia – feedback from patients and healthcare specialists

Co-author: **Daisy Volmer**, *University of Tartu*

To improve medication adherence of geriatric polypharmacy patients, a smart pillbox has been developed. It is a device (connected to an app), supporting correct use of medicines. The app could be managed by patients and/or family members/caregivers/ healthcare specialists. In addition it would display clinically relevant potential adverse drug reactions and interactions with medicines, supplements and food. It has the potential to forward medicines administration data to healthcare professional.

The aim of this study was to evaluate interest and need towards smart pillbox amongst patients, caregivers and healthcare specialists in Estonia.

Electronic survey among patients, caregivers (N=198) and healthcare specialists (N=112) was conducted to identify potential problems with medication adherence and expectations for the smart device.

Results revealed that about 75% of geriatric patients (mean age 65) use more than three medications daily. About 20% admitted problems with adherence. Issues were mainly related to decrease of cognitive and physical ability, thus patients would benefit from the device. On the contrary, healthcare specialist see problems with medication adherence in 90% of patients, which may refer to patients evaluating regularity of medication use higher than it actually is. Benefits of the device pointed out by specialists were safer medication use (64%) and reminders before patient runs out of medications (88%). Patients prioritized light and sound notifications (64%), as well as SOS-button for calling an ambulance (54%).

Smart systems have a great potential to support elderly polypharmacy patients that live at home. Information from the device can be interpreted by healthcare professionals to monitor and increase medication adherence of patients.

Helena Balažic, *University of Tartu, University of Ljubljana, Slovenia*

Stability and safety of alginate/poly(ethylene oxide) nanofibrous scaffolds for tissue engineering

Co-authors: **K. Põhako**, *University of Tartu*; **I. Laidmäe**, *University of Tartu*; **Š. Zupančič**, *University of Ljubljana*; **K. Kogermann**, *University of Tartu*

Electrospun nanofibers are known to be suitable as scaffolds for tissue engineering due to their high surface area to volume ratio and high porosity. Since nanofibers for tissue engineering purposes should be biodegradable, biocompatible and should not cause any immunogenic response, they are often produced from natural polysaccharides such as sodium alginate (Na-Alg). As these are hard to electrospin on their own, synthetic polymers, such as poly(ethylene oxide), PEO are usually added. Mixture of Na-Alg and PEO is especially interesting because they are both water-soluble and non-toxic solvents can be used for electrospinning, but these scaffolds need to be stabilised for tissue engineering purposes using cross-linking methods.

We tested different crosslinking methods (ionic cross-linking, UV-irradiation together with chemical crosslinker, γ-irradiation) in order to assess which method or combination of them is the most effective to stabilise nanofibrous scaffolds made from combination of Na-Alg and PEO to be used in physiologically relevant environment (37ºC buffered phosphate-saline pH 7.4). The interactions between the polymers and possible structural changes were determined using DSC (differential scanning calorimetry) and FTIR spectrometry (Fourier-transform infrared spectrometry). Additionally, we tested the safety of stabilized nanofiber matrices using hamster kidney fibroblasts.

The most effective stabilisation method was a combination cross-linking method consisting both ionic crosslinking of Na-Alg with CaCl2 and UV irradiation together with 4-hydroxybenzophenone. Both methods separately were not able to stabilize the scaffolds. Furthermore, too long time of UV – irradiation and γ – irradiation damaged the scaffolds as polymers started to degrade. The viability of fibroblasts in medium with tested mats was comparable to their viability when incubated with control biocompatible mats.

To sum up, electrospun Alg/PEO scaffolds have potential to be used for tissue engineering purposes, as they are stable in biologically relevant conditions and they are not toxic to the fibroblast cells.

Helene Tigro, *Tallinn University*

The synthesis and characterization of Bri2 BRICHOS coated magnetic particles and their application to protein fishing: Identification of novel binding proteins

Co-authors: **Nina Kronqvist**, *Karolinska Institute*; **Axel Abelein**, *Karolinska Institute*; **Lorena Galan-Acosta**, *Karolinska Institute*; **Gefei Chen**, *Karolinska Institute*; **Michael Landreh**, *Karolinska Institute*; **Alexey Lyashkov**, *National Institute on Aging*; **Miguel A. Aon**, *National Institute on Aging*; **Luigi Ferrucci**, *National Institute on Aging*; **Ruth Shimmo**, *Tallinn University*; **Jan Johansson**, *Karolinska Institute*; **Ruin Moaddel**, *National Institute on Aging*

Alzheimer's disease is an amyloid disease in which the amyloid beta peptide forms neurotoxic oligomers that can cause neuronal dysfunction and eventually form amyloids that end up in the extracellular plaques. Human integral membrane protein 2B (ITM2B or Bri2) is a member of the BRICHOS family, proteins that efficiently prevent amyloid beta 42 aggregation via a unique mechanism. The identification of novel Bri2 BRICHOS client proteins could help elucidate signaling pathways and determine novel targets to prevent or cure amyloid diseases. To identify Bri2 BRICHOS interacting partners, we carried out a 'protein fishing' experiment using recombinant human (rh) Bri2 BRICHOS-coated magnetic particles, which exhibit essentially identical ability to inhibit amyloid beta 42 fibril formation as free rh Bri2 BRICHOS, in combination with proteomic analysis on homogenates of human neuroblastoma SH-SY5Y cells. We identified 70 proteins that had more significant interactions with rh Bri2 BRICHOS relative to the corresponding control particles. Three previously identified Bri2 BRICHOS interacting proteins were also identified in our 'fishing' experiments. The binding affinity of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the top 'hit', was calculated and was identified as a strong interacting partner. Enrichment analysis of the retained proteins identified three biological pathways: Rho GTPase, heat stress response and pyruvate, cysteine and methionine metabolism. The study confirmed that the novel application of 'protein fishing' is a viable approach that in combination with proteomics offers a significant advantage in the identification of new interacting partners and provides insight into novel mechanisms.

Ines Vaide, *University of Tartu*

How we treat patients with acquired haemophilia A (AHA) with recombinant porcine factor VIII

Co-authors: **R. Klamroth**, *Vivantes Klinikum im Friedrichshain*; **I. Vaide**, *University of Tartu*; **C. Kubicek-Hoffmann**, *Vivantes Klinikum im Friedrichshain*; **M. Orlovic**, *Vivantes Klinikum im Friedrichshain*; **E. Langer**, *Labor Berlin*; **S. Gottstein**, *Vivantes Klinikum im Friedrichshain*

Treatment of bleeds in patients with AHA with bypassing agents is often difficult and unpredictable. Monitoring of the effect in routine clinical practice is not possible. A recombinant porcine factor VIII B-domain-deleted product (rpF8; OBIZUR) is approved for treatment of bleeding episodes in adults with AHA. The recommended high initial dose of 200 IU/kg bodyweight is a matter of debate. We report our approach to the treatment of patients with AHA with rpF8.

Methods Retrospective chart review of patients with AHA treated with rpF8 from April 2016 to September 2019.

13 major bleeds in 12 patients, median age 78(38-92), were treated with rpF8.In one bleed (gastrointestinal) rpF8 was second-line treatment and in 12 bleeds (muscle, soft tissue) first line. Good efficacy was seen in 12 bleeds. rpF8 loading doses of 50 U/kg bodyweight increased F8 activity 55%-112% within 1h. Subsequent median doses were 2x 25 - 50 IU/kg bodyweight/day for 1 and 7 days with F8 trough level above 30%. Duration of treatment was adjusted the type of bleeding. No rpF8-related adverse events were reported. Two patients died in the hospital (one of sepsis, one of pneumonia after aspiration). All patients received 1g tranexamic acid concomitantly and prednisolone for immunosuppression. 1 of 12 patients received a rpF8 loading dose of 100 U/kg bodyweight due to a life threatening throat and tongue bleed. Here we observed no increase of F8 activity (1% at baseline to 2% after 1h) and treatment was switched to bypassing agents. rpF8 inhibitor -titer measurement in this patient revealed an anti-porcine titer of 13,8 BU (human titre 4,8 BU). All other patients had anti-porcine titers between 0,4 and 0,8 BU.

rpF8 showed good haemostatic efficacy in 12/13 bleeds in 11/12 patients with lower doses than in the registration study. There was a close correlation between the measured F8 levels and hemostatic efficacy. We recommend an initial dose of 50 U rpF8/kg bodyweight and monitoring of F8 levels to trough levels of at least 30%. In no or low increase of F8 levels relevant rpF8 inhibitor- titres are likely.

Ines Vaide, *University of Tartu*

How to monitor Drug effectiveness in Hemophilia Patients in the new era of treatment. Emicizumab - a bispecific antibody

Co-authors: **Timea Szanto**, *University of Helsinki, Helsinki University Hospital*; **Ines Vaide**, *University of Tartu*; **Annukka Jouppila**, *Clinical Research Institute HUCH*; **Marja Lemponen**, *Helsinki University Hospital*; **Riitta Lassila**, *University of Helsinki, Helsinki University Hospital*

Introduction. Novel hemophilia non-factor therapy with emicizumab(emi) bridges FIXa with FX to trigger tenase activity. Measuring the effects of emi compared to how we have used to monitor the effectiveness of treatment with classical Factor replacement therapy is limited. The balance between thrombosis and bleeding is an important aspect to ensure certainty of treatment success. We aimed to monitor the role of emi in the coagulation during treatment. Methods: Seven patients were included. Repeated analyses at once-weekly emi loading and 3-12 -month maintenance followed ROTEM in the presence or absence of corntrypsin inhibitor (CTI), APTT-based factor analysis, prothrombin fragments F1+2 (ELISA).

Results. Global coagulation assays: In ROTEM, emi only shortened the clotting times of InTEM in citrated blood and emi strengthened the clot profile in CTI-citrated blood. The contact-activating NaTEM depicted the emi effect during follow-up, while CTI eliminated it completely. Circulating F1+2 did not increase. Emi shortened APTT and did not interfere with chrmogenic FVIII reagent (bovine). APTT-based one-stage assays detected elevated FVIII>FXI>FXII, but not the FIX or FX levels. We want to highlight that a lower than on-label emi dose managed to control the bleeding tendency with a thrombogenic clinical propensity in one patient, coagulation activity reached to same levels according to biomarkers and ROTEM as the other patients with the routine dosing scheme.

Conclusion. We provide evidence that ROTEM and especially NaTEM enable the laboratory assessment of emi effects. These tools are clinically relevant in the operating theatre and emergency settings and may solve the incidental dilemmas of abnormal coagulation during emi therapy.

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Edijs Vavers, *Latvian Institute of Organic Synthesis, Latvia*

Reduced expression of the R2 subunit of metabotropic GABA receptors in habenula is associated with increased seizure susceptibility in sigma-1 receptor knockout mice

Co-authors: **Gundega Stelfa**, *Latvia University of Life Sciences and Technologies*; **Baiba Svalbe**, *Latvian Institute of Organic Synthesis*; **Karlis Vilks**, *Latvian Institute of Organic Synthesis*; **Liga Zvejniece**, *Latvian Institute of Organic Synthesis*; **Maija Dambrova**, *Latvian Institute of Organic Synthesis*

Sigma-1 receptor is a unique ligand-regulated intracellular integral membrane-bound protein principally located at the juxtaposition of endoplasmic reticulum and mitochondria-associated membrane. The growing body of evidence suggests its significant involvement in the modulation of seizures. In our study we aimed to investigate the possible interaction between sigma-1 receptor and metabotropic G-protein-coupled GABA-B and ionotropic GABA-A receptors by using sigma-1 receptor knockout mice. In addition, we evaluated knockout mice susceptibility to chemoconvusant-induced seizures. Quantitative PCR, Western blotting and immunohistochemistry of free-floating sections were used to assess the expression of subunits of GABA-B and GABA-A receptors in different brain structures. Our results demonstrate that genetic inactivation of the sigma-1 receptor decreased the expression of R2 subunit of GABA-B receptor in habenula. No significant difference of GABA-A receptor expression in brain was observed between wild-type and knockout animals. By using intravenous pentylenetetrazol and (+)-bicuculline infusion-induced acute seizure models, we show significantly decreased seizure threshold in sigma-1 receptor knockout mice. Our study provides strong evidence of the interaction between sigma-1 receptor and metabotropic GABA receptors. The reduced inhibitory GABA neurotransmission, especially in the medial habenula, is associated with a higher susceptibility to seizures due to the inactivation of sigma-1 receptor. Our results confirm that sigma-1 chaperone is a valuable target for the development of novel antiseizure drugs and warrants further investigation of involvement in G-protein-coupled GABA-B receptor-related signalling mechanisms.

Inga Villa, *University of Tartu*

Associations of impulsivity with food intake in a longitudinal birth cohort study

Co-authors: **Denis Matrov**, *Tallinn University*; **Triin Kurrikoff**, *University of Tartu*; **Inga Villa**, *University of Tartu*; **Katre Sakala**, *Tallinn University, University of Tartu, National Institute for Health Development*; **Aleksander Pulver**, *Tallinn University*; **Toomas Veidebaum**, *National Institute for Health Development*; **Ruth Shimmo**, *Tallinn University*; **Jaanus Harro**, *University of Tartu*

Impulsivity is associated with unhealthy diet, substance abuse and higher body weight (Bénard et al, Am J Clin Nutr 2019;109, VanderBroek-Stice et al, Appetite 2017;112, Eensoo et al, Psychopharmacology (Berl) 2004;172). Whether these associations extend to specific nutrients is largely unknown.

The aim of the study was to ascertain a possible longitudinal association between adaptive and maladaptive impulsivity dimensions, cardiorespiratory fitness (CRF) and dietary intake.

The sample included two birth cohorts of the Estonian Children Personality Behaviour and Health Study (www.ecpbhs.ee). Impulsivity and dietary intake were measured 3 times in both cohorts on subjects of ages 15 to 33 years. Maximum power output on cycle-ergometer test was used as an indicator of CRF.

Impulsivity was measured by a 24 item Likert-type Adaptive and Maladaptive Impulsivity Scale (AMIS). Impulsivity scores were predicted from the food diaries (48 h and 72 h diaries followed by interview) data converted into nutrient categories according to the Finnish Micro-Nutrica and Estonian NutriData databases. Linear mixed-effects approach was used to model the time-dependence between observations.

Lower maladaptive impulsivity was associated with higher CRF (β = -0.07; 95% CI: -0.12; -0.03). Higher maladaptive impulsivity was associated with lower dietary intake of zinc (β = -0.10; 95% CI: -0.15; -0.06) and vegetables (β = -0.04; 95% CI: -0.07; -0.01) and higher intake of sodium (β = 0.06; 95% CI: 0.02; 0.10). Vitamin B6 was positively associated with adaptive impulsivity (β = 0.04; 95% CI: 0.01; 0.07).

Conclusively, food choice may affect the biochemistry of the central nervous system and therefore regulate the manifestations of impulsivity.

Kairi Tiirik, *University of Tartu*

Electrospun porcine gelatine matrix as an alternative to pig skin in an artificial biofilm model

Co-authors: **Liis Preem**, *University of Tartu*; **Kadi Sagor**, *University of Tartu*; **Marta Putrinš**, *University of Tartu*; **Tanel Tenson**, *University of Tartu*; **Karin Kogermann**, *University of Tartu*

Insufficient infection treatment in chronic wounds is associated more and more with the presence of biofilm, thus, novel biofilm targeting wound care products are being developed. It is of importance to have appropriate analytical methods that are suitable for testing these applications for their antibiofilm properties. While taking into consideration the physical, mechanical and biopharmaceutical properties of wound care products.

The aim of the present study was to develop and compare in vitro and ex vivo biofilm models for assessing the antibiofilm properties of electrospun antimicrobial wound dressings. In vitro model was created using thermally crosslinked electrospun porcine gelatine (GEL) matrix as an artificial skin and for ex vivo model pig ear skin was used. Different pathogenic bacteria isolated from infected wounds was used to develop a biofilm. The model was set up in well-plates, where on top of GEL matrix or pig skin bacterial dispersion was added and chloramphenicol (CAM)-loaded electrospun wound dressings were applied. These systems were incubated for 24 and 48h, subsequently planktonic bacteria were removed, biofilm disrupted and quantified.

The results show that GEL matrix is suitable to be used as an artificial skin in in vitro biofilm model, as bacteria adhered to its surface and formed a biofilm up to 108CFU/mL. Application of CAM-loaded wound dressings effectively inhibited or reduced the biofilm formation equally in both models.

To conclude, designed in vitro model on top of GEL matrix allows to compare and evaluate the antibiofilm properties of electrospun wound dressings. Also helps to characterize novel wound care products which will result in their more successful design and development.

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Karin Valmse, *Tallinn University of Technology*

Nano-ions act as promising inhibitors of pancreatic lipase

Co-authors: **Pavel Kerner**, *Tallinn University of Technology*; **Robert Risti**, *Tallinn University of Technology*; **Aivar Lõokene**, *Tallinn University of Technology*; **Riina Aav**, *Tallinn University of Technology*

Pancreatic lipase, a key enzyme in the hydrolysis of dietary triglycerides (fats), is a potential drug target for the treatment of obesity. Reducing pancreatic lipase activity by inhibitors leads to less uptake of energy-rich fatty acids and monoglycerides. In addition to the anti-obesity effect, inhibitors of pancreatic lipase may reduce postprandial hypertriglyceridemia and improve conditions of glycemic control in type 2 diabetic patients. The lipase acts in a complex environment at the surface of insoluble triglycerides, and its activity is influenced primarily by bile salts and colipase. Despite extensive investigations, there is only one approved pancreatic-inhibiting drug (Orlistat) on the market.

Here, we present a new type of inhibitor of pancreatic lipase – a hybrid organic-inorganic nano-ion that reduce lipase activity in triglyceride emulsions. Nanometer sized anions have been shown to interact with biomolecules [1] and may accumulate at lipid/water interfaces where they can interact with pancreatic lipase. The most promising results have been obtained with the urea based macrocycle – hemicucurbituril (cycHC[8]), whose barrel-like structure binds inorganic ions and forms a nano-ion (cycHC[8]-ion)[2]. Our data suggest that for inhibition of lipase activity, accumulation of a cycHC8-ion at the surface of triglyceride/water and the participation of the bile salt taurodeoxyholate is required, suggesting formation of a ternary complex pancreatic lipase/cycHC8-ion/taurodeoxycholate.

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Karola Peebo, *North Estonia Medical Centre, Psychiatry Clinic*

First-Episode Psychosis Integrative Treatment: Estonian Experience

Co-authors: **Erika Saluveer**, *North Estonia Medical Centre, Psychiatry Clinic*; **Harri Küünarpuu**, *North Estonia Medical Centre, Psychiatry Clinic*; **Teele Orgse**, *North Estonia Medical Centre, Psychiatry Clinic*; **Jaanus Harro**, *Tallinn University, University of Tartu*

Early intervention approach in the treatment of first-episode psychosis has been shown to be more effective on psychopathological, functional, and quality-of-life outcome measures compared to treatment as usual. Information about the long-term effects is still limited. This study presents further evidence on the short- and long-term benefits of early intervention for first-episode psychosis. A sample of 199 first-episode psychosis patients was formed to assess the efficacy of the early intervention programme in Tallinn, Estonia in 2004-2008. One hundred and seven patients were available for assessment after two years. The long-term effects of the intervention were assessed in a registry-based ten-year follow-up. One hundred and sixteen patients who received the intervention were included in the long-term follow-up; their results were compared with a retrospectively formed control group (n=114). Patients included in the early intervention programme achieved substantial symptomatic improvement after 6 months of treatment (BPRS score reduction >50%). Patients had significant functional improvement (GAF score improved significantly after 6 months) and the quality of life after 12 months was significantly higher than at the beginning of treatment (Q-les-Q score). Employment was increased by 14% (43.9% to 57.9%) after 2 years. Ten-year follow-up showed that patients in the intervention group had spent more time working during the follow-up period and had almost two times larger incomes than patients in the control group. Significantly fewer patients in the intervention group had been in supported housing compared to the control group. This study concluded that the early intervention treatment for first-episode psychosis has positive short- and long-term effects.

Katre Sakala, *National Institute for Health Development, University of Tartu, Tallinn University*

Association between platelet MAO activity and lifetime substance use in a longitudinal birth cohort study

Co-authors: **Kairi Kasearu**, *University of Tartu*; **Urmeli Katus,** *University of Tartu*; **Toomas Veidebaum**, *National Institute for Health Development*; **Jaanus Harro**, *University of Tartu*

Platelet monoamine oxidase (MAO) activity, a marker of central serotonergic capacity, has been associated with a variety of problem behaviours. Surprisingly, any link between platelet MAO activity and illicit drug initiation, use or addiction has not been established. Thus, we have examined platelet MAO activity, and drug use initiation and lifetime drug use in a longitudinal birth cohort study. The sample included both birth cohorts (original n = 1238) of the Estonian Children Personality Behaviour and Health Study. Longitudinal association from age 15 to 25 years between platelet MAO activity and lifetime drug use was analyzed by mixed effects regression models. Differences at ages 15, 18 and 25 were compared by t-tests. Cox proportional hazard regression analysis was used to assess the association between platelet MAO activity and the age of substance use initiation. Male subjects who reported at least one drug use event had lower platelet MAO activity compared to non-users, both in cross-sectional and longitudinal analysis, while in females, platelet MAO activity was not associated with drug use. Males with low platelet MAO activity had started to use drugs at a younger age. Moreover, in male subjects experimenting with illicit drugs only once, low platelet MAO activity was also associated with higher risk at a younger age. In contrast, the age of first experience with alcohol was not associated with platelet MAO activity, possibly reflecting the low social barrier to alcohol in the society. Conclusively, the association of low platelet MAO activity with illicit drug use is primarily owing to expression of excessive risk-taking behaviour at early age.

Kelli Somelar, *University of Tartu*

Development of depression-like behavior and altered hippocampal neurogenesis in a mouse model of chronic neuropathic pain

Co-authors: **Monika Jürgenson**, *University of Tartu;* **Külli Jaako**, *University of Tartu*; **Kaili Anier,** *University of Tartu*; **Anu Aonurm-Helm**, *University of Tartu*; **Liga Zvejniece**, *Latvian Institute of Organic Synthesis*; **Alexander Zharkovsky**, *University of Tartu*

Clinical observations have indicated a connection between chronic pain and psychiatric disorders such as anxiety and major depression. The connection has been studied in rodent models, where neuropathic pain is induced via neuropathic injury. In these models, animals develop depression-like and anxiety behaviors. However, the underlying neurobiological mechanisms linking chronic pain and depression are not yet fully understood. Adult neurogenesis in the hippocampus is a fundamental process related to brain plasticity and impaired neurogenesis has been associated with the development of mood disorders and cognitive impairments.

Our study aimed to elucidate the underlying long-term changes in brain plasticity, with a focus on alterations in hippocampal neurogenesis, induced by neuropathic pain in mice at a time point when depression-like behavior has already developed.

We found that manifestation of anxiety- and depression-like behavior as well as cognitive impairment co-occur with decreased survival of newly generated cells but not with impaired proliferative activity in the dentate gyrus of the hippocampus. Moreover, we detected an impaired differentiation of newly generated cells into mature neurons and a shift towards increased differentiation into astroglial cells, accompanied with signs of reactive microgliosis.

Our findings indicate that a reduction in mature functional neurons, rather than reduced proliferation or decreased number of neuronal progenitor cells, is the long-term change in hippocampal plasticity that manifests in neuropathic pain conditions after depression-like behavior has developed.

Kristi Kallas, *Tallinn University*

The relationship of maternal care and exploratory behaviour in the rat

Co-authors: **Marianna Školnaja,** *Tallinn University*; **Aleksander Pulver**, *Tallinn University*; **Ruth Shimmo**, *Tallinn University;* **Jaanus Harro**, *University of Tartu*

Rats exhibit differences in naturally occurring maternal care and this can affect offspring behaviour. Exploration presents a vital adaptive strategy that channels the interaction of anxiety and curiosity. Rats display persistent differences in their behaviour in the exploration box test (Mällo et al., Behav. Brain Res. 2007; 177, 269-281) that are associated with variability in many neurochemical mechanisms, most notably dopaminergic (O'Leary et al., Pharmacol. Res. 2016; 113, 739-746). We have studied whether maternal care by the dams is associated with their exploratory behaviour, and whether this affects exploration in offspring. Total of 29 litters were observed for maternal care, the offspring (n=320) and both parents were tested in the exploration box. Low arched-back nursing/licking and grooming (ABN/LG) dams showed less object investigation but no other difference in exploration. Female offspring of high ABN/LG dams had higher overall exploratory behaviour. In contrast, no such a difference was found in male offspring. High ABN/LG dam group also had less pups than low maternal care group but litter size as a covariate did not affect the difference of low and high ABN/LG female offspring in exploratory behaviour. In female offspring, exploratory behaviour correlated with maternal (r_s=0.37; $p<0.001$) but not with paternal exploration (r_s=0.08; p=0.31). Exploration in male offspring correlated neither with maternal (r_s=-0.06; p=0.45) nor paternal (r_s=0.01; p=0.93) exploratory behaviour. These findings suggest that while in F1 generation exploration in male rats is not significantly associated with parental phenotype or maternal care, in females such relationships exist, possibly helping to explain how differences in male and female exploratory behaviour emerge.

Margus Kanarik, *University of Tartu*

Long-term neuronal metabolic activity in mice with deficient serotonin synthesis

Co-authors: **M. Shkolnaya**, *Tallinn University, Tallinn University of Technology*; **A. G. Schmitt-Böhrer**, *University of Würzburg*; **K. P. Lesch**, *University of Würzburg*; **J. Harro**, *University of Tartu*

Disturbances in serotonergic (5-HT) neurotransmission are inherent to several psychopathological conditions e.g., depression, anxiety, impulsivity, and aggression. The rate of cerebral 5-HT synthesis is set by tryptophan hydroxylase-2 (Tph2), thus mice with inactive Tph2 gene are suitable to study associations between pathological behaviour and compromised 5-HT transmission (K.P. Lesch et al, Philos Trans R Soc Lond B Biol Sci 367, 2012). In order to find out the brain regions mediating aberrant serotonergic neurotransmission and behaviour, the activity of cytochrome oxidase (COX), a mitochondrial respiratory chain enzyme indicative of long-term neuronal activity (F.E. Gonzalez-Lima, Ed., Cytochrome Oxidase in Neuronal Metabolism and Alzheimer's Disease, Plenum Press, New York, 1998), was measured in 32 brain regions of male and female Tph2 knockout (KO), heterozygous (HET) and wild-type (WT) mice. Female HET mice had higher COX activity than males in frontal cortical, accessory olfactory, cingulate, and thalamic regions. Female WT mice had higher COX activity than males in piriform cortex. Female WT and HET mice had higher COX activity than males in hippocampal and raphe nuclei. Male KO mice had higher COX levels compared to WT and HET males in cingulate, hippocampal and raphe nuclei, with metabolic activity comparable to female mice. In posterior cingulate/retrosplenial cortex female KO mice had markedly higher COX activity compared to males, this was also the only region were Tph2 inactivation led to an increase in COX activity in females. Conclusively – female mice have higher long-term neuronal activity in several brain regions, male mice tend to be more affected by the inactivation of the Tph2 gene, resulting in an increase in neuronal energy metabolism.

Maris-Johanna Tahk, *University of Tartu*

Measuring of fluorescent ligand binding to M4 muscarinic receptors

Co-authors: **Tõnis Laasfeld**, *University of Tartu*; **Jane Torp**, *University of Tartu*; **Ago Rinken**, *University of Tartu*

Muscarine as an active pharmacological substance was found by O.Schmiedeberg and R.Koppe in 1869 in Tartu [1]. Since then, the M1 and M4 muscarinic acetylcholine receptors (mAChRs) have emerged as promising drug targets for the treatment of symptoms of neurodegenerative and neurodevelopmental disorders [2]. As orthosteric binding sites of all mAChR subtypes (M1-M5) are structurally conserved, allosteric ligand binding and biased signaling may enable to achieve subtype selectivity. Progress in fluorescence-based methods have opened new possibilities to discover and characterize ligands with favorable and unique properties. Dr. Keller group at the University of Regensburg have synthesized several ligands, which are suitable for fluorescence based assays [3]. UR-CG072 and UR-MK342, both work well in fluorescent anisotropy assay (FA) with baculovirus particles to M4 receptors, having affinities in the low nanomolar range and also enabling screening of unlabelled ligands. The ligand binding can also be measured in live-cells with fluorescence microscopy while image quantification is achieved by using machine learning based algorithms. Using CHO-K1 cells expressing M4 receptors and TAMRA-labelled UR-CG072, we could monitor ligand binding kinetics in live cells and characterize the affinities of competitive ligands. This assay system enables to study kinetic percularities of different ligands and discover allosteric modulations, which are essential for development of subtype-selective ligands of mAChR.

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Marten Vares, *University of Tartu*

Vulnerability to chronic stress in rats with low positive affectivity and high expression of hedonic behaviour

Co-authors: **M. Kanarik**, *University of Tartu*; **K. Kõiv**, *Institute of Psychology, University of Tartu*; **M. Vares**, *University of Tartu*; **K. Anja**, *University of Tartu*; **K. Laugus**, *University of Tartu*; **M. Metelitsa**, *University of Tartu*; **H. Tigro**, *Tallinn University*; **R. Shimmo**, *Tallinn University*; **J. Harro**, *University of Tartu*

Rats with lower inherent positive affectivity (PA), assessed by lower level of 50-kHz ultrasonic vocalizations (USV) are more vulnerable to stress. In apparent contrast, free-fed rats with persistently higher consumption of sucrose are more sensitive to stress. To understand how these effects comprise stress vulnerability, we compared in a single experiment the effects of chronic stress in rats with innate differences in PA and hedonic response.

Male Wistar rats (n=95) underwent 2-week tickling sessions and 3-week sucrose consumption tests in order to divide them into 4 groups of 20 rats each: HC-HSuc, HC-LSuc, LC-HSuc, and LC-LSuc. Half of the animals were submitted to 5-week chronic variable stress (CVS) regimen. CVS was followed by behavioural tests and lastly a single dose of amphetamine (AMPH; 1 mg/kg; IP) was administered to all animals with recording of 50-kHz USV-s and locomotor activity in a standard cage for 20 min.

CVS decreased weight gain in weeks 4-5 of CVS. In elevated plus-maze, CVS reduced entries to open arms and ratio of open/total arm entries in LC-HSuc while, increasing these parametres in HC-HSuc-rats. CVS reduced AMPH induced 50-kHz USVs only in LC groups, and statistically significantly only in LC-HSuc rats. In frontal cortex (FC), reduction in 5-HIAA levels was observed in the LC-HSuc group, and an increase was detected in NMN levels in HC-HSuc group. Stressed HC-HSuc rats had higher concentrations of α- and β-alanine, tryptophan, and isoleucine in FC.

We have confirmed, in a common experiment, the previous separate findings that individual differences in both PA and hedonic response can contribute to stress resilience and vulnerability. The highest vulnerability to stress was found if low PA and high expression of hedonic behaviour coincided.

Raimonds Lozda, *University of Latvia, Latvia*

Serotonin sulfate as a potential biomarker of drug efficacy in depression

Co-authors: **Santa Purviņa, MD, PhD**, *Institute of Science of P.Stradins Clinical University Hospital*; **Dainis Krieviņš, MD, PhD**, *Institute of Science of P.Stradins Clinical University Hospital*

Introduction. According to the latest meta-analysis, the overall prevalence of depression in COVID-19 patients is 45%.

According to the literature, presently used antidepressants do not differ significantly and are associated with less than 50% response after the first treatment attempt. The detection of indoleamine metabolites in the CSF remains an essential method for evaluating the efficacy of a drug in clinical trials. However, healthcare professionals highly value a less invasive approach due to patient safety and clinical convenience. Therefore, our special attention was paid to the final phase of serotonin (5-HT) degradation by sulfotransferases (SULT) and the final biotransformation product, serotonin sulfate (5-HT-SO4), which crosses the CSB and thus at least partially mimics the CNS-specific 5-HT metabolism.

Aims. Our scientific team has already carried out a preliminary study. Our original study aimed to test the hypothesis that 5-HT-SO4 can be detected in human plasma by the modern HPLC-MS / MS method and to develop this method.

Results. The study ended with the first-time detection of naturally occurring 5-HT-SO4 in human plasma samples from healthy volunteers.

Conclusions. The method's sensitivity to identify individual changes in the compound was observed in healthy volunteers stimulated with hydroxytryptophan, but most of the results were below the detection limit. In 2019, the HPLC MS / MS method was reworked and validated according to the latest industry standards. According to unpublished data, this method solved significant problems identified during the development of the original method. In addition, the validated method has a higher sensitivity than the original one. The above study and unpublished data reveal the feasibility of further studies.

Robert Risti, *Tallinn University of Technology*

A calorimetric approach for investigating anti-hypertriglyceridemic drugs in a native-like environment

Co-authors: **Aivar Lõokene**, *Tallinn University of Technology*; **Ly Villo**, *Tallinn University of Technology*

Lipoprotein lipase (LPL) is a key enzyme in triglyceride metabolism and a potential drug target for the treatment of hypertriglyceridemia, a condition shown to be causally related to cardiovascular diseases and pancreatitis. The complex regulation mechanism of LPL has made it difficult to mimic the physiological environment, under which LPL acts, in vitro.

Herein, we introduce a calorimetric method for determining LPL activity that uses undiluted human plasma as a substrate and overcomes several limitations of other well-known techniques. Isothermal-titration calorimetry (ITC) allows continuous monitoring of the lipolytic reaction. The high sensitivity of the ITC assay allows for measurements on the picomolar scale and both initial rates and kinetic for complete hydrolysis of plasma lipids can be studied. We used this approach to compare plasma samples from randomly selected donors and patients with type II diabetes. Our data indicated that plasmas with similar triglyceride concentrations can exhibit vastly different LPL activity. This could have implications in personal medicine, where exogenous LPL activity could be measured in plasma samples for identifying subjects whose triglyceride levels are increased due to difficulties for LPL to perform its function. We also used the versatility of the ITC system to investigate how a potential therapeutic agent against hypertriglyceridemia, an apolipoprotein C-II mimetic peptide (18A-CII-a), affects LPL activity in human plasma. Our measurements revealed that the 18A-CII-a peptide is an efficient stimulator of LPL activity and increases the amount of available substrate for LPL.

In summary, we present a novel calorimetric method for quantitative measurements of LPL activity and interactions in human plasma.

Tõnis Tokko, *University of Tartu*

Drunk driving is associated with anger and aggression and mediated/ moderated by the serotonin transporter gene promoter polymorphism

Co-authors: **Diva Eensoo**, *National Institute for Health Development*; **Kadi Luht-Kallas**, *Estonian Academy of Security Sciences*; **Jaanus Harro**, *University of Tartu*

Road traffic injuries are a serious public health issue. Risk-taking in traffic has been associated with impulsivity, aggression, and alcohol use. The serotonin transporter gene promoter polymorphism (5-HTTLPR) has been associated with impulsivity, alcohol use, and traffic accidents. The aim of this study was to examine how serotonin transporter genotype could mediate or moderate possible associations between drunk driving, impulsivity, anger, and aggression.

A sub-sample (n = 402 males) of the Estonian Psychobiological Study of Traffic Behaviour (EPSTB) with mean age (SD) = 41.2 (13.7) filled out Adaptive and Maladaptive Impulsivity Scale (AMIS), Driving Anger Scale (DAS33), Buss – Perry Aggression Questionnaire and AUDIT. L'/l' homozygotes were compared with the s' allele carriers (n = 338; l'/l', 32.5%; s'/l', 51.2%; s'/s', 16.3%). Traffic violation data (5-year period) were obtained from the police database.

Drunk drivers (n = 17, 4%) had significantly higher disinhibition (maladaptive impulsivity) (mean(SD) = 19.3(4.3) vs 16.7(4.7), t (381) = -2.2, p = 0.029, physical aggression (19.1(7.9) vs 16.0(5.5), t (375) = -2.2, p = 0.030), hostility (18.8(5.1) vs 16.1(4.8), t (375) = -2.2, p = 0.026) and significantly more other traffic violations (70.6% vs 32.5%, χ^2 = 10.53, p = 0.001) compared to drivers with no drunk driving violation. 5-HTTLPR was not directly associated with drunk driving, but path analysis revealed indirect association of l/l homozygotes and drunk driving via higher driving anger, physical aggression and AUDIT score (RMSEA = 0.007, CFI = 1.00, and TLI = 0.99).

In conclusion, variation in the serotonergic system appears to be a mediating/ moderating factor of associations between drunk driving, anger, and aggression measures.

Triin Kurrikoff, *University of Tartu*

Low platelet monoamine oxidase (MAO) activity associates with higher alcohol consumption, with higher BMI and lower consumption of sugar and sweets

Co-auhtors: **Inga Villa,** *University of Tartu*; **Katre Sakala**, *University of Tartu, National Institute for Health Department, Tallinn University*; **Jaanus Harro**, *University of Tartu*

Serotonin plays a role in many brain processes regulating behaviour and physiological processes, including appetite (Müller 2010; Academic Press, London). However, there are almost no studies about association of one widely studied marker for the central serotonin system, platelet monoamine oxidase (MAO) activity, and nutrition or BMI.

Our study sample was the Estonian Children Personality Behaviour and Health Study (http://www.ecpbhs.ee/). The consumption of pure alcohol and sugar and sweets was assessed at age 25 with a 72 h diet record using the Estonian NutriData food consumption database (versions 4.0–7.0, National Institute for Health Development, Estonia). Subjects were divided into three groups according to their platelet MAO values: 25% of with the lowest platelet MAO value, 25% with the highest platelet MAO value and 50% of subjects who had medium platelet MAO value.

Subjects from the group with the lowest platelet MAO value had the highest proportion of subjects with present alcohol dependence, the highest consumption of pure alcohol in grams, the highest BMI (although there was no difference in skinfolds, and females from the same group had the lowest daily energy intake) and the lowest consumption of sugar and sweets in grams. The association of alcohol intake and platelet MAO was not a confound by smoking: Both males and females with the lowest quartile of the platelet MAO value, who smoked less than 10 cigarettes per day, had the highest consumption of pure alcohol (8.9 g in the lowest quartile, 6.7 g with medium value and 4.5 g in the highest quartile).

Thus, low platelet MAO activity, that is often associated with higher alcohol consumption, is also associated with lower consumption of sugar and sweets and higher BMI.

Urmeli Katus, *University of Tartu*

Association between antisocial behaviour involving police contact, dietary intake and obesity in a longitudinal birth cohort study

Co-authors: **Katre Sakala**, *University of Tartu, National Institute for Health Development, Tallinn University*; **Inga Villa**, *University of Tartu*; **Inge Ringmets**, *University of Tartu*; **Jaanus Harro**, *University of Tartu*

Antisocial behaviour (ASB) is characterised by lack of empathy and social sensitivity, irresponsibility, disobedience, impulsivity and elevated reward seeking (Molero Jurado et al, Front Psychol 2017; 8: 170, Maneiro et al, Pers Indiv Diff 2017; 104: 417–422, Morgan et al, Pers Indiv Diff 2014; 63: 122–127). We previously found an association between fixation to rewards and obesity (Katus et al, Neurosci Lett 2020; 735: 135158), thus we next analysed the association between ASB involving police contact, dietary intake and measures of obesity, from adolescence to young adulthood.

The sample included both birth cohorts (originally n = 1238) of the Estonian Children Personality Behaviour and Health Study. The association between ASB, dietary intake and measures of obesity was assessed using the linear mixed-effects regression models. Models for dietary intake were adjusted to body weight and physical activity. Associations at ages 15, 18 and 25 years were analysed using the independent sample t-test. According to the regression models, subjects with ASB involving police contact (n=212) had a significantly ($p < 0.005$ for interaction) greater increase per year in body weight, body mass index (BMI), waist circumference, hip circumference, waist-to-hip ratio and waist-to-height ratio from 15 to 25 years of age. Throughout the study period, daily energy intake, protein, lipid and carbohydrate intake in grams were higher ($p < 0.005$) among subjects with ASB. Subjects with ASB also had higher ($p < 0.05$) body weight, BMI, waist circumference, waist-to-hip ratio, waist-to-height ratio, daily energy intake, protein, lipid and carbohydrate intake at ages 15, 18 and 25 years. Conclusively, ASB was associated with dietary intake and measures of obesity from adolescence to young adulthood.