# POLYMORPHISM OF *SULT2A1* GENE AND PLASMA LEVELS OF DEHYDROEPIANDROSTERONE SULFATE (DHEAS) IN INDIVIDUALS WITH NEUROCOGNITIVE DISORDER

**Nikola Balić, 2nd year student, diploma study Experimental biology, University of Zagreb Faculty of Science, Zagreb**

Supervisor: Lucija Tudor, Ruđer Bošković Institute, Zagreb

INTRODUCTION: The neurosteroid dehydroepiandrosterone sulfate (DHEAS) plays a crucial role in various brain functions, such as learning, memory, behavior, neurogenesis and neuroprotection. The research linked endogenous DHEAS levels to dementia and cognitive functions in aging. Cognitive dysfunctions were associated with both decreased and increased DHEAS concentrations in dementia patients, although the observed beneficial effects on cognition varied across studies. The study investigated the differences in the DHEAS plasma concentration and genotype distribution of rs2637125 polymorphism in *SULT2A1* gene, coding for the enzyme sulfotransferase 2A1, between 213 subjects with mild and 91 subjects with severe neurocognitive disorder, as well as the association of rs2637125 polymorphism with DHEAS concentration in plasma and their association with neurocognitive disorder severity.

MATERIALS AND METHODS: Dementia was diagnosed according to DSM-5 criteria, and cognitive symptoms were evaluated using mental state examination tests. Plasma and DNA were extracted from the blood samples of the subjects. The ELISA method was employed to measure plasma DHEAS concentration, while genotyping was performed using real-time PCR. The research received approval from the Ethics Committee of the Vrapče Psychiatry Clinic and the Ruđer Bošković Institute and was conducted in adherence to the Helsinki Declaration (1964).

RESULTS AND DISCUSSION: The study did not find significant association of *SULT2A1* polymorphism with severity of neurocognitive disorder, nor with DHEAS concentration in plasma of individuals with mild and severe neurocognitive disorder. No significant correlation between plasma DHEAS concentration and age or cognitive scores was found in subjects with dementia and MCI, except for the ADAS-Cog scale in subjects with dementia.

CONCLUSION: These results indicate that the rs2637125 polymorphism of the *SULT2A1* gene does not affect DHEAS concentration in plasma and is not related to dementia development. Moreover, the concentration of DHEAS in the plasma is not related to the age, gender and severity of the patient's cognitive impairment.

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# BENEFITS AND RISKS OF USING PARTICULAR DIETARY SUPPLEMENTS IN CANCER PATIENTS

**Mia Bašić, study of Pharmacy, University of Split School of Medicine, Split**
Supervisor: Diana Gujinović, University of Split School of Medicine, Split

INTRODUCTION: Taking into account the knowledge gap on dietary supplement use in cancer patients[1], who often reach for more "natural" preparations in public pharmacies[2], the aim of this study was to present the latest evidence on the potential benefits and safety profile of particular dietary supplements in patients being treated for various malignant diseases.

METHODS: The PubMed database was reviewed in March and July 2023; by using general keywords from the MeSH thesaurus, and then the names of different supplements of interest for which there is data on the frequency of use in such patients, or previously published relevant findings. The search was limited to a five-year period (2018-2023). We included only articles reporting studies on human subjects regardless of study design. Studies focused on food, animal experiments, cancer risks and prevention, and different interventions changing the sensitivity of cancer cells to nutraceuticals were excluded.

RESULTS AND DISCUSSION: We found evidence of potential benefits of berberine and epigallocatechin-3-gallate used along with radiotherapy, beta-glucan, and curcumin with doxorubicin, and probiotics after surgical resection of gastrointestinal cancer. Recently, nanotechnology-based delivery systems have received tremendous attention as novel approaches for overcoming challenging solubility, bioavailability, targeting, and toxicity of antitumor natural products[3]. So far, curcumin, astaxanthin, quercetin and rutin, as well as omega 3 fatty acids, have been successfully prepared as nanoformulations.

CONCLUSION: In order for clinical recommendations to be once put into practice, more high-quality studies on the benefits and harms of using dietary supplements in oncology patients are needed, as well as standardization of useful products in terms of correct formulation and dosage, and recognition of all indications for which they could be applicable.

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# BRAIN - DERIVED NEUROTROPHIC FACTOR (BDNF): POTENTIAL THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE

**Kristina Bišof, 5th year student of Medical Biochemistry, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb**

Supervisor: Marcela Konjevod, PhD, Laboratory of Molecular Neuropsychiatry, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb

INTRODUCTION: Alzheimer's disease (AD) is a progressive neurodegenerative disease. Neurobiological changes associated with AD appear decades prior to symptom onset. In some cases, mild cognitive impairment (MCI) precedes AD. Earlier diagnosis, before significant cognitive decline, would allow for earlier intervention and a better quality of life for those affected. The aim of this study was to investigate the role of brain-derived neurotrophic factor (BDNF) as a potential biomarker and therapeutic target for AD.

MATERIALS AND METHODS: This study included 153 participants (69 AD patients, 84 MCI patients). Cognitive decline was assessed by psychometric tests. Whole blood samples were processed in order to obtain platelet-poor plasma, which has been used for the determination of BDNF concentration. Genomic DNA and total RNA were isolated from peripheral blood. Genotyping of *BDNF* polymorphisms (rs6265 and rs56164415), and determining relative *BDNF* expression were carried out on the ABI Prism 7000 Sequencing Detection System apparatus. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethical Committee of the University Psychiatry Hospital Vrapče.

RESULTS AND DISCUSSION: Plasma BDNF concentration was significantly increased in AD patients compared to MCI subjects, while negative correlation was observed between plasma BDNF concentration and psychometric test scores. Furthermore, lower *BDNF* gene expression was noticed in patients with AD compared to MCI subjects. These findings might suggest compensatory neuroprotective mechanisms. A higher frequency of allele A (rs6265) was detected in AD patients, while subjects with the GG genotype (rs6265) and carriers of allele T (rs56164415) performed better on psychometric tests, identifying alleles A and C as risk alleles.

CONCLUSION: Identification of risk alleles may elucidate genetic factors contributing to the development of AD. Even though published data is heterogeneous, BDNF might represent a useful peripheral biomarker for monitoring cognitive decline and/or represent a potential therapeutic target. However, further studies with age-matched healthy controls are necessary.

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# THE ASSOCIATION OF *BDNF* VAL66MET AND C270T POLYMORPHISMS AND BDNF PLASMA CONCENTRATION WITH DEPRESSION AND TREATMENT RESPONSE

**Juraj Crneković, 5th year, study of Pharmacy, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb**
Supervisor: Lucija Tudor, Ruđer Bošković Institute, Zagreb

INTRODUCTION: Major depressive disorder is a common psychiatric disorder with complex pathophysiology. Current therapeutic approaches mostly involve antidepressants such as escitalopram, a selective serotonin reuptake inhibitor, and vortioxetine, which, in addition to SERT inhibition, also affects serotonin receptors. It has been proposed that lack of BDNF, which is essential for neural plasticity, is involved in the development of depression. *BDNF* polymorphisms, including Val66Met and C270T, have been associated with BDNF expression and psychiatric disorders, including depression. The aim of this study was to investigate the association of the *BDNF* Val66Met and C270T polymorphisms and plasma BDNF concentrations with the development and severity of depression, and the effect of antidepressant drugs.

MATERIALS AND METHODS: Depression and its severity were evaluated according to DSM-5 criteria and using HAMD-17 and MADRS scales. Plasma and DNA were extracted from the blood samples of the 120 healthy subjects and 119 subjects with depression. Genotyping was performed using real-time PCR, while plasma BDNF concentration was measured with the ELISA method before and after 4 weeks of therapy. The research was approved by the Ethics Committee of the University Hospital Center Zagreb and Ruđer Bošković Institute.

RESULTS AND DISCUSSION: The research confirmed the decreased concentration of BDNF in the plasma of depressed patients compared to controls. Both vortioxetine and escitalopram, proved to be effective antidepressants, but only vortioxetine increased BDNF plasma concentration after 4 weeks. The Val66Met polymorphism, in contrast to the C270T, affected the basal concentration of BDNF in plasma, but none of the investigated polymorphisms have been associated with the development and severity of depression, or to response to therapy.

CONCLUSION: The decrease of plasma BDNF in subjects with depression and its elevation after 4 weeks of vortioxetine therapy showed that BDNF could be used as potential peripheral biomarker of depression and therapeutic target.

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# STUDENTS' PROJECT OF DRUG DEVELOPMENT: TrkB AGONIST AS NEW POTENTIAL TREATMENT FOR DEPRESSION IN ALZHEIMER'S DISEASE

**Asja Fajtović**, **Ivana Trbojević, Mislav Vrdoljak,** 5th year, study of Pharmacy, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb
**Ivana Prpić**, 5th year, study of Medicinal Biochemistry, University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb
Supervisor: Lidija Bach-Rojecky, University of Zagreb Faculty of Pharmacy and Biochemistry

INTRODUCTION: Besides cognitive impairment, untreated depression exerts high negative impact on therapeutic outcomes in patients with Alzheimer's disease (AD). The aim of this project is to develop novel TrkB agonist which will ameliorate depressive-like behavior and improve cognitive funtions in preclinical model of AD.

MATERIALS AND METHODS: After designing the molecule named imiadep, molecular docking experiments revealed high affinity for TrkB receptors, and pharmacodynamic profile was confirmed on SH-SY5Y human neuroblastoma cells. *In vivo* pharmacokinetic and toxicokinetic studies were conducted on C57BL/6 mice. Proarrhythmic potential was determined on guinea pig hearts, while genotoxicity was tested with Ames test. *In vivo* studies were conducted on adult 3xTg-AD mice to test antidepressant activity and influence on cognitive funtions employing the model of the chronic mild stress and Morris water maze and passive avoidance test, respectively. *Post-mortem* analysis of Aβ plaques was performed by immunohistochemical staining of brain slices. All experiments were conducted in accordance with EU guidelines (Directive 2010/63) and were approved by the local Ethics committee.

RESULTS AND DISCUSSION: Imiadep demonstrated high affinity for TrkB receptor, slow dissociation rate, good blood-brain barrier permeability and bioavailability. Toxicity studies revealed gastrointestinal side effects in higher doses. Pharmacokinetic profile was acceptable, with intensive hepatic metabolism (CYP2D6 oxidation and glucuronidation), and renal elimination. Drug and metabolites showed no proarrhythmic potential and no signs of genotoxicity. The behavioral and memory tests showed significant reduction of depression-like behaviors and slight improvement in cognitive function in mice treated with imiadep compared to positive control (sertraline). Furthermore, immunohistochemical analysis revealed fewer Aβ plaques in brain slices than control group.

CONCLUSION: Preclinical *in vitro* and *in vivo* studies showed that imiadep, the first oral TrkB agonist, has an acceptable pharmacological profile with efficacy in the treatment of depressive and cognitive symptoms in transgenic animal model of AD. These results suggest that imiadep could be a new antidepressant for AD patients.

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# ASSESSMENT OF TRANSTHYRETIN EXPRESSION, A POTENTIAL CNS NEURO-PROTECTIVE AGENT, IN DIFFERENT EXPERIMENTAL APPROACHES OF A WELL-ESTABLISHED RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

**Robert Kranjčec, 5th year student, study of Medicine, University of Zagreb School of Medicine, Zagreb**

Supervisor: Ana Babić Perhoč, Department of Pharmacology, University of Zagreb School of Medicine & Croatian Institute for Brain Research, Zagreb

INTRODUCTION: Sporadic Alzheimer's disease (sAD) is a neurodegenerative disorder characterized by a complex multifactorial pathogenesis, presenting challenges in animal modeling of the disease. Here, we investigated the effect of intracerebroventricular administration of streptozotocin[1] (STZ-icv) on transthyretin (TTR), a protein produced in the liver and choroid plexus, which has been attributed with a neuroprotective role in sAD[3]. Our aim was to investigate the relationship between STZ-icv-induced sAD and TTR dysfunction, and to examine TTR's neuroprotective capacity in this model.

MATERIALS AND METHODS: 3-month-old male C57BL/6J mice (n=42), were stratified into seven experimental groups to receive icv vehicle (control) or STZ at varying administration protocols (unilateral/bilateral, low/high dose). Cognitive function was evaluated using the Novel Object Recognition (NOR) test 30 days post-injection. Leptomeningeal amyloidosis was analysed using Congo Red staining and CNS TTR expression by immunohistochemistry. Ethical approval: CLASS:UP/I-332-01/22-01/47; UP-NUMBER:525-09/589-23-5.

RESULTS AND DISCUSSION: STZ animals showed decreased TTR expression within the CP; however, a detectable level of TTR was retained across all groups, indicating that STZ did not completely suppress TTR expression. TTR expression increased in the periventricular region of the hypothalamus in STZ-treated mice, suggesting a possible compensatory response to STZ-icv-induced injury. Leptomeningeal amyloid deposition was more pronounced in STZ-receiving groups and the effect was dose-dependent, supporting STZ's proamyloidogenic effect[2]. In the NOR task, STZ-mice demonstrated an exploration ratio closer to 0.5, suggesting a detrimental effect of STZ on recognition memory. Notably, control groups also manifested diminished exploration, indicating potential overcomplexity in the NOR task design.

CONCLUSION: Results demonstrate STZ's ability to alter the expression of TTR in the CP and other regions of the brain without causing total suppression. This discovery is significant for the upcoming phases of the project, where further suppression of TTR through viral gene silencing and stabilization of transthyretin in the sAD model will be investigated, thereby enhancing our understanding of TTR's role in sAD.

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**Project title**: The role of transthyretin in sporadic Alzheimer's disease-associated leptomeningeal and cerebrovascular amyloidosis and neuroprotective potential of a brain directed tafamidis prodrug – TransADamis. **Funding**: Pfizer Inc., Global TTR Amyloidosis Research ASPIRE grant

# GLUCOCORTICOIDS: CLOSE TO OR STILL FAR FROM COMPLETE UNDERSTANDING OF THEIR EFFECTS

**Laura Kunjašić, study of Pharmacy, University of Split School of Medicine, Split**
Supervisor: Diana Gujinović, University of Split School of Medicine, Split

INTRODUCTION**:** Considering the clinical significance of corticosteroids, a unique and very complex group of drugs with a limiting safety profile, the aim of this study was to identify novel insights into mechanisms of numerous genomic, but also rapid non-genomic effects, about which little is known[1].

METHODS: The PubMed database was reviewed in April 2023 using the MeSH thesaurus; first associating terms related to glucocorticoids (GCs) with phrases implying recent findings, and then with terms "non-genomic" and "nongenomic". To ensure that the data is up-to-date, the search was limited to a five-year period (2018-2023). We did not set the study design as an exclusion criterion.

RESULTS AND DISCUSSION: Since GC responses were demonstrated to be species- and cell-type-specific, achieving tissue-specific drug delivery might boost the efficacy and safety of GC therapy[2]. Also, the beneficial effects of GCs may be enhanced by designing formulations that target specific non-coding RNAs, recently shown to also be involved in the regulation of transcriptional activity of the GC receptor[3]. Emerging evidence indicates that GC non-genomic actions have been implicated in neuronal, immune, behavioral, metabolic, and ion-regulatory physiologic systems. GINA 2021 Strategy Report implemented these findings by recommending ICS-formoterol as the starting treatment even in mild disease since it was shown that GCs, along with delayed effects, can also rapidly enhance the effects of bronchodilators. Calcium mobilization, ROS, and muscle tone were identified as their non-genomic targets[4]. Also, recent findings on the glucocorticoids' impact on immune cells through regulatory T cells[5], paradoxical pro-inflammatory effects, and metabolic alterations, all suggest potential changes in GC treatment in the future.

CONCLUSION: Characterizing and understanding of these multiple mechanisms will be critical for developing GC therapies, used across all medical specialties, that are more targeted and have reduced adverse effects, which might not restrict their use to an important extent.

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# SINGLE-WALLED CARBON NANOTUBES CHEMICALLY FUNCTIONALIZED WITH POLYETHYLENE GLYCOL IN TRAUMATIC BRAIN INJURY IN MICE: EFFECTS ON TISSUE REPAIR AND SYNAPTIC PLASTICITY

**Viktorija Mihalić, 2nd year, diploma study of Sanitary Engineering, University of Rijeka School of Medicine, Rijeka**
Supervisor: Petra Dolenec, University of Rijeka School of Medicine, Rijeka

INTRODUCTION**:** Traumatic brain injury (TBI) is one of the main causes of death and disability in young people and represents an important public health and socioeconomic problem. However, effective neuroprotective therapy still does not exist. The aim of this research was to investigate the impact of single-walled carbon nanotubes functionalized with polyethylene glycol (SWCNT-PEG) on neuronal loss, neurodegeneration, myelination, microglial and astrocytes response, and synaptic changes in the mice brain, five weeks after TBI.

MATERIALS AND METHODS: Single moderate TBI was induced using a lateral fluid percussion injury model. SWCNT-PEG or control solution (PEG) was injected into the injury site one week after the TBI or sham injury. Mice were sacrificed and brains were prepared for histological and immunohistochemical methods. The use of animals was approved by the Faculty's Animal Welfare Committee and by the Veterinary Department of the Ministry of Agriculture, and has been conducted in the accordance with the Croatian Animal Protection Act, which has been matched with the current European Union legislation.

RESULTS AND DISCUSSION: Results indicated a significant loss of neurons in parietal cortex (PC), disturbances in myelin integrity in white matter tracts, and the presence of neurodegeneration after TBI. The application of SWCNT-PEG resulted in reduced neuronal loss and possibly reduced pathological changes in white matter tracts. No significant effects of TBI or SWCNT-PEG application on astrocytes and the number of microglia cells were detected in PC and hippocampus, although morphological changes of microglia were noticed in PC. Synaptic changes were not detected in the PC, but decrease in synaptophysin signal was significant in the hippocampus after TBI, however, the administration of SWCNT-PEG did not significantly affect these changes. The results did not indicate negative effects of SWCNT-PEG on brain tissue.

CONCLUSION: This preliminary research indicates the neuroprotective potential of a single application of SWCNT-PEG after the TBI and possible impact on microglia.

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# GENERATING A NOVEL PARKINSON'S DISEASE ANIMAL MODEL BY INTRASTRITAL APPLICATION OF DIABETOGENIC SUPSTANCE

**Vito Papić, 4th year, study of medicine, University of Zagreb School of Medicine, Zagreb**
Supervisor: Jelena Osmanović Barilar, University of Zagreb School of Medicine, Zagreb

INTRODUCTION: Type 2 diabetes has recently been proposed as a risk factor for Parkinson's disease (PD) with shared similarities in dysregulated metabolic pathways[1]. The aim of this project is to examine whether metabolic dysfunction caused by direct application of a diabetogenic substance, streptozotocin (STZ)[2], to the corpus striatum, a brain region affected in PD, can induce symptoms characteristic of PD (motor deficit, cognitive and behavioral alterations)[1].

MATERIALS AND METHODS: Adult Male Wistar rats were intrastriatally administered with STZ (unilaterally or bilaterally, 0.75 or 1.5 mg/kg per striatum; STZ) or vehicle (CTR). One month later, cognitive, behavioural, metabolic, and motor functions were tested by Rota Rod, Novel Object Recognition, Passive Avoidance, Morris Water Maze, and CatWalk. Levels of tyrosine hydroxylase (TH), insulin receptor (IR), AMPA receptor (AMPAR) and choline acetyltransferase (ChAT) were measured in the hippocampus and striatum using the western blot technique.

RESULTS AND DISCUSSION: STZ rats showed deficits in motor functions, spatial learning and memory, fear conditioning, and memory recognition. The deficit was found more pronounced after bilateral administration in comparison to unilateral. STZ decreased levels of TH and IR in the stratum independently of the administration method and dose, while TH levels remained unchanged in the hippocampus. On the other hand, the levels of AMPAR and ChAT were found increased only after unilateral STZ administration in the striatum while in the hippocampus increment of AMPAR levels were seen after bilateral and unilateral application of the low STZ dose.

CONCLUSION: Results demonstrate the development of hallmark PD symptoms (motor, cognitive, and neuropathological) after intrastriatal STZ administration, indicating its possible use as a new PD model, especially after bilateral application of a low STZ dose, due to the impairment being greater the higher the dose administered.

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# ORAL MANIFESTATIONS OF SJÖGREN'S SYNDROME: TREATMENT OPTIONS

**Lana Premuž, 5th year, study of Dental Medicine, University of Zagreb School of Dental Medicine, Zagreb**
**Antonija Vlahek, 5th year, study of Dental Medicine, University of Zagreb School of Dental Medicine, Zagreb**
Supervisor: Kristina Peroš, University of Zagreb School of Dental Medicine, Zagreb

INTRODUCTION: Sjögren's syndrome (SS) is a chronic, inflammatory, autoimmune disease causing irreversible exocrine gland damage. Hyposalivation, the most important oral symptom of SS, is experienced by impacted individuals and causes xerostomia (a sensation of oral dryness) as well as problems with speech, swallowing and eating. Patients with SS are at risk of developing oral mucosal inflammation and progressive dental decay. Currently, treatment of primary SS focuses on relieving symptoms rather than altering the course of the disease. The aim of this study is to give an overview of safe and efficient oral treatments for SS.

MATERIALS AND METHODS: A systematic literature search was conducted from October to November 2023. Databases included were: PubMed, Cochrane, Proquest, Serial solutions and Wiley Online Library. The keywords were „Sjögren's syndrome", „oral treatment", and „xserostomia" without timespan limits. Two researchers were looking for recommendations, assessment, development and evaluations of published studies.

RESULTS AND DISCUSSION: Systematic search identified 23 relevant trials all of which studied possible oral treatments of Sjögren's syndrome. 13 of screened trials were randomized controlled studies, 6 of them were clinical trials, 2 were reviews and there was 1 systematic review as well as 1 case report.

CONCLUSION: Possible oral treatments of Sjögren's syndrome include: artificial saliva, sialendoscopy, cholinergic agents such as pilocarpine and cevimeline, seletalisib, lozenges of malic acid, sodium carvonate oral spray, iguratimod, corticosteroid irrigation and traditional Chinese medicine. In some studies, little to moderate effect has been shown by rituximab, but its cost and questionable effectiveness makes it non recommendable to be used as an oral treatment. Ineffective oral treatments of Sjögren's syndrome include: tocilizumab, omega-3 and vitamin E supplementation and low-laser therapy. Promising results as oral treatments of SS have been shown by: Lactobacillus acidophilus and propionate, combination of prednisone and hydroxychloroquine and monoclonal antibodies against CD20, BAFF, BAFF receptors. However, further studies are necessary.

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# REGION-SPECIFIC DISTRIBUTION AND ACTIVATION OF INSULIN RECEPTOR SIGNALING AFTER ACUTE INTRANASAL INSULIN ADMINISTRATION IN THE RAT BRAIN

**Leonarda Vlahov, 1st year, student of diploma study Molecular biology, University of Zagreb Faculty of Science, Zagreb**
Supervisor: Ana Knezović, University of Zagreb School of Medicine, Department of Pharmacology, Zagreb

INTRODUCTION: Impaired insulin signaling is associated with many neurodegenerative diseases. New insights point to a role of insulin in brain metabolism, but the molecular mechanisms of insulin central effects and its distribution after intranasal (IN) administration are still unknown. This project investigates the time-dependent insulin distribution in different brain areas after IN administration and its effect on insulin signaling activation.

MATERIALS AND METHODS: Male Wistar rats were given regular insulin intranasally in both nostrils (10 μL per nostril, 2 IU in total) and animals were sacrificed 3, 7.5, 15, 30, 60 and 120 minutes after administration. Insulin and glucose concentration were measured in plasma and CSF, while levels of insulin, insulin receptor substrate (IRS) and its phosphorylated forms (pIRS307 and pIRS612) were measured in olfactory and respiratory epithelia (OE and RE), olfactory bulb (OFB), cerebellum (CB), brain stem (BS), hypothalamus (HPT), hippocampus (HPC), striatum (S) and frontal, parietal and temporal cortex (FC, PC, TC).

RESULTS AND DISCUSSION: Compared to the control, insulin concentration was found increased at earliest time point (3 min) in all observed brain regions and epithelia and remained increased after 7.5 min in TC and CB and in epithelia after 7.5, 15 and 30 min. Plasma and CSF glucose was found unaltered in all time-points. IRS was found activated (p/t IRS612): immediately after IN administration (3 min) in TC and S, in HPC, CB and OFB 7.5 min after, and in FC and PC, 30 min after IN administration. Activation was immediately followed by inactivation (p/t IRS307) in these regions. IRS was found unaltered in HPT in all of the time-points and inhibited in BS.

CONCLUSION: Insulin was distributed immediately after IN application throughout the brain and it was quickly utilized, without any peripheral glucose concentration changes. Activation of insulin signaling varies across the brain regions pointing to their different responses to insulin.

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# A COMPARISON OF CLINICAL GUIDELINES AND COCHRANE SYSTEMATIC REVIEWS ON THE PHARMACOTHERAPY OF HYPERTENSION

**Sara Vuko, 5th year student, study of Pharmacy, University of Split School of Medicine, Split**
Supervisor: Darko Modun, University of Split, School of Medicine, Split

INTRODUCTION: Hypertension is one of the most prevalent diseases in the modern world and contributes markedly to cardiovascular deaths. Many patients worldwide need to have well-established guidelines on this topic. Guidelines by the American College of Cardiology (ACC)/American Heart Association (AHA) and the European Society of Hypertension (ESC)/European Society of Hypertension are created by a number of experts in the field trying to yield optimal recommendations to fit the needs of millions of patients. Cochrane Reviews is a tool addressing various questions in medical research based only on the quality of evidence with predefined criteria. However, it is finding its way into drug prescribing practice. The aim of this study was to compare Cochrane Reviews and the two guidelines regarding the therapy of hypertension.

MATERIALS AND METHODS: We investigated the following parameters and cofactors: threshold, first-line treatments, single pill formulations, comorbidities, age, race, as cofactors therapy-resistant form of hypertension, and cardiovascular risk calculation.

RESULTS AND DISCUSSION: Unlike Cochrane Reviews, both AHA and ESH in their treatment strategy put emphasis on single-pill combinations in the initial treatment, to improve adherence. Moreover, they recommend different first-line treatments, based on the severity of hypertension and cofactors, while Cochrane Reviews recommends thiazide diuretics in every circumstance or does not address a particular cofactor.

CONCLUSION: Evidently, there is more room for treatment alterations for patients with more special needs and underlying conditions by following guidelines than Cochrane Reviews.

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