

CROATIAN INTERNATIONAL PUBLICATIONS

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Ristić S¹, Čizmarević NS², Sepčić J³, Kapović M², Peterlin B⁴. Angiotensin-converting enzyme insertion/deletion gene polymorphism in multiple sclerosis: a meta-analysis. *Neurol Sci.* 2016 Aug 27. [Epub ahead of print]

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The activity of angiotensin-converting enzyme (ACE) has been increased in the blood and cerebrospinal fluid of multiple sclerosis (MS) patients. In addition, there has been suppression of disease development in experimental autoimmune encephalomyelitis after blockade of ACE. These findings suggest that ACE may play a role in the MS pathogenesis. Since the previous studies investigating the association between the insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene and MS reported contradictory results, we performed a meta-analysis of four studies conducted in European populations of Slavic origin (1062 patients and 1067 controls) using the Comprehensive Meta-analysis 3.0 software. The results demonstrated that the ACE I/D polymorphism had no statistically significant association with an increased MS risk (all $p \geq 0.05$) under all genetic comparison models: (1) allelic (D vs. I), (2) recessive (DD vs. ID + II), (3) dominant (DD + ID

vs. II), and (4) additive (DD vs. ID vs. II). This meta-analysis indicates that the ACE I/D polymorphism is not associated with susceptibility to MS in Europeans of Slavic origin. Further studies with larger sample sizes from genetically different populations are warranted.

Mišir M^{1,2}, Renić M³, Mihalj M², Novak S², Drenjančević I². Is shorter transient middle cerebral artery occlusion (t-MCAO) duration better in stroke experiments on diabetic female Sprague Dawley rats? *Brain Inj.* 2016 Aug 19:1-7. [Epub ahead of print]

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AIM: To determine optimal duration of transient middle cerebral artery occlusion (t-MCAO) for a stroke model in female diabetic Sprague-Dawley (SD) rats. METHODS: Streptozotocin-induced type-1 diabetic SD female rats ($n = 25$, 12 weeks old, five groups; $n = 5$ per group) were subjected to different duration of t-MCAO (20, 30, 45, 60 and 90 minutes) followed by reperfusion. A control group of rats without diabetes ($n = 5$) was subjected to 30 minutes of t-MCAO followed by reperfusion. Twenty-four hours after reperfusion, infarct volumes were evaluated by 2,3,5-

triphenyltetrazolium chloride (TTC) staining. RESULTS: Intra-ischaemic reductions of regional cerebral blood flow (rCBF) were similar in all groups (68-75% of baseline values). Reperfusion was significantly impaired in the 90-minute ischaemia group (56-62% vs 80-125% in other groups). Twenty minutes of t-MCAO induced a small infarct ($3 \pm 5\%$ of ischaemic hemisphere). Thirty minutes of ischaemia produced a significantly larger infarct ($46 \pm 6\%$). In the 45 and 60 minute groups, ischaemia infarct was $52 \pm 5\%$ and $59 \pm 3\%$ of the ischaemic hemisphere, respectively. Ischaemia of 90' led to a massive stroke ($89 \pm 6\%$ of ischaemic hemisphere encompassing the whole striatum ($22 \pm 3\%$) and almost the whole MCA irrigated cortex area ($67 \pm 6\%$)). Thirty minutes of t-MCAO did not produce stroke in the control group. CONCLUSION: The diabetic rat stroke model should be different from the non-diabetic, because female type-1 diabetic SD rats are highly sensitive to brain ischaemia and it is necessary to significantly shorten the duration of t-MCAO, optimally to 30 minutes.

Duvnjak L¹, Blaslov K². Statin treatment is associated with insulin sensitivity decrease in type 1 diabetes mellitus: A prospective, observational 56-month follow-up study. J Clin Lipidol. 2016 Jul-Aug;10(4):1004-10

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BACKGROUND: Statins are effective in the primary and secondary prevention of cardiovascular events in individuals with and without diabetes. Emerging evidence, however, suggests that statins might reduce insulin sensitivity and secretion in healthy population and in type 2 diabetes. OBJECTIVE: We aimed to investigate the effect of statin therapy introduction on insulin sensitivity in patients with type 1 diabetes mellitus (T1DM). METHODS: This prospective observational 56-month long study included 832 randomly selected T1DM patients aged 25 to 61 years. Uncontrolled dyslipidemia and clinician-perceived need for treatment, rather than randomization, were basis for individuals being started on either atorvastatin or simvastatin (10-40 mg); N = 345, 41.47%. Patients on statin treatment were compared with those unexposed to statin. Insulin sensitivity was assessed using equation derived from euglycemic-hyperinsulinemic clamp studies-estimated glucose disposal rate. RESULTS: Patients who started statin therapy (N = 345,

59.42% atorvastatin and 40.58% simvastatin) experienced a greater decrease in insulin sensitivity (19.27% vs 12.82% P < .001) and metabolic control deterioration compared with statin-free group. The risk of decrease in insulin sensitivity attributable to statin use was 36.7% (hazard ratio 1.36; 95% confidence interval 1.31-1.43) after adjustment for age, gender, disease duration, smoking status, and the concomitant antihypertensive therapy. CONCLUSION: Although there is still a lack of a clear molecular explanation on the adverse effects of statin therapy on insulin sensitivity, we showed that it deteriorates insulin sensitivity in T1DM. The cardiovascular benefits of statin treatment might outweigh the risk of developing insulin resistance, but, the possible metabolic control worsening merits to be considered.

Peric Kacarevic Z¹, Snajder D², Maric A³, Bijelic N⁴, Cvijanovic O⁵, Domitrovic R⁶, Radic R⁷. High-fat diet induced changes in lumbar vertebra of the male rat offsprings. Acta Histochem. 2016 Aug 27. [Epub ahead of print]

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In obesity, bone marrow adiposity increases and proinflammatory cytokines excretion activates RANK/RANKL/OPG system, which leads to increased bone resorption. The aim of this study was to analyze trabecular and cortical bone parameters in animals exposed to the high-fat

diet in utero and after lactation. Skeletal organ of interest was the fifth lumbar vertebra, which is not exposed to bio-mechanical loading in rats. Further aims were to determine TNF- α and IL-6 serum concentrations, and the intensity of the TNF- α immunohistochemical staining in the bone marrow. Ten female Sprague Dawley rats, nine weeks old, were randomly divided in two groups and fed either standard laboratory chow or food rich in saturated fatty acids during five weeks, and then mated with genetically similar male subjects. After birth and lactation male offsprings from both groups were divided in four subgroups depending on the diet they were fed until twenty-two weeks of age. The highest cholesterol and triglyceride concentration were found in both groups of offsprings fed with high-fat diet. The lowest trabecular bone volume, lowest trabecular number and highest trabecular separation were found in offsprings fed with high-fat diet of mothers on standard laboratory chow. The same group of offsprings was also characterized by the highest intensity of TNF- α immunostaining in the bone marrow and the highest TNF- α serum concentration, which suggest that this proinflammatory cytokine has interfered with bone metabolism, possibly by stimulation of bone resorption, which led to inadequate trabecular bone development and bone modeling of the fifth lumbar vertebra.

Šafranko ŽM¹, Balog T¹, Musa M², Bujak IT³, Sobočanec S⁴. The effect of 17 β -estradiol on sex-dimorphic cytochrome P450 expression patterns induced by hyperoxia in the liver of male CBA/H mice. *Mol Cell Biochem.* 2016 Aug 31. [Epub ahead of print]

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The aim of this study was to determine whether treatment of male CBA/H mice with 17 β -estradiol (E2) had protective effect on survival and hepatic oxidative damage of lipids and proteins against hyperoxia. Furthermore, we wanted to explore the effect of E2 treatment on the expression of sex-specific cytochrome P450 isoforms, and their possible involvement in E2-induced resistance to

hyperoxia. Lipid peroxidation and protein carbonylation were analysed spectrophotometrically and were used as a measure of lipid and protein oxidative damage. Real-time PCR and western blot analysis were used to measure both gene and protein expression levels of Cyp2E1, Cyp7B1 and Cyp2A4, respectively. We found that treatment of male CBA/H mice with E2 increased survival upon hyperoxia exposure, and provided protection against hepatic lipid and protein oxidative damage. Hyperoxia had feminizing effect on the expression of sex-specific CYPs, which resembled the lifespan-promoting conditions. E2 administration had the opposite effect on the expression pattern of these CYPs in hyperoxic versus normoxic conditions. Results of this research proposed possible male strategy in adaptive response to oxidative stress, which may finally result in their longer lifespan.

Ćurko-Cofek B¹, Kezele TG¹, Marinić J², Tota M², Čizmarević NS³, Milin Č³, Ristić S³, Radošević-Stašić B⁴, Barac-Latas V¹. Chronic iron overload induces gender-dependent changes in iron homeostasis, lipid peroxidation and clinical course of experimental autoimmune encephalomyelitis. *Neurotoxicology.* 2016 Aug 26;57:1-12. [Epub ahead of print]

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To analyze iron- and gender-dependent mechanisms possibly involved in pathogenesis of multiple sclerosis (MS) in this study we evaluated the effects of iron overload (IO) on iron status and lipid peroxidation processes (LPO) in tissues of female and male DA rats during chronic relapsing experimental autoimmune encephalomyelitis, a well-established MS animal model. Rats were treated by iron sucrose (75mg/kg bw/day) or with saline solution during two weeks before the sensitization with bovine brain homogenate in complete Freund's adjuvant. Clinical signs of EAE were monitored during 29 days. Serum and tissues of CNS and liver were sampled before immunization and at day 13th post immunization (during acute phase of EAE). The

determination of ferritin, iron, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) and evaluation of histopathology were performed by ELISA, ICP spectrometry and immunohistochemistry. Results showed that IO in female EAE rats accelerated the onset of disease. In contrast, in male rats it accelerated the progression of disease and increased the mortality rate. During acute phase of EAE female IO rats sequestered more Fe in the liver, spinal cord and in the brain and produced more ferritin than male EAE rats. Male rats, however, reacted on IO by higher production of MDA or 4-HNE in the neural tissues and showed greater signs of plaque formation and gliosis in spinal cord. The data point to sexual dimorphism in mechanisms that regulate peripheral and brain iron homeostasis and imply that men and women during MS might be differentially vulnerable to exogenous iron overload.

