cleavage that releases the second HCV protease NS3, without this cleavage the virus cannot replicate any further. The lack of an automatable screening assay has been a major problem in identifying chemicals against HCV NS2. Herein, a cell-based assay for HCV NS2 cis-protease activity was validated, miniaturized and automatized for 384 well plate for high throughput screening (HTS). A targeted library of 2000 cysteine protease inhibitors were tested against the HCV NS2 protease activity. A counterscreen was developed and used to filter out the false positive hits. The remaining hits were validated in a secondary screen using the HCV cell culture system with full length and subgenomic replicons. Using bioinformatics techniques, a series of structure – activity relationship (SAR) were tested further. We identified chemical scaffolds with potential different modes of action due to their different effect on the NS2-NS3 site processing. In conclusion, it is presented the first cell based HTS assay for HCV NS2 cysteine protease activity which identified new chemicals confirming HCV NS2 as a druggable target.

P.09-047-Tue
Moderate physical activity alters cardiac lipid metabolism of male rats on high fructose diet

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Metabolic syndrome is a growing health problem worldwide associated with modern day diet, rich in saturated fat and sugar. Increase in fructose consumption contributes to ectopic lipid storage which in turn is a risk factor for development of type 2 diabetes and cardiovascular disease. Defective substrate utilization in the heart caused by systemic derangements in metabolic syndrome leads to cardiac dysfunction. Exercise is shown to alleviate most of the symptoms related to this metabolic disorder. The aim of this study was to analyze the impact of low intensity exercise on molecular mechanism of cardiac free fatty acid (FFA) transport and metabolism and serum lipid profile of fructose fed male rats. Male Wistar rats were divided into control group and two groups that received 10% fructose for 9 weeks, one of which was additionally exposed to low intensity exercise. Concentration of circulating FFA, triacylglycerol (TAG), total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were measured as well as TAG content in heart tissue. Protein content of fatty acid translocase CD36, carnitine palmitoyl transferase 1 (CPT1) and Lipin1 in cardiac lysate and CD36 content in plasma membrane were evaluated. High fructose diet elevated serum TAG and showed a trend of increase of total cholesterol ($P = 0.066$). Exercise didn't change TAG and total cholesterol, but FFA were decreased compared to control group. Although there was no change in protein expression of CD36 transporter, exercise raised the level of CD36 in plasma membrane compared to fructose group. Exercise increased protein expression of both CPT1, mitochondrial transporter of FFA, and Lipin1, enzyme involved in TAG synthesis, compared to control group. High fructose consumption changed serum lipid levels, whereas low intensity exercise had greater influence on increasing the transport and metabolism of FFA in the heart.

P.09-048-Wed
Oxadiazon-induced toxicity in HCT116 cells: involvement of oxidative stress and apoptosis

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Pesticides are chemical compounds used to control organisms considered to be harmful in agriculture and also in other sectors. They are ubiquitous in the environment and exposure to these compounds is suspected to increase the incidence of certain pathologies especially, cancer. At present, exposure to pesticides through food is attracting increasing interest. In this study, we were interested in an Oxadiazole family herbicide, Oxadiazon. Although many studies are available regarding its environmental and ecological impact, very little is known about its toxicity in the mammalian system. The aim of this study was to investigate the toxic effect of Oxadiazon on intestine using an in vitro model (HCT116). Therefore, we evaluated the cell viability, elucidated the generation of free radicals, measured the mitochondrial membrane potential, and valued DNA fragmentation. Our results showed that Oxadiazon is cytotoxic to HCT116. It causes damage through the generation of free radicals and induces lipid peroxidation and DNA damage. We also demonstrated that such effects can be responsible for Oxadiazon-induced apoptosis.

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Investigation of the nucleotide metabolism and growth of *Mycobacterium smegmatis* under different genotoxic stress conditions

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The fidelity of replication is essential for every organism to maintain their genome integrity. The fine-tuned pool of deoxyribonucleoside 5'-triphosphates (deoxynucleotides, or dNTPs) is a critical factor that contributes to the accurate DNA synthesis. It has been shown that perturbation of the well conserved balance of dNTPs results in elevated mutation rate. Interestingly, the pathogenic *Mycobacterium tuberculosis* (*M. tuberculosis*) has an especially low *in vitro* mutation rate although it lacks the mismatch repair enzymes. However, *in vivo* samples from patients often exhibit resistance to more than one drugs, caused exclusively by various single-nucleotide mutations. Based on these observations, our aim is to establish the relationship between different types of genotoxic stresses and the deoxynucleotide pool size and balance in *Mycobacterium smegmatis (M. smegmatis)*. As a valid model of *M. tuberculosis*, we used the non-pathogenic *M. smegmatis*, since they share the same DNA metabolic and repair routes. Several stresses are important in the lifecycle of *M. tuberculosis*: these are oxidative stress, ionizing radiation, nutrient starvation, alkylating agents, hypoxia and currently used antimycobacterial drugs. We defined appropriate treatment period based on the growth curve of *M. smegmatis*. The concentration of chemical agents used for the treatment were specified to inhibit cell growth. dNTP concentration following genotoxic treatments were measured applying a fluorescent-based DNA-polymerization method. Our results showed that different environmental stresses cause changes in the dNTP pool size: for antimycobacterial agents there are several orders of magnitude increase in the