and/or 500 μM 5-ASA and then exposed to a combination of cytokines (IL-1α, TNF-α, IFN-γ) for a certain period of time. Nitric oxide (NO) was measured by a fluorimetric assay whereas prostaglandin E_2 (PGE_2) was evaluated by using a competitive immunoassay. The protein levels of iNOS, COX-2 and IkB-α were analyzed by Western blotting. Our data showed that both C3G and Resveratrol were able to inhibit cytokine-induced inflammation in intestinal cells, in terms of NO, PGE_2 and IL-8 production and of iNOS and COX-2 expressions, at a much lower concentration than 5-ASA, suggesting a higher anti-inflammatory efficiency of C3G and Resveratrol. Interestingly, neither of the above mentioned compounds prevents IκB-α degradation, suggesting the involvement of another cell signaling pathway. Therefore, the ability of these compounds to suppress cytokine-induced STAT1 phosphorylation has been studied. As a matter of fact, polyphenols can reach high concentrations in the gastrointestinal tract which make Cyanidin-3-glucoside and Resveratrol promising nutraceuticals, able to give complementary benefits in the context of inflammatory bowel disease.

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Involvement of Reactive Oxygen Species (ROS) in skeletal muscle function during ageing: Study in a model of isolated single skeletal muscle fibre

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Reactive oxygen species (ROS) are constantly produced in skeletal muscle and the hypothesis that ROS are involved in the process of ageing is extensively accepted. Several studies indicate that ROS might be responsible of different adaptive responses that lead to maintain muscle mass and function. However, during ageing these adaptive responses are attenuated or disrupted and ROS might be the key regulators of this patho-physiological process.

We study the effect of ROS in skeletal muscle using an *ex-vivo* physiological model, the single skeletal muscle fibre isolated from the *Flexor Digitorum Brevis* muscle. This model is suitable for the use of specific detectors for different ROS to monitor, in real time, intracellular ROS production in single skeletal muscle fibres.

We have demonstrated that contractile activity generates a net intracellular increase of ROS in single muscle fibres and the effect was abolished when glutathione was replenished. In addition, contractile activity produces an intracellular increase of nitric oxide in fibres and this effect was attenuated by treatment with nitric oxide synthase inhibitors. Moreover, contractile activity induces an intracellular increase of superoxide in muscle fibres and this effect was abolished by treatment with superoxide scavengers. In another study we have demonstrated that passive elongation of skeletal muscle fibres from young mice induced an increase in intracellular superoxide with no increase in intracellular nitric oxide. In contrast, in fibres from old mice passive elongation induced an increase in intracellular nitric oxide with no change in superoxide production. Recently, we have proved that ROS are increased in fibres from old mice at rest and, surprisingly, no further increase in ROS generation during contractile activity. The defect in short-term adaptations to contractions reported in old mice may be related to a diminished or absent increase in the muscle generation of ROS that accompanies contractile activity.

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Evidence of an interplay between ER stress/UPR and mitochondria in human hepatic cells treated with the antiretroviral drug Efavirenz

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Altered function of the endoplasmic reticulum (ER) manifested as accumulation of misfolded/unfolded proteins and/or depletion of $[Ca^{2+}]_{er}$, giving rise to a state of "ER-stress", has been related to various hepatic diseases including drug-induced hepatotoxicity. Efavirenz, a non-nucleoside analog reverse transcriptase inhibitor, is a cornerstone of the current combined anti-HIV1 therapy. Despite being generally safe, it has been associated with several adverse events including liver damage. We have recently reported involvement of mitochondrial dysfunction in this event, using human hepatic cells exposed to clinically relevant concentrations of EFV. In parallel, these cells display markers of ER-stress and UPR activation. Here, we analyzed the relation between EFV-induced ER stress and mitochondrial (dys)function in the same model. Primary human hepatocytes, the human hepatoma cell line Hep3B and rho^0 cells generated in Hep3B background (phenotype lacking functional mitochondria) were exposed to EFV (10 and 25μM) EFV for 24h. The concentration-dependent increase in both mRNA and protein expression of GADD153/CHOP (CCAAT/enhancer binding protein) and GRP78 (Glucose-regulated protein 78) was largely diminished in rho^0 cells. Similarly, unlike WT cells, rho^0 cells displayed no increase in the ER-signal (fluorescence microscopy). The specific interconnection between ER-stress and mitochondria was also shown by studying calcium levels. EFV exposure resulted in a decrease in $[Ca^{2+}]_m$ and an increase in $[Ca^{2+}]_c$, which differs from the action of a classic-stressor such as thapsigargin. Moreover TEM experiments revealed that EFV-treated cells exhibited a higher level of contact (closer location) between mitochondria and ER (both displaying altered morphology). In conclusion, human hepatic cells treated with clinically relevant concentrations of Efavirenz present markers of ER-stress with a specific involvement of mitochondria in this effect. These findings expand our knowledge of the mechanisms that trigger ER-stress and throw light on the mitochondria/ER interplay in drug-induced hepatic challenge with specific relevance for the patients undergoing EFV-containing therapy.

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SESSION 11

Beneficial role of nitric oxide in cholesterol-induced steatohepatitis and LPS-induced endotoxemia

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