

uninfected) related to this criterion produced 3 false negative and 2 false positive results, which proves that PDE/PME ratio may serve as a biomarker for HIV-1 staging and diagnosis.

**Discussion/Conclusion:** There are several biochemical processes which affected by the HIV infection could be responsible for the changes in phospholipid metabolism. What they have in common is that they are critically dependent on the structural formation of membrane proteins and their local environment, the membrane lipid. While not everything is known about these metabolic pathways, the PDE/PME ratio still can be used as a diagnostic criterion ( $PDE/PME < 0.35$  for infected cells). HIV-1 infection can be monitored noninvasively using 3D Chemical Shift Imaging to acquire 31P spectroscopic data from lymph nodes or other infected organs. Based on our preliminary results 31P 3D Chemical Shift Imaging could be used for non-invasive monitoring of HIV-1 infection (including monitoring patients with AIDS).

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### NMR analysis of the human follicular fluid composition

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**Purpose/Introduction:** Follicular Fluid (FF) is the environment of the oocyte during its maturation in vivo. It is a derivative of the sanguineous plasma and secretions synthesized in the wall of the follicle. Oocyte maturation process depends on FF composition, however exist few studies where all their components are determined. The exhaustive identification and comparison of compounds constitutive the FF can help to distinguish oocytes with greater capacity to be fertilized and, in this way, we may to obtain a greater rate of pregnancies in Fertilization in Vitro treatments. The objective of this work is to identify and (when possible) to quantify the metabolites presents in Follicular Fluid by means of high resolution Nuclear Magnetic Resonance. We analyzed FF samples of healthy ovum donors as well as patients subject to infertility treatments.

**Subjects and Methods:** For this study a total of 44 FF samples was used, 14 patients treated for artificial insemination (>35 years) and 30 donors (<35 years). These samples have been obtained by transvaginal puncture guided by ultrasound scan.

<sup>1</sup>H NMR mono-dimensional (<sup>1</sup>H 1D using several water suppression and diffusion techniques) and two-dimensional NMR homo and heteronuclear spectra (2D TOCSY, 1H-13C HSQC, CPMG, DOSY 2D) have been tested and recorded. The spectra were acquired in a 11.1 T high resolution NMR instrument equipped with cryo-probe. Several acquisition temperatures were tested but the temperature was set at 298 K in most of the spectra in order to avoid as much as possible sample degradation without loss resolution.

**Results:** Signals from a great amount of metabolites and broad peaks belonging to the most abundant macromolecules were present in each FF sample. DOSY experiments were extremely useful for separating resonances as function of the diffusion velocity of the different molecules. Thus DOSY feature has allowed to obtain spectra exclusively with macromolecules signals (much lower diffusion velocity than small metabolites). The CPMG experiments allowed us to eliminate the broad signals of the macromolecules given their fast transversal relaxation, that is their high correlation time or slow movement.

**Discussion/Conclusion:** A significant amount of signals and molecules, similarly to the ones found in serum, were assigned and identified with some specific and interesting variations. The spectra showed a large variation in some particular chemical shifts with the time, probably due to fast degradation of the sample. Significant quantitative differences were found between both groups of samples.

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### CB<sub>1</sub> cannabinoid receptor controls intermediary metabolism in rat hippocampal slices

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CB<sub>1</sub> cannabinoid receptors (CB<sub>1</sub>Rs) control neural transmission and plasticity, therefore they serve as attractive therapeutic targets (Mackie K, 2006, *Annu Rev Pharmacol Toxicol* 46:101). For instance, CB<sub>1</sub>R activation affords neuroprotection against brain insult (Jackson SJ et al., 2005, *J Neurol Sci* 233:21). Prevention of neuronal death may involve the control and rescue of the basic metabolic functions of the brain tissue. Although it has been shown that activation of CB<sub>1</sub>Rs with its selective agonist WIN55212-2 (WIN) decreases glucose utilization in hippocampal tissue (Pontieri et al., 1999, *Neuropsychopharmacol* 21:773), it remains to be determined if intermediary metabolism is also controlled by CB<sub>1</sub>Rs.

Hippocampal slices (400µm thick, from Wistar rats, 8 weeks old) were superfused for 3 hours (3 mL/min, 37 °C) with a Krebs solution containing 5.5 mM [U-<sup>13</sup>C]glucose and 5.5 mM sodium [2-<sup>13</sup>C]acetate, which is oxidized in glial cells, and gassed with 95%O<sub>2</sub>/5%CO<sub>2</sub>. 4-aminopyridine (4AP, 50 µM) was included to allow slice stimulation. The CB<sub>1</sub>R receptor agonist WIN (1 µM) and/or antagonist AM251 (0.5 µM) were tested in this system. Perchloric acid extracts of the slices were lyophilized, dissolved in D<sub>2</sub>O and pH adjusted to 7.0. Incorporation of <sup>13</sup>C atoms into different carbon positions of metabolites (metabolite isotopomers) were determined by <sup>13</sup>C-NMR spectroscopy (Varian Unity-500 spectrometer, 5mm broadband NMR probe). A <sup>13</sup>C isotopomer analysis of glutamate and GABA was performed to evaluate the role of the CB<sub>1</sub>Rs in the control of intermediary metabolism.

The CB<sub>1</sub>R agonist WIN significantly decreased the metabolism of both [2-<sup>13</sup>C]acetate (11.6+/-2.0%) and [U-<sup>13</sup>C]glucose (11.2+/-3.4%) in the tricarboxylic acid (TCA) cycle that contributes to the glutamate pool. WIN also significantly decreased [U-<sup>13</sup>C]glucose (11.7+/-4.0%) but not [2-<sup>13</sup>C]acetate metabolism contributing to the pool of GABA. These effects of WIN were prevented by the selective CB<sub>1</sub>R antagonist AM251.

Together, our results suggest that CB<sub>1</sub>Rs are able to control hippocampal intermediary metabolism in both neuronal and glial compartments, which supports their role in neuroprotection.

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### <sup>23</sup>Na multiple quantum filtered NMR characterisation of Na<sup>+</sup> binding and dynamics in animal cells

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**Purpose / Introduction:** <sup>23</sup>Na NMR is a quite useful technique for biological studies, despite the fact that part of the <sup>23</sup>Na resonance is often not detectable in conventional spectra because of quadrupolar effects. However, when the nucleus bound state is not at the extreme narrowing motional limit and