INTRODUCTION
Pathological altered renal physiology resulting from acute kidney injury (AKI) or tubular acidosis is associated with a perturbation of renal pH [1]. Clinical biomarker of kidney damage, like blood urea nitrogen (BUN) and serum creatinine denote kidney damage only after a significant loss (50%) of renal function has occurred [2]. Therefore, newer approaches able to provide reliable and non-invasive surrogate biomarkers of kidney injury as pH levels would have considerable clinical relevance. Magnetic Resonance Imaging is a primary diagnostic and imaging technique. Recently, we proposed that Iopamidol, a clinically-approved radiopaque X-Ray contrast agent (CA), can be used as a pH-responsive CA for MRI-CEST (Chemical Exchange Saturation Transfer) investigations [3]. In this study, we investigated the use of Iopamidol to monitor the disease evolution “in vivo” by imaging pH variations in glycerol-induced AKI model evaluating the effect induced by two different glycerol dosages.

METHODS
AKI model was induced in BALB/c mice (n=8, Charles River) by intramuscular injection of a 50% glycerol solution (8 mL/kg body weight and 6 ml/kg b.w.) into the inferior hind limbs. Healthy mice (n=6) were used to assess the pH base levels. A clinical dose of Iopamidol (0.75 g Iodine / kg b.w.) was injected via a catheter into the tail vein. CEST images were acquired on a 7 T scanner Avance 300 (Bruker BioSpin) using a fast spin-echo sequence preceded by a saturation pulse (3 μT, 5 sec). CEST spectra were interpolated by smoothing splines and the ratio of the saturation transfer effects at 4.2 and 5.5 ppm was used for the calculation of the pH values. Animals were imaged at the following time points: day 0 and after the glycerol injection at days 1, 3, 7, 14 and 21. Mice were then sacrificed and paraffin-fixed biopsy kidney samples were sectioned (5 μm); luminal hyaline casts, tubular damage and dilatation were assessed by light microscopy to evaluate renal histology.

RESULTS AND DISCUSSION
In control mice we observed, 15 min after Iopamidol injection, a mean pH value of 6.7. In AKI mice, pH evolution follows a slight increase in the first days (mean pH values 7.1 and 7.3 after 1 and 3 days, respectively). After pH values start to decrease (pH = 6.9 after 1 week, pH = 6.8 after 2 weeks) reverting to control values (pH = 6.7 after 3 weeks) (Fig. 1). This evolution closely followed the time evolution BUN levels with a peak after 3 days, a recovery starting after one week and with the complete restoration at day 21. AKI induced by the lower glycerol dose resulted in a lower increase of pH values during the first (pH=6.9) and third day (pH = 7.1) after damage induction. The kidney damage evolution was also observed by looking at the number of pixels showing a CEST effect: during the increase of the disease damage, this number reduced, to increase again with the recover of the kidney functionality. Kidney regions showing no CEST contrast after the AKI onset correlated well with the lesion stage associated with the tubular injury.

CONCLUSIONS
Iopamidol, a MRI-CEST CA allows to image in vivo renal pH and to follow the kidney damage evolution and recovery in a glycerol-induced AKI model. The pH changes closely followed the amount of acute renal damage induced by two different glycerol doses.

REFERENCES