Combined ASL Perfusion Imaging, BOLD Imaging and $^{31}$P NMR Spectroscopy of the Leg in a Rat Model of Peripheral Arteriopathy

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INTRODUCTION:
Peripheral arteriopathy is a major clinical entity. In this disease, gene therapy offers interesting therapeutic perspectives but also has spurred the development of new, totally non-invasive and fully quantitative evaluation tools [1,2]. Using such methodology, the objective of the present work was to investigate the relationship between energy metabolism and perfusion during the recovery phase of exercise and to characterize the natural history of chronic arteriopathy in a rat model.

MATERIALS AND METHODS:
Iliac artery excision followed 7 days later by femoral ligation was performed in male Sprague-Dawley rats weighing 344±32g~400g (n=6). Comparable animals underwent a sham-operation and constituted the control group (n=5). At successive time-points after surgery, at weeks 1,2,3 and 5, the two groups underwent two NMR examinations in a 4T spectrometer, the first to evaluate oxidative phosphorylation by $^{31}$P NMRS, the second to measure muscle perfusion and capillary oxygenation by ASL-NMRI. Under pentobarbital anesthesia, isometric contractions were induced using percutaneous electrodes and a Compex current generator. The protocol consisted in 1-min stimulation at 100Hz with supra-maximal intensity. The rat paw was attached to the pedal of a custom-built ergometer and the force developed by the rat leg was continuously recorded. $^{31}$P spectroscopy was carried out with a surface coil facing the calf. The creatine rephosphorylation time constant (TCr) was calculated from the analysis of series of 8 s spectra (4 transients) collected during recovery from exercise. The following day, exactly the same stimulus procedure was applied to perform perfusion imaging with a SATIR sequence [3], using a discrete cosine body transmitter coil and an orthogonal surface receiver coil below the calf. Perfusion profiles were monitored over 4 min post exercise. Quantitative perfusion data were obtained from the analysis of the differences in leg muscle signal intensity between pairs of selectively and non-selectively labelled images. Peak (maximum) post-exercise perfusion and time-perfusion integrals, which probably better describe the hyperaemic response, were determined. In addition, for each pair of images, mean signal intensity was carried out with a surface coil facing the calf. The creatine rephosphorylation time constant (TCr) was calculated from the analysis of series of 8 s spectra (4 transients) collected during recovery from exercise. The following day, exactly the same stimulus procedure was applied to perform perfusion imaging with a SATIR sequence [3], using a discrete cosine body transmitter coil and an orthogonal surface receiver coil below the calf. Perfusion profiles were monitored over 4 min post exercise. Quantitative perfusion data were obtained from the analysis of the differences in leg muscle signal intensity between pairs of selectively and non-selectively labelled images. Peak (maximum) post-exercise perfusion and time-perfusion integrals, which probably better describe the hyperaemic response, were determined. In addition, for each pair of images, mean signal intensity was taken and plotted over time. Due to the T2 weighting of the single-shot RARE sequence, it provided a "capillary" BOLD index. The BOLD effect was expressed as the ratio of actual signal intensity to the the end-recovery signal, taken as a reference.

RESULTS:
In the ischemic rats, the time-force integral was initially lower than in the sham-operated group (0.32±0.03 N.s vs 0.40±0.08 N.s, p=0.02) and remained lower throughout the observation period. Mitochondrial ATP production, as evaluated by post-exercise TCr, was dramatically prolonged at the early stage of the disease (251.5±159.5 s vs 88.1±9.9 s, p=0.015) but progressively improved and, by week 5, was no longer different between arteriopathic rats and control animals (99.4±9.7 s vs 91.3±8.7 s, NS). Perfusion, expressed either as peak perfusion or time-perfusion integral, was also severely impaired immediately after the induction of ischemia (Time-perfusion integral: 16.4±7.3 ml/100g vs 68.3±12.9 ml/100g; p<0.001). In striking contrast to TCr, perfusion of arteriopathic rats only improved moderately over time (Time-perfusion integral at week 5: 35.1±9.9 ml/100g; p=0.005 vs week1). Figure 1 suggests an explanation for the de-correlation of TCr and perfusion evolutions, in the course of the disease. The pseudo-hyperbolic relationship between TCr and perfusion indicates that at very high flow rates, as elicited by our electro-stimulation paradigm in control rats, mitochondrial ATP production is maximum and perfusion-independent. In contrast, in the ischemic model, mitochondrial ATP production is evidently limited by perfusion. Interestingly, it would appear that spontaneous angiogenesis proceeded only until it corrected cell hypoxia and normalized TCr (backward travel of horizontal segment of the curve) but not to restore small vasodilatory capacity (vertical segment of the curve). This is further substantiated by the BOLD data analysis. Early into recovery, a BOLD effect was observed in control rats (3.8±1.6% signal increase, p<0.005 vs week1). Figure 1 suggests an explanation for the de-correlation of TCr and perfusion evolutions, in the course of the disease. The pseudo-hyperbolic relationship between TCr and perfusion indicates that at very high flow rates, as elicited by our electro-stimulation paradigm in control rats, mitochondrial ATP production is maximum and perfusion-independent. In contrast, in the ischemic model, mitochondrial ATP production is evidently limited by perfusion. Interestingly, it would appear that spontaneous angiogenesis proceeded only until it corrected cell hypoxia and normalized TCr (backward travel of horizontal segment of the curve) but not to restore small vasodilatory capacity (vertical segment of the curve). This is further substantiated by the BOLD data analysis. Early into recovery, a BOLD effect was observed in control rats (3.8±1.6% signal increase, p<0.005), which correlated to perfusion ($R^2=0.41$, p<0.0001; see Fig.2). In the arteriopathic group, to the contrary, the BOLD effect was negative (-1.15±2.2%, p<10$^{-6}$) indicating that, at no point in time, was perfusion in excess relative to oxygen demand, as occurs in controls.

CONCLUSION:
The truly non-invasive set-up of this protocol, including the muscle electro-stimulation and the measurement of force development, made it possible to monitor the natural adaptations of muscle perfusion and energy metabolism in a model of chronic arteriopathy, over several weeks. Parallel evaluation of mitochondrial ATP production and vasodilatory response to exercise identified that spontaneous angiogenesis in the ligature-ablation arteriopathic model was sufficient to progressively normalize muscle energetics but not to restore the vasodilatory reserve elicited by electro-stimulation in control animals. The last statement is supported by the confrontation between ASL and BOLD data.

REFERENCES: