Regular physical exercise stimulates endothelium-dependent NO-mediated vasorelaxation and reduces the progression of atherosclerotic lesions in hypercholesterolemic apolipoprotein E-deficient mice (apoE<sup>−/−</sup>)

Pellegrin Maxime<sup>1,2</sup>, Houdayer Christophe<sup>3</sup>, Berthelot Alain<sup>1</sup>, Laurant Pascal<sup>1,2</sup>

<sup>1</sup>EA 3921 Optimisation Métabolique et Cellulaire, Université de Franche-Comté, France
<sup>2</sup>Groupe Physiologie et Biologie de la Cellule Sportive, Université de Franche-Comté, France
<sup>3</sup>Laboratoire de Biologie du Développement et de la Reproduction, CHU Besançon, France

Introduction
Physical activity has been shown to slow the initiation and progression of atherosclerotic disease (i), the major cause of death in industrialized countries. However, the mechanisms mediating the atheroprotective effects of exercise are not clearly defined. Endothelial dysfunction represents a key early step in the development of atherosclerosis and is also involved in plaque progression and the occurrence of atherosclerotic complications. The hallmark of endothelial dysfunction is impaired endothelium-dependent vasodilatation, which is mediated by nitric oxide (NO) (ii). The aim of this study was to explore the beneficial effects of training on both NO-dependent vasorelaxation and atherosclerotic plaque size in the apoE<sup>−/−</sup> mice.

Methods
20-week-old male apoE<sup>−/−</sup> mice treated for 9 weeks with a lipid-rich Western-type diet were divided into two groups: exercise group (apoE<sup>−/−</sup> X ; n=10) and sedentary group (apoE<sup>−/−</sup> S ; n=11). The mice in the exercise group underwent a 9-week endurance swimming program (50 min/day ; 5 days/week). Aortic root was used for morphometric (quantitative) determination of the atherosclerotic plaque area. Relaxation of the thoracic aorta was examined in vitro in vascular rings precontracted with phenylephrine (10<sup>−7</sup> M). Endothelium-dependent relaxation was assessed by cumulative concentration-response curves for acetylcholine (10<sup>−9</sup> - 10<sup>−4</sup> M) or calcium ionophore A23187 (10<sup>−8</sup> - 3.10<sup>−5</sup> M) (receptor- and nonreceptor-mediated endothelium- and NO-dependent agonists respectively). Endothelium-independent relaxation was evaluated by cumulative concentration-response curves for the NO donor DEA-NONOate (10<sup>−8</sup> - 10<sup>−4</sup> M) or the non-specific vasodilator papaverine (10<sup>−6</sup> - 10<sup>−4</sup> M).

Results
Our results showed that atherosclerotic lesion areas in the aortic roots were reduced in apoE<sup>−/−</sup> X (5679.6 µm² ± 3445.4 vs 44337.5 µm² ± 10488.8, p<0.05; Fig 1). Endothelium-dependent NO-mediated relaxation to acetylcholine was shifted to the left in aorta of training apoE<sup>−/−</sup> mice (Fig 2). Maximal endothelium-dependent relaxation (I<sub>max</sub>) was higher than in the sedentary group (I<sub>max</sub> values : 86.7% ± 3.0 vs 64.5% ± 6.2, p<0.05; Fig 2). The sensitivity (pD<sub>2</sub>) to acetylcholine was increased in apoE<sup>−/−</sup> X (pD<sub>2</sub> values: 7.06 ± 0.06 vs 6.73 ± 0.10, p<0.05). Cumulative concentration-response curves to papaverine was also shifted to the left in trained ApoE<sup>−/−</sup> mice (pD<sub>2</sub> values: 5.92 ± 0.11 vs 5.54 ± 0.10, p<0.05). Aortic rings exhibited similar responses to DEA-NONOate and calcium ionophore.

Discussion/Conclusion
The main findings of this study are that regular physical exercise in apoE<sup>−/−</sup> stimulates endothelium-dependent NO-mediated vasorelaxation and prevents the progression of atherosclerotic lesions. Exercise training could inverse endothelium-dependent vasorelaxation by at least 3 mechanisms 1) improvement of the receptor’s sensitivity to acetylcholine; 2) higher post-transductional activity with probably activation of endothelial NOSynthese (eNOS); 3) greater availability of the guanylate cyclase/cGMP system in vascular muscle. In conclusion, our findings confirm that exercise have an important role in preventing atherosclerotic cardiovascular disease.

References