# Analysis of cholesterol export from purified endosomes in NPC cellular models

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## LAY SUMMARY

At the cellular level, the Niemann-Pick type C disease is characterized by the accumulation of cholesterol and other lipids in sub-cellular organelles, called late endosomes and lysosomes. Even if the genes (npc1 and npc2) involved in the pathology are known, the molecular mechanisms by which these proteins mediate the export of cholesterol (and possibly other lipids) from late endosomes/lysosomes remain unclear.

The main goal of my research project was to characterize *in vitro* the mechanism of cholesterol export from purified endosomes and to elucidate of the role of the molecular players in the process, including NPC1 and NPC2 proteins with the aim to clarify the direct role of these proteins in cholesterol export from the organelles.

During the fellowship period, I set up an in vitro assay to measure export of cholesterol from isolated endosomes and I used this assay to compare the kinetics of export in endosomes purified from NPC model cells (NPC1 or NPC2). According to these measurements, I did not observe any difference in the kinetics of cholesterol export in NPC cells compared to control cells. These results indicate that at least on isolated organelles, NPC1 and NPC2 proteins seem not directly implicated in cholesterol export. On the other hand, results are compatible with an indirect- or perhaps a regulatory-role of NPC1/NPC2 in intra-cellular cholesterol traffic.

I also performed experiments in order to clarify the mechanism of cyclodextrin action at the level of endosomes, specifically focusing of the function of the lipid lysobisphosphatidic acid (LBPA), which is an endosome/lysosomes-specific crucial for cholesterol traffic in the endosomes. Experimental results indicate that HPCD treatment strongly modulates LBPA content of endosomes. Physiological significance of these results is still unclear and I am currently involved in characterizing the relevance of these changes in lipid composition on HPCD action in NPC cells.

I am confident that my project, which will continue after the end of this fellowship, will contribute to a better characterization of the molecular processes at the basis of the disease and the mechanism of action of potentially efficient treatments, like cyclodextrin. I hope that basic knowledge on the mechanism of the disease will ultimately help in finding a cure for Niemann-Pick, which is the final goal of our research and our efforts.