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HUMAN NUCLEAR LIPID MEMBRANE: LIPID COMPOSITION & DYNAMICS REVEALED BY NMR

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Muscular dystrophy diseases, breast and kidney cancers are linked to malformation of the nuclear envelope (NE). Mechanisms involved in the maintenance of NE morphology are based on proteo-lipid interactions. Recently, the physical properties of specific lipids has highlighted their essential role in NE assembly process of a sea urchin model. To provide molecular insight in how the nuclear architecture is regulated, we used NMR as a quantitative method to investigate the lipid composition and the dynamics of the NE. Nuclei extraction were performed from HEK 293T human kidney cells. A physical extraction based on a pressure treatment and a sucrose gradient was used and improved considerably the nuclei yield, and afforded obtaining a high quantity of intact nuclei (NE lipids) required for NMR experiments, with a minimum of cell debris, or the ER and Golgi compartments. The nuclear lipids were then extracted from the pure nuclei using a modified Folch method. Liquid-state NMR experiments showed that the NE is composed of a complex mixture of phospholipids and with phosphatitylcholine present in a higher proportion compared to other membrane organelles. Reconstructed nuclear lipid extract membranes were analysed by solid-state NMR and exhibit atypical physical properties. The lamellar gel-fluid phase transition temperature was found very low and broad at -10 ± 15°C, possibly due to the presence of numerous lipid species and unsaturated acyl chains. Furthermore, at 25°C, reconstructed nuclear lipid membrane was found to be more rigid than classical model membranes suggesting a larger bilayer thickness. Finally, reconstructed nuclear lipid liposomes have shown a very important prolate deformation in a magnetic field, which is unusual for biological membranes and suggests an important curvature elasticity.