Adipocyte-derived extracellular vesicles: the quest for a proper isolation protocol

Authors:
Lisa Mennens, REVAL– Rehabilitation Research Center, Hasselt University, Diepenbeek, Belgium. BIOMED Biomedical Research Center, Hasselt University, Diepenbeek, Belgium. NIMO– Human Biology, Maastricht University, Maastricht, The Netherlands., lisa.mennens@uhasselt.be
Baharak Hosseinkhani, Laboratory of Angiogenesis and Vascular Metabolism– Center for Cancer Biology (CCB), VIB and Department of Oncology, Leuven Cancer Institute (LKI), KU Leuven, Leuven, Belgium. BIOMED Biomedical Research Center, Hasselt University, Diepenbeek, Belgium., baharak.hosseinkhani@kuleuven.be
Johan Jocken, NIMO– Human Biology, Maastricht University, Maastricht, The Netherlands., johan.jocken@maastrichtuniversity.nl
Kenneth Verboven, REVAL– Rehabilitation Research Center, Hasselt University, Diepenbeek, Belgium. BIOMED Biomedical Research Center, Hasselt University, Diepenbeek, Belgium., kenneth.verboven@uhasselt.be

Background: In obesity, excessive adipose tissue accumulation leads to ectopic lipid deposition, insulin resistance (IR) and dysfunctional interorgan crosstalk, in which extracellular vesicles (EVs) became known as important mediators. In the obese state, the cargo of adipocyte-derived EVs (adEVs) might play a distinct role in the development of obesity-related IR.
Methods: Human multipotent adipose-derived stem cells (hMADS) were isolated from subcutaneous adipose tissue biopsies of males with/without obesity. Cells were differentiated and transferred to EV isolation medium (DMEM/Ham's F12 with 3% 300kDa EV-depleted FBS). After 48h, EV isolations were performed using 100kDa ultrafiltration and size exclusion chromatography. Obtained adEVs were characterized using NTA and mass spectrometry.
Results: NTA data showed that adEVs from differentiated hMADS of donors with obesity resulted in a notable lower yield as opposed to cells from a lean donor (mean yield: $9.60x10^9$ vs $1.85x10^{10}$, respectively). However, adEVs from donors with obesity had a larger mean diameter and interestingly, also a higher protein concentration (mean size: 154nm vs 114nm; mean protein concentration: 0.37µg/µL vs 0.48µg/µL, respectively). Preliminary data (n=1) of these analyses showed the presence of adipocyte-specific markers such as FABP4, adiponectin, lipoprotein lipase, …, EV-markers CD9 and CD63, as well as markers specific for insulin signalling (insulin receptor, insulin-degrading enzyme, insulin-like growth factors, …). As expected, an upregulation in proteins related to fatty acid metabolism, transport and lipid biosynthesis were found in differentiated compared to undifferentiated hMADS. In adEVs from lean donors, proteins related to the regulation of lipid and cholesterol storage were upregulated, while those of donors with obesity showed upregulation peptide cross-linking, muscle cell migration and extracellular structure organization pathways.
Conclusion: Our preliminary data shows differences in adEV yield, size and content based on their donor's metabolic state, hereby suggesting a role in regulating metabolism. However, further measurements are necessary to make robust conclusions.
Funding: This work was supported by the Research Foundation – Flanders (FWO grant number 11C4423N to Lisa Mennens).