Extracellular Vesicle Secretion Dynamics and lipid composition in human multipotent adipose-derived stem cells from Lean and Obese donors

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Introduction: Obesity is characterized by disturbed interorgan crosstalk and lipid metabolism, in which adipocyte-derived extracellular vesicles (adEVs) could contribute by mobilizing lipids and products of lipolysis.

Methods: Subcutaneous human multipotent adipose-derived stem cells (hMADS) from male donors with/without obesity were incubated for 48h in DMEM/Ham's F12 with 3% 300kDa EV-depleted FBS pre- and post-differentiation. adEVs were isolated from conditioned media using 100kDa ultrafiltration and size exclusion chromatography and characterized using nanoparticle tracking analysis and cryoEM. Basal lipolysis was assessed by measuring free glycerol concentrations following 3h *ex vivo* incubation of donor-specific mature adipocytes. Preliminary lipidomics using LC-MS was done after lipid extraction using the MTBE protocol, and data analysis was performed in the Lipostar software. Data are mean fold changes ± SEM.

Results: Before differentiation, hMADS from lean donors secreted 38% more adEVs as compared to hMADS from donors with obesity (0,62±0,09; $p<0,05$). Compared to the lean group, adipogenic differentiation induced an increased adEV secretion in the obesity group (pre: 0,62±0,09; post: 1,07±0,14; $p<0,01$) up to the level of the lean group. Particles size did not differ between groups or differentiation

levels (~ 162±4nm). In the obesity group, a significantly lower *ex vivo* basal lipolysis in mature adipocytes was also observed (9,55±1,6 vs 12,3±2,7µmol/L; $p<0,05$). Preliminary lipidomics of adEVs revealed a general enrichment of glycerolipids, glycerophosphocholines and phosphosphingolipids in all samples. As expected, triacylglycerols increased post-differentiation compared to pre-differentiation, independent of metabolic phenotype, while no difference in saturation degree between donor phenotypes was detected. Pre-differentiation, lean donors revealed a relative enrichment of phosphosphingolipids and glycerophosphocholines compared to donors with obesity, while post-differentiation, this effect was reversed. Principle Component Analysis demonstrated clustering of both post-differentiation adEV samples, independent of metabolic state.

Conclusion: Secretion of adEVs varies with the donor's metabolic state, with lower adEV secretion and mature adipocyte lipolysis in donors with obesity. Adipogenic differentiation restored adEV levels to those of lean donors and increased triacylglycerols in adEVs in both groups, irrespective of adEV size. These findings suggest a link between metabolic state, lipolytic function, and adEV secretion, warranting further investigation through proteomics and extensive lipidomics.

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