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Adipocyte-derived exosomes induce NASH development *in vitro*

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1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is associated with visceral obesity. Adipocyte atrophy and accompanying inflammation of visceral adipose tissue (AT) lead to the secretion of a plethora of factors that once taken up by the liver, exacerbate the development of non-alcoholic steatohepatitis (NASH) [1]. Yet, the exact mechanisms behind the interplay between AT and the liver, and the potential role of exosomes is not fully understood.

2 Aim

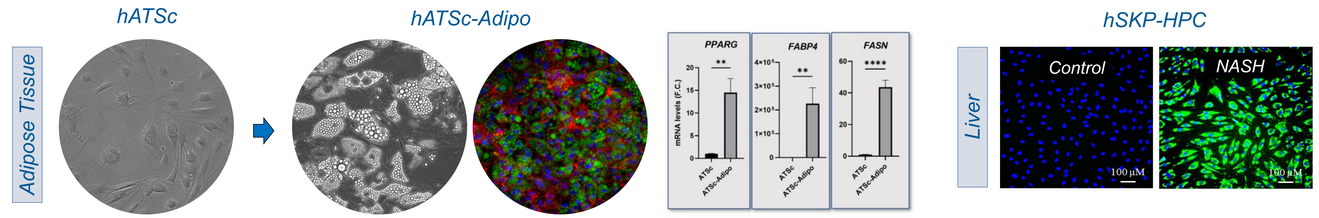
To investigate the potential steatogenic and inflammatory effects that exosomes derived from adipocytes have on the liver using multipotent human stem cell-based *in vitro* models, mimicking both healthy and dysregulated adipose tissue and the hepatic parenchyma.

3 Method

Human adipose-derived stromal cells (**hATSc**) were isolated from liposuction material after ethical approval and informed consent of the patients. Upon expansion, the cells were differentiated towards adipocyte-like cells (**hATSc-Adipo**). To mimic the microenvironment of visceral adipose tissue (AT) in steatosis and NASH patients, these cells were exposed for 24 hours to a cocktail of multiple factors (*steatosis: palmitic acid, insulin, glucose; NASH: palmitic acid, insulin, glucose, IL1β, TNFα, IL6, TGFβ*), identified from analysis of AT samples of patients with histologically proven steatosis or NASH [2] and mimicking hyperglycemic and hypertriglyceridemic blood levels of obese patients. Upon a recovery wash period of 24 hours, exosomes were isolated from the cell supernatants of ATSc-Adipo cultures. The steatogenic and inflammatory effects of the isolated exosomes were evaluated using human skin-derived precursors (hSKP) differentiated towards hepatic cells (hSKP-HPC) [3].

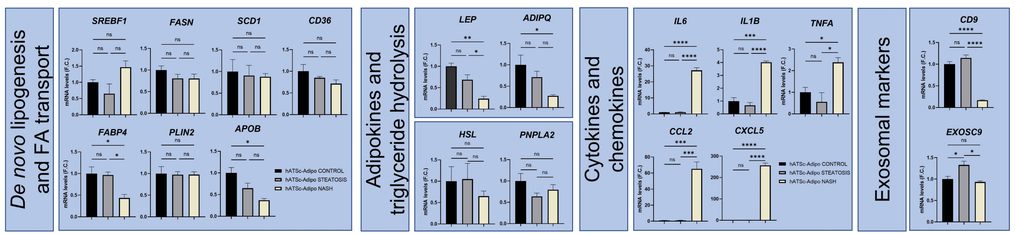
4 Results

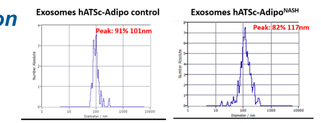
(A) *Human tissue-derived multipotent stem cells can be used to model cellular processes in the liver and adipose tissue*



Human multipotent hATSc can be efficiently differentiated *in vitro* into functional adipocyte-like cells (hATSc-Adipo). Multipotent hSKP isolated from small human skin segments can be differentiated towards hepatic cells (hSKP-HPC) that are highly responsive to NASH-inducing factors as we have previously documented [4].
[Green: BODIPY 493/503 (green); red: Phalloidin-iFluor 594]

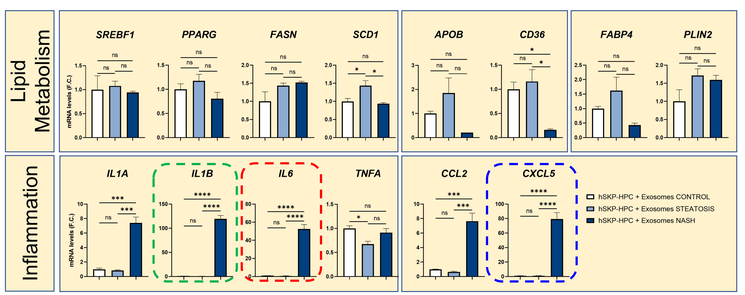
(B) *hATSc-Adipo exposed to steatosis- and NASH-inducing factors mimic adipose tissue dysregulation*





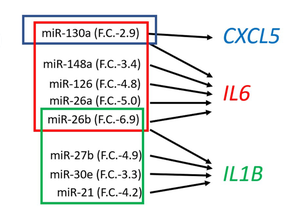
hATSc-Adipo exposed to steatosis- or NASH-specific triggers show no, or modest modulation of genes playing a role in lipid metabolism. The NASH cocktail, however, induces a strong upregulation of inflammatory genes and a downregulation of exosomal markers, which does not affect size and concentration of isolated exosomes.

(C) *hSKP-HPC exposed to exosomes from dysregulated hATSc-Adipo show inflammatory response*



hSKP-HPC exposed to exosomes isolated from hATSc-Adipo previously exposed to NASH-inducing factors, show a strong modulation of inflammatory markers, but no pronounced indication of lipid metabolism markers leading to intracellular lipid accumulation.

(D)



Multiple miRNAs from blood exosomes of patients with NASH [5] have been identified to regulate *CXCL5, IL6, IL1B* based on the analysis of putative binding sites (http://www.targetscan.org/). Investigation of these miRNAs in the presented *in vitro* system is ongoing.

5 Conclusions

Our data show that adipocyte-like cells derived from human ATSc can mimic cellular responses specific of inflamed AT during NASH. The exosomes secreted by these cells induce the expression of steatogenic and inflammatory markers in an established hepatic *in vitro* system. This study shows the value of human-based *in vitro* cell systems in the investigation of complex pathophysiological processes, such as the interplay between AT and the liver during the onset of NASH.

6 References

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One-way ANOVA with *post hoc* Tukey's multiple comparisons test (*, **, *** and ****; p ≤ 0.05, p ≤ 0.01, p ≤ 0.001 and p ≤ 0.0001)

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Leerstoel Mireille Aerens
voor de ontwikkeling van proefdiervrije methoden

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