Investigating the Role of Extracellular Traps in Adipose Tissue Dysfunction Under Environmental Stressors: Implications for Metabolic Disease Development and Treatment

Background:

The environment has fundamentally influenced physiological and metabolic adaptations of living organisms. Environmental stressors, including nutrient excess and pollutants, such as endocrine-disrupting chemicals (EDCs), challenge homeostasis and metabolic health. Throughout evolution, dietary fluctuations have driven adaptations of flexible mechanisms essential for energy balance [1]. Adipose tissue, a dynamic metabolic organ, evolved primarily to store energy and regulate systemic homeostasis under variable environmental pressures.

Today, sedentary lifestyles, excessive nutrient intake, and EDC exposure impair adipose tissue function, promoting chronic inflammation and metabolic diseases such as obesity, type 2 diabetes, and metabolic syndrome [2]. Brown adipose tissue (BAT), crucial historically for survival via non-shivering thermogenesis [3,4], now faces functional decline due to high-fat diets and thermoneutral environments, shifting toward a white adipose tissue (WAT)-like phenotype [5].

WAT, the main lipid storage depot, also undergoes significant structural and functional alterations in response to nutrient excess, characterized by adipocyte hypertrophy, hyperplasia, chronic inflammation, and dysregulated adipokine secretion [6,7]. Additionally, lipophilic EDCs such as bisphenol A and phthalates accumulate in adipose tissue through diet and air exposure, exacerbating systemic inflammation, particularly in individuals with elevated adiposity [8].

During my master's thesis, I investigated how a high-fat diet (HFD) triggers adipocyte inflammation and induces their trans-differentiation into a neutrophil-like, senescent, and pro-inflammatory phenotype. These adipocytes displayed increased neutrophil-like markers, extracellular DNA release, and nuclear alterations without undergoing cell death, a phenomenon resembling neutrophil extracellular traps (NETs). S100A9 protein emerged as a potential mediator of adipocyte extracellular traps (Adipo-ETs), contributing to chronic inflammation linked to nutrient excess. Given that EDCs induce adipose changes like HFD and heighten diabetes risk, they may similarly promote Adipo-ET formation.

Aims and PhD project plan:

The aim of the project is to validate the previously observed inflammatory mechanisms in humans, with a focus on the functional relevance of Adipo-ETs in the pathogenesis of metabolic dysfunctions such as obesity and T2D under environmental stress. This is necessary because inflammatory activation is faster in mice due to immune system differences [9].

AIM 1 – In vitro validation of Adipo-ET formation and inflammatory phenotype.

AIM 1.1 – 2D models: The acquisition of a neutrophil-like inflammatory phenotype and the formation of Adipo-ETs will be assessed by means of in vitro models on human adipose (hiBAT) and differentiated mesenchymal stem cells (MSCs) exposed to nutritional stress (high-fat levels) and relevant EDCs. Immune-metabolic interaction will also be assessed by co-culturing with human THP1 M1 macrophages. Molecular biology analyses such as qPCR, Western blot, immunofluorescence, and ELISA will assess secretory inflammatory activity, extracellular DNA release, and Adipo-ETs. The causal role and therapeutic potential of S100A9 and PADI4 will be explored using transfection, siRNA, and pharmacological inhibition. Further RNAi or CRISPR editing of key genes (Padi4, Mpo), will identify other key nodes in the inflammatory cascade. Markers of cell senescence will be investigated, and cell viability will be assessed by flow cytometry to exclude cytotoxic effects.

AIM 1.2 – 3D models: A three-dimensional model will also be developed to better reproduce adipose tissue structure compared to 2D models. The molecular analyses described above will also be applied in this context.

AIM 2 – In vivo validation and therapeutic targeting.

Use of S100A9 knock-out mice with selective ablation in adipose tissues (AT-S100A9-KO), to determine the causative role of the protein in Adipo-ET formation and adipose tissue dysfunction. By comparing wild-type (WT) and AT-S100A9-KO mice, both under basal conditions and under metabolic and environmental stress (HFD and exposure to EDCs), the effects on the inflammatory response and insulin sensitivity will be assessed, using immunofluorescence, qPCR, and immunoblotting techniques. Finally, the therapeutic potential of S100A9 inhibition to limit the formation of Adipo-ETs will be explored.

AIM 3 – Data analysis, thesis writing, and dissemination.

Thesis writing and dissemination of results.

Statistical analysis. Sample size estimation will be performed prior to experiments ($\alpha = 0.05$; power ≥ 0.80) using expected differences based on preliminary results or literature. Normality of data distribution will be evaluated (Kolmogorov-Smirnov or Shapiro-Wilk tests), followed by appropriate parametric (Student's t-test, ANOVA) or non-parametric (Mann-Whitney, Kruskal-Wallis) analyses. When multiple comparisons are performed, adjustments (Bonferroni, Holm-Sidak corrections) will be applied. Statistical significance will be set at $p < 0.05$, using GraphPad Prism.

Expected results:

The results of this project could clarify how environmental stressors, including excess nutrients and exposure to relevant EDCs, promote adipose tissue dysfunction through Adipo-ETs formation. Confirmation of the S100A9's role would open new therapeutic perspectives to prevent and treat metabolic complications associated with obesity and T2D.

	Year 1	Year 2	Year 3
AIM 1.1:	Month 1-9		
AIM 1.2:	Month 6–12	Month 13–15	
AIM 2:		Month 13–24	Month 25–30
AIM 3:			Month 30–36

References:

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