CHALLENGES IN ISOLATING EXTRACELLULAR VESICLES FROM HUMAN PLASMA BY DENSITY GRADIENT ULTRACENTRIFUGATION

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Cardiovascular disease (CVD) remains the leading cause of both morbidity and mortality worldwide, with atherosclerosis being the primary cause of many heart and vascular health-related disorders [1]. To enhance the understanding of the pathogenesis of atherosclerosis and develop preventive strategies, the research focuses on the lipoproteins and relatively understudied extracellular vesicles (EVs) particles. Extracellular vesicles and lipoproteins are prevalent in human plasma and possess similar physicochemical properties, making their isolation and independent analysis particularly challenging in atherosclerosis research. In this work, a density gradient ultracentrifugation–based methodology was developed to isolate and separate EVs from lipoproteins in human plasma.

This cross-sectional study enrolled 201 consecutive participants (aged 40–61, both sexes) from the national CVD prevention program between April and July 2024. The study was approved by the Vilnius Regional Biomedical Research Ethics Committee (Approval No. 2023/9-1518-998), affiliated with Vilnius University. A 10–40% density gradient solution was prepared, and samples were ultracentrifuged at an average of 118,700 g for 40 h at 4 °C. To facilitate potential clinical applicability, a plasma sample volume of only 500 µL was used. Total protein (TP) concentration was measured in each gradient fraction to assess material distribution and guide the selection of EV-enriched fractions for downstream analyses. This analysis validated the method and illustrated the expected distribution of proteins across the gradient. EVs were predominantly detected in fractions with moderate protein content, whereas lipoproteins and soluble plasma proteins were associated with high protein peaks (Figure 1). This was further confirmed by Western blot analysis, which identified apolipoprotein B in fractions 1–5 and EVs CD81 marker in fractions 6–9. However, co-isolation with apolipoprotein A-I was observed, as it was detected across fractions 2–10.

Despite the successful isolation of extracellular vesicles and the detection of EVs markers by Western blot, translating this methodology into clinical practice remains challenging. The low EVs concentration obtained from clinically feasible plasma volumes, together with co-isolation of apolipoprotein A-I, highlights persistent limitations in EVs yield and purity. Further optimization is required to support reliable downstream analyses and clinical applications.

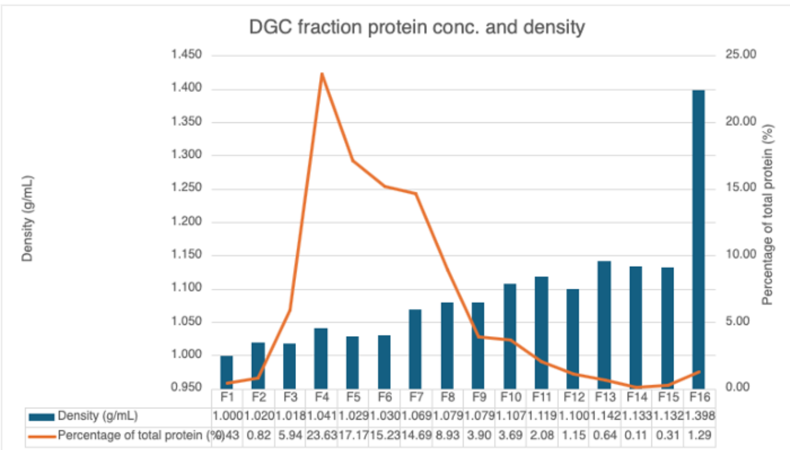

Fig. 1. Density and total protein percentage in each fraction after density gradient centrifugation.

Keywords: EVs, atherosclerosis, biomarker

[1] B. A. Stark et al., “Global, regional, and national burden of cardiovascular diseases and risk factors in 204 countries and territories, 1990-2023,” Journal of the American College of Cardiology, vol. 86, no. 22, pp. 2167–2243, Sep. 2025, doi: 10.1016/j.jacc.2025.08.015.