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# Proatherogenic potential of oxidative modifications in LDL

Kiseleva D.[1, 2]*, Ziganshin R.[3], Kuzmin V.[4], Cherednichenko V.[1], Khovantseva U.[1], Bogatyreva A.[1], Markina Yu.[1], Markin A.[1, 5]

[1] *Laboratory of Cellular and Molecular Pathology of Cardiovascular System, Petrovsky National Research Centre of Surgery, Moscow, Russia*

[2] *Department of Biophysics, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia*

[3] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*

[4] *Biotechnological Faculty, Lomonosov Moscow State University, Moscow, Russia*

[5] *Medical Institute, Peoples' Friendship University of Russia Named after Patrice Lumumba (RUDN University), Moscow, Russia*

* *kiseleva.dg@gmail.com*

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*Motivation and Aim*: Atherosclerosis is a chronic vascular disease and is one of the main causes of acute cardiovascular events. All currently existing theories that explain the process of the initial atherosclerotic lesion formation underline the important role of low-density lipoprotein (LDL) in the development of this pathology. For more than 20 years, it has been emphasized that not LDL itself, but rather its oxidized modifications (oxLDL) [1], cause an increased accumulation of cholesterol in the cells of the subendothelial intimal layer, including macrophages, which occurs through specialized LOX-1 receptors [2]. However, it is difficult to conduct experiments with oxLDL *in vitro* for the following reasons:

- Actual oxLDL level represent a small percentage of total LDL in plasma;
- In order to obtain a sufficient amount of oxLDL for research purposes, the native LDL fraction from the blood plasma of healthy donors is artificially modified, for example, by $CuSO_4$ or lipoxygenase, which has a destructive effect on both lipids and proteins in LDL, and cannot reproduce actual modifications found in patients;
- Experimental data comparing the effect of native, aggregated and oxLDL show conflicting results regarding the accumulation of cholesterol in cells, without confirming the existing hypothesis;

The measurement of oxLDL in the plasma of patients and the correlations with clinical data, namely the risk for development of atherosclerosis is based on enzyme-linked immunosorbent assay (ELISA), i.e. on protein modifications. Each ELISA kit has various antigens, moreover, the modification of only Apolipoprotein B (ApoB) is most frequently measured.

It is also known that patients with type 2 diabetes mellitus (T2DM) suffer from an active progression of atherosclerosis, which increases mortality from cardiovascular diseases (CVD) by 4–5 times in patients in this group. Hyperglycemia promotes the production of reactive oxygen species in mitochondria, increasing the intracellular accumulation of advanced glycation end products, and eventually leads to oxidative modification of LDL [3].

Thus, the purpose of this study was to analyze the existing modifications in native LDL samples isolated from the plasma of patients with T2DM and chronic CVD, and their connection with the accumulation of cholesterol by macrophages *in vitro*.

*Methods and Algorithms*: LDL were isolated from the plasma of 15 patients using a standard protocol for sequential ultracentrifugation with solutions of various densities (LEC of the Petrovsky National Research Center of Surgery, Approval No. 5, 11 December 2022). The cholesterol-to-protein ratio in THP-1 cells was carried out using the CHOLESTEROL liquicolor kit, the protein level was determined by the Lowry method. Cells were previously stimulated to differentiate into M0 macrophages by phorbol-12-myristate 13-acetate (PMA) treatment. Liquid chromatography and mass spectrometry was performed with an Ultimate 3000 Nano LC System coupled to the Orbitrap Tribrid Lumos mass spectrometer. Data analysis was performed in MaxQuant 2.4.2.0, Perseus 2.0.11 and with Python.

*Results*: 38 post-translational modifications as well as their combinations in 151 proteins were analyzed. The results are presented in Fig. 1.

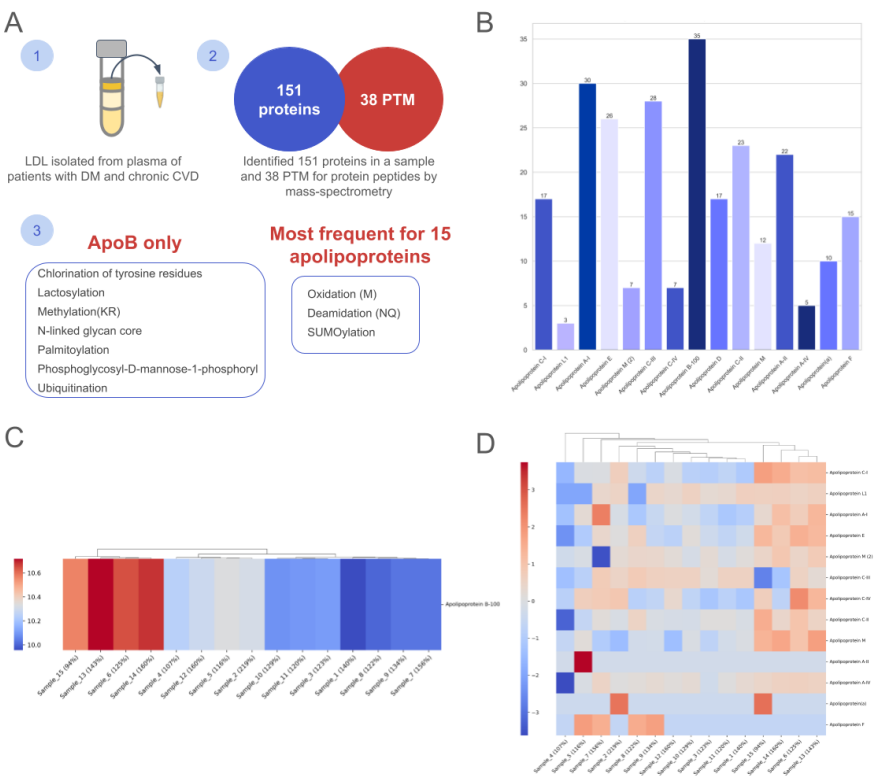


**Fig. 1.** Post-translational modifications (PTM) of 15 apolipoproteins detected in LDL samples from patients with T2DM and chronic CVD. A. General approach to the analysis; 38 modifications were found in 151 proteins. The figure shows the most common modifications in 15 apolipoproteins, and specifies those that are unique to ApoB. B. Barplot illustrates the frequency of PTM in apolipoproteins, i.e. the number of modification types that occur in the protein. C. Clustermap for the oxidation of Met, as the most common modification of ApoB in LDL samples depending on the accumulation of cholesterol by cells. D. Clustermap for the oxidation of Met, as the most common modification of apolipoproteins (excepting ApoB) in LDL samples depending on the accumulation of cholesterol by cells

LDL particles have a complex morphology. ApoB is the main structural protein for the lipid spherical particle, however, other apolipoproteins, as well as certain blood plasma proteins, also bind to the surface of LDL. Since the apolipoprotein group is the major group of LDL structure, this work shows results for 15 of the 151 proteins detected. The main modifications that occur in these proteins are shown in Fig. 1A. Apolipoproteins B, A-I, C-III and E are most frequently susceptible to modification (Fig. 1B). The most common modification found among all apolipoproteins is the oxidation of Met, rather than Cys or Lys, which are detected after *in vitro* artificial modification of LDL or measured by ELISA kits [4]. However, neither ApoB nor the other apolipoproteins showed a relationship between the percentage of cholesterol accumulation in cells and the quantity of Met residues oxidation (Fig. 1C, D). Other modifications that are associated with protein oxidation [5, 6] also do not affect the percentage of cholesterol accumulation by macrophages. Statistically significant Pearson correlations were found between the percentage of accumulation and the relative abundance (rIBAQ) of such proteins in the sample as DAP kinase 1 (0.74), Myomegalin (0.71), Suprabasin (0.67) and Spearman correlation for Fibronectin (0.68).

*Conclusion*: Out of the 38 detected post-translational modifications in LDL that could occur during the oxidation of methionine, lysine, cysteine, tyrosine or other amino acids, none of them affected the increased accumulation of cholesterol by macrophages. The authors suggest that accumulation and inflammation are induced by other proteins that form an LDL particle, which may include: DAP kinase 1, Myomegalin, Suprabasin, Fibronectin.

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