



1 Article

2 Spectroscopic Signature of Red Blood Cells in the 3 D-galactose-Induced Accelerated Aging Model

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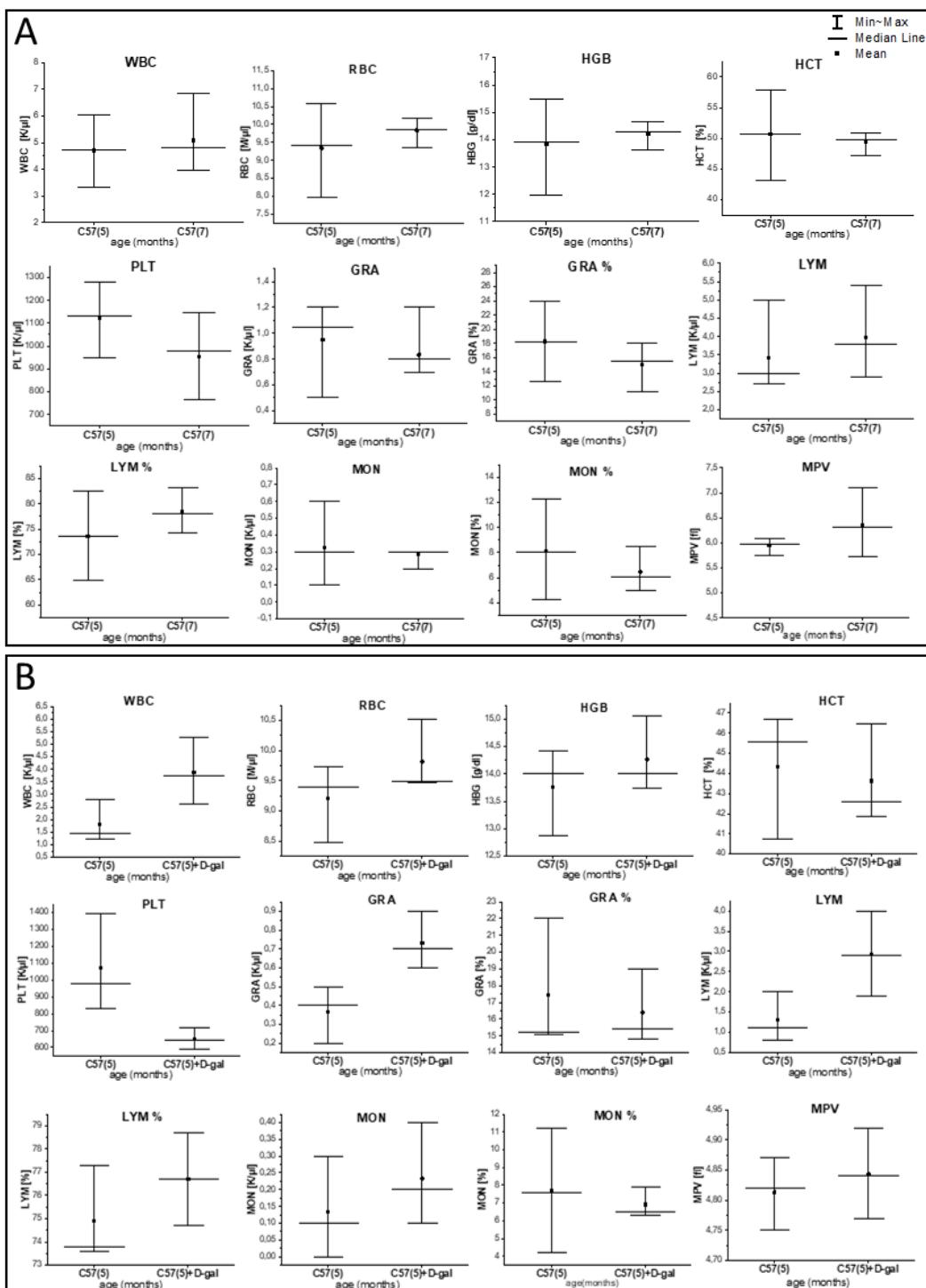
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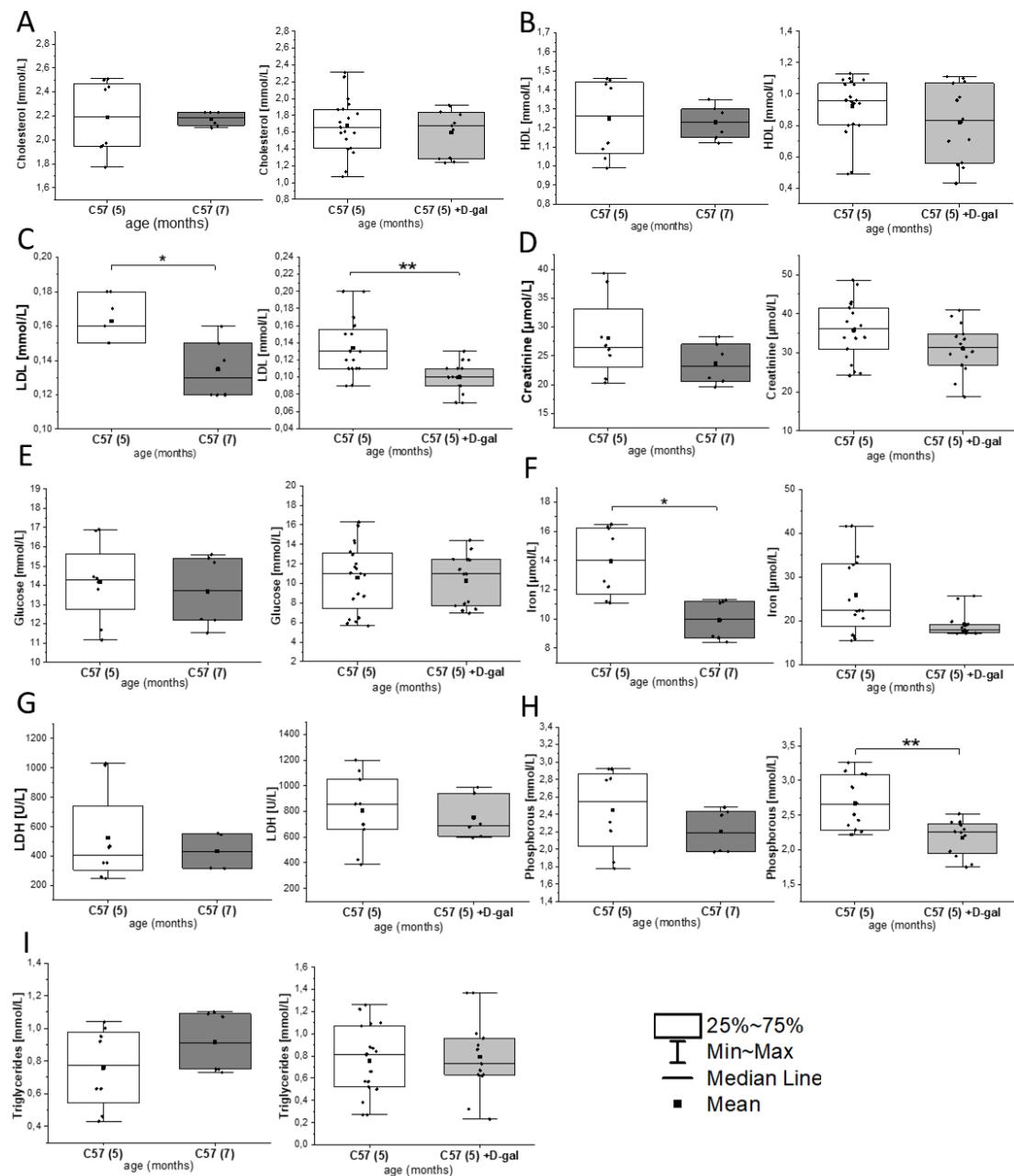
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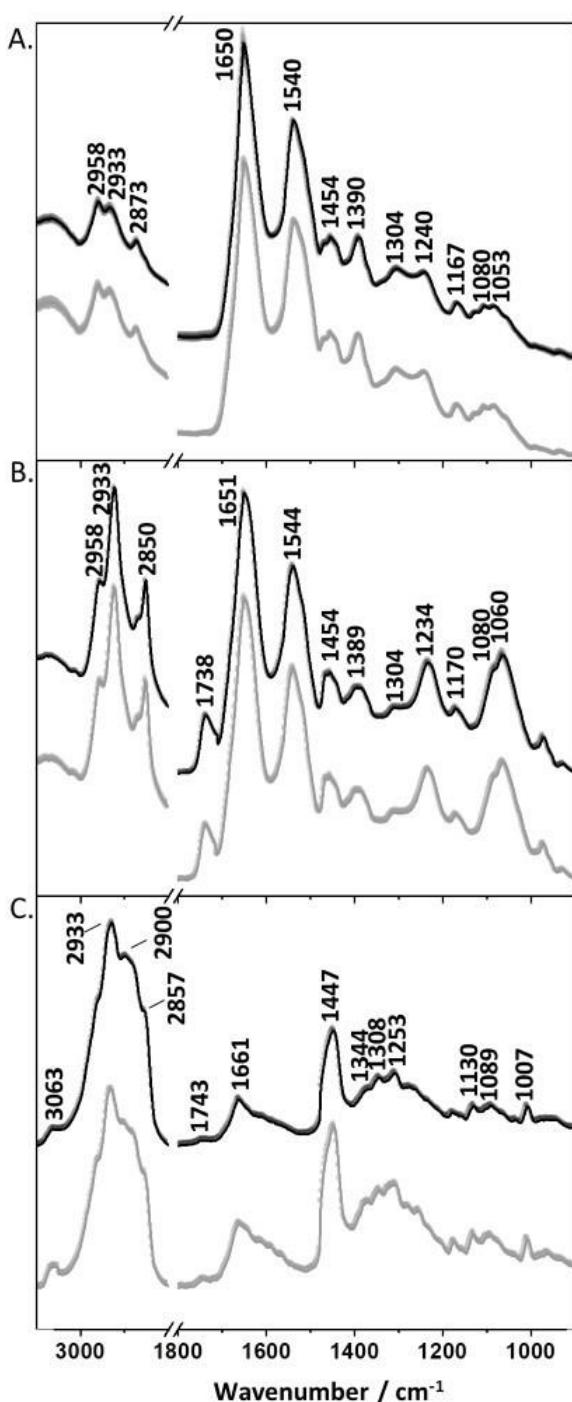
45 **Figure S1.** Changes in blood morphological parameters in natural aging and D-gal model. WBC, RBC, HGB, HCT, PLT, GRA, LYM,
46 MON, MPV for (A) 5- and 7-month-old male C57BL/6J mice (N=4 and N=6, respectively) in natural aging and (B) 5-month-old male
47 C57BL/6J mice in D-galactose induced aging model (N=3) with control group (N=3). Data distribution is presented as interval plots
48 (mean value, median, min-max whiskers). Statistical significance of the obtained values was tested with Mann-Whitney test.

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51 **Figure S2.** Biochemical parameters in blood plasma in natural aging and D-gal model. (A) Cholesterol, (B) HDL, (C) LDL, (D) creatinine, (E) glucose, (F) iron, (G) LDH, (H) phosphorous and (I) triglycerides level for 5- and 7-month-old male C57BL/6J mice (N=4 and N=3, respectively) in natural aging and 5-month-old male C57BL/6J mice in D-galactose induced aging model (N=7) with control group (N=10). Data distribution is presented as box (median and interquartile range, min-max whiskers). Statistical significance of the obtained values was tested with Mann-Whitney test (*p < 0.05; **p < 0.01).



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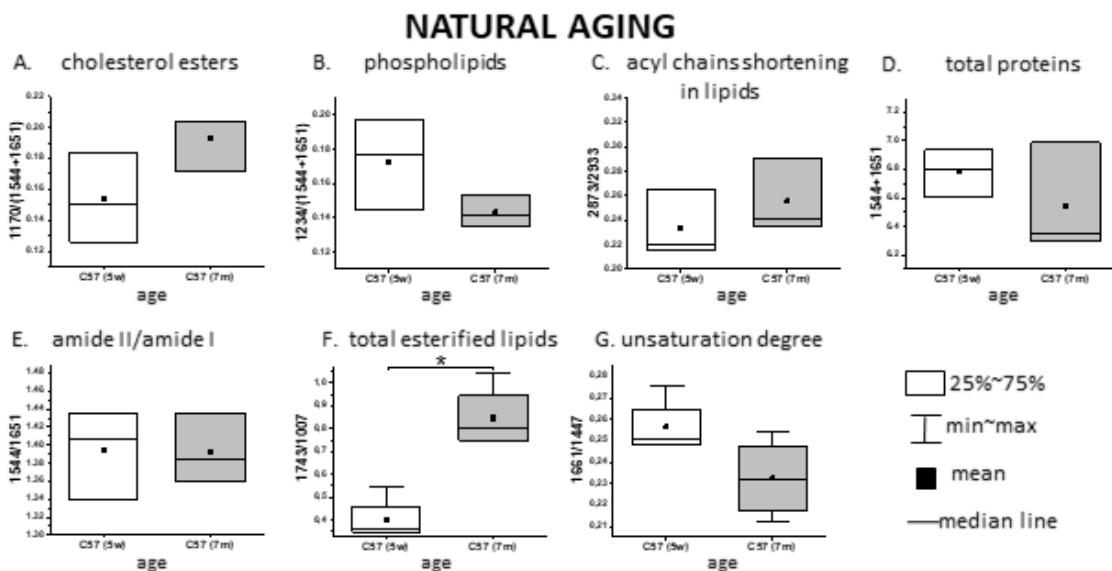
57 **Figure S3.** Averaged FTIR and Raman spectra with SD for RBCs and their membranes in D-gal model.
58 FTIR spectra of intact RBCs
59 (A) and isolated membranes (B) and Raman spectra of isolated membranes (C) taken from control (grey) and D-galactose induced
accelerated aging mice (black).

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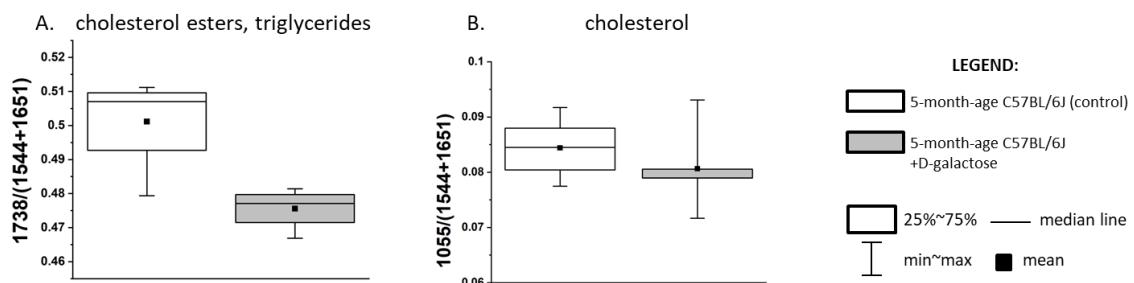
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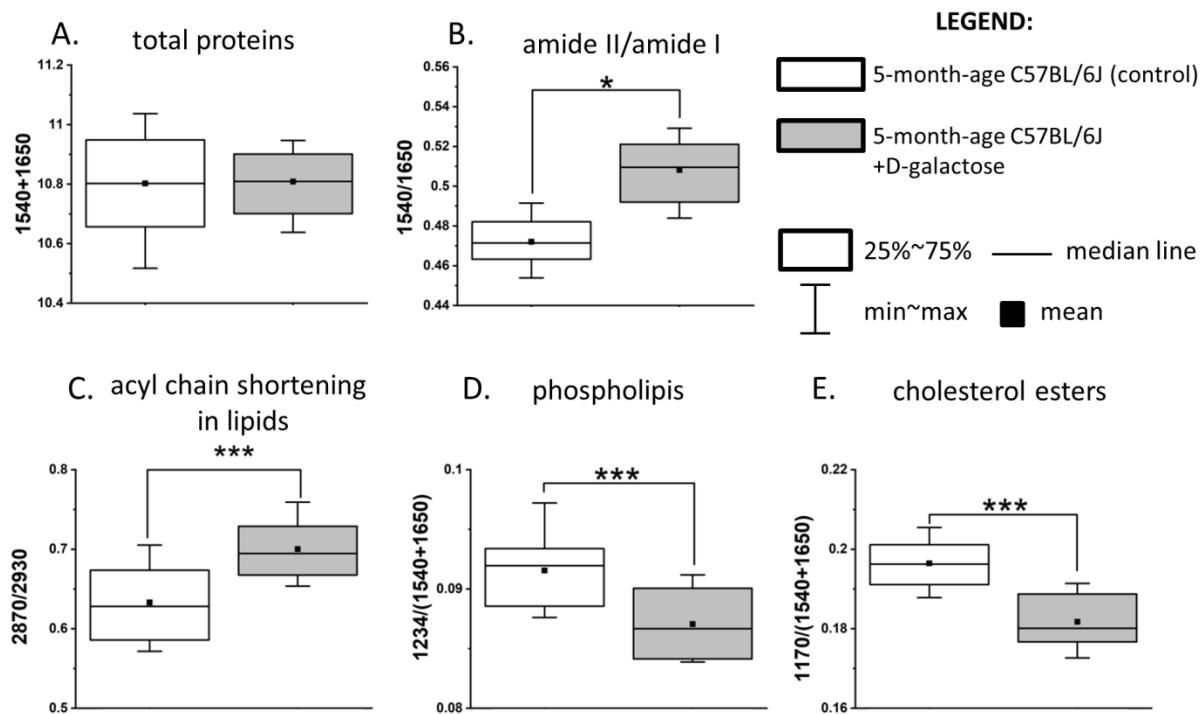
65 **Figure S4.** Spectroscopically – derived biochemical profile of RBC membranes in natural aging.

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67 **Figure S5.** Alterations in biochemical composition of RBC membranes due to the D-galactose induced accelerated aging (C57BL/6)
68 and D-galactose-fed C57BL/6J mice). Ratios calculated for the integral absorbances in the ATR-FTIR spectra. **Integration regions for**
69 **IR bands:** amide I – 1651 cm⁻¹ (1687–1605 cm⁻¹); amide II – 1544 cm⁻¹ (1560–1502 cm⁻¹); C=O stretch- 1738 cm⁻¹ (1758–1727 cm⁻¹),
70 1055 cm⁻¹ (1076–1034 cm⁻¹).

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CHANGES IN PROFILE OF RBCs



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73 **Figure S6.** Alterations in biochemical composition of intact RBCs due to the D-galactose-induced accelerated aging (C57BL/6J and
 74 D-galactose-fed C57BL/6J mice). Ratios calculated for the integral absorbances in the ATR-FTIR spectra. **Integration regions for IR**
 75 **bands:** amide I – 1651 cm^{-1} ($1687\text{--}1605\text{ cm}^{-1}$); amide II – 1544 cm^{-1} ($1560\text{--}1502\text{ cm}^{-1}$); CH_2 symmetric stretch – 2933 cm^{-1} ($2863\text{--}2847$
 76 cm^{-1}); CH_3 asymmetric stretch – 2873 cm^{-1} ($2965\text{--}2936\text{ cm}^{-1}$); PO_2^- asymmetric stretch – 1236 cm^{-1} ($1261\text{--}1214\text{ cm}^{-1}$); $-\text{CO}\text{--O}\text{--C}$ stretch
 77 – 1167 cm^{-1} ($1191\text{--}1144\text{ cm}^{-1}$).

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