Hypercholesterolemia triggers innate immune dysbalance and transforms brain infarcts after ischemic stroke

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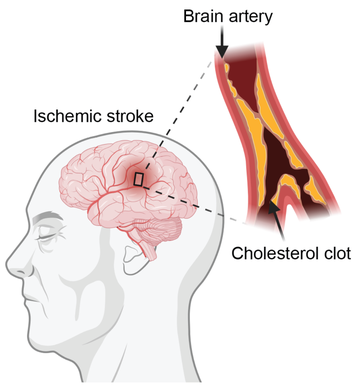
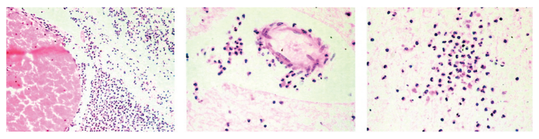
Background and Aims

Post-stroke early activation of neutrophils contributes to intensive neuroinflammation and worsens disease outcomes. Other pre-existing patient conditions can modify the extent of their activation during disease, especially hypercholesteremia. However, whether and how increased circulating cholesterol amounts can change neutrophil activation responses very early after stroke has not been studied. In this study, we investigated the effect of high-fat diet-induced hypercholesterolemia on the activation of neutrophils and brain infarcts in a mouse model of transient ischemic stroke.



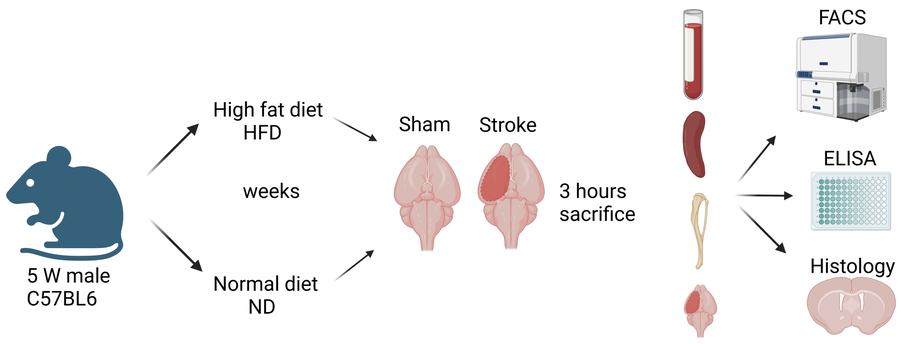
Neutrophils in the brain of stroke patient (Price et al 2004, Stroke).



Aim: Neutrophil activation in therapeutic time window of 3 hours?

Methods

To investigate the effect of increased circulating cholesterol on neutrophil activation within the first three hours after stroke, we fed wild-type C57BL/6 male mice with a Western-style high-fat diet (HFD) or normal diet (ND) for six weeks and then induced stroke by transient occlusion of the middle cerebral artery. The activation of immune cells and plasma levels of cytokines were analyzed using flow cytometry. The amount of plasma neutrophil extracellular traps (NETs) was measured using citH3-DNA complex ELISA. Brain infarct volumes were quantified by analysis of cresyl violet stained histological sections.



Results

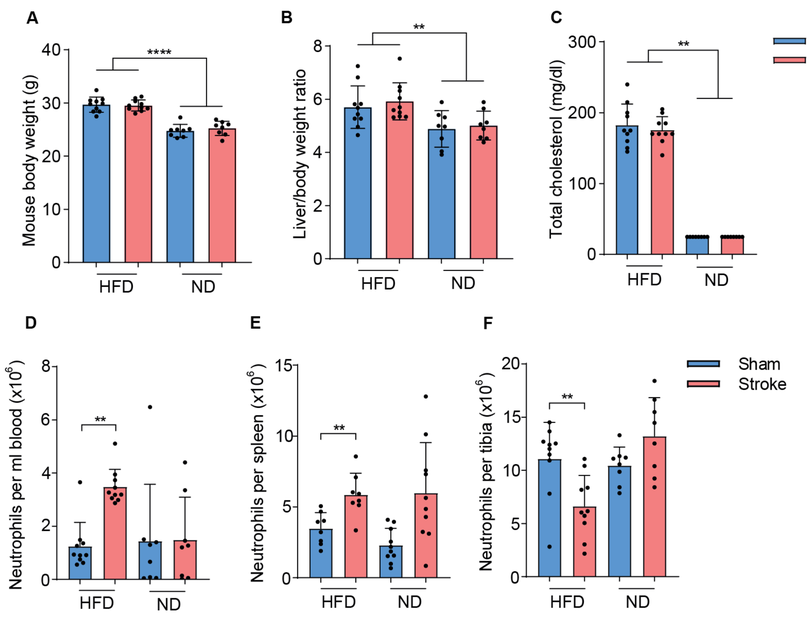


Figure 1. HFD increases circulating cholesterol levels and neutrophil numbers in stroke mice. A. Total body weight of sham-operated or stroke mice fed on HFD or ND. **B.** Liver-body weight ratio of HFD or ND mice after 3 hours of stroke or sham operation. **C.** Plasma amounts of cholesterol in HFD and ND mice 3 hours after stroke or sham-operation. **D**. The number of neutrophils in blood, **E.** spleen and **F**. tibial bone marrow of HFD and ND mice 3 hours after stroke or sham-operation. Data are mean ±s.d., statistical analyses were performed by Mann-Whitney U test **P<0.01, ****P<0.0001, N=8-10 mice per group. HFD=high fat diet, ND=normal diet.

Results

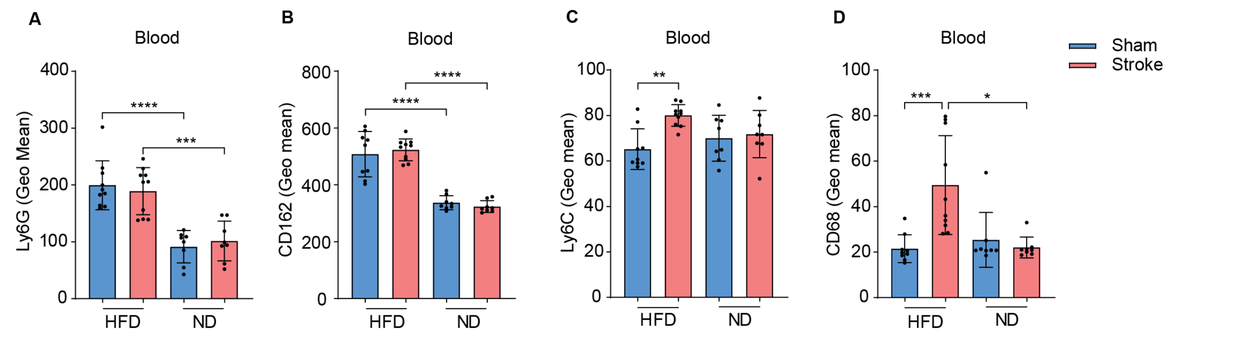


Figure 2. HFD triggers blood neutrophil and monocyte activation in stroke mice. A. Mean fluorescence intensity (MFI) of Ly6G and **B.** CD162 on blood neutrophils in HFD and ND mice after stroke or sham operation. **C.** MFI of Ly6C and **D.** CD68 on monocytes in HFD and ND mice after stroke or sham operation. **E.** Mean fluorescence intensity (MFI) of Ly6G and **F.** CD162 on splenic neutrophils in HFD and ND mice after stroke or sham operation. **G.** MFI of Ly6C and **H.** CD68 on monocytes in HFD and ND mice after stroke or sham operation. Data are mean ±s.d., statistical analyses were performed by ordinary one-way ANOVA for multiple comparisons, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. N=8-9 mice per group. HFD=high fat diet, ND=normal diet.

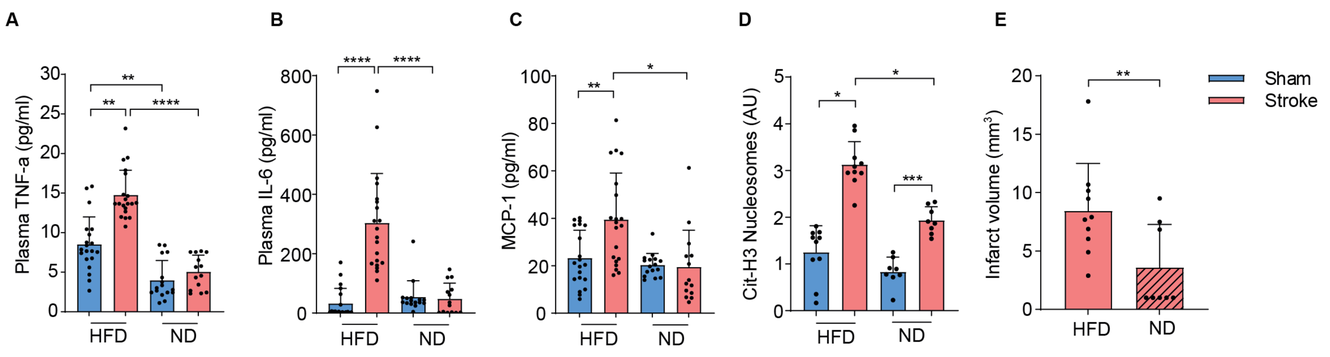


Figure 3. HFD triggers the release of inflammatory cytokines, NETs and increases brain infarct volumes after stroke. A-C. The amounts of plasma TNF-a, IL-6 and MCP-1 in HFD and ND-treated mice after stroke or sham operation. **D.** The plasma amounts of cit-H3 nucleosomes in HFD and ND mice after stroke or sham operation. **E.** The quantitative analysis of infarct volume using cresyl violet stained histological brain sections in HFD and ND mice. Data are mean ±s.d., statistical analyses were performed by ordinary one-way ANOVA for multiple comparisons or the Mann-Whitney U test for two-group comparisons. *P<0.05, **P<0.01, ****P<0.0001. N=8-9 mice per group.

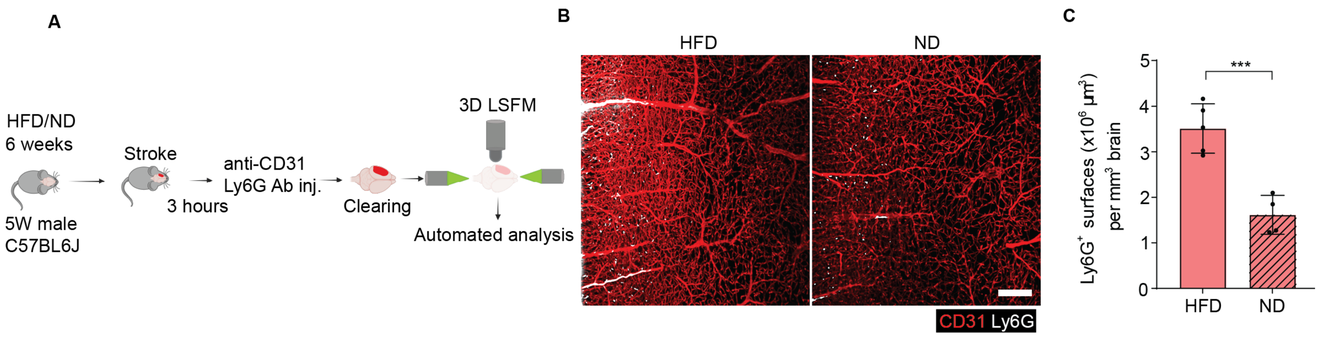
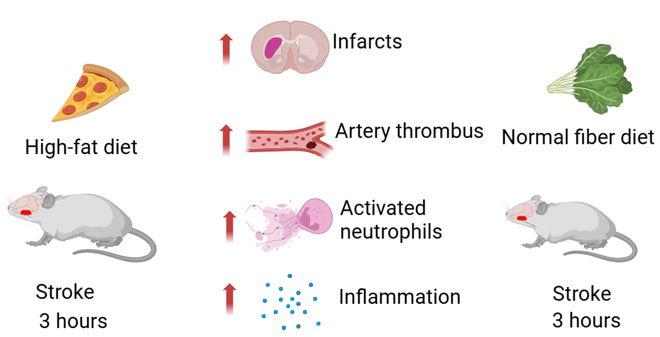


Figure 4. HFD triggers neutrophil accumulation in brain vasculature after stroke. A. The schematic of 3D-LSFM brain imaging of HFD and ND stroke mice. **B.** 3D rendering of ipsilateral brain hemispheres after stroke in HFD and ND mice showing higher frequencies of infiltrated neutrophils in HFD mice. Red=CD31, White=Ly6G, scale bar=200 um. **C.** The total surface volumes of Ly6G in the ischemic hemispheres of HFD and ND mice. Data are mean ±s.d., statistical analyses were performed by Mann-Whitney U test for two-group comparisons. ***P<0.001, N=4-5 mice per group. HFD= high-fat diet, ND= normal diet, LSFM=light-sheet fluorescence microscopy.

Conclusions and outlook



1. Hypercholesteremia-driven neutrophil activation in combination with clinical investigations might be used as a predictive marker of stroke severity in patients.
2. Future studies identifying underlying mechanisms of neutrophil activation are needed to develop strategies to reduce inflammatory brain injury in stroke patients.

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