Journal of Clinical and Translational Hepatology **2023** vol. 11(5) | 1282–1285
DOI: 10.14218/JCTH.2023.00094

Research Letter

Early but Not Late Exercise Training in Mice Exacerbates Hepatic Inflammation in Developing Nonalcoholic Fatty Liver Disease

Artemiy Kovynev[1,2#], Zhixiong Ying[1,2#], Joost M. Lambooij[3,4], Hendrik J.P. van der Zande[3], Bruno Guigas[3], Patrick C.N. Rensen[1,2] and Milena Schönke[1,2*]

[1]Division of Endocrinology, Department of Medicine, Leiden University Medical Center, Leiden, The Netherlands; [2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands; [3]Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands; [4]Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, The Netherlands

Received: 1 March 2023 | **Revised:** 25 April 2023 | **Accepted:** 10 May 2023 | **Published online:** 19 June 2023

Citation of this article: Kovynev A, Ying Z, Lambooij JM, van der Zande HJP, Guigas B, Rensen PCN, *et al*. Early but Not Late Exercise Training in Mice Exacerbates Hepatic Inflammation in Developing Nonalcoholic Fatty Liver Disease. J Clin Transl Hepatol 2023;11(5):1282–1285. doi: 10.14218/JCTH.2023.00094.

Over 2 billion people worldwide are estimated to have nonalcoholic fatty liver disease (NAFLD), defined by excess hepatic fat. Commonly, progression into nonalcoholic steatohepatitis (NASH) is characterized by the onset of liver inflammation following exacerbated steatosis.[1] This suggests that reducing liver inflammation (e.g. through lifestyle interventions involving exercise training) may come secondary to a reduction of steatosis. However, both the metabolic and inflammatory processes involved in NAFLD are under circadian control and could respond differently to exercise at different times of day.[2]

To investigate the time-of-day–dependent effect of exercise training on NAFLD amelioration in the early disease stages we trained high-fat high-cholesterol (HFHC)-fed APOE*3-Leiden cholesteryl ester transfer protein (CETP) mice during their early or late active period. This mouse model was chosen because of its humanized lipid metabolism and its ability to develop all hallmarks of human NAFLD upon HFHC feeding.[3] The animals were treadmill trained five times per week for 8 weeks at either Zeitgeber time (ZT)13 (E-RUN) or ZT22 (L-RUN). Corresponding sedentary animals (E-SED and L-SED) were put into empty cages at the same time to control for experiment-induced stress. After 8 weeks, all mice were killed at the same circadian time (ZT17) on the day after the last exercise training bout to allow for comparisons between all four groups and to reduce the confounding effect of the acute exercise (Supplementary File 1).

At the end of the study, body weight (Fig. 1A) and lean body mass (Supplementary Fig. 1A) were similar in all mice, but trained mice had gained less fat mass than sedentary mice (Fig. 1B), indicating a measurable exercise effect. Fasting plasma glucose, which when elevated is independently positively associated with the risk of developing NAFLD,[4] was unchanged among the groups (Fig. 1C). Furthermore, no differences in hepatic steatosis, NAFLD activity score, plasma alanine aminotransferase (ALT) levels, portal inflammation and liver weight were observed between the groups (Fig. 1D–F and Supplementary Fig. 1D, E), likely owing to an overall limited potential to improve these parameters in early stages of steatosis without signs of NASH. Accordingly, liver triglyceride (TG), total cholesterol (TC), and phospholipid (PL) levels (Fig. 1G–I) as well as plasma TG and TC (Supplementary Fig. 1B, C) levels remained unchanged in the exercising and sedentary groups regardless of the time of training.

Surprisingly, exercise training had a time-of-day specific impact on liver inflammation, challenging the notion that hepatic inflammation merely follows the level of steatosis. In livers collected at the same circadian timepoint 1 day after the last training, flow cytometry analysis of isolated hepatic immune cells revealed an unexpected hepatic increase in the total number of leukocytes, neutrophils, and monocytes in response to early training that did not reflect increased blood immunocyte levels (Fig. 2A–C and Supplementary Fig. 2C–F). Notably, blood leukocytes were even significantly decreased in E-RUN compared to E-SED at the same time liver leukocytes were elevated (Supplementary Fig. 2C), consistent with migration of these cells to the liver. Late training, however, had no effect on the immune cell populations in blood or liver. The increase of specific cell populations following early training may indicate disease acceleration, as infiltrating neutrophils are associated with NAFLD development and disease progression.[5,6] In line with that, infiltrating monocytes, which are recruited to the liver partly through

Abbreviations: Adgre1, adhesion G protein-coupled receptor E1; ALT, alanine aminotransferase; CETP, cholesteryl ester transfer protein; E-RUN, early running; E-SED, early sedentary; HFHC, high-fat high-cholesterol; *Il1b*, interleukin-1β; L-RUN, late running; L-SED, late sedentary; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NK, natural killer (cells); PL, phospholipid; TC, total cholesterol; TG, triglyceride; *Tnf*, tumor necrosis factor; ZT, Zeitgeber time.

[#]Contributed equally to this work.

[*]**Correspondence to:** Milena Schönke, Division of Endocrinology, Department of Medicine, Leiden University Medical Center, Albinusdreef 2, 2333BG Leiden, The Netherlands. ORCID: https://orcid.org/0000-0003-2030-6958. Tel: +31-715268188, E-mail: m.schoenke@lumc.nl

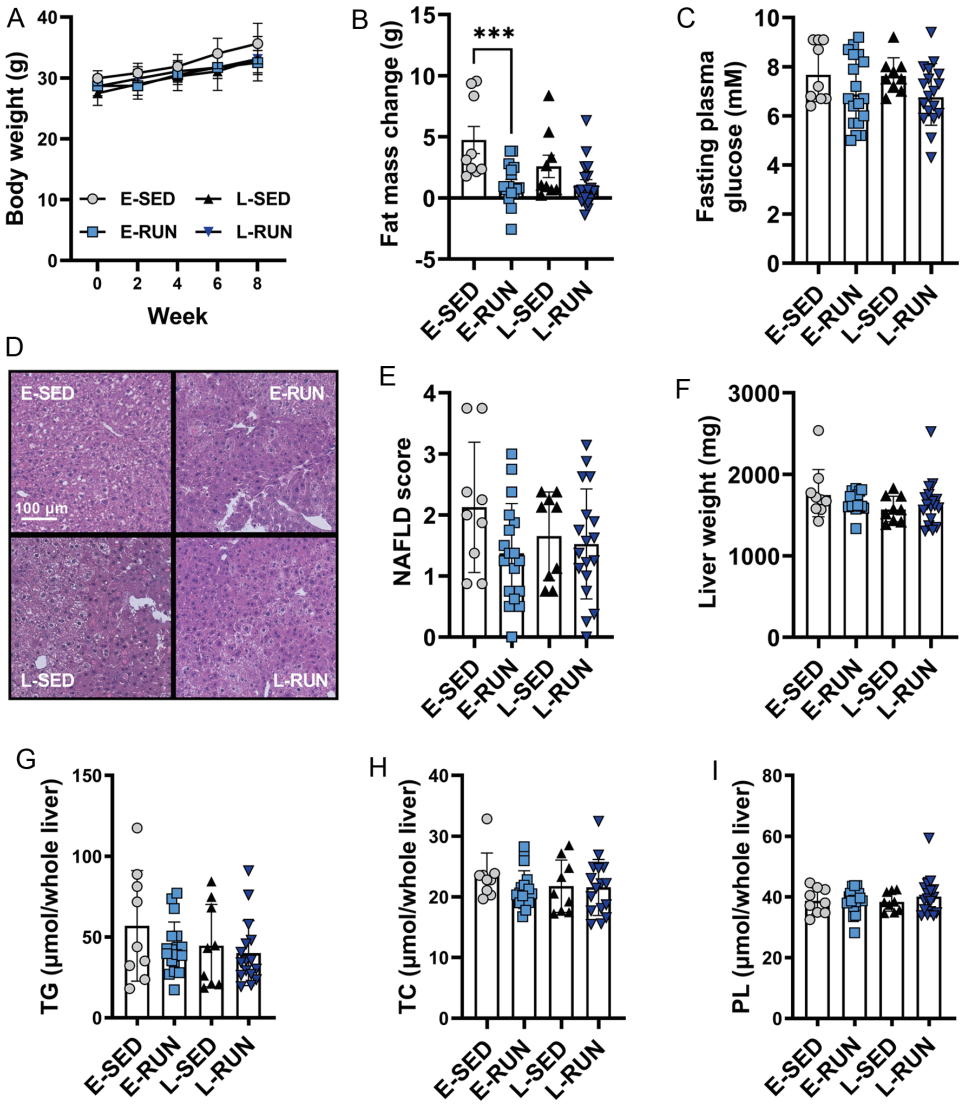

Fig. 1. Early and late exercise training both improved body composition but not developing liver steatosis in APOE*3-Leiden.CETP mice. (A–C) Over 8 weeks of training, body weight (A) and changes of fat mass (B) were monitored, and fasting plasma glucose was measured after 8 weeks (C). (D–E) Representative liver images are shown (D) that were used to assess the NAFLD score (E). (F–I) Liver weight (F), liver triglyceride (G), total cholesterol (H), and phospholipid (PL) content (I) were assessed. ***p<0.001, one-way analysis of variance, n=9–18. E-SED, early sedentary; E-RUN, early running; L-SED, late sedentary; L-RUN, late running; TG, triglycerides; TC, total cholesterol; PL, phospholipids.

hepatocyte-derived stress signals such as interleukin (IL)-1β and tumor necrosis factor alpha (TNFα), differentiate into proinflammatory macrophages that contribute to tissue damage and the loss of resident macrophages.[7] Interestingly, early training also increased the number of natural killer (NK) cells in the liver (Fig. 2D). Although the contribution of these cells to NAFLD development and progression to NASH remains controversial, they can produce large quantities of proinflammatory cytokines such as interferon gamma.[8] Taken together, early training led to an inflammatory response in the liver characterized by an increase of proinflammatory and tissue damage-associated cell populations.

An increase in hepatic inflammation following early training was also confirmed by gene expression analysis in isolated hepatic immune cells. Gene expression of the secreted proinflammatory factors IL-1β (*Il1b*) and TNF-α (*Tnf*) was increased after early training but not after late training (Fig. 2E, F). Similarly, the expression of the macrophage marker F4/80 (*Adgre1*) was increased following early training (Fig. 2G). In line with that, early training also increased the expression of *Tnf, Il1b* and *Adgre1* in whole liver tissue (Fig. 1D–F).

It is not clear whether the observed increase of liver inflammation with early training is beneficial or detrimental in NAFLD development. As the number of circulating immune cells as well as their activity exhibits a circadian rhythm in mice and humans, exercise in the early active phase may stimulate cell migration into the liver at the time these cells are most prone to migrate into peripheral organs.[9] Consequently, one could speculate that by stimulating liver inflammation in developing steatosis, early training activates a rapid alert system that supports disease resolution. Conversely, it has been shown that early exercise can acutely worsen metabolic diseases as seen in people with obesity and type 2 diabetes where early high intensity cycling elicited

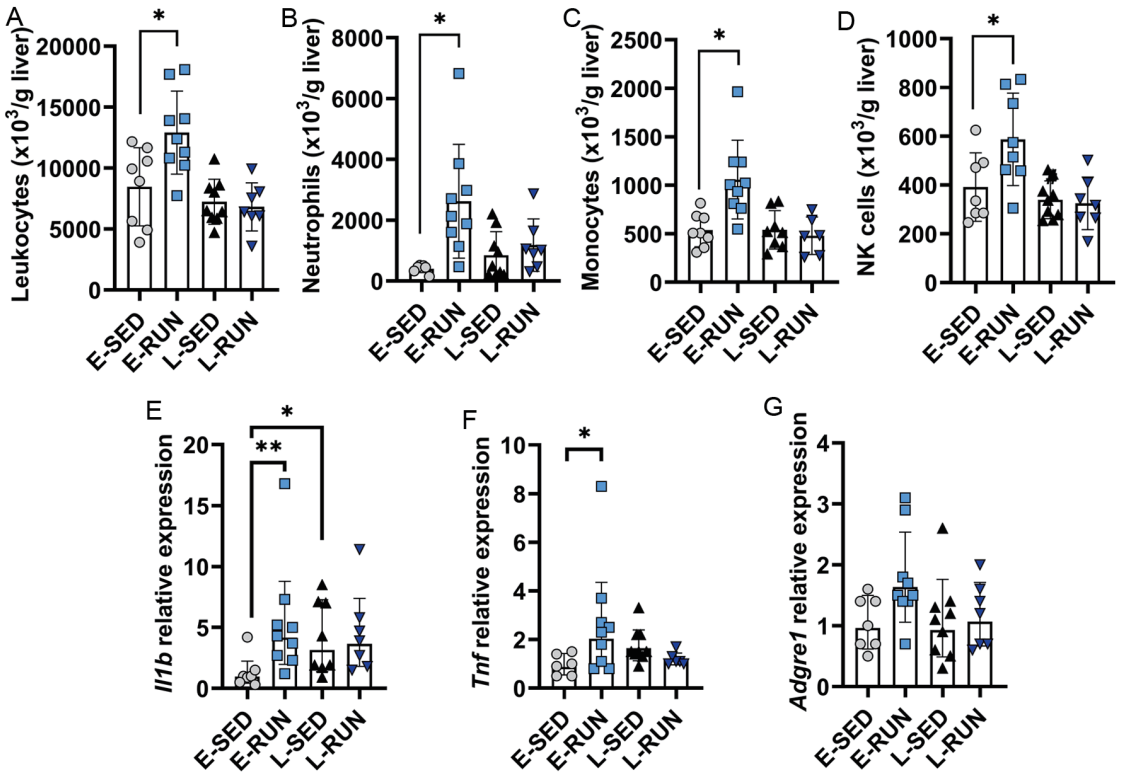

Fig. 2. Early exercise promotes distinct changes in liver immune cell populations and inflammatory markers in developing steatosis. (A–D) The total number of liver leukocytes (A), neutrophils (B), monocytes (C) and NK cells (D) was determined after 8 weeks of treadmill training. (E–G) Gene expression of *Il1b* (E), *Tnf* (F) and *Adgre1* (G) was assessed in isolated liver immune cells and shown relative to E-SED. *p<0.05, **p<0.01, one-way analysis of variance, n=7–9. E-SED, early sedentary; E-RUN, early running; L-SED, late sedentary; L-RUN, late running; NK, natural killer (cells); *Il1b*, interleukin-1β; *Tnf*, tumor necrosis factor; *Adgre1*, adhesion G protein-coupled receptor E1.

unfavorable blood glucose spikes that did not occur with late exercise.[10] Accordingly, our findings could indicate that early training accelerated disease progression while late exercise training potentially targets liver steatosis and inflammation at a later disease stage. However, while not affecting liver lipid levels, the hepatic gene expression of Srebp1c (*Srebf1*), the mediator of insulin-induced fatty acid synthesis, was downregulated with both early and late training (Supplementary Fig. 1G), suggesting that the regulation of metabolic and inflammatory disease drivers may not be synchronized. Future studies need to assess the translatability of our findings to advanced disease stages and to human NAFLD. Notably, we observed distinct inflammatory modulation already at an early disease stage with a low NAFLD score, low grade hepatic steatosis and before the disease becomes inflammation-driven. This may present a previously underappreciated inflammation-targeted treatment opportunity in a large part of the population at risk for NASH. In summary, we showed that early and late exercise training in a mouse model of NAFLD differently influenced liver inflammation in developing steatosis. An unexpected increase in liver inflammation was observed with early exercise training.

Acknowledgments

We thank Trea Streefland and Reshma Lalai (Div. of Endocrinology, Dept. of Medicine, LUMC, Leiden, the Netherlands) for their excellent technical assistance. We also thank Lars Hoeve, Sjahnaaz Bholai and Jack Brouwer for their technical contributions.

Funding

This study was financed by a grant from the Novo Nordisk Foundation to MS (NNF18OC0032394). ZY was supported by a full-time PhD scholarship from the China Scholarship Council.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (AK, ZY, PCNR, MS), acquisition of data (AK, ZY, JML, HJPZ, MS), analysis and interpretation of data (AK, ZY, JML, HJPZ, BG, PCNR, MS), drafting of the manuscript (AK, ZY, MS), critical revision of the manuscript for important intellectual content (JML, BG, PCNR), and study supervision (BG, PCNR, MS). All authors have made a significant contribution to this study and have approved the final manuscript.

References

[1] Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med 2018;24(7):908–922. doi:10.1038/s41591-018-0104-9, PMID:29967350.
[2] Mazzoccoli G, De Cosmo S, Mazza T. The Biological Clock: A Pivotal Hub in Non-alcoholic Fatty Liver Disease Pathogenesis. Front Physiol 2018;9:193. doi:10.3389/fphys.2018.00193, PMID:29662454.
[3] van den Hoek AM, Verschuren L, Caspers MPM, Worms N, Menke AL, Princen

HMG. Beneficial effects of elafibranor on NASH in E3L.CETP mice and differences between mice and men. Sci Rep 2021;11(1):5050. doi:10.1038/s41598-021-83974-8, PMID:33658534.
[4] Zou Y, Yu M, Sheng G. Association between fasting plasma glucose and nonalcoholic fatty liver disease in a nonobese Chinese population with normal blood lipid levels: a prospective cohort study. Lipids Health Dis 2020;19(1):145. doi:10.1186/s12944-020-01326-3, PMID:32563249.
[5] Bertola A, Bonnafous S, Anty R, Patouraux S, Saint-Paul MC, Iannelli A, *et al*. Hepatic expression patterns of inflammatory and immune response genes associated with obesity and NASH in morbidly obese patients. PLoS One 2010;5(10):e13577. doi:10.1371/journal.pone.0013577, PMID:21042596.
[6] Hoogerland JA, Staels B, Dombrowicz D. Immune-metabolic interactions in homeostasis and the progression to NASH. Trends Endocrinol Metab 2022;33(10):690–709. doi:10.1016/j.tem.2022.07.001, PMID:35961913.
[7] Daemen S, Gainullina A, Kalugotla G, He L, Chan MM, Beals JW, *et al*. Dynamic Shifts in the Composition of Resident and Recruited Macrophages Influence Tissue Remodeling in NASH. Cell Rep 2021;34(2):108626. doi:10.1016/j.celrep.2020.108626, PMID:33440159.
[8] Martínez-Chantar ML, Delgado TC, Beraza N. Revisiting the Role of Natural Killer Cells in Non-Alcoholic Fatty Liver Disease. Front Immunol 2021;12:640869. doi:10.3389/fimmu.2021.640869, PMID:33679803.
[9] Pick R, He W, Chen CS, Scheiermann C. Time-of-Day-Dependent Trafficking and Function of Leukocyte Subsets. Trends Immunol 2019;40(6):524–537. doi:10.1016/j.it.2019.03.010, PMID:31109762.
[10] Savikj M, Gabriel BM, Alm PS, Smith J, Caidahl K, Björnholm M, *et al*. Afternoon exercise is more efficacious than morning exercise at improving blood glucose levels in individuals with type 2 diabetes: a randomised crossover trial. Diabetologia 2019;62(2):233–237. doi:10.1007/s00125-018-4767-z, PMID:30426166.