Role of gut-liver axis in circadian exercise and dietary interventions to improve metabolic health

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Chapter 9

Summary, Samenvatting, List of publications, Curriculum Vitae and Acknowledgements

Summary

The prevalence of obesity and associated metabolic disorders, such as metabolic dysfunction-associated steatotic liver disease (MASLD), type 2 diabetes (T2D) and atherosclerotic cardiovascular disease (ASCVD), have been increasing at alarming rate during the last decades. Although some pharmaceutical options to treat obesity, MASLD and T2D are emerging, lifestyle interventions aimed at weight loss are still the recommended first-line treatment. While many different lifestyle interventions are available to facilitate weight loss, the guidelines and advice for individuals with overweight and obesity are complex and sometimes contradictory. Accordingly, the aim of this thesis was to optimize the existing lifestyle interventions, such as exercise training and changes in the diet, and elucidate the mechanisms via which said interventions can be improved.

In **chapter 1**, I introduced the pathogenesis and mechanisms of MASLD development, the main focus of this thesis, as well as the current challenges around the treatment of MASLD. Currently the most effective way to target MASLD is via prolonged weight loss. Previous research has established that there is a need for lifestyle interventions that are sustainable in the long term and also maximize the interventions' benefits to reduce body weight. Literature search revealed that specific timing of certain activities during the day, such as exercise or food intake, may have long-lasting beneficial metabolic consequences, by making better use of circadian fluctuations of the body, aligning timing of activities with the organs' circadian clocks. Another way to improve lifestyle interventions appears to be through beneficial modulation of the gut microbiome, which has been causally linked with weight gain and obesity. Interventions likely need to revert the gut microbiome towards a "healthy" state, to ensure weight loss, and prevent otherwise likely weight regain. Specifically, an increase in bacteria that can convert fiber into short-chain fatty acids (SCFAs) is linked to positive metabolic outcomes.

In addition to being metabolic disorders, MASLD, T2D and ASCVD are also inflammatory in their nature. Therefore, in **chapter 2,** we have reviewed the existing evidence of the impact of timing of exercise training on inflammation. We concluded that late, but not early, exercise is associated with higher circulating levels of interleukin-6 (IL-6) in humans. While IL-6 may have both pro- and anti-inflammatory properties, IL-6 that is released by muscles after exercise induces an anti-inflammatory response only. Additionally, late exercise increases both the cell count and the relative abundance of NK cells and T cell subpopulations in

circulation, a protective mechanism to repair muscle damage. Overall, late exercise training may thus be more beneficial than early exercise due to higher anti-inflammatory effects. Nonetheless, we also found that most studies on timing of exercise training are poorly controlled. Usually, they only include one sex and one exercise bout, and apply a range of different types of exercise, highlighting the need for better and more well-controlled studies on this subject.

To gain better understanding of the influence of circadian exercise on inflammation in early stages of MASLD, in **chapter 3** we used male APOE*3-Leiden.CETP mice, a well-established model for human-like lipid metabolism and obesity-associated metabolic diseases when fed a high-fat high-cholesterol (HFHC) diet. We exercise-trained these mice at an age of 10-12 weeks, either in the beginning (early runners) or the end (late runners) of their active period, daily, for 8 weeks. Since this experimental set up allowed us to study the very early stages of MASLD without development of substantial steatosis, neither early or late running affected fat accumulation in the liver. Curiously, however, liver inflammation was increased in the early runners, but not in the late runners. These data suggest that exercising in the late active period, which would be in the evening for humans, may be more beneficial to prevent the onset or counteract diet-induced MASLD.

In **chapter 4**, we next investigated the impact of timing of exercise on fully developed MASLD, by using older APOE*3-Leiden.CETP male mice that exercised for 12 weeks. Strikingly, late but not early exercise decreased liver fat accumulation, body weight and fat gain. Mechanistically, late but not early exercise changed the diversity of the gut microbiome, increasing the abundance of bacteria that can convert dietary fiber into SCFAs. To investigate if these changes in the microbiome of late-exercising mice are causal to the protective effects observed on the liver, we transplanted the fecal microbiome of mice that exercised late into mice that did not exercise. The mice that received fecal transplantation of late exercising mice showed reduction in liver fat accumulation and body weight gain compared to mice that received fecal transplantation of sedentary mice, confirming that the observed beneficial effects of late exercise are at least partially mediated via the changes in the gut microbiome. Taken together, our findings of **chapters 3** and **4** show that late exercise training may be more beneficial than early exercise to reduce body weight and body fat, but also liver inflammation and liver steatosis, involving the gut-muscle axis.

Besides timing of exercise, other important aspects are the frequency and intensity of exercise, especially given that too frequent or intense exercise may negatively affect the gut integrity. Therefore, in **chapter 5** we investigated the impact of moderate exercise for 5 days per week and vigorous exercise for 3 days per week on the gut integrity and gut microbiome composition in male wild-type mice on a non-obesogenic regular chow diet. Surprisingly, we found that frequent moderate exercise resulted in more pronounced damage to the gut wall structure than less frequent vigorous exercise. Simultaneously, mice that less frequently vigorously exercised had a higher abundance of gut bacteria producing butyrate, which is a SCFA that can strengthen the gut wall. From these data we concluded that larger breaks between exercise sessions give the gut more time to recover, while an increase in butyrate-producing bacteria in the gut serves as an additional protective mechanism for the gut wall.

As in both **chapters 4** and **5** the beneficial changes induced by late exercise were connected to an increase in the abundance of gut bacteria that ferment dietary fiber into SCFAs, we hypothesized that combining late exercise with a diet enriched in dietary fiber may lead to further improvement of MASLD. Therefore, in **chapter 6** we exercise-trained male APOE*3-Leiden.CETP mice, fed a high-fat high-cholesterol diet with or without the addition of 10% inulin as dietary fiber, daily in the late active period, for 8 weeks. Both late exercise training and dietary fiber supplementation reduced fat mass gain and lowered plasma glucose levels. Only the combination treatment, however, induced fat loss, and decreased plasma triglyceride and cholesterol levels compared to sedentary control mice. Late exercise training with and without supplementation with fiber had a similar ameliorating effect on the MASLD score. Dietary fiber supplementation was mainly responsible for remodeling the gut microbial composition, with an increase in the relative abundance of SCFA-producing bacteria, while, surprisingly, late exercise training alone and with dietary fiber resulted in the highest increase of SCFA production. We concluded that the combination of late exercise training and dietary fiber supplementation decreases fat mass and improves glucose and lipid homeostasis, but does not have an additional synergistic positive effect on liver health compared to exercise training alone.

Increasing dietary fiber consumption is just an example of dietary lifestyle interventions that can induce weight loss. Recently, various diets aimed at calorie restriction, such as intermittent fasting or 5:2 fasting, appeared as new and effective

ways for body weight management. While they were initially believed to activate metabolic remodeling, recent studies showed that their benefits in fact result from caloric restriction. Consequently, fasting-mimicking diets (FMD), which restrict food intake to ~700 kcal/day for 4-7 days, appeared as a mode that both induces caloric restriction and mimics the metabolic effects of fasting. Since the impact of FMD on the gut microbiome has not been studied before, in **chapter 7**, we set out to study the impact of a year-long, 5 days per month FMD intervention on the gut microbiome in participants with obesity and T2D. Notably, the provided diet contained higher amount of fiber than the daily recommended fiber intake. One fasting bout resulted in a major remodeling of the gut microbiome with an increase in the abundance of SCFA-producing bacteria. After completion of year-long FMD intervention, long-lasting changes in the gut microbiome we observed, especially on the functional level, with an increase in the abundance of bacterial genes responsible for the production of butyrate. This coincided with improvement of glycemic management in 53% of the participants, compared to an improvement of only 8% of the participants in the control group. Overall, we concluded that FMD is safe for the gut microbiome and induces a long-term positive gut microbiome remodeling.

Finally, in **chapter 8**, we summarized and discussed the findings of this thesis in the broader context of scientific literature. Overall, our findings show that there is great potential for optimizing exercise and dietary regiments to combat metabolic diseases. These modulations largely act via manipulation of the gut microbiome composition. Specifically, late exercise training and supplementation with dietary fiber shift the gut microbiome towards bacteria that have higher SCFA production. This, in turn, results in more body fat loss and lower liver steatosis. However, all of these findings still need to be verified in humans, where additional factors including chronotype and food quality, play a role in the observed outcomes. After finishing a lifestyle intervention program, weight regain is also likely, and stable and healthy microbiome may prevent or at least slow down this weight regain. This is also why there is a bright future for lifestyle interventions even with the emergence of potent anti-obesity drugs, such as Semaglutide or Tirzepatide. Such lifestyle interventions should be used before, during and likely also after treatment with these new drugs, to both enhance weight loss and prevent weight regain.