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Journal

Liver International, 44(2)

ISSN

1478-3223

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Publication Date

2024-02-01

DOI

10.1111/liv.15769

Peer reviewed

HHS Public Access

Author manuscript

Liver Int. Author manuscript; available in PMC 2025 February 01.

Published in final edited form as:

Liver Int. 2024 February ; 44(2): 532–540. doi:10.1111/liv.15769.

Exercise training improves serum biomarkers of liver fibroinflammation in patients with metabolic dysfunction-associated steatohepatitis

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Abstract

Background & Aims: Exercise training is recommended for all patients with metabolic dysfunction-associated steatotic liver disease (MASLD) and may reverse liver fibrosis. Whether exercise training improves liver fibrosis without body weight loss remains controversial. We further investigated this relationship using serum biomarkers of liver fibroinflammation in a post hoc analysis of an exercise trial where patients did not lose significant body weight.

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Author contributions: All authors contributed in the following manner and 1) provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, 2) drafted the article or revised it critically for important intellectual content and 3) provided final approval of the version to be published.

Clinical trial registration number: NCT03518294

Ethics approval statement: All patients provided informed consent prior to being included in the study and the study was approved by the Penn State Health Institutional Review Board (Study 8507). All research was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines and Penn State Health local regulatory requirements.

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Methods: In the NASHFit Trial, patients with metabolic dysfunction-associated steatohepatitis (MASH) were randomized to receive either moderate-intensity aerobic exercise training or standard clinical care for 20 weeks. Mediterranean-informed dietary counseling was provided to each group. Change in serum biomarkers was measured and compared between the two groups.

Results: Exercise training led to improvement in serum biomarkers of liver fibroinflammation, including 1) ≥17 IU/L reduction in ALT in 53% of individuals in the exercise training group compared to 13% in the standard clinical care group ($p<0.001$; mean reduction 24% vs. 10% respectively) and 2) improvement in CK18 (−61 vs. +71 ng/mL, $p=0.040$). ALT improvement ≥17 IU/L was correlated with ≥30% relative reduction in MRI-measured liver fat and PNPLA3 genotype.

Conclusion: Exercise training improves multiple serum biomarkers of liver fibroinflammation at clinically significant thresholds of response without body weight loss. This study provides further evidence that exercise training should be viewed as a weight neutral intervention for which response to intervention can be readily monitored with a widely available non-invasive biomarkers that can be applied at the population level.

Lay summary

- The NASHFit Trial investigated the effects of exercise training on liver health in patients with metabolic dysfunction-associated steatohepatitis (MASH) who did not experience significant weight loss.
- Exercise training led to significant improvements in multiple liver fibroinflammation biomarkers.
- These findings suggest that exercise can improve liver health even without weight loss and can be monitored using widely accessible biomarkers.

Keywords

nonalcoholic fatty liver disease; fatty liver; steatotic liver disease; physical activity; cardiorespiratory fitness

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formally known as nonalcoholic fatty liver disease (NAFLD), is one of the most common causes of chronic liver disease affecting approximately 30% of the world's population.[1] MASLD rates continue to rise in parallel with the obesity pandemic, as body mass index is an independent dose-responsive predictor of hepatic steatosis.[2] Furthermore, common metabolic comorbidities, including type two diabetes mellitus, are associated with increased risk of developing MASLD.[3] Previous literature has shown concomitant metabolic syndrome to be key in the pathogenesis of hepatic steatosis, prompting the nomenclature change to include a metabolic association.[4–6] The increasing prevalence of MASLD is of particular concern as patients can develop a histologically characterized subtype of MASLD known as metabolic dysfunction-associated steatohepatitis (MASH), previously

called nonalcoholic steatohepatitis (NASH). MASH is associated with increased risk of advanced liver fibrosis, cirrhosis, and hepatocellular carcinoma.[7]

As a result, the cost of treating MASLD/MASH is an expensive proposition for the global healthcare system. In the United States alone, the annual direct cost of MASLD is just over $100 billion, with individuals with advanced MASH contributing nearly half this cost.[8] These healthcare costs are due to both greater healthcare resource utilization and higher direct medical and non-medical costs. Importantly, this economic burden increases with disease severity or complications.[9]

Despite the well-known high economic impact of MASLD, there remains no regulatory agency approved cure or effective drug treatment, although several promising therapeutics are on the horizon.[10] Accordingly, lifestyle modifications such as dietary changes and increasing physical activity are the mainstay of management and will likely remain so even when an approved drug therapy is widely available.[11–15] Physical activity, particularly exercise training, has been shown to reduce liver fat measured by magnetic resonance imaging (MRI).[16] Moreover, exercise training can reduce liver inflammation and can lead to histologic changes.[17]

Given the increasing rate of MASLD/MASH, it is crucial to have an accurate and widely-available non-invasive biomarker to gauge histologic changes as it is liver fibrosis stage that is known to drive clinical outcomes and remains the target of all interventions, drug or lifestyle-based.[18,19] Past research has elucidated that a reduction in alanine aminotransferase (ALT) by at least 17 U/L is significantly associated with histologic changes,[20] providing a reliable non-invasive method to assess fibrosis response to intervention in individuals with MASLD/MASH. The NASH Fibrotic Index (NFI)[21] was also recently published, providing the researcher and clinician alike a novel reliable tool to non-invasively determine the risk of fibrotic NASH, defined histologically as NASH + NAFLD Activity Score (NAS) ≥4 + liver fibrosis (F) stage ≥2.

Debate remains if exercise can improve liver fibrosis in the absence of clinically significant weight loss,[14] and at this point in time, it is generally accepted that body weight loss is required to improve liver histology,[22] even with emerging evidence recently suggesting the benefit of exercise training on non-invasive MASH biomarkers is independent of clinically significant body weight loss.[23] Weight-neutral interventions remain of particular interest given most patients with MASH are unable to achieve significant weight loss. For these reasons, there remains a clear gap in knowledge of high significance. The primary aim of our study was to examine the impact of an exercise training program on established biomarkers of liver fibroinflammation, in patients who did not experience a significant change in body weight.

Patients and Methods

Study design and population

We conducted a post-hoc analysis of the previously published 20-week NASHFit Trial (NCT03518294)[24] which investigated the efficacy moderate-intensity aerobic exercise

training in comparison to standard clinical care. Of the 28 patients enrolled, 24 patients completed the exercise trial between May 2018 and February 2021. Paired samples were available for 23 patients for this analysis [exercise n=15) and standard of care (n=8)]. All patients provided informed consent prior to being included in the study and the study was approved by the Penn State Health Institutional Review Board (Study 8507). All research was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines and Penn State Health local regulatory requirements. Previous papers have detailed the specifics about eligibility criteria, recruitment, randomization, sample size and other research methods completed as a part of this study.[24,25] Briefly, sedentary adults with biopsy-confirmed MASH using the NASH Clinical Research Network histological scoring system were included in this study.[26] Patients were excluded if they had excessive alcohol consumption, other chronic liver disease, uncontrolled diabetes or if there was a concern about their ability to complete regular exercise sessions. Once enrolled and baseline testing was completed, individuals with MASH were randomized 2:1 to receive either exercise training intervention or standard clinical care.[20] No stratified randomization was performed. Patients in the intervention group completed moderate-intensity aerobic exercise sessions five times each week, for 30 minutes each session. Standard of care control patients continued their clinical care at the discretion of their treating medical provider, and all received standard lifestyle education. Digital therapeutic monitoring and direct supervision of exercise training sessions ensured compliance with the study protocol. Both study groups received Mediterranean-informed dietary counseling. No clinically significant changes in body weight (mean body weight loss was 2.8%, which is well below the 5% accepted threshold) were observed with this intervention.

Statistical Analysis

The analysis of the primary endpoint (proportion of patients achieving ≥17 IU/L reduction in ALT) was performed with the use of a chi-squared test. Continuous variables were analyzed using paired t-tests and other categorical variables were analyzed again by the chi-squared test or Fisher's exact test where appropriate. Statistical significance was determined by two-sided p-values of <0.05. Pearson's correlation coefficients were calculated between ≥17 IU/L reduction in ALT and clinical variables from the NASHFit Trial. SAS (Cary, NC) Version 9.4 was used for all statistical analysis.

Results

Baseline characteristics

Twenty-four patients completed the NASHFit Trial. ALT at study entry and study completion was measured for 23 patients (15 exercise, 8 standard). Mean age was 52 +/− 11 years (range 25 to 69 yrs.). Mean body weight was 101 +/− 18 kg and mean body mass index (BMI) was 33.8 +/− 5.0 kg/m^2. Thirteen patients were female (57%), 70% had hypertension, 61% had hyperlipidemia and 39% had diabetes. Liver fibrosis stage at study entry was 56% (n=13) F0/F1 fibrosis, 22% F2 (n=5), 17% F3 (n=4) and 4% F4 (n=1). Overall study cohort FNI was 49%. Baseline characteristics were similar between the exercise and the standard of care groups (Table 1). Specifically, the groups were well matched for age, sex, BMI, metabolic disease, MASH stage and FNI.

Change in MASH biomarkers following exercise training

There was a significant improvement in ALT in individuals who completed the exercise training program when compared to those who received standard clinical care. ALT was reduced by −24% (−14 +/− 14 IU/L) for the exercise group versus −10% reduction (−6 +/− 16 IU/L, p=0.060) for standard clinical care. The majority (53%) of exercise patients achieved the clinically significant threshold of response of ≥17 IU/L, which is validated to surrogate for liver fibrosis improvement,[20] compared to 13% in the standard clinical care group (p<0.001) (Figure 1). Achieving this threshold of serum ALT improvement was positively correlated with ≥30% relative reduction in MRI-PDFF (r=0.44, p=0.109), PNPLA3 GG genotype (r=0.45, p=0.108) or one G allele substitution (PNPLA3 GG or GC genotype) in PNPLA3 (r=0.57, p=0.033), and negatively correlated with hyperlipidemia (r=−0.58, p=0.031). Figure 2 further depicts the relationship between PNPLA3 genotype and achieving the desired threshold of ALT response. Additionally, the reduction in ALT was seen in parallel with an improvement in cytokeratin (CK) 18 (−61 +/− 45 vs. + 70 +/− 143 IU/L, p=0.040) (Table 2).

Importantly, 33% of individuals achieved both ALT reduction of at least 17 IU/L and at least a 30% relative reduction in MRI-proton density fat fraction (PDFF), another non-invasive threshold of clinically meaningful difference which surrogates for histologic improvement in MASH and liver fibrosis.[27–29] Of the patients who achieved at least 17 IU/L reduction in ALT, 53% also achieved at least 30% relative reduction in MRI-PDFF. No patient in the standard of group achieved both a reduction in ALT of at least 17 IU/L and a relative reduction in MRI-PDFF of 30% or greater. The FNI also was differentially impacted by exercise training where an 18% reduction was observed following exercise intervention compared to no change with standard clinical care (p=0.10).

As reported previously,[17] no clinically significant changes in body weight occurred in either group (Table 3). Mean body weight loss was 2.8%. Furthermore, sensitivity analysis excluding the three subjects who achieved body weight loss of 5% or more did not change the original conclusions; 50% achieved ALT reduction of at least 17 IU/L with exercise training vs. 13% with standard clinical care (p<0.001) and 33% achieved both ≥17 IU/L and ≥30% relative MRI-PDFF reduction with exercise training vs. 0% with standard clinical care.

Exercise training subjects also had better glycemic control than standard of care subjects with significant reductions in fasting glucose (p=0.039) and hemoglobin A1c (p=0.006) observed. Liver volume was reduced (−286 +/− 228 vs. +52 +/− 163 cc, p=0.039) in parallel with improvement in MRI-PDFF (absolute change −4.9 +/− 5.8 vs. +1.2 +/− 2.8%, p=0.012).

Discussion

In this post hoc analysis of adults with MASH enrolled in the NASHFit Trial, 20-weeks of moderate intensity aerobic exercise training improved serum biomarkers of liver fibroinflammation compared to standard clinical care. Notably, 53% of exercise training patients achieved a reduction in ALT of ≥17 IU/L, which is a validated biomarker

of liver fibrosis response. Exercise training also improved other biomarkers of liver fibroinflammation, including 1) at least 30% relative reduction in MRI-PDFF, 2) the composite of ALT reduction ≥17 IU/L and ≥30% relative reduction in MRI-PDFF 3) CK-18 and 4) FNI. Exercise training also improved other key indices of liver and metabolic health, including glycemic control, body composition and metabolic disease control. Notably, these findings were observed without clinically significant body weight loss. This was observed in parallel with improvements in MRI-measured liver fat, body composition and metabolic disease control. Collectively, the results of this analysis strongly support that exercise training should not only be offered to every individual with MASLD/MASH, but that every effort should be made to provide resources and necessary support required for an individual to successfully perform the exercise required to improve their chronic liver disease.

Recently published evidence suggests that exercise training can achieve amounts of liver fat reduction similar to or even exceeding the effect size seen with early phase MASH drug trials,[16,30–33] and at thresholds widely considered to be a meaningful treatment effect because this surrogates to improvement in liver fibrosis.[29] Our study not only further validates this important finding, but adds to our understanding of the weight-neutral benefits of exercise training because we demonstrated other serum biomarkers of liver fibroinflammation to be improved at clinically meaningful thresholds. When considered in the bigger picture of how we currently approach clinical management of the individual with MASLD/MASH, exercise training should no longer be viewed as a vehicle to achieve body weight loss; discussions with our patients should move beyond what they see on the scale and instead focus on the many clinical benefits seen without weight loss, including the loss of liver fat and improvement in biomarkers of liver fibroinflammation. This is important for several reasons. One, most patients with MASLD/MASH fail to meet body-weight loss targets with lifestyle intervention alone, making weight-neutral interventions like exercise training more meaningful to support. Two, fibrosis remains most closely tied to clinical outcomes, including both liver and non-liver related[34] and arresting or improving liver fibrosis remains the goal of all therapeutic interventions, including those that are lifestyle related.

This study also demonstrated the feasibility of using multiple serum biomarkers of liver fibroinflammation that are widely clinically available to monitor treatment response to exercise training. Given the limitations of liver biopsy including sampling error, cost, procedural complications, and significant variability in pathologist interpretation,[35] using liver biopsy to monitor treatment response is unreasonable. Having an accurate, reliable non-invasive method to monitor treatment response remains of much interest not only in clinical trials, but also in the routine day-to-day management of the MASLD patient. This is the first study to show feasibility in measuring serial ALT values across a validated clinical threshold extrapolated from MASH drug trials in patients undergoing exercise intervention. The data suggests that ALT response (with a goal of at least 17 IU/L improvement) can be widely used by healthcare providers as a low-cost, readily accessible test to employ in the routine monitoring of treatment response to lifestyle intervention. Moreover, this study is the first to show a differential impact of exercise training on the risk of fibrotic MASH where a nearly 20% reduction in risk was observed.

The relationship between exercise training and genetic risk for MASLD remains one of much interest.[36] Multiple recent reports suggest that moderate-to-high amounts of total physical activity (exercise is a subtype of physical activity that is planned, structured and repetitive and performed with a goal in mind[37]) decrease the risk of incident MASLD.[38–41] In fact, when ≥3000 Metabolic Equivalent of Tasks (MET)-mins/week are completed, the risk of incident disease can be reduced by just over 1/3 for individuals at-risk for MASLD as defined by polygenic risk scores. While a polygenic risk score was not calculated for this post hoc analysis and would have been helpful to further define the individuals most likely to respond to exercise training given our knowledge that multiple genes are implicated in MASLD/MASH pathogenesis,[42] the relationship between PNPLA3 genotype and improvement in serum biomarkers of fibroinflammation was investigated. There was a strong correlation in individuals who achieved the clinically meaningful threshold of at least 17 IU/L reduction with PNPLA3 GG or GC genotype. In other words, even in individuals at the greatest genetic risk for not MASLD development but also disease progression and cardiovascular disease events,[43–45] exercise training overcame the increased genetic risk and led to clinically meaningful improvement in multiple serum biomarkers. These findings are important because as move towards a future of precision medicine where individualized treatment plans are created, understanding how response to any therapy, including exercise training, which will always be recommended even when a regulatory agency approved drug becomes widely available, will be required to develop the most effective individualized treatment plans we all envision for our patients with MASLD/MASH. It also remains to be determined whether exercise training will enhance the clinical benefit of the drugs currently in the development pipeline which are genetically based, including those which target PNPLA3.[46]

This study has many strengths, including the analysis of paired samples from a highly rigorous, randomized controlled clinical trial conducted in a well-phenotyped population of patients with MASH. Sensitivity analysis based on widely accepted clinically significant body weight loss thresholds provide further confidence in our conclusions. Possible limitations include the modest sample size, a lack of powering for histologic outcomes (optional paired biopsies were performed in four patients to demonstrate feasibility for the next phase of study) and the study duration which could not capture long-term clinical outcomes. Post hoc analyses are also limited in that they can be exploratory in nature as the outcomes of interest in the present study were not pre-specified in the original study protocol.

In conclusion, this post hoc analysis of a randomized controlled exercise intervention trial in adults with biopsy-confirmed MASH demonstrated that exercise training significantly improves serum biomarkers of liver fibroinflammation at clinically significant thresholds of response. Importantly, the improvement in multiple biomarkers occurred without clinically significant changes in body weight and in concert with improvement in MRI-PDFF at amounts validated for histologic improvement. This study provides further evidence that exercise training should be viewed as a weight neutral intervention for which response to intervention can be readily monitored with a widely available non-invasive biomarkers that can be applied at the population level.

Acknowledgments

No manuscript writing assistance was used in preparation of this work.

Grants and Financial Support:

Research reported in this publication was supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under Award Number K23DK131290 and L30DK118601. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

This project was also funded, in part, under a grant with the Pennsylvania Department of Health using Tobacco CURE Funds. The Department specifically disclaims responsibility for any analyses, interpretations, or conclusion.

The study was also supported by NIH/NCATS Grant UL1TR000127 and UL1TR002014.

RL receives funding support from NCATS (5UL1TR001442), NIDDK (U01DK061734, U01DK130190, R01DK106419, R01DK121378, R01DK124318, P30DK120515), and NHLBI (P01HL147835).

Conflict of Interest:

Dr. Stine receives or has received research support from Astra Zeneca, Galectin, Noom, Inc, Novo Nordisk, and Zydus Therapeutics. Dr. Stine consults for Novo Nordisk and SAB Therapeutics.

Dr. Loomba serves as a consultant or advisory board member for Arrowhead Pharmaceuticals, AstraZeneca, Bird Rock Bio, Boehringer Ingelheim, Bristol-Myer Squibb, Celgene, Cirius, CohBar, Conatus, Eli Lilly, Galmed, Gemphire, Gilead, Glympse bio, GNI, GRI Bio, Intercept, Ionis, Janssen Inc., Merck, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Pfizer, Prometheus, Sanofi, Siemens, and Viking Therapeutics. In addition, his institution has received grant support from Allergan, Boehringer-Ingelheim, Bristol-Myers Squibb, Cirius, Eli Lilly and Company, Galectin Therapeutics, Galmed Pharmaceuticals, GE, Genfit, Gilead, Intercept, Grail, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, NuSirt, Pfizer, pH Pharma, Prometheus, and Siemens. He is also co-founder of Liponexus, Inc.

All other authors have no relevant conflicts of interest to report.

Data availability statement:

Data from the NASHFit study is available publicly and can be found at: https://www.icpsr.umich.edu/web/pages/

Abbreviations:

ALT	alanine aminotransferase
BMI	body mass index
CK	cytokeratin
MASH	metabolic dysfunction-associated steatohepatitis
MASLD	metabolic dysfunction-associated steatotic liver disease
MET	metabolic equivalent of task
MRI	magnetic resonance imaging
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis

PDFF proton density fat fraction

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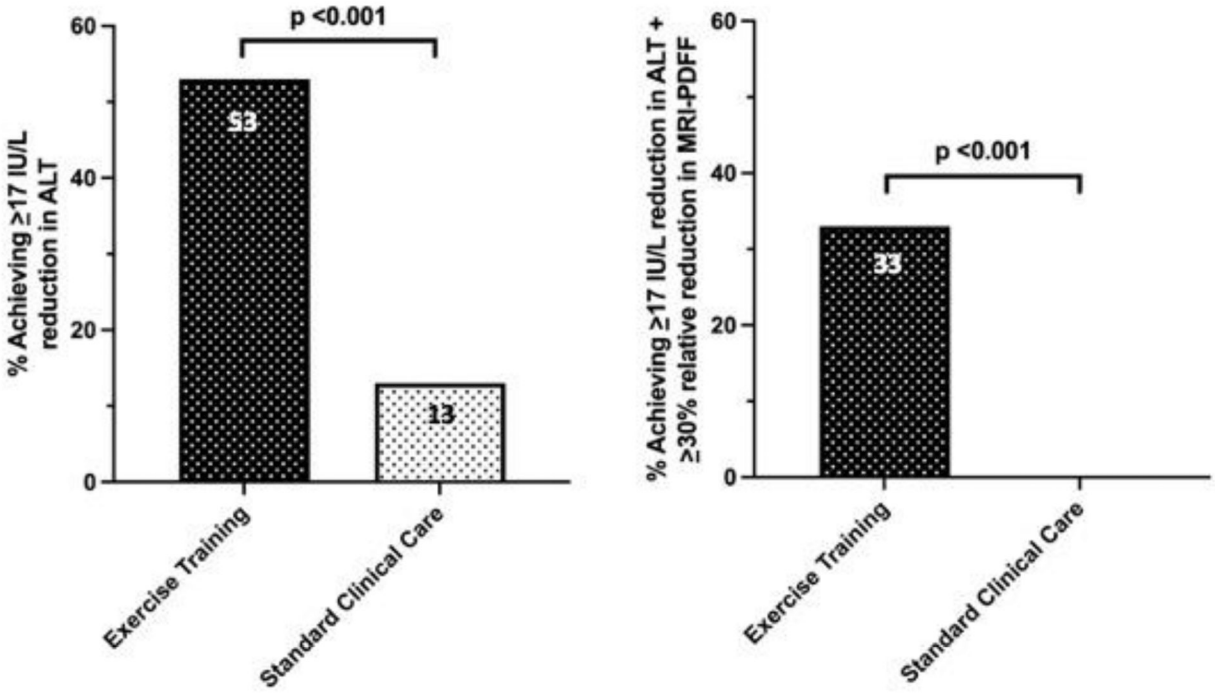

Figure 1.

Exercise training improves multiple biomarkers of liver fibroinflammation.

(A)53% of exercise training individuals achieved ALT reduction of at least 17 IU/L compared to 13% of standard of care individuals.

(B)No standard of care individual achieved improvement in both serum and imaging biomarkers as measured by ≥17 IU/L ALT reduction + ≥30% relative reduction in MRI-PDFF compared to 53% of exercise training individuals.

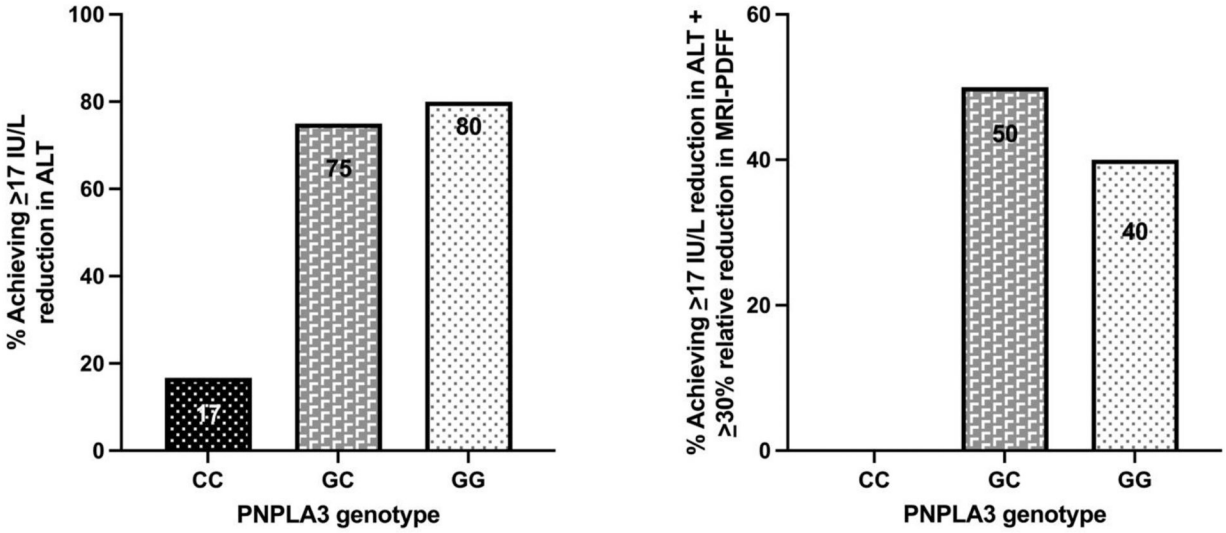

Figure 2.

PNPLA3 genotype impacts exercise response as measured by biomarkers of liver fibroinflammation.

Individuals with the G-allelic substitution were more likely to respond to exercise training and improve (A) serum biomarkers and (B) both serum and imaging biomarkers.

Table 1.

Baseline comparisons between Exercise and Standard Clinical Care participants

	Standard Clinical Care (n=8)	Exercise (n=15)
Demographics		
Age, yrs.	46.1 (11.0)	55.2 (10.6)
Female sex, n (%)	3 (38)	10 (67)
BMI, kg/m^2	34.6 (5.3)	33.4 (4.9)
Body weight, kg	108.6 (23.0)	97.2 (13.2)
Metabolic risk		
Comorbidities, n (%)		
Diabetes	3 (38)	6 (40)
Hyperlipidemia	5 (63)	9 (60)
Hypertension	5 (63)	14 (93)
Hemoglobin A1c, %	6.2 (1.1)	6.4 (1.3)
Glucose (fasting), mg/dL	135.6 (54.5)	131.2 (36.6)
HOMA-IR	9.5 (6.2)	14.3 (11.5)
VO_2peak, mL/kg/min	24.6 (5.5)	20.6 (5.3)
Body fat, %	40.5 (11.6)	43.7 (7.6)
NASH phenotyping		
PNPLA3 genotype, n (%)		
CC	2 (25)	6 (40)
GC	4 (25)	4 (27)
GG	2 (25)	5 (33)
Medications		
Vitamin E, n (%)	2 (25)	3 (20)
GLP-1 agonist, n (%)	0 (0)	1 (7)
Non-invasive tests		
FIB-4	1.73 (2.11)	1.32 (0.48)
NFS	−1.54 (1.88)	−1.51 (0.98)
Serum biomarkers		
Adiponectin, ng/mL	3641 (2004)	3726 (1099)
CK-18, IU/L	126 (118)	425 (345)
Imaging biomarkers		
Liver fat (MRI-PDFF), %	21.7 (11.6)	19.7 (5.8)
NAS	5.0 (0.5)	5.1 (1.0)
Steatosis	2.4 (0.5)	2.5 (0.7)
Lobular inflammation	1.4 (0.5)	1.4 (0.6)
Hepatocyte ballooning	1.3 (0.5)	1.2 (0.4)
Fibrosis stage, n (%)		

	Standard Clinical Care (n=8)	Exercise (n=15)
0/1	4 (50)	9 (60)
2	3 (38)	2 (13)
3	0 (0)	4 (26)
4	1 (12)	0 (0)

BMI=body mass index; GLP=glucagon like peptide; HOMA-IR=homeostatic model assessment for insulin resistance; MRI=magnetic resonance imaging; NAFLD=nonalcoholic fatty liver disease; NAS=NAFLD Activity Score; PDFF=proton density fat fraction; VO_2=oxygen consumption

*Continuous variables reported as mean +/− SD,

**No subjects were taking pioglitazone or obeticholic acid

***all p-values were >0.05 for baseline characteristics between groups

Table 2-

Outcome measures: Biomarkers

	Control (n=8)			Exercise (n=15)			
	Baseline	**Post**	**Within group p-value**	**Baseline**	**Post**	**Within group p-value**	**Between group p-value**
Serum Biomarkers							
Adiponectin, ng/mL	3641 (2004)	3482 (1728)	0.868	3726 (1099)	3802 (1231)	0.866	0.409
CK18, IU/L	126 (118)	168 (198)	0.653	425 (345)	364 (334)	0.741	0.040
≥17 IU/L reduction in ALT, n (%)		1 (13)			8 (53)		<0.001
Imaging biomarkers							
MRI-PDFF liver fat, %	21.7 (11.6)	22.9 (13.3)	0.851	19.7 (5.8)	15.4 (4.9)	0.038	0.012
≥30% relative reduction in MRI-PDFF, n (%)		1 (13)			5 (36)		0.016
Combined serum + imaging biomarkers							
≥17 IU/L reduction in ALT + ≥30% relative reduction in MRI-PDFF, n (%)		0 (0)			5 (36)		<0.001

ALT=alanine aminotransferase; CK=cytokeratin; FGF=fibroblast growth factor, FIB-4=Fibrosis-4 index; MRI=magnetic resonance imaging; NAFLD=nonalcoholic fatty liver disease; NFS=NAFLD Fibrosis Score; PAI=plasminogen activator inhibitor, PDFF=proton density fat fraction

*Reported as mean +/− SD

Table 3-

Outcome measures: Metabolic risk

	Control (n=8)			Exercise (n=15)			
	Baseline	**Post**	**Within group p-value**	**Baseline**	**Post**	**Within group p-value**	**Between group p-value**
Anthropometry and Body composition							
BMI, kg/m^2	34.6 (5.3)	35.0 (5.4)	0.880	33.4 (0.6)	32.8 (0.3)	0.729	0.129
Body weight, kg	108.6 (23.0)	110.1 (23.3)	0.896	97.2 (13.2)	94.9 (12.3)	0.625	0.051
Waist circumference, in	45.8 (4.9)	46.3 (4.8)	0.835	43.7 (3.5)	43.0 (0.6)	0.596	0.069
VAT, lbs.	6.9 (2.7)	7.2 (2.8)	0.830	5.4 (1.5)	5.2 (1.1)	0.678	0.032
Body fat, %	40.5 (11.6)	38.4 (11.1)	0.724	43.7 (7.6)	42.3 (8.0)	0.628	0.374
Liver volume, cc	2545 (640)	2597 (570)	0.866	2267 (445)	2131 (418)	0.405	0.039
Cardiorespiratory fitness							
VO_2peak, mL/kg/min	24.6 (5.5)	22.7 (3.2)	0.429	20.6 (5.3)	23.7 (6.5)	0.167	0.055
Biochemistry							
Glucose (fasting), mg/dL	136 (55)	156 (89)	0.059	131 (37)	117 (29)	0.247	0.039
Hemoglobin A1c, %	6.2 (1.1)	6.6 (1.7)	0.555	6.4 (1.3)	6.0 (0.8)	0.348	0.006
HOMA-IR	9.5 (6.2)	12.6 (14.9)	0.618	14.3 (11.5)	9.2 (6.5)	0.160	0.168
Insulin, IU/mL	27.3 (14.5)	31.1 (29.9)	0.763	41.1 (27.9)	33.4 (20.1)	0.400	0.442
Lipids							
Total cholesterol, mg/dL	203 (37)	184 (25)	0.278	187 (49)	187 (51)	0.986	0.350
LDL, mg/dL	120 (33)	109 (33)	0.554	109 (40)	109 (41)	0.964	0.476
HDL, mg/dL	43 (12)	40 (12)	0.614	43 (9)	44 (7)	0.712	0.188
Triglyceride, mg/dL	222 (94)	228 (108)	0.921	172 (64)	172 (76)	0.998	0.982

ALT=alanine aminotransferase; AST=aspartate aminotransferase, BMI=body mass index; HDL= high density lipoprotein; HOMA-IR=homeostatic model assessment of insulin resistance; LDL=low density lipoprotein; VAT=visceral adipose tissue; VO2=oxygen uptake

*Reported as mean +/− SD