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ORIGINAL ARTICLE

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Greater hepatic lipid saturation is associated with impaired glycaemic regulation in men with metabolic dysfunction-associated steatotic liver disease but is not altered by 6 weeks of exercise training

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Abstract

Aims: To examine the impact of impaired glycaemic regulation (IGR) and exercise training on hepatic lipid composition in men with metabolic dysfunction-associated steatotic liver disease (MASLD).

Materials and Methods: In Part A (cross-sectional design), 40 men with MASLD (liver proton density fat fraction [PDFF] ≥5.56%) were recruited to one of two groups: (1) normal glycaemic regulation (NGR) group (glycated haemoglobin [HbA1c] < 42 mmol·mol^{-1} [<6.0%]; $n = 14$) or (2) IGR group (HbA1c ≥ 42 mmol·mol^{-1} [≥6.0%]; $n = 26$). In Part B (randomized controlled trial design), participants in the IGR group were randomized to one of two 6-week interventions: (1) exercise training (EX; 70%–75% maximum heart rate; four sessions/week; $n = 13$) or (2) non-exercise control (CON; $n = 13$). Saturated

(SI; primary outcome), unsaturated (UI) and polyunsaturated (PUI) hepatic lipid indices were determined using proton magnetic resonance spectroscopy. Additional secondary outcomes included liver PDFF, HbA1c, fasting plasma glucose (FPG), homeostatic model assessment of insulin resistance (HOMA-IR), peak oxygen uptake (VO_2 peak), and plasma cytokeratin-18 (CK18) M65, among others.

Results: In Part A, hepatic SI was higher and hepatic UI was lower in the IGR versus the NGR group ($p = 0.038$), and this hepatic lipid profile was associated with higher HbA1c levels, FPG levels, HOMA-IR and plasma CK18 M65 levels ($r_s \geq 0.320$). In Part B, hepatic lipid composition and liver PDFF were unchanged after EX versus CON ($p \geq 0.257$), while FPG was reduced and VO_2 peak was increased ($p \leq 0.030$). ΔVO_2 peak was inversely associated with Δhepatic SI ($r = -0.433$) and positively associated with Δhepatic UI and Δhepatic PUI ($r \geq 0.433$).

Conclusions: Impaired glycaemic regulation in MASLD is characterized by greater hepatic lipid saturation; however, this composition is not altered by 6 weeks of moderate-intensity exercise training.

KEYWORDS
exercise intervention, fatty liver disease, liver, glycaemic control

1 | INTRODUCTION

Excessive hepatic lipid accumulation and accompanying cardiometabolic dysfunction, recently renamed 'metabolic dysfunction-associated steatotic liver disease' (MASLD),[1] is a leading risk factor for type 2 diabetes.[2] The coexistence of these diseases accelerates the progression to metabolic dysfunction-associated steatohepatitis (MASH),[3] a more advanced form of liver disease which predisposes to premature cardiovascular and liver-related mortality.[4] Accumulating evidence, however, suggests that the composition rather than the quantity of hepatic lipids may be central to the hepatic and cardiometabolic consequences of hepatic steatosis.[5] Specifically, preclinical research has implicated saturated hepatic lipids as more lipotoxic and (poly)unsaturated hepatic lipids as more protective.[6–8]

Hepatic lipid composition assessment has traditionally required liver biopsy,[9,10] however, advances in proton magnetic resonance spectroscopy (^{1}H-MRS) now permit this assessment non-invasively.[11–13] These studies show greater hepatic lipid saturation and/or lower hepatic lipid unsaturation/polyunsaturation in both MASLD and type 2 diabetes populations alongside associations with insulin resistance.[11,12,14] This more 'lipotoxic' lipid profile could underpin the relationship between MASLD, type 2 diabetes and a more aggressive liver disease trajectory. In support of this, Roumans et al.[12] recently attributed this lipid profile to elevated de novo lipogenesis (DNL), a process directly stimulated by hyperglycaemia and hyperinsulinaemia, which exclusively produces saturated fatty acids (SFAs).[15] Whether glycaemic regulation is specifically related to hepatic lipid composition in MASLD requires further investigation.

Lifestyle modification, including exercise, remains the primary treatment option for MASLD.[16] These guidelines are based on evidence that exercise training, independent of weight loss, can decrease hepatic lipids as well as providing other cardiometabolic benefits[17,18]; however, the effects of exercise on hepatic lipid composition are less clear.[19] A small single-arm trial reported that 7 days of moderate-intensity walking increased hepatic lipid polyunsaturation in people with MASLD,[20] while another study found that 4 weeks of moderate-intensity cycling did not alter hepatic lipid composition in people with obesity.[21] The impact of exercise on hepatic lipid composition in people with established MASLD and impaired glycaemic regulation (IGR) requires attention.

This study had two aims: (1) to examine the association between ^{1}H-MRS-measured hepatic lipid composition and glycaemic regulation in men with MASLD and (2) to determine the impact of 6 weeks of moderate-intensity aerobic exercise training on hepatic lipid composition in men with MASLD and IGR. We hypothesized that men with MASLD and IGR would have higher hepatic lipid saturation, and lower hepatic lipid unsaturation and polyunsaturation, compared to those with normal glycaemic regulation (NGR). Furthermore, 6 weeks of exercise training would decrease the proportion of saturated hepatic lipids and increase the proportion of unsaturated and polyunsaturated hepatic lipids.

2 | MATERIALS AND METHODS

2.1 | Study design and ethical approval

The DELIVER (Diabetes, Exercise and LIVER fat) study was a two-part clinical trial conducted across three research sites (Loughborough, Leicester and Nottingham) in the East Midlands, United Kingdom. Part

A involved a cross-sectional comparison of two groups of men with MASLD: (1) an NGR group and (2) an IGR group. Part B was a randomized controlled trial consisting of 6 weeks of moderate-intensity exercise training in the IGR group. The study protocol is shown in Supplementary Figure S1. Ethical approval was granted by a National Health Service (NHS) research ethics committee (18-EM-0161) and the research was conducted in accordance with the Declaration of Helsinki (2013). All participants provided written informed consent and the study was prospectively registered (NCT04004273).

2.2 | Participants

Participants were inactive men (self-reported sex) with MASLD, aged 30–75 years, and living with overweight or obesity. MASLD was defined as per the updated diagnostic criteria, that is, elevated hepatic steatosis (proton density fat fraction [PDFF] ≥5.56% measured via ^{1}H-MRS[22]) with at least one of five cardiometabolic risk factors and the absence of excessive self-reported alcohol consumption (<30 g·day^{-1}) or other secondary aetiologies.[1,16] NGR was defined as glycated haemoglobin (HbA1c) <42 mmol·mol^{-1} (<6.0%), while IGR was defined as HbA1c ≥42 mmol·mol^{-1} (≥6.0%). Participants in the IGR group with type 2 diabetes were eligible if their condition was managed through lifestyle and/or metformin only. The full eligibility criteria and further details on each criterion are outlined in the Supplementary Methods S1.

2.3 | Procedures

2.3.1 | Cross-sectional analyses: Part A

Participants were assessed across two study visits separated by at least 1 week to allow for physical activity and dietary monitoring. Visits commenced after an overnight fast with participants having abstained from caffeine, alcohol, and exercise for 24 h. Participants standardized their dietary intake before Visits 1 and 2.

Prospective participants attended Visit 1 at the Sir Peter Mansfield Imaging Centre, Nottingham, UK, where anthropometric indices were assessed. Participants then underwent a combined abdominal magnetic resonance imaging (MRI) and liver ^{1}H-MRS scan to assess hepatic lipid composition (^{1}H-MRS), liver PDFF (^{1}H-MRS), subcutaneous abdominal adipose tissue (ScAT; MRI) and visceral adipose tissue (VAT; MRI). Eligible participants' 7-day device-measured physical activity (GENEactiv, Activinsights Ltd, Cambs, UK) and dietary intake (3-day records) were assessed in the following week (see Supplementary Methods S1).

Participants then attended Visit 2 at the Leicester Diabetes Centre, Leicester, UK. This visit involved a fasting venous blood sample, medical evaluation and peak oxygen uptake (VO_2 peak) test (see Supplementary Methods S1). Participants were subsequently assigned to the NGR ($n = 14$) or IGR ($n = 26$) group based on HbA1c eligibility.

2.3.2 | Six-week randomized controlled trial: Part B

The IGR group progressed to Part B and were randomized 1:1 to one of two 6-week interventions: (1) exercise training (EX; $n = 13$) or (2) control (CON; $n = 13$). Randomization sequence generation, concealment and allocation were conducted by an independent trial statistician (G.W.) using an online tool (http://randomization.com). Randomization was stratified by ethnicity ('White' and 'Other') and used permuted blocks of random sizes.

For the EX intervention, participants performed four exercise training sessions per week for 6 weeks to achieve current MASLD recommendations.[18] Exercise sessions comprised of moderate-intensity continuous walking or cycling exercise, defined as 70%–75% of age-predicted maximum heart rate and/or a self-determined rating of perceived exertion score of 13–14 (Borg 6–20 scale[23]). At least one session per week was supervised by the research team, while the remaining sessions were completed unsupervised with a heart rate monitor (A300; Polar Electro, Kempele, Finland) used to self-regulate exercise intensity and confirm adherence. Session duration progressed from 35 min in Week 1 to 50 min in Week 6, including a 5-min warm up and 10-min cool down at a self-selected intensity. Participants in the CON group received no intervention and maintained usual lifestyle habits.

During Week 5, repeat assessments of physical activity and dietary intake were conducted. Post-intervention assessments (Visits 3 and 4) were performed 48 and 72 h after the final session in Week 6, consisting of identical measures to Visits 1 and 2 (pre-intervention assessments).

2.3.3 | Magnetic resonance acquisition and analysis

All MR measurements were performed using a 3.0T Philips Ingenia MRI system with a 32-channel Philips SENSE XL torso coil. Two-point modified Dixon scans and an in-house automated segmentation algorithm (MATLAB R2020a; The MathWorks Inc., Natick, MA, USA) were used to quantify volumes of ScAT, VAT, and VAT-to-total abdominal adipose tissue (TAT; ScAT + VAT) ratio.[24]

The ^{1}H-MRS spectra were acquired using a Stimulated Echo Acquisition Mode-localized, single-voxel sequence. Single breath-holds, with and without water suppression, were used for the assessment of liver PDFF, while high-sensitivity spectra were acquired over six breath-holds for determination of hepatic lipid composition. All ^{1}H-MRS spectra were processed and analysed off-line by an experienced researcher (S.J.B.) in a blinded fashion using a home-developed MATLAB script (MATLAB R2020a; The MathWorks Inc). Liver PDFF and hepatic lipid composition indices of saturation (SI), unsaturation (UI), and polyunsaturation (PUI) were subsequently calculated using externally validated equations.[11,22] The ^{1}H-MRS acquisition and post-processing procedures are described in further detail in the Supplementary Methods S1.

2.3.4 | ^{1}H-MRS method validation

Before the study, an in-house ^{1}H-MRS validation experiment for hepatic lipid composition and PDFF assessment was performed using lipid-water phantoms. The experiment confirmed the validity of our ^{1}H-MRS method, demonstrating strong correlations (Pearson's *r*) and agreement (Bland–Altman) between the measured and expected values for both PDFF and the lipid composition indices (Supplementary Figures S2 and S3). The full methods and results of the validation experiment are described in the Supplementary Methods and Results S1, respectively.

2.3.5 | Biochemical analyses

Blood samples were collected, processed and analysed for plasma concentrations of glucose, HbA1c, insulin, non-esterified fatty acids, triacylglycerol, high-density lipoprotein, low-density lipoprotein, total cholesterol, liver function tests, C-reactive protein, interleukin-6, and cytokeratin-18 (CK18) M30 and M65 (detailed in Supplementary Methods S1). Homeostasis model assessment of insulin resistance (HOMA-IR) and adipose tissue insulin resistance index (Adipo-IR) were subsequently calculated.[25,26]

2.4 | Outcomes

The primary outcomes were differences in hepatic SI between the NGR and IGR groups (Part A), and differences in the pre- to post-intervention change in hepatic SI after EX versus CON (Part B). All other analyses in Part A and B were secondary outcomes (see the Supplementary Methods for the full list S1).

2.5 | Sample size

Sample size calculations were performed by a trial statistician (G.W.) based on the original ^{1}H-MRS validation study.[11] A total of 28 participants ($n = 14$ per group) were required in Part A assuming a 5% difference in hepatic SI (SD 4%) with 80% power and a 5% alpha error rate. Based on the same data, 24 participants were required for Part B, inflated to 26 ($n = 13$ per intervention) to allow for expected drop-out (10%).

2.6 | Statistical analysis

Data were analysed using SPSS Statistics v27 (SPSS Inc., IL, USA), with normality assessed using histograms and box plots. Baseline characteristics of all study groups are reported as mean ± SD for normally distributed data, median (interquartile range) for non-normally distributed data, and number (percentage) for categorical data.

In Part A, differences in primary and secondary outcomes between the NGR and IGR groups were assessed using independent samples *t*-tests and Mann–Whitney tests, as appropriate. Given the pilot nature of the study and firm hypotheses, one-tailed tests were used for hepatic lipid composition indices. Associations between hepatic lipid composition indices and other study outcomes were examined using Pearson's *r* or Spearman's *rho* where appropriate.

In Part B, generalized linear models with a normal distribution and identity link function were used to assess differences in the change (post- minus pre-intervention values) in primary and secondary outcomes between the EX and CON interventions. Intervention group was included as the explanatory variable, while ethnicity and baseline (pre-intervention) values for each outcome were included as covariates. Data are presented as adjusted means with 95% confidence intervals for each group and the intervention effect (EX minus CON). Associations between changes in the hepatic lipid composition indices and changes in other study outcomes were explored using Pearson's *r*.

An additional sensitivity analysis was performed for Part A using multiple imputation for missing values.[27] For Part B, the primary analysis was conducted using a complete-case approach, while an intention-to-treat analysis was also performed using the same multiple imputation method. Effect sizes (ES) for comparisons are described using Cohen's *d*.[28] Statistical significance was set at an alpha level of $p < 0.05$.

3 | RESULTS

3.1 | Participant flow and missing data

The study CONSORT diagram is presented in Figure 1. Between 30 October 2018 and 20 July 2022, 190 individuals were screened for eligibility and 40 participants were enrolled in the study. For Part A, 14 participants were allocated to the NGR group, while 26 participants were allocated to the IGR group. All participants in the IGR group then enrolled in Part B, with 13 participants randomized to EX and 13 participants randomized to CON. During EX, two participants discontinued the intervention due to the exercise intensity and COVID-19 pandemic, respectively, thus 11 participants completed the exercise training. Two participants in the NGR group did not complete the maximal exercise test due to adverse events (described below in Section 3.4), therefore, VO_2 peak data are presented for $n = 12$. Due to technical issues during MRI acquisition, ScAT, VAT, and VAT:TAT data are available for 36 participants ($n = 12$ for NGR and $n = 24$ for IGR) in Part A and 19 participants ($n = 8$ for EX and $n = 11$ for CON) in Part B.

3.2 | Cross-sectional analyses: Part A

Participant characteristics of the NGR and IGR groups are shown in Table 1. Demographic and physical characteristics were similar between groups, except for VAT and the VAT:TAT ratio, which tended to be higher in the IGR group. For the primary outcome,

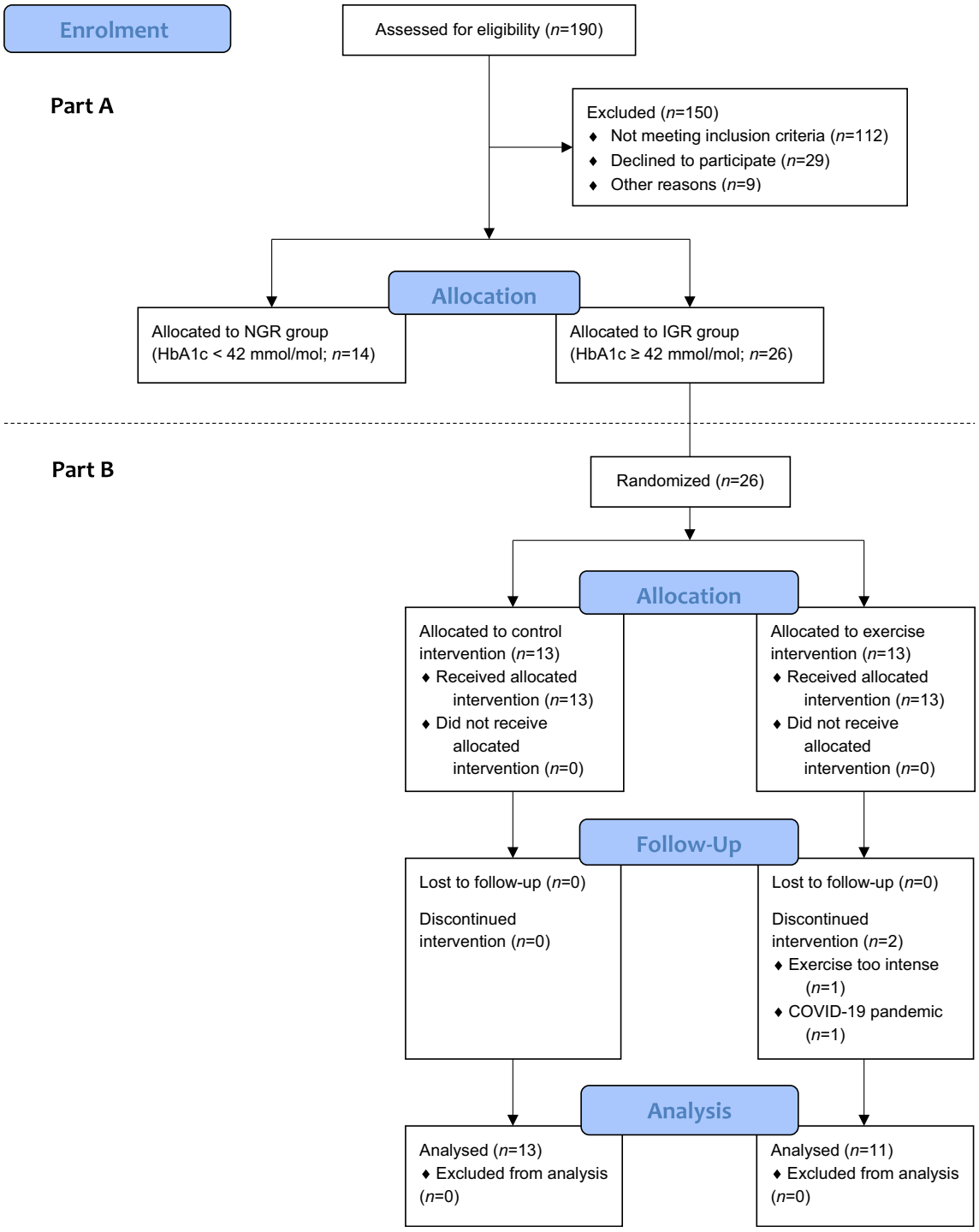

FIGURE 1 Study CONSORT flow diagram. HbA1c, glycated haemoglobin; IGR, impaired glycaemic regulation; NGR, normal glycaemic regulation.

hepatic SI was higher in the IGR versus NGR group (Supplementary Figure S4A), while hepatic UI was lower in the IGR group (Supplementary Figure S4B). Hepatic PUI and liver PDFF were similar between groups (Supplementary Figure S4C-D). HbA1c and circulating glucose were higher in the IGR versus NGR group, while circulating insulin concentrations and insulin resistance indices were not different between groups.

No differences in blood pressure, circulating lipids, liver enzymes, or markers of systemic inflammation were evident between groups. Furthermore, circulating CK18 M30 was similar in the two groups, while circulating CK18 M65 was higher and the CK18 M30:M65 ratio tended to be lower in the IGR versus the NGR group (Table 1). Habitual physical activity and dietary intake were similar in the two groups (Supplementary Table S1). The sensitivity analysis showed the same

TABLE 1 Participant characteristics of the normal glycaemic regulation and impaired glycaemic regulation groups (Part A).

Variable	NGR ($n = 14$)	IGR ($n = 26$)	p value	Effect size (d)
Ethnicity				
White European	12 (85.7)	20 (77.0)		
South Asian	2 (14.3)	5 (19.2)		
Other	0 (0.0)	1 (3.8)		
Age, years[a]	57 (20)	63 (32)	**0.395**	0.27
Anthropometry, body composition and cardiorespiratory fitness				
Body weight, kg	109.6 ± 18.5	105.2 ± 16.6	0.443	0.26
BMI, $kg{\cdot}m^{-2}$	34.8 ± 4.9	34.0 ± 4.1	0.601	0.18
Waist circumference, cm	113.7 ± 11.6	113.4 ± 10.5	0.955	0.02
Body fat, %	33.9 ± 4.4	32.3 ± 5.5	0.337	0.32
ScAT, mL	4132 ± 1586	3521 ± 1050	0.176	0.49
VAT, mL[a]	2281 (1080)	2714 (1322)	0.087	0.58
VAT:TAT ratio, AU	0.39 ± 0.10	0.46 ± 0.09	0.057	0.70
Absolute VO_2 peak, $L{\cdot}min^{-1}$	3.10 ± 0.71	2.84 ± 0.57	0.433	0.41
Relative VO_2 peak, $mL{\cdot}kg{\cdot}min^{-1}$	28.5 ± 4.6	27.3 ± 4.8	0.438	0.27
Hepatic lipids				
SI, %[a]	86.9 (7.2)	89.8 (4.0)	**0.038**	**0.59**
UI, %[a]	13.1 (7.2)	10.2 (4.0)	**0.038**	**0.59**
PUI, %[a]	2.0 (4.6)	0.9 (2.5)	**0.188**	0.29
Liver PDFF, %	14.8 ± 7.7	17.6 ± 7.2	0.273	0.37
Glycaemic regulation and insulin sensitivity				
HbA1c, $mmol{\cdot}mol^{-1}$[a]	39 (6)	51 (14)	**< 0.001**	**2.83**
HbA1c, %[a]	5.7 (0.5)	6.8 (1.2)	**< 0.001**	**2.83**
Glucose, $mmol{\cdot}L^{-1}$[a]	5.6 (0.6)	7.4 (1.1)	**< 0.001**	**1.91**
Insulin, $pmol{\cdot}L^{-1}$[a]	76 (76)	81 (46)	0.364	0.29
HOMA-IR, AU[a]	3.3 (3.0)	4.8 (2.7)	0.411	0.26
Adipo-IR, AU[a]	39.1 (27.8)	36.9 (32.3)	0.863	0.06
Blood pressure, circulating lipids, and other circulating proteins				
SBP, mmHg	135 ± 9	141 ± 12	0.117	0.53
DBP, mmHg	92 ± 9	95 ± 10	0.368	0.30
TAG, $mmol{\cdot}L^{-1}$[a]	1.43 (1.13)	2.15 (1.17)	0.239	0.38
NEFA, $mmol{\cdot}L^{-1}$	0.46 ± 0.17	0.57 ± 0.20	0.100	0.56
Total cholesterol, $mmol{\cdot}L^{-1}$	4.69 ± 1.34	4.25 ± 0.88	0.218	0.42
HDL, $mmol{\cdot}L^{-1}$	0.99 ± 0.15	0.93 ± 0.21	0.336	0.32
LDL, $mmol{\cdot}L^{-1}$	2.72 ± 1.04	2.39 ± 0.78	0.269	0.37
ALT, $U{\cdot}L^{-1}$[a]	33.6 (14.7)	36.8 (22.0)	0.379	0.28
AST, $U{\cdot}L^{-1}$	42.4 ± 11.8	42.5 ± 10.3	0.964	0.02
AST:ALT ratio, AU[a]	1.13 (0.72)	1.06 (0.25)	0.335	0.31
GGT, $U{\cdot}L^{-1}$[a]	30.9 (10.2)	35.7 (17.7)	0.173	0.44
CRP, $mg{\cdot}L^{-1}$[a]	1.71 (1.57)	1.30 (1.88)	0.478	0.23
IL-6, $pg{\cdot}mL^{-1}$[a]	2.82 (2.66)	1.67 (1.46)	0.444	0.24
CK18 M30, $IU{\cdot}L^{-1}$[a]	105 (141)	142 (121)	0.349	0.30
CK18 M65, $IU{\cdot}L^{-1}$[a]	149 (132)	227 (253)	**0.016**	**0.82**
CK18 M30:M65 ratio, AU[a]	0.68 (0.32)	0.59 (0.20)	0.057	0.63

Note: Categorical data are presented as frequency (percentage) and continuous data are presented as mean ± SD, or as median (interquartile range) where data were non-normally distributed. For the NGR and IGR groups respectively, $n = 12$ and $n = 24$ for MRI data, and $n = 12$ and $n = 26$ for VO_2 peak data (^{1}H-MRS data were available for the full sample). Bold values indicate statistical significance at $p < 0.05$.

Abbreviations: Adipo-IR, adipose tissue insulin resistance index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AU, arbitrary units; BMI, body mass index; CK18, cytokeratin-18; CRP, C-reactive protein; DBP, diastolic blood pressure; GGT, gamma-glutamyl transferase; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; IGR, impaired glycaemic regulation; IL-6, interleukin-6; NEFA, non-esterified fatty acids; NGR, normal glycaemic regulation; PDFF, proton density fat fraction; PUI, polyunsaturation index; SBP, systolic blood pressure; ScAT, subcutaneous abdominal adipose tissue; SI, saturation index; TAG, triacylglycerol; TAT, total adipose tissue; UI, unsaturation index; VAT, visceral adipose tissue; VO_2 peak, peak oxygen uptake.

[a]Indicates non-parametric analyses were performed.

pattern of results and interpretation for all group differences (Supplementary Table S2).

In the whole cohort, higher HbA1c and circulating glucose were associated with greater hepatic SI and lower hepatic UI (Figure 2A–D). Furthermore, HOMA-IR was positively associated with hepatic SI (Figure 2E) and inversely associated with hepatic UI (Figure 2F) and PUI (*rho* = −0.316; $p = 0.047$). Circulating CK18 M65 concentrations were positively associated with hepatic SI (*rho* = 0.327; $p = 0.040$) and inversely associated with hepatic UI and PUI (*rho* ≤ −0.325; $p = 0.040$). Consequently, a lower CK18 M30:M65 ratio was associated with higher hepatic SI (*rho* = −0.398; $p = 0.011$) and lower hepatic UI (*rho* = 0.398; $p = 0.011$). BMI and liver PDFF were inversely related to hepatic PUI (*rho* ≤ −0.333; $p \leq 0.036$).

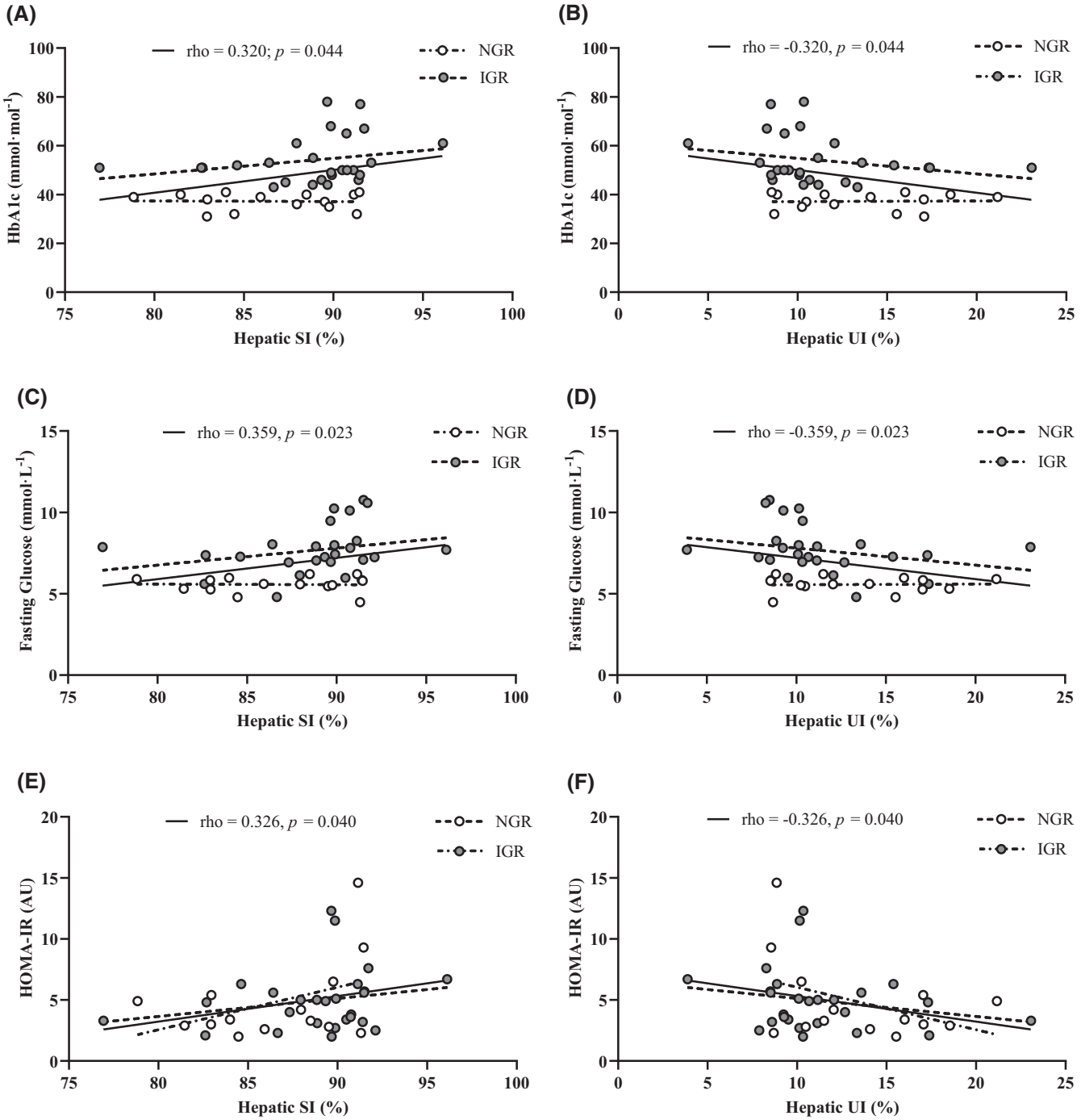

FIGURE 2 Associations of glycaemic parameters with the hepatic saturation index (SI; A,C,E) and unsaturation index (UI; B,D,E) in the normal glycaemic regulation (NGR) and impaired glycaemic regulation (IGR) groups combined ($n = 40$). Data were analysed using Spearman's rank order correlation analyses (*rho*). AU, arbitrary units; HOMA-IR, homeostatic model assessment of insulin resistance.

TABLE 2 Baseline (pre-intervention) participant characteristics of the exercise training and control groups (Part B).

Variable	EX ($n = 11$)	CON ($n = 13$)
Ethnicity, *n* (%)		
White European	9 (81.8)	10 (76.9)
South Asian	2 (18.2)	2 (15.4)
Other	0 (0.0)	1 (7.7)
Type 2 diabetes, *n* (%)	8 (72.7)	10 (76.9)
Metformin use, *n* (%)	7 (63.6)	7 (53.8)
Age, years	61 (17)	63 (18)
Anthropometry, body composition and cardiorespiratory fitness		
Body weight, kg	101.2 (28.5)	98.5 (14.8)
BMI, $kg{\cdot}m^{-2}$	34.1 (5.6)	31.9 (4.1)
Waist circumference, cm	113.8 ± 10.6	111.2 ± 9.9
Body fat, %	32.7 ± 4.7	30.9 ± 5.9
ScAT, mL	3741 ± 1303	3303 ± 640
VAT, mL	3389 ± 1119	2769 ± 871
VAT:TAT ratio, AU	0.48 ± 0.08	0.45 ± 0.09
Absolute VO_2 peak, $L{\cdot}min^{-1}$	3.07 ± 0.55	2.77 ± 0.53
Relative VO_2 peak, $mL{\cdot}kg{\cdot}min^{-1}$	28.7 ± 4.0	27.4 ± 4.3
Exercise capacity, s	734 ± 203	652 ± 286
Hepatic lipids		
SI, %	90.7 (4.2)	89.3 (5.0)
UI, %	9.3 (4.2)	10.7 (5.0)
PUI, %	0.6 (1.6)	1.0 (3.3)
Liver PDFF, %	21.1 ± 7.2	13.9 ± 5.9
Glycaemic regulation and insulin sensitivity		
HbA1c, $mmol{\cdot}mol^{-1}$	51 (20)	50 (6)
HbA1c, %	6.8 (1.8)	6.7 (0.5)
Glucose, $mmol{\cdot}L^{-1}$	7.9 (1.5)	7.2 (1.7)
Insulin, $pmol{\cdot}L^{-1}$	84 ± 24	73 ± 24
HOMA-IR, AU	5.2 ± 1.6	3.8 ± 1.3
Adipo-IR, AU	50.5 (35.5)	34.9 (12.7)
Blood pressure, circulating lipids and other circulating proteins		
SBP, mmHg	143 ± 14	136 ± 20
DBP, mmHg	98 ± 10	92 ± 10
TAG, $mmol{\cdot}L^{-1}$	2.18 (0.82)	2.00 (1.31)
NEFA, $mmol{\cdot}L^{-1}$	0.60 ± 0.19	0.57 ± 0.17
Total cholesterol, $mmol{\cdot}L^{-1}$	4.00 ± 1.05	4.41 ± 0.77
HDL, $mmol{\cdot}L^{-1}$	0.93 ± 0.21	0.98 ± 0.16
LDL, $mmol{\cdot}L^{-1}$	2.20 ± 0.87	2.44 ± 0.66
ALT, $U{\cdot}L^{-1}$	36.7 (19.2)	32.6 (20.7)
AST, $U{\cdot}L^{-1}$	39.8 ± 9.2	43.1 ± 10.8
AST:ALT ratio, AU	1.03 (0.23)	1.12 (0.36)
GGT, $U{\cdot}L^{-1}$	35.8 (17.4)	35.0 (21.8)
CRP, $mg{\cdot}L^{-1}$	1.47 (2.00)	1.18 (1.90)
IL-6, $pg{\cdot}mL^{-1}$	1.79 (1.42)	1.50 (1.24)
CK18 M30, $IU{\cdot}L^{-1}$	150 (98)	114 (98)
CK18 M65, $IU{\cdot}L^{-1}$	297 (259)	206 (151)
CK18 M30:M65 ratio, AU	0.57 ± 0.21	0.56 ± 0.13

Note: Categorical data are presented as frequency (percentage) and continuous data are presented as mean ± SD, or as median (interquartile range) where data were non-normally distributed. For the EX and CON groups, respectively, $n = 8$ and $n = 11$ for MRI data (^{1}H-MRS data were available for the full sample).

Abbreviations: Adipo-IR, adipose tissue insulin resistance index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AU, arbitrary units; BMI, body mass index; CK18, cytokeratin-18; CON, control; CRP, C-reactive protein; DBP, diastolic blood pressure; EX, exercise training; GGT, gamma-glutamyl transferase; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin-6; NEFA, non-esterified fatty acids; PDFF, proton density fat fraction; PUI, polyunsaturation index; SBP, systolic blood pressure; ScAT, subcutaneous abdominal adipose tissue; SI, saturation index; TAG, triacylglycerol; TAT, total adipose tissue; UI, unsaturation index; VAT, visceral adipose tissue; VO_2 peak, peak oxygen uptake.

TABLE 3 Pre- to post-intervention changes and the intervention effects (exercise training minus control) after the 6-week intervention period.

Variable	Pre- to post-intervention change (95% CI)		Intervention effect		
	EX ($n = 11$)	CON ($n = 13$)	EX minus CON	*p* value	Effect size, *d*
Anthropometry, body composition and cardiorespiratory fitness					
Body weight, kg	−1.1 (−2.1, 0.0)	−0.4 (−1.3, 0.6)	−0.7 (−2.0, 0.6)	0.273	0.40
BMI, $kg \cdot m^{-2}$	−0.3 (−0.7, 0.0)	−0.1 (−0.4, 0.2)	−0.2 (−0.6, 0.2)	0.363	0.35
Waist circumference, cm	−2.5 (−4.0, −1.1)	−1.1 (−2.4, 0.3)	−1.4 (−3.2, 0.3)	0.096	0.59
Body fat, %	−1.3 (−2.3, −0.3)	−0.4 (−1.4, 0.5)	−0.9 (−2.1, 0.4)	0.166	0.50
ScAT, mL	−281 (−676, 114)	−58 (−378, 262)	−223 (−682, 236)	0.342	0.40
VAT, mL	−235 (−450, −20)	−112 (−295, 71)	−123 (−377, 131)	0.344	0.40
VAT:TAT ratio, AU	0.00 (−0.02, 0.03)	0.00 (−0.02, 0.02)	0.00 (−0.03, 0.03)	0.969	0.02
Absolute VO_2 peak, $L \cdot min^{-1}$	0.02 (−0.08, 1.12)	−0.10 (−0.20, 0.00)	0.12 (−0.01, 0.24)	0.067	0.68
Relative VO_2 peak, $mL \cdot kg \cdot min^{-1}$	0.4 (−0.5, 1.4)	−0.9 (−1.7, 0.0)	1.3 (0.1, 2.4)	**0.030**	**0.80**
Exercise capacity, s	72 (8, 136)	25 (−32, 82)	47 (−30, 125)	0.230	0.47
Hepatic lipids					
SI, %	−2.6 (−5.2, 0.1)	−0.8 (−3.0, 1.4)	−1.8 (−4.8, 1.3)	0.270	0.40
UI, %	2.6 (−0.1, 5.2)	0.8 (−1.4, 3.0)	1.8 (−1.3, 4.8)	0.270	0.40
PUI, %	0.5 (−0.7, 1.7)	0.1 (−0.9, 1.1)	0.4 (−1.1, 1.8)	0.620	0.18
Liver PDFF, %	−2.0 (−5.0, 1.0)	0.2 (−2.5, 3.0)	−2.2 (−6.2, 1.6)	0.257	0.45
Glycaemic regulation and insulin sensitivity					
HbA1c, $mmol \cdot mol^{-1}$	−0.3 (−2.1, 1.5)	0.3 (−1.2, 1.9)	−0.6 (−2.8, 1.5)	0.538	0.23
HbA1c, %	−0.02 (−0.19, 0.15)	0.03 (−0.11, 0.17)	−0.05 (−0.25, 0.14)	0.601	0.20
Glucose, $mmol \cdot L^{-1}$	−0.8 (−1.4, −0.3)	0.2 (−0.3, 0.6)	−1.0 (−1.7, −0.3)	**0.006**	**1.11**
Insulin, $pmol \cdot L^{-1}$	−23 (−65, 18)	15 (−23, 52)	−38 (−88, 12)	0.140	0.55
HOMA-IR, AU	−2.1 (−5.3, 1.0)	1.4 (−1.4, 4.3)	−3.5 (−7.6, 0.5)	0.083	0.68
Adipo-IR, AU	−9.2 (−23.6, 5.1)	1.7 (−11.8, 15.1)	−10.9 (−28.2, 7.1)	0.235	0.44
Blood pressure, circulating lipids and other circulating proteins					
SBP, mmHg	−2 (−7, 3)	−6 (−11, −1)	4 (−3, 10)	0.282	0.40
DBP, mmHg	−6 (−10, −2)	−3 (−7, 0)	−3 (−8, 2)	0.284	0.40
TAG, $mmol \cdot L^{-1}$	−0.43 (−0.67, −0.18)	−0.62 (−0.84, −0.40)	0.19 (−0.10, 0.49)	0.194	0.47
NEFA, $mmol \cdot L^{-1}$	0.00 (−0.13, 0.14)	−0.03 (−0.15, 0.10)	0.03 (−0.13, 0.20)	0.712	0.13
Total cholesterol, $mmol \cdot L^{-1}$	0.01 (−0.29, 0.30)	−0.11 (−0.37, 0.15)	0.12 (−0.24, 0.47)	0.512	0.24
HDL, $mmol \cdot L^{-1}$	0.04 (−0.01, 0.09)	0.04 (0.02, 0.08)	0.00 (−0.06, 0.05)	0.891	0.05
LDL, $mmol \cdot L^{-1}$	0.09 (−0.14, 0.31)	0.06 (−0.14, 0.26)	0.03 (−0.24, 0.30)	0.849	0.07
ALT, $U \cdot L^{-1}$	−0.3 (−7.3, 6.7)	−5.8 (−12.1, 0.5)	5.5 (−2.9, 13.9)	0.203	0.47
AST, $U \cdot L^{-1}$	0.3 (−5.2, 5.8)	−2.7 (−7.7, 2.4)	3.0 (−3.9, 9.6)	0.392	0.31
AST:ALT ratio, AU	0.03 (−0.11, 0.17)	0.06 (−0.08, 0.19)	−0.03 (−0.20, 0.15)	0.744	0.12
GGT, $U \cdot L^{-1}$	−4.7 (−9.3, −0.1)	−4.4 (−8.5, −0.3)	−0.3 (−5.8, 5.2)	0.916	0.04
CRP, $mg \cdot L^{-1}$	3.66 (−1.18, 8.50)	6.21 (1.89, 10.53)	−2.55 (−8.37, 3.28)	0.391	0.32
IL-6, $pg \cdot mL^{-1}$	−0.18 (−1.25, 0.88)	0.03 (−0.94, 1.00)	−0.21 (−1.51, 1.08)	0.741	0.12
CK18 M30, $IU \cdot L^{-1}$	−14 (−55, 28)	−1 (−39, 36)	−13 (−63, 38)	0.631	0.18
CK18 M65, $IU \cdot L^{-1}$	−49 (−114, 16)	−24 (−80, 33)	−25 (−103, 52)	0.517	0.24
CK18 M30:M65 ratio, AU	0.07 (−0.05, 0.19)	0.05 (−0.06, 0.16)	0.02 (−0.12, 0.16)	0.773	0.10

Note: Data were analysed using generalized linear models with a normal distribution and identity link function, and are presented as mean (95% confidence interval) change from baseline adjusted for pre-intervention values and ethnicity. For the EX and CON groups, respectively, $n = 8$ and $n = 11$ for MRI data (^{1}H-MRS data were available for the full sample). Bold values indicate statistical significance at $p < 0.05$.

Abbreviations: Adipo-IR, adipose tissue insulin resistance index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AU, arbitrary units; BMI, body mass index; CK18, cytokeratin-18; CON, control; CRP, C-reactive protein; DBP, diastolic blood pressure; EX, exercise training; GGT, gamma-glutamyl transferase; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin-6; NEFA, non-esterified fatty acids; PDFF, proton density fat fraction; PUI, polyunsaturation index; SBP, systolic blood pressure; ScAT, subcutaneous abdominal adipose tissue; SI, saturation index; TAG, triacylglycerol; TAT, total adipose tissue; UI, unsaturation index; VAT, visceral adipose tissue; VO_2 peak, peak oxygen uptake.

3.3 | Six-week randomized controlled trial: Part B

Baseline characteristics of the two intervention groups are presented in Table 2. Collectively, participants were predominantly White European (79.2%), had type 2 diabetes (75.0%) and were taking metformin (58.3%). Baseline characteristics were similar in each group (Table 2, Supplementary Table S3), except for PDFF, circulating glucose and HOMA-IR, which were lower in the CON versus the EX intervention group (Table 2). During EX, participants attended 23.1 ± 1.1 of 24.0 (96.2%) training sessions. Participants' mean heart rate during exercise was 118 ± 6 bpm (73.0% of age-predicted maximum heart rate), while their total weekly exercise volume increased from 148 ± 19 min/week in Week 1 to 197 ± 79 min/week in Week 6. Changes in habitual physical activity and dietary intake across the intervention period were similar in the two groups, except for moderate-to-vigorous physical activity bouts ≥5 min and ≥10 min, which were increased during EX compared to CON (Supplementary Table S4). Furthermore, dietary mono- and polyunsaturated fatty acid intake were increased and decreased after EX and CON, respectively (Supplementary Table S4).

Table 3 shows the intervention responses. Across the 6 weeks, aerobic fitness (relative VO_2 peak) was improved with EX compared with CON. Intervention responses were similar between groups for all hepatic lipid composition indices (Supplementary Figure S5A–C) as well as other anthropometric/adiposity-related outcomes. Given baseline differences in liver PDFF between EX and CON, an additional *post-hoc* sensitivity analysis was conducted for the hepatic lipid composition indices, with baseline PDFF included as a covariate. The same results were obtained, albeit with marginally smaller estimates (hepatic SI: −1.1% [−4.5, 2.2%], $p = 0.505$, ES = 0.26; hepatic UI: 1.1% [−2.2, 4.5%], $p = 0.505$, ES = 0.26; hepatic PUI: 0.3% [−1.2, 1.9%], $p = 0.681$, ES = 0.16).

Changes in HbA1c, circulating insulin and Adipo-IR were similar in the two interventions, whereas circulating glucose concentrations were reduced, and HOMA-IR tended to be reduced after EX compared to CON (Table 3). No differences were observed in changes in blood pressure, circulating lipids, liver enzymes, systemic inflammatory markers and CK18 neoepitopes between the two interventions (Table 3). The intention-to-treat sensitivity analysis showed the same pattern of results and interpretation for all intervention responses (Supplementary Table S5).

Correlation analysis (EX and CON combined) revealed that the change in relative VO_2 peak was inversely associated with changes in hepatic SI (Supplementary Figure S6A) and positively associated with changes in hepatic UI and PUI (Supplementary Figure S6B,C). Similar associations were observed between absolute VO_2 peak and hepatic SI ($r = -0.442$; $p = 0.031$), UI ($r = 0.442$; $p = 0.031$) and PUI ($r = 0.400$; $p = 0.053$).

3.4 | Adverse events

Ten non-serious adverse events occurred across the study, with three related to baseline cardiac arrythmias, five related to cardiac arrythmias during the pre-intervention VO_2 peak test and two related to chest tightness during training sessions. Three incidental findings were identified during the pre-intervention MRI scan, which were deemed benign upon referral.

4 | DISCUSSION

Using validated ^{1}H-MRS-derived indices of hepatic lipid composition, this study demonstrated that men with MASLD and IGR had higher hepatic lipid saturation and lower hepatic lipid unsaturation compared to those with NGR, whereas hepatic lipid polyunsaturation was similar regardless of glycaemic status. Furthermore, 6 weeks of moderate-intensity exercise training had minimal impact on hepatic lipid composition.

Hepatic lipid composition is thought to be central to the metabolic and hepatic consequences of hepatic steatosis in MASLD, such as insulin resistance and hepatic fibro-inflammation.[5] Accordingly, in our MASLD population, we observed a higher hepatic SI and lower hepatic UI in individuals with IGR versus individuals with NGR. Additionally, this lipid profile was associated with markers of glycaemic dysregulation and insulin resistance. These findings corroborate previous studies reporting elevated saturated hepatic lipids in populations with MASLD, type 2 diabetes, and obesity with high versus low HOMA-IR.[10,12,14] Notably, these previous observations have been made against comparator groups with markedly lower liver fat content (2.2%–5.0%). In our study, the differences in hepatic SI and UI were evident despite high liver PDFF in both study groups, highlighting a close relationship between hepatic lipid composition and glycaemic regulation in MASLD.

Mechanistically, elevated hepatic DNL may contribute to these observed relationships, given that this pathway exclusively produces palmitate (saturated fatty acid; [SFA]) and is upregulated by hyperglycaemia and hyperinsulinaemia.[15] Hepatic DNL is disproportionately elevated in MASLD,[29,30] and positively correlates with both 24-h circulating glucose concentrations and the liver SFA fraction.[12,29] Conversely, genetic forms of steatotic liver disease are characterized by preserved metabolic function, lower DNL and increased hepatic polyunsaturated lipids.[10,31] Consequently, it is plausible that hyperglycaemia in our participants with MASLD and IGR could be driving the greater accumulation of saturated hepatic lipids through increased hepatic DNL. In turn, a greater production of hepatic SFAs and their associated lipotoxic lipid intermediates may exacerbate glycaemic dysregulation and insulin resistance in a continual cycle.[6] Mechanistic studies are required for confirmation.

Another novel finding was that circulating CK18 M65 concentrations, a marker of total hepatocyte cell death,[32] were higher in the IGR versus NGR group, and were directly associated with a higher hepatic SI and lower hepatic UI. CK18 is the major intermediate filament protein in the liver which holds strong potential as a biomarker for MASH, including fibrotic MASH.[32] While observational, our data imply that greater hepatic injury in conditions of glycaemic dysregulation could be related to higher hepatic lipid saturation and lower

unsaturation. In support of this, human MASH and fibrosis is characterized by a further enrichment of saturated hepatic lipids and depletion of polyunsaturated hepatic lipids,[9,33,34] while in preclinical studies, SFAs promote hepatocellular stress, inflammation, apoptosis and fibrogenesis.[6,7,35] Collectively, these data could help explain the more aggressive liver disease trajectory in coexisting MASLD and type 2 diabetes.[3]

Contrary to our hypothesis, 6 weeks of exercise training did not appreciably alter hepatic lipid composition compared to CON. Albeit in a sample with less severe metabolic dysfunction, one previous study also reported a lack of change in hepatic saturated lipid composition, despite a more intense exercise programme (70% $\dot{V}O_2$ peak) over 4 weeks.[21] Conversely, our data contrast with those showing an increase in hepatic PUI after 7 consecutive days of treadmill walking (85% maximum heart rate) in individuals with MASLD.[20] The latter study was limited, however, by the absence of a control group, and the higher PUI could reflect an effect of the last exercise bout on fatty acid mobilization/uptake, as observed in rodents.[19] Nevertheless, it is noteworthy that the absolute change in hepatic SI after our exercise intervention was −2.6%, which is similar to the difference observed between the IGR and NGR groups (2.9%) in our study. Consequently, our sample size may have been insufficient to detect these potential subtle yet meaningful changes as the study was powered on larger expected differences (5.0%).

We also found that the change in liver PDFF, which reflects total hepatic lipid, was similar in the EX compared to the CON intervention. This was despite a control-adjusted absolute change of −2.1% after EX, which matches a previous meta-analysis summarizing exercise training studies in individuals with MASLD where significant weight loss was not achieved.[17] However, this modest change in liver PDFF and the lack of change in body weight, in addition to a relatively short intervention period, may explain why more substantial changes in hepatic lipid composition were not observed. Indeed, two recent studies involving a 24-week comprehensive lifestyle intervention[36] and an 8-week low-energy diet[37] reported significant decreases in hepatic lipid saturation where the average weight loss was 9 kg. Longer-term lifestyle interventions with larger reductions in body weight may be required for more substantial alterations in hepatic lipid composition in MASLD.

One previous study noted positive associations between baseline aerobic fitness and hepatic PUI in individuals with MASLD.[14] Interestingly, we found that VO_2 peak was improved after EX compared with CON, and the change in VO_2 peak was inversely associated with the change in hepatic SI and positively associated with the change in hepatic UI and PUI. This could suggest that exercise training regimens optimized to achieve the greatest improvements in $\dot{V}O_2$ peak may promote the largest shifts towards a more favourable hepatic lipid profile. Improvements in whole-body aerobic capacity partly reflect improved oxidative capacity of multiple tissues, including the liver.[38] Thus exercise-induced improvements in hepatic mitochondrial function and fatty acid oxidative capacity,[38] which share a reciprocal relationship with hepatic DNL,[39] could partly explain these observed associations.

Strengths of this study include the novel comparisons in an MASLD population, with varying degrees of glycaemic regulation, and the robust randomized controlled trial design and intervention adherence in Part B. Furthermore, we internally validated our ^{1}H-MRS measurement of hepatic lipid composition. However, the validation experiment did identify some degree of bias and variability, particularly with regard to the hepatic PUI. Although this would not impact the interpretation of the study findings, further in vivo validation is required against gold standard liver biopsy assessment. Additionally, although a multi-ethnic population was recruited, the inability to generalize our findings to women is a limitation of the study. Importantly, the decision to recruit men was based on the recognition that MASLD and related cardiovascular morbidity is more prevalent and severe in men compared with women[40] and on funding constraints which demanded a homogenous sample. Further limitations include the observational nature of Part A, preventing causality from being established, and the potential for undetected differences in dietary intake to have confounded hepatic lipid composition data (due to poor sensitivity of diet records). Additionally, while our cross-sectional analyses were powered *a priori*, larger studies are needed to further explore factors associated with differences in hepatic lipid composition. In Part B, baseline differences in liver PDFF and glycaemic parameters between the intervention groups may have dampened our ability to detect intervention effects. Equally, although our sample size was informed by formal calculations, our ES data indicate an intervention effect may have potentially been detectable with a larger sample.

In conclusion, this study demonstrates that glycaemic dysregulation in MASLD is characterized by greater hepatic lipid saturation and lower hepatic lipid unsaturation, a lipid profile that is associated with markers of insulin resistance and hepatic injury. Conversely, 6 weeks of moderate-intensity exercise training does not appreciably alter hepatic lipid composition in men with MASLD and IGR, despite small numerical differences potentially beginning to emerge. These findings support the notion that this more 'lipotoxic' hepatic lipid profile could contribute to the relationship between MASLD, type 2 diabetes, and a worse liver disease prognosis, while exercise training alone with minimal changes in body weight/adiposity may be insufficient to impact hepatic lipid composition. Future research should unpick the mechanisms linking glycaemic status and hepatic lipid composition and explore the impact of longer-term exercise and/or pharmacological interventions on hepatic lipid composition in MASLD.

AUTHOR CONTRIBUTIONS

Jack A. Sargeant, Penny Gowland, David R. Webb, Melanie J. Davies, Guruprasad P. Aithal and James A. King were involved in the conception and design of the study. Scott A. Willis, Sundus Malaikah, Stephen J. Bawden, Aron P. Sherry, Jack A. Sargeant, Nicole A. Coull, Christopher R. Bradley, Alex Rowlands, Iyad Naim, Ghazala Waheed and James A. King contributed to the acquisition and/or analysis of the study data. Scott A. Willis, Sundus Malaikah, Gaël Ennequin, Thomas Yates, David J. Stensel, Guruprasad P. Aithal and James A. King were involved in the interpretation of the study data. Scott A. Willis, Sundus Malaikah and James A. King drafted the manuscript, while all authors critically revised the manuscript for important intellectual content. Furthermore, all authors approved the final version of

the manuscript and agree to be accountable for all aspects of the work. James A. King is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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