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VERY LOW-VOLUME INTERVAL TRAINING IMPROVES NONALCOHOLIC FATTY LIVER DISEASE FIBROSIS SCORE AND CARDIOMETABOLIC HEALTH IN ADULTS WITH OBESITY AND METABOLIC SYNDROME

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Non-alcoholic fatty liver disease (NAFLD) and cardiometabolic disorders are highly prevalent in obese individuals. Physical exercise is an important element in obesity and metabolic syndrome (MetS) treatment. However, the vast majority of individuals with obesity do not meet the general physical activity recommendations (*i.e.* 150 min of moderate activity per week). The present study aimed to investigate the impact of a highly time-saving high-intensity interval training (HIIT) protocol (28 min time requirement per week) on NAFLD fibrosis (NFS) and cardiometabolic risk scores in obese patients with MetS and elevated NFS values. Twenty-nine patients performed HIIT on cycle ergometers (5 × 1 min at an intensity of 80 – 95% maximal heart rate) twice weekly for 12 weeks and were compared to a control group without exercise (CON, n = 17). Nutritional counseling for weight loss was provided to both groups. NFS, cardiometabolic risk indices, MetS z-score, cardiorespiratory fitness (VO_{2max}) and body composition were assessed before and after intervention. The HIIT (–4.3 kg, $P < 0.001$) and CON (–2.3 kg, $P = 0.003$) group significantly reduced body weight. There were no significant group differences in relative weight reduction (HIIT: –3.5%, CON: –2.4%). However, only the HIIT group improved NFS (–0.52 units, $P = 0.003$), MetS z-score (–2.0 units, $P < 0.001$), glycemic control (HbA1c: –0.20%, $P = 0.014$) and VO_{2max} (+3.1 mL/kg/min, $P < 0.001$). Decreases in NFS (–0.50 units, $P = 0.025$) and MetS z-score (–1.4 units, $P = 0.007$) and the increment in VO_{2max} (3.3 mL/kg/min, $P < 0.001$) were significantly larger in the HIIT than in the CON group. In conclusion, only 28 min of HIIT per week can elicit significant improvements in NFS and a several cardiometabolic health indices in obese MetS patients with increased NFS grades. Our results underscore the importance of exercise in NAFLD and MetS treatment and suggest that our low-volume HIIT protocol can be regarded as viable alternative to more time-consuming exercise programs.

Key words: *obesity, interval training, non-alcoholic fatty liver disease, physical activity, metabolic syndrome, cardiopulmonary exercise test, blood pressure, liver fibrosis*

INTRODUCTION

The prevalence of obesity has markedly increased across the globe over the last decades. Obesity is linked with an elevated risk of developing a number of health issues (1). If obesity is accompanied by an accumulation of further cardiometabolic risk factors, including hyperglycemia, dyslipidemia and hypertension (*i.e.* the metabolic syndrome, MetS), the risk of developing cardiovascular diseases (CVD) and certain types of cancer as well as for mortality is additionally significantly increased (2, 3). Additionally, it has been reported that a large proportion of obese people suffer from non-alcoholic fatty liver disease (NAFLD), which is characterized by an increased fat storage in the liver independent of alcohol consumption (4). Non-alcoholic fatty liver disease can progress to chronic liver inflammation and lead to serious liver damage, such as hepatic fibrosis and cirrhosis (4). Due to its strong association with other established cardiometabolic risk factors (5), it has been considered the hepatic component of MetS (6). Consequently, the development and evaluation of viable strategies to treat obesity and related disorders is currently more relevant than ever.

Dietary modifications, particularly the reduction of caloric intake, and the uptake of physical exercise are cornerstones in obesity, MetS and NAFLD treatment (7-11). It has been shown that regular exercise may offer protection against the development of MetS and NAFLD (12, 13). Furthermore, the degree of cardiorespiratory fitness (CRF) was found to be strongly inversely associated with cardiovascular problems and mortality (13, 14). However, the vast majority of obese individuals (15) and patients with NAFLD (16) do not meet the recommended physical activity (PA) amount (*i.e.* ≥ 150 min/week of moderate aerobic exercise (7)). Given that 'perceived lack of time' is one of the most

frequently mentioned barriers to regular PA in the general population (17) as well as particularly among obese individuals (18) and NAFLD patients (16), recent research has focused on developing more time-saving exercise modalities.

In this context, high-intensity interval training (HIIT) has emerged as an attractive alternative to traditional moderate-intensity continuous aerobic training (MICT). High-intensity interval training is characterized by short intense exercise bouts separated by low-intensity recovery periods. Research has shown that HIIT can evoke beneficial effects on cardiometabolic risk indices in overweight and obese populations after only a few weeks (19). Additionally, it has been demonstrated that HIIT provides similar or even superior effects for reducing body fat mass than MICT (20). Data on the effects of HIIT on NAFLD are still very limited but initial studies (21-26), including a first meta-analysis (27), have revealed beneficial impact on intrahepatic fat (IHF) levels.

'Low-volume' HIIT is a rather novel, particularly time-efficient sub-type of interval training, that typically requires < 30 min/session (28). Recently, studies from our group revealed that a highly time-saving low-volume HIIT protocol (14 min/session) effectively improved CRF and cardiometabolic risk profiles in untrained normal-weight individuals (29) and obese MetS patients (30, 31). However, data on the effects of low-volume HIIT on NAFLD in obese MetS patients are still lacking.

Therefore, the present study aimed to investigate the impact of our very low-volume HIIT protocol compared to a control group without exercise (CON) on NAFLD fibrosis score (NFS) and cardiometabolic risk indices in obese individuals diagnosed with MetS and increased NFS values. On the basis of results from our previous investigations (30, 31), we expected that our very low-volume HIIT protocol would significantly improve NFS and cardiometabolic risk factors in obese MetS patients.

MATERIALS AND METHODS

Study design

The present investigation was a sub-project of a larger randomized controlled study (ClinicalTrials.gov number: NCT03306069), focusing to evaluate the impact of interval training on various clinical outcomes in obese MetS patients (30, 31). In the principal trial, patients were allocated to different training groups (including very low-volume HIIT) or a control group without exercise, only obtaining nutritional advice to support weight loss (standard care). All patients assigned to the CON group were given the opportunity to participate in an exercise program at our Training Center after termination of the study. Sample size calculation and randomization procedures of the main study were previously reported (30). The current sub-project aimed to specifically investigate the impact of our very low-volume HIIT protocol on NAFLD and cardiometabolic risk indices in a sub-group of the principle study (*i.e.* obese MetS patients with increased NFS). Primary outcome was NFS, further outcomes of interest were MetS z-score, maximal oxygen uptake (VO_{2max}) and body composition.

All patients were fully informed about the study objectives and methods, which conformed to the Helsinki Declaration. Written consent was obtained from each participant. The study protocol was approved by the ethical committee of the Friedrich-Alexander University Erlangen-Nurnberg (approval number: 210_17B).

Patients

Advertisements posted in local newspapers and flyers laid out in surrounding medical practices were used to recruit patients for the study. Inclusion criteria for the main study were previously described (30) and included: age ≥ 18 years, obesity (BMI ≥ 30 kg/m^2), diagnosis of MetS (32) and a self-reported sedentary lifestyle. To be included in the current sub-project, patients additionally needed to exhibit at least an intermediate NFS, using the cutoff value > –1.455, as previously suggested (33, 34). Exclusion criteria for this sub-project were: clinical diagnosis of heart disease, cancer, severe orthopaedic conditions or other conditions that might prohibit safe participation in physical exercise, pregnancy and NFS values < –1.455. All patients agreed not to change their medication intake without medical clearance and consulting the principal investigator and not to make major changes in their lifestyle habits during the study period to minimize confounding effects.

Health examinations

Baseline examination was performed 1 week prior to the onset of the intervention. Patients were asked to present in an overnight-fasted state at our research laboratory and to abstain from alcohol consumption as well as from intense physical activities for at least 24 h preceding the examination. During the examination, patients were carefully screened to ensure safe participation in exercise training and to exclude the presence of viral infections that could influence the primary outcome NFS (*e.g.* hepatitis B or C). Post-examination was conducted within the first week after final exercise session. Examinations were carried out single-blinded (*i.e.* investigators involved in data collection were not aware of patients' group allocation). All assessments were performed in a controlled laboratory environment with stable ambient conditions and stringently standardized as described in the following.

Measurements of blood pressure

Patients were first asked to empty their bladder and subsequently to rest seated for a duration of 5 min. Thereupon, measurements of blood pressure (BP) were performed using an automatic upper-arm blood pressure monitor (M5 professional, Omron, Mannheim, Germany). Two measurements in a row were conducted on both arms with a 60-second break in between each measurement. For further analysis, the averaged values of the arm with the higher BP measurements were used.

Blood sampling

Blood samples were collected through venipuncture of the patient's antecubital arm vein into different tubes with the help of a disposable cannula (S-Monovette, Sarstedt, Nuermbrecht, Germany). The subsequent analyses were carried out at the laboratories of the University Hospital Erlangen as previously described in detail (30). Analyses included serum values of glucose (GLU), triglycerides (TG), total-cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), liver biochemistry (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations, bilirubin, albumin and gamma glutamyl transpeptidase (GGT)), glycosylated hemoglobin A_{1c} (HbA_{1c}) and blood count.

Measurements of body composition

For body composition measurements, patients were still in a fasted state and again instructed to empty their bladder if necessary. Measurements were carried out using a multi-frequency segmental bioelectrical impedance analysis (BIA) device (seca mBCA 515, Seca, Hamburg, Germany). The validity of the BIA-device has been previously compared against

the 4-compartement reference method for body composition assessment and demonstrated to provide precise results in obese individuals (35). Patients' waist circumference (WC) was obtained in upright position, to the nearest millimeter, using a measuring tape.

Determination of NFS and MetS z-score

NFS was calculated based on the following formula (33):
–1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelets (× 109/L) – 0.66 × albumin (g/dL)

The diagnosis of diabetes was given when fasting glucose was ≥ 126 mg/dL or the participant was treated with anti-diabetic drugs. Impaired fasting glucose (IFG) was defined when fasting glucose concentration ranged between 100 – 125 mg/dL (36). According to Angulo *et al.* (33), the NFS cutoff value of < –1.455 rules out advanced fibrosis, while values of –1.455 to 0.676 indicate intermediate and > 0.676 advanced fibrosis, respectively. Previous research has demonstrated a strong association between NFS and MetS (6, 37) and proper validity to predict the presence of advanced liver fibrosis in obese to morbidly obese patients (38).

MetS z-score was calculated based on the following formulas (39), including the variables sex (M: males, F: females), WC, mean arterial blood pressure (MAB), serum concentrations of GLU, TG, and HDL-C:
Males:
[(40 – HDL)/9.0] + [(TG – 150)/81.0] + [(GLU – 100)/11.3] + [(WC – 102)/7.7] + [(MAB – 100)/9.1]
Females:
[(50 – HDL)/14.1] + [(TG – 150)/81.0] + [(GLU – 100)/11.3] + [(WC – 88)/9.0] + [(MAB – 100)/9.1]

Cardiopulmonary exercise test

Patients performed a standardized ramp incremental exercise test on an electronically braked cycle ergometer (Corival cpet, Lode, Groningen, Netherlands) to measure VO_{2max}, maximal power output (W_{max}) and maximal heart rate (HR_{max}). After brief familiarization, the initial load was set at 50 W and subsequently gradually increased (25 W/2 min in females; 30 W/2 min in males) until volitional exhaustion. Using this approach, exhaustion was typically achieved within 8 – 12 min in all patients as previously recommended for cardiopulmonary exercise testing (40).

HR was measured continuously in real time by means of a 12-lead ECG system (custo cardio 110, custo med, Ottobrunn, Germany). Oxygen uptake during the exercise test was measured using an open-circuit breath-by-breath gas analyzer (Metalyzer 3B-R3, Cortex Biophysik, Leipzig, Germany). Ventilatory threshold (VT) was determined using the V-slope method (*i.e.* plotting carbon dioxide output versus oxygen uptake) to determine submaximal aerobic capacity (41).

Nutritional counseling and physical activity assessment

All patients obtained comprehensive nutritional counseling from a qualified dietitian in a one-on-one conversation at study entry. Dietary advices were based on the international guidelines for obesity treatment (42). Additionally, patients received recipes and food lists to support adherence to the dietary recommendations. Nutritional intake was analyzed using 3-day food records (Nutri-Science, Freiburg, Germany) at study onset and during the final week of intervention. Caloric and macronutrient intakes as well as alcohol consumption were evaluated using the software Prodi 6 expert (Nutri-Science, Freiburg, Germany). Daily life physical activity was assessed using activity records that were completed concomitantly with the dietary protocols (*i.e.* 3 days each at study start and during the final intervention week).

High-intensity interval training (HIIT)

The low-volume HIIT protocol was in accordance to the protocol established by Reljic *et al.* (29) and performed as previously described (30, 31). In brief, the HIIT sessions were performed on electronically braked cycle ergometers (Corival cpet, Lode, Groningen, Netherlands) and consisted of a 2 min warm-up phase, 5 interval bouts of 1 min at 80 – 95% HR_{max} divided by 1 min recovery periods of low intensity and a concluding 3 min cool-down (corresponding to 14 min session time in total). All sessions were supervised by certified instructors, who ensured that patients reached their individual target HR values. Patients were equipped with chest strap HR monitors (Polar H7 heart rate sensor, Polar Electro Oy, Kempele, Finland), which allowed continuous HR tracking during the training session. Heart rate values of each session were recorded and subsequently evaluated using a specific HR-analysis software (Polar Team, Polar Electro Oy, Kempele, Finland). Training was performed twice weekly with a minimum of 1 day recovery between sessions for a duration of 12 weeks.

Statistical analysis

All analyses were conducted as per protocol using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). Initially, data distribution was analyzed using the Shapiro-Wilk test. In case of normal distribution, two-way repeated-measures ANOVAs were performed to investigate main effects of group and time as well as interaction effects between both factors. Homogeneity of variance was checked with the Levene's test. If significant main or interaction effects were found, *post hoc* paired and unpaired t-tests were conducted to analyze pre-post-intervention changes within-groups and between-group post-intervention differences. If data were not normally distributed, log or square root transformation was done and the same aforementioned statistical tests were used with the transformed values. If the transformation did not lead to data normalization, the non-parametric Friedman test was performed and Wilcoxon's and Mann-Whitney tests were used for corresponding *post-hoc* comparisons. Effect sizes, including partial eta-squared (η^2) for ANOVA and Kendall's co-efficient of concordance (W) for the Friedman test, were calculated and classified as follows: small ≤ 0.01, medium ≥ 0.06, and large ≥ 0.14 for η^2, and small ≤ 0.10, medium ≥ 0.30, and large ≥ 0.50 for W (43). The significance level was $P < 0.05$ for all statistical analyses. All data are reported as means ± standard deviation (SD) and pre-/post-intervention changes are displayed with 95% confidence intervals (95% CI).

RESULTS

In the main study (30), a total number of 163 patients were initially screened. After the screening and baseline examination, 154 patients remained for randomization (n = 2: did not meet inclusion criteria, n = 5: withdraw, n = 2: excluded due to medical reasons). Of these, n = 40 were assigned to the HIIT group, n = 40 to the CON group and n = 77 to other exercise modalities (data not shown because not objective of the present investigation). Fifteen patients dropped out (HIIT: n = 8; CON: n = 7). A detailed study flow chart (including dropout reasons) is displayed in *Fig. 1*. Of the remaining 65 patients who completed

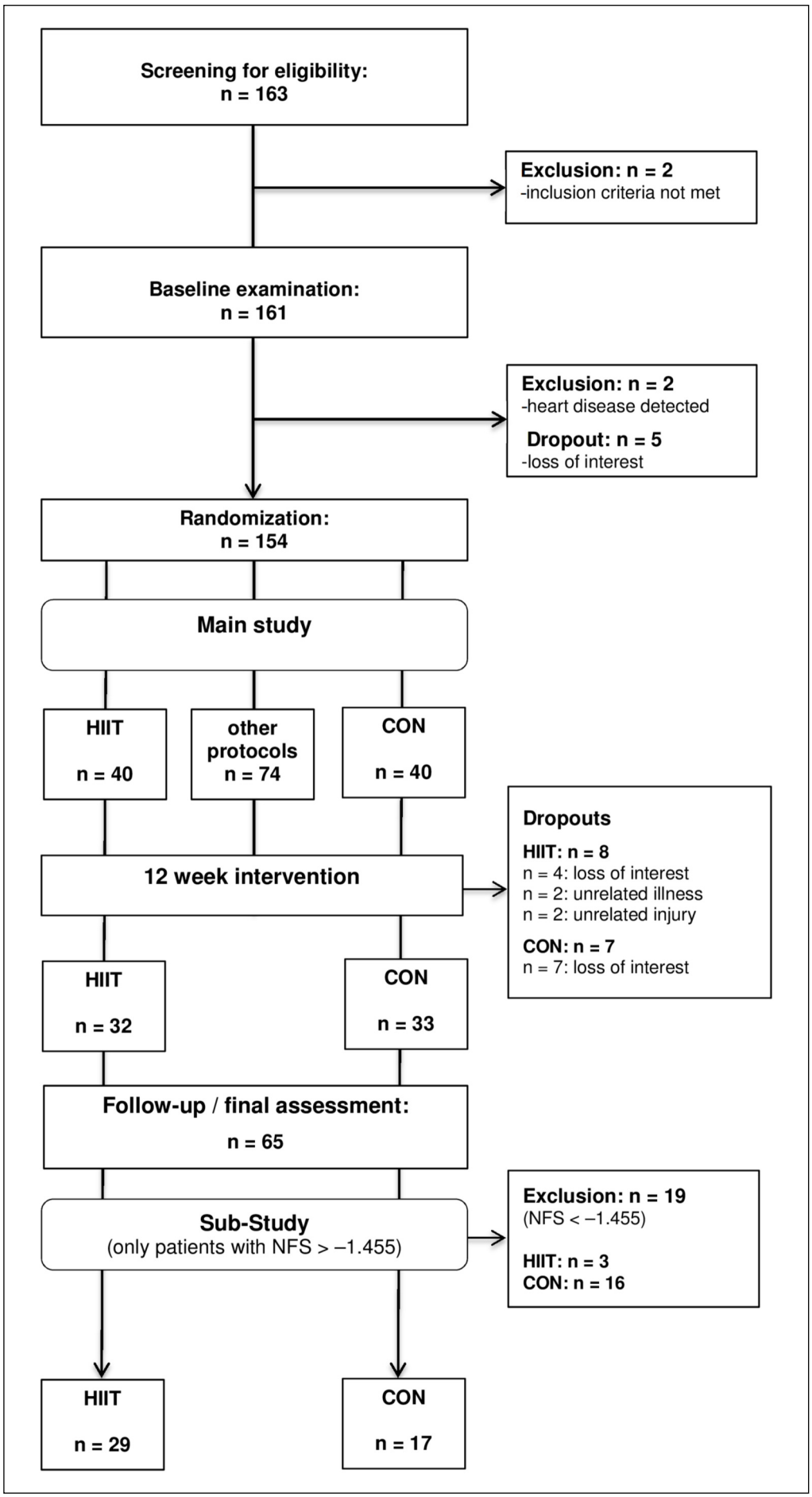

Fig. 1. Study flow chart.

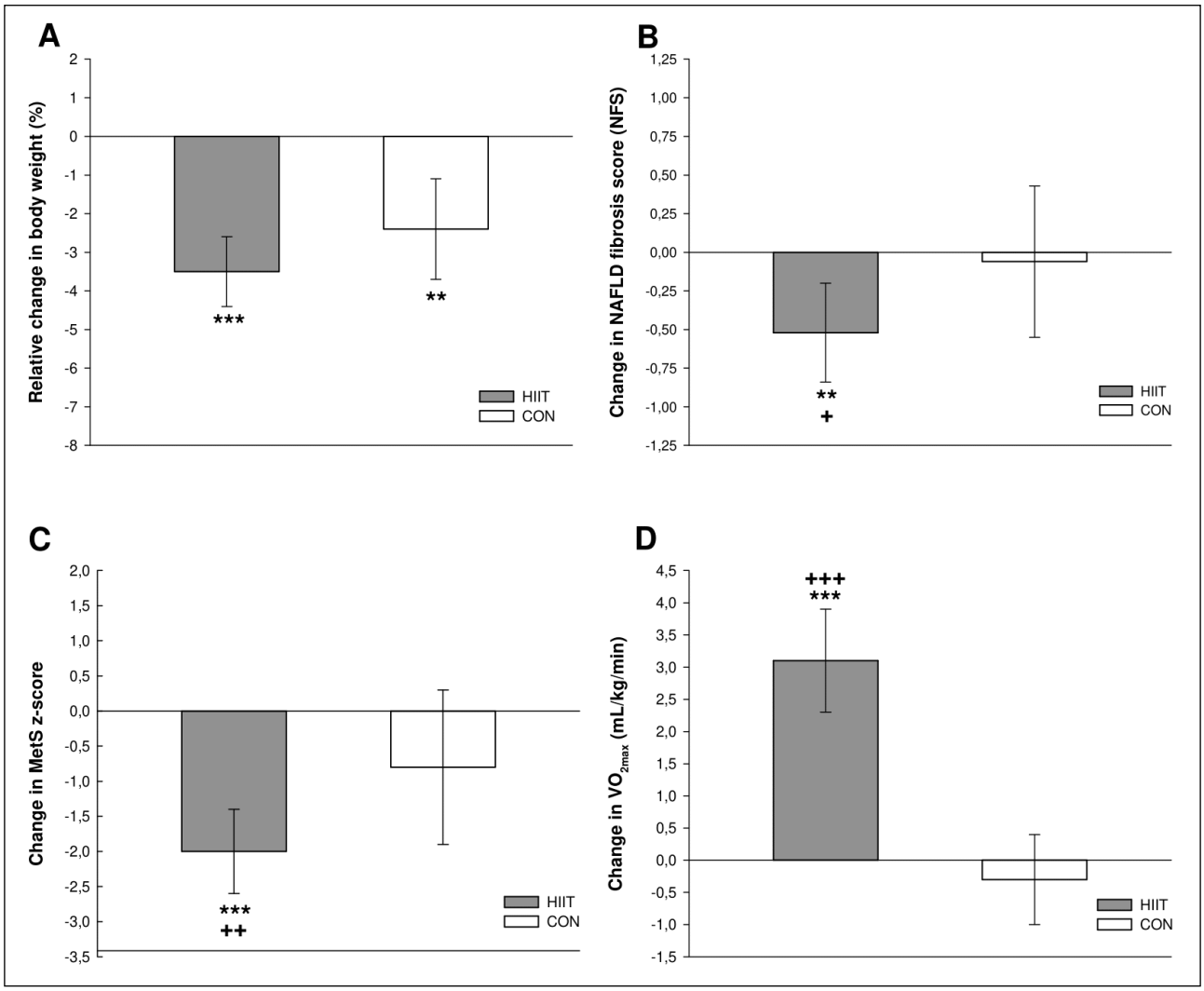

Fig. 2. Changes in body weight (A), NAFLD fibrosis score (NFS) (B), MetS z-score (C), and VO_{2max} (D), in the HIIT group and the control group. HIIT, high-intensity interval training group; CON, control group; NAFLD, non-alcoholic fatty liver disease; MetS, metabolic syndrome; VO_{2max}, maximal oxygen uptake. **($P < 0.01$), ***($P < 0.001$): significant change from pre-intervention; $^{+}$($P < 0.05$), $^{++}$($P < 0.01$), $^{+++}$($P < 0.001$): significant difference compared to the CON group.

Table 1. Anthropometric and body composition data before and after the intervention.

Variable	High-intensity interval training group (HIIT) (n = 29)				Control group (n = 17)				Group differences in Δ	
	Baseline	**Post**	**Δ (95%CI)**	***P***	**Baseline**	**Post**	**Δ (95%CI))**	***P***	**Mean (95%CI)**	***P***
Body weight (kg)	126.5±27.9	122.3±27.8	–4.3 (–3.1, –5.4)	< 0.001	106.1±19.1	103.7±19.8	–2.3 (–0.9, –3.8)	0.003	–1.9 (–0.1, –3.7)	0.039
Fat mass (kg)	56.5±17.3	53.1±17.5	–3.3 (–2.0, –4.7)	< 0.001	51.1±11.6	49.4±12.5	–1.6 (–0.1, –3.1)	0.053	–1.7 (0.3, –3.8)	ND
Fat mass (%)	44.3±7.1	42.9±8.2	–1.3 (–0.5, –2.2)	0.003	48.1±5.9	47.5±6.3	–0.6 (0.3, –1.5)	0.203	–0.7 (0.6, –2.0)	ND
Fat free mass (kg)	70.1±15.3	69.2±15.6	–0.9 (–0.2, –1.6)	ND	55.0±11.3	54.2±11.2	–0.9 (0.1, –1.5)	ND	–0.2 (0.9, –1.3)	ND
Total body water (L)	52.3±11.2	51.6±11.4	–0.7 (–0.2, –1.2)	0.050	41.5±8.2	40.7±8.2	–0.7 (–0.1, –1.3)	0.053	0 (0.8, –0.8)	ND
Waist circumference (cm)	123.5±18.2	117.1±16.9	–6.3 (–4.6, –8.1)	< 0.001	112.6±13.6	112.6±14.8	0 (–2.7, 2.7)	0.982	–6.3 (–3.4, –9.7)	< 0.001

Baseline and post-intervention data are expressed as mean ± SD. Pre-/post-intervention changes (Δ) and group differences in Δ are presented as mean and 95% confidence intervals (95% CI). ND, not determined because no significant main/interaction effect.

the study, n = 29 of the HIIT group (52.1 ± 9.6 years), and n = 17 of the CON group (56.7 ± 9.8 years), fulfilled the specific criteria to be included in this sub-project (*i.e.* NFS > –1.455), resulting in a final total sample size of n = 46, with a gender distribution of n = 12 women (41%) and n = 17 men (59%) in the HIIT group and n = 14 women (82%) and n = 3 men (18%)

Table 2. Non-alcoholic fatty liver disease (NAFLD) fibrosis score (NFS), NFS components and liver biochemistry variables before and after the intervention.

Variable	High-intensity interval training group (HIIT) (n = 29)				Control group (n = 17)				Group differences in Δ	
	Baseline	Post	Δ (95%CI)	*P*	Baseline	Post	Δ (95%CI)	*P*	Mean (95%CI)	*P*
NFS	0.01±0.10	–0.50±0.10	–0.52 (–0.2, –0.8)	0.003	0.53±1.70	0.47±1.70	–0.06 (0.2, –0.3)	0.632	–0.5 (0, –0.9)	0.025
BMI (kg/m²)	40.9±7.8	39.6±7.8	–1.3 (–1.0, –1.7)	< 0.001	39.4±5.3	38.5±5.6	–0.9 (–0.4, –1.4)	0.003	–0.5 (0.1, –1.1)	ND
ALT (U/L)	35±22	29±10	–6 (0, –12)	0.042	32±19	27±16	–5 (0, –11)	0.078	–0.2 (8, –9)	ND
AST (U/L)	28±11	26±11	–2 (0, –5)	ND	31±13	29±14	–2 (2, –6)	ND	0 (5, –5)	ND
AST/ALT ratio	0.93±0.31	0.94±0.22	0 (–0.1, 0.1)	ND	1.18±0.61	1.28±0.68	0.1 (0, 0.2)	ND	–0.1 (0.1, –0.2)	ND
Platelet count (×10⁹/L)	226±47	238±62	12 (–5, 28)	ND	218±68	229±76	10 (–5, 25)	ND	–1 (25, –22)	ND
Diabetes/IFG (n/%)	18 (62%)	12 (41%)	–6 (–21%)	< 0.001	12 (71%)	12 (71%)	0 (0)	1.00	6 (21%)	ND
GGT (U/L)	35±18	32±17	–3 (2, –7)	0.252	31±23	24±15	–7 (–1, –12)	0.017	4 (–3, 11)	ND
Albumin (g/L)	42±2	42±2	0 (–1, 1)	ND	41±4	40±.3	–1 (0, –2)	ND	1 (0, 2)	0.044
Bilirubin (μmol/L)	10.9±4.8	11.5±4.5	0.5 (–0.5, 1.5)	ND	14.0±10.4	14.0±10.9	0 (–1.7, 1.7)	ND	0.4 (–1.5, 2.4)	ND

Baseline and post-intervention data are expressed as mean ± SD. Data for diabetes/IGF prevalence are shown as absolute and percentage value. Pre-/post-intervention changes (Δ) and group differences in Δ are presented as mean and 95% confidence intervals (95% CI). BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; IFG, impaired fasting glucose; ND, not determined because no significant main/interaction effect.

in the CON group, respectively. No significant relationship between gender and changes in primary outcome was detected. Thus, the data of both women and men were considered together in all analyses.

Anthropometric and body composition data

A significant main effect of time was observed for body weight ($P < 0.001$, $\eta^2 = 0.55$), fat mass ($P < 0.001$, $\eta^2 = 0.35$), body fat percentage ($P = 0.004$, $\eta^2 = 0.17$), WC ($P < 0.001$, $\eta^2 = 0.29$) and body water ($P < 0.002$, $W = 0.21$). A significant interaction effect was found for body weight ($P = 0.039$, $\eta^2 = 0.09$) and WC ($P < 0.001$, $\eta^2 = 0.29$). *Post hoc* tests revealed that both groups significantly reduced body weight (HIIT: –4.3 kg, 95% CI: –5.4 to –3.1 kg, $P < 0.001$; CON: –2.3 kg, 95% CI: –3.8 to –0.9 kg, $P = 0.003$). There were no significant differences ($P = 0.078$) in relative weight loss between the HIIT (–3.5%, 95% CI: –4.4 to –2.5%) and the CON group (–2.4%, 95% CI: –3.7 to –1.0%) (shown in *Fig. 2a*). Fat mass (–3.3 kg, 95% CI: –4.7 to –2.0 kg, $P < 0.001$), body fat percentage (–1.3 %, 95% CI: –2.2 to –0.5 %, $P = 0.003$) and WC (–6.3 cm, 95% CI: –8.1 to –4.6 cm, $P < 0.001$) were only significantly decreased in the HIIT group (shown in *Table 1*).

Nutritional analysis and physical activity assessment

Three patients (HIIT: n = 2, CON: n = 1) did not submit two complete food records and were thus excluded from nutritional evaluation. Mean daily energy and macronutrient intakes at baseline were: 2398 ± 808 kcal, 107 ± 40 g protein, 102 ± 42 g fat and 255 ± 90 g carbohydrates in the HIIT group, and 2545 ± 859 kcal, 106 ± 28 g protein, 120 ± 61 g fat and 244 ± 78 g carbohydrates in the CON group. Both groups reduced daily energy intake from baseline to follow-up (HIIT: –581 kcal, 95% CI –780 to –383 kcal, $P < 0.001$; CON: –460 kcal, 95% CI –602 to –317 kcal, $P < 0.001$) without significant group differences. In both, the HIIT and CON group, the reduction in caloric intake was primarily realized through decreased carbohydrate ingestion (HIIT: –71 g, 95% CI –99 to –43 g, $P < 0.001$; CON: –65 kcal, 95% CI –92 to –37 g, $P < 0.001$). However, daily protein (HIIT: –14 g, 95% CI –19 to –9 g, $P < 0.001$; CON: –13 kcal, 95% CI –18 to –7 g, $P < 0.001$) and fat intakes (HIIT: –23 g, 95% CI –31 to –15 g, $P < 0.001$; CON: –16 kcal, 95% CI –28 to –5 g, $P < 0.001$) also decreased significantly in both groups. In both groups, mean alcohol consumption did not change significantly from baseline (HIIT: 3.8 g, 95% CI 1.7 to 5.8 g; CON: 2.6 g, 95% CI 0.6 to 4.7 g) to follow-up (HIIT: 2.4 g, 95% CI 0.8 to 4.0 g; 1.5 g, 95% CI 0.1 to 2.9 g). None of the patients consumed alcohol on daily basis or in amounts exceeding the recommended upper levels of alcohol (*i.e.* 10 g/day for women and 20 g/day for men, respectively) (44). Prior and during the study period, none of the patients engaged in structured exercise or vigorous physical activities (*i.e.* ≥ 6.0 METS, as previously defined (7)). There were no significant changes in the amount of moderate daily physical activity (*i.e.* 3.0 to 5.9 METS (7)) assessed baseline (HIIT: 5.4 min/day, 95% CI 3.7 to 7.1 min/day; CON: 5.0 min/day, 95% CI 2.5 to 7.4 min/day) and within the last week of the intervention (HIIT: 5.1 min/day, 95% CI 3.7 to 6.5 min/day; CON: 5.7 min/day, 95% CI 3.2 to 8.3 min/day).

Non-alcoholic fatty liver disease fibrosis score and liver biochemistry variables

Significant main time effects were detected for NFS ($P = 0.016$, $\eta^2 = 0.13$) and its components BMI ($P < 0.001$, $\eta^2 = 0.56$), ALT ($P = 0.008$, $\eta^2 = 0.15$) and hyperglycemia ($P = 0.045$, $\eta^2 = 0.09$). There was a trend for a group-by-time interaction for NFS ($P = 0.050$, $\eta^2 = 0.09$). In addition, there was a significant main effect of time for GGT ($P < 0.012$, $\eta^2 = 0.14$). *Post hoc* tests revealed that NFS (–0.52 units, 95% CI –0.84 to –0.19 units, $P = 0.003$) (*Fig. 1b*), BMI (–1.3 kg/m², 95% CI: –1.7 to –1.0 kg/m², $P < 0.001$), ALT (–6.0 U/L, 95% CI –11.8 to –0.2 U/L, $P = 0.042$) and the prevalence of hyperglycemia (–21%, $P = 0.012$) were significantly decreased in the HIIT group after the

Table 3. Non-alcoholic fatty liver disease (NAFLD) fibrosis score (NFS) grading among patients before and after the intervention.

Variable	**High-intensity interval training group (HIIT)** (n = 29)		**Control group** (n = 17)	
	Baseline	**Post**	**Baseline**	**Post**
Low risk < –1.455	0 (0%)	5 (17%)	0 (0%)	0 (0%)
Intermediate risk –1.455 to 0.676	22 (76%)	20 (69%)	13 (76%)	13 (76%)
High risk > 0.676	7 (24%)	4 (14%)	4 (24%)	4 (24%)

Data are expressed as absolute numbers and percentages in parentheses.

Table 4. Cardiometabolic risk variables before and after the intervention.

Variable	**High-intensity interval training group (HIIT)** (n = 29)				**Control group** (n = 17)				**Group differences in Δ**	
	Baseline	**Post**	**Δ (95%CI)**	***P***	**Baseline**	**Post**	**Δ (95%CI)**	***P***	**Mean (95%CI)**	***P***
MetS z-score	4.00±3.65	1.96±3.31	–2.0 (–1.5, –2.6)	< 0.001	3.20±2.60	2.42±2.58	–0.8 (0.3, –1.8)	0.134	–1.4 (–0.4, –2.4)	0.007
Systolic BP (mmHg)	141±14	130±13	–11 (–7, –14)	< 0.001	134±16	134±12	–1 (6, –8)	0.794	–10 (–3, –16)	0.005
Diastolic BP (mmHg)	91±12	84±9	–7 (–4, –10)	< 0.001	85±14	85±10	0 (–4, 4)	0.916	–7 (–2, –11)	0.006
MAB (mmHg)	108±11	99±9	–8 (–6, –10)	< 0.001	101±14	101±10	0 (–5, 5)	0.864	–8 (–3, –12)	0.002
Glucose (mmol/L)	5.7±0.9	5.5±0.7	–0.2 (0.1, –0.4)	0.210	6.0±1.1	5.6±1.1	–0.4 (0.1, –0.9)	0.064	0.2 (–0.6, 0.2)	ND
HbA_{1c} (%)	5.7±0.5	5.5±0.3	–0.2 (0, –0.3)	0.014	5.8±0.9	5.4±1.0	–0.3 (0.5, –0.8)	0.205	0 (–0.3, 0.3)	ND
Triglycerides (mmol/L)	1.6±0.6	1.6±0.6	0 (–0.2, 0.2)	ND	1.7±0.9	1.5±0.7	–0.2 (0, –0.5)	ND	0.2 (–0.5, 0.1)	ND
Cholesterol (mmol/L)	5.6±0.8	5.6±0.9	0 (–0.3, 0.3)	ND	5.9±1.0	5.5±0.7	–0.4 (–0.2, –0.7)	ND	0.4 (0, –0.9)	ND
HDL-C (mmol/L)	1.2±0.3	1.3±0.3	0.1 (0, 0.1)	ND	1.4±0.3	1.3±0.3	–0.1 (0, –0.1)	ND	0.1 (0, 0.2)	ND
LDL-C (mmol/L)	3.9±0.7	3.8±0.7	–0.1 (0.2, –0.3)	ND	3.9±0.8	3.6±0.7	–0.3 (–0.1, –0.5)	ND	0.2 (–0.6, 0.2)	ND
LDL/HDL ratio	3.3±0.9	3.0±0.9	–0.3 (0.1, –0.5)	ND	2.9±0.8	2.8±0.8	–0.1 (0.1, –0.2)	ND	–0.2 (0.2, –0.5)	ND

Baseline and post-intervention data are expressed as mean ± SD. Data for diabetes/IGF prevalence are shown as absolute and percentage value. Pre-/post-intervention changes (Δ) and group differences in Δ are presented as mean and 95% confidence intervals (95% CI). MetS, metabolic syndrome; BP, blood pressure; MAB, mean arterial blood pressure; HbA_{1c}, glycosylated hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ND, not determined because no significant main/interaction effect.

intervention period. In the CON group, there were significant reductions in BMI (–0.9 kg/m^2, 95% CI: –1.4 to –0.4 kg/m^2, P = 0.003) and GGT (–6.5 U/L, 95% CI: –11.7 to –1.3 U/L, P = 0.017). NFS decrease was significantly larger in the HIIT group compared to the CON group (–0.50 units, 95% CI: –0.9 to –0.1 units, P = 0.025) (shown in *Fig. 2b*, *Table 2*). In the HIIT group, the prevalence among patients who had high risk of advanced fibrosis dropped by 10% from pre- to post-intervention and 17% of patients moved into the low risk NFS categorization after the training period. In the CON group, the mean prevalence rates in the three NAFLD risk grades remained unchanged (shown in *Table 3*).

Cardiometabolic risk variables

Significant group-by-time interactions and main effects of time were evident for MetS z-score (P < 0.015, $\acute{\eta}^2$ = 0.13 and P < 0.001, $\acute{\eta}^2$ = 0.41), systolic BP (P = 0.005, $\acute{\eta}^2$ = 0.17 and P = 0.001, $\acute{\eta}^2$ = 0.22, respectively), diastolic BP (P = 0.006, $\acute{\eta}^2$ = 0.16 and P = 0.004, $\acute{\eta}^2$ = 0.18, respectively) and MAB (P = 0.002, $\acute{\eta}^2$ = 0.20 and P = 0.001, $\acute{\eta}^2$ = 0.23, respectively). Moreover, there were significant main time effects for fasting glucose (P = 0.014, $\acute{\eta}^2$ = 0.13) and HbA_{1c} (P = 0.030, $\acute{\eta}^2$ = 0.10). *Post hoc* tests showed that post-intervention MetS z-score (–2.0 units, 95% CI –2.6 to –1.5 units, P < 0.001) (shown in *Fig. 2c*), systolic BP (–11 mmHg, 95% CI –14 to –7 mmHg, P < 0.001), diastolic BP (–7 mmHg, 95% CI –10 to –4 mmHg, P < 0.001) MAB (–8 units, 95% CI –10 to –6 mmHg, P < 0.001) and HbA_{1c} (–0.20%, 95% CI –0.32 to –0.04%, P = 0.014) were significantly decreased in the HIIT group (shown in *Table 4*). MetS z-score reduction was significantly larger in the HIIT group than in the CON group (–1.4, 95% CI –2.4 to –0.4 units, P = 0.007).

Cardiorespiratory fitness

There were significant interaction and time effects for absolute VO_{2max} (P < 0.001, $\acute{\eta}^2$ = 0.41 and P < 0.013, $\acute{\eta}^2$ = 0.14, respectively), relative VO_{2max} (P < 0.001, $\acute{\eta}^2$ = 0.40 and P < 0.001, $\acute{\eta}^2$ = 0.32, respectively), absolute W_{max} (P < 0.001, $\acute{\eta}^2$ = 0.59 and P < 0.001, $\acute{\eta}^2$ = 0.29, respectively), relative W_{max} (P <

Table 5. Cardiorespiratory fitness data before and after the intervention.

Variable	High-intensity interval training group (HIIT) (n = 29)				Control group (n = 17)				Group differences in Δ	
	Baseline	**Post**	**Δ (95%CI)**	***P***	**Baseline**	**Post**	**Δ (95%CI)**	***P***	**Mean (95%CI)**	***P***
VO_{2max} (L/min)	2.52±0.65	2.77±0.68	0.25 (0.16, 0.36)	< 0.001	1.80±0.58	1.71±0.56	–0.09 (–0.01, –0.16)	0.027	0.33 (0.21, 0.46)	< 0.001
VO_{2max} (mL/kg/min)	20.5±5.1	23.5±6.0	3.1 (2.1, 3.9)	< 0.001	17.1±4.8	16.9±5.5	–0.3 (0.4, –1.0)	0.378	3.3 (2.1, 4.6)	< 0.001
W_{max} (W)	157±46	181±45	24 (19, 29)	< 0.001	124±47	117±42	–7 (–2, –13)	0.018	31 (23, 39)	< 0.001
W_{max} (W/kg)	1.3±0.4	1.6±0.5	0.3 (0.2, 0.3)	< 0.001	1.2±0.4	1.2±0.4	0 (–0.1, 0)	0.632	0.3 (0.2, 0.4)	< 0.001
Power at VT (W)	59±26	86±28	27 (21, 33)	< 0.001	57±29	57±30	0 (–4, 5)	0.916	–6.3 (–3.4, –9.7)	< 0.001

Baseline and post-intervention data are expressed as mean ± SD. Pre-/post-intervention changes (Δ) and group differences in Δ are presented as mean and 95% confidence intervals (95% CI); VO_{2max}, maximal oxygen uptake; W_{max}, maximal power output; VT, ventilatory threshold; ND, not determined because no significant main/interaction effect.

0.001, η^2=0.47 and $P < 0.001$ η^2=0.42, respectively) and power output at VT ($P < 0.001$, $\eta^2 = 0.52$ and $P < 0.001$, $\eta^2 = 0.52$, respectively). In the HIIT group, absolute VO_{2max} (+250 mL, 95% CI 160 to 360 mL, P < 0.001), relative VO_{2max} (+3.1 mL/kg/min, 95% CI 2.1 to 3.9 mL/kg/min, P < 0.001), absolute W_{max} (+24 W, 95% CI 19 to 29 W, P < 0.001), relative W_{max} (+0.3 W/kg, 95% CI 0.2 to 0.3 W/kg, P < 0.001) and VT-performance (+27 W, 95% CI 21 to 33 W, P < 0.001) increased significantly post intervention. By contrast, the CON group displayed significant decreases in absolute VO_{2max} (–90 mL, 95% CI –160 to –10 mL, P = 0.027) and W_{max} (–7 W, 95% CI –13 to –2 W, P = 0.018) post-intervention (shown in *Table 5*). VO_{2max} increase was significantly greater in the HIIT compared to the CON group (+3.3 mL/kg/min, 95% CI 2.1 to 4.6 mL/kg/min, $P < 0.001$) (shown in *Fig. 2d*).

Training data and adverse events

In the HIIT group, the mean HR peak value that was reached at the end of each exercise interval corresponded to 93 ± 5% of HR_{max}, verfiying that the specified exercise intensity was achieved. Adherence rate (the percentage of the scheduled training sessions that the patients completed) was high (94 ± 8%), indicating that HIIT was well tolerated and accepted by the patients. There were no adverse events during the entire intervention period.

DISCUSSION

Although there is growing evidence that HIIT may have a beneficial impact on liver fat (25, 43), data on the impact of (very) low-volume HIIT protocols on hepatic health are still very scarce. To our knowledge, this study was the first to investigate the responses to a very low-volume HIIT protocol involving less than 30 min of total exercise per week on hepatic outcomes in obese MetS patients. The key findings were as follows: (i) as little as 28 min of very low-volume HIIT per week (involving a total of 10 min of vigorous exercise per week) induced significant improvements in NFS, MetS z-score and HbA_{1c}, and (ii) despite a similar relative amount of weight loss compared with the CON group, only patients in the HIIT group experienced significant improvements in NFS and cardiometabolic risk indices, underpinning the crucial role of physical exercise in improving hepatic and cardiometabolic health - even when applied in very small doses.

Non-alcoholic fatty liver disease is one of the most widespread liver conditions in highly-industrialized countries and its prevalence ranges between 50 – 90% in obese individuals (4). Moreover, a close relationship has been reported between NAFLD and metabolic abnormalities (5, 6). In accordance with the literature, we found that 58% of patients included in our main study (30) exhibited increased NFS grades (*i.e.* the sample of the present sub-study) and that patients' NFS values were significantly correlated with their MetS z-score values ($r = 0.25$, $P = 0.018$). Patients diagnosed with both NAFLD and MetS display a particularly high risk for the development of severe health problems (5, 6) and thus, these patient collectives need special attention.

There is strong evidence that exercise, even in the absence of weight loss, may improve NAFLD and cardiometabolic risk outcomes (7, 12). To date, there are no specific exercise guidelines for NAFLD and the majority of studies that have investigated the hepatic benefit of exercise (6, 10, 12, 46) have adopted the general PA guidelines for weight loss and prevention of weight regain, recommending at least 150 min/week of moderate-intensity activity (7). Nonetheless, a large proportion of individuals worldwide - in particular obese cohorts and NAFLD patients - do not meet these guidelines, mainly due to time constraints (16-18), and thus, the advocated 150 min of physical activity per week may not be a realistic goal for many adults. Additionally, a meta-analysis has recently indicated that reductions in liver fat can also be achieved with exercise volumes of < 150 min/week, although apparently to a smaller extent then > 150 min of moderate to vigorous exercise are accumulated per week (27).

In this context, it has been suggested that HIIT may be an attractive alternative for NAFLD patients (27, 45) with first studies demonstrating promising results. In a pioneering study, Hallsworth *et al.* (21) observed significant reductions in IHF (–27%), ALT (–3 U/L) and fat mass (–1.8 kg) after 12 weeks of HIIT (3 exercise sessions/week of 30 – 40 min duration) in obese patients. Studies that compared HIIT with MICT demonstrated that both exercise modalities similarly led to reduced relative IHF levels in the range of 14 – 37% (22, 23) in overweight and obese individuals, without significant impact on body composition. Recently, it was reported that 8 weeks of HIIT (3 weekly sessions of ~40 min duration) improved intrahepatic triglyceride content (–2.3%), ALT (–4.1 U/L), VO_{2max} (+5.2 mL/kg/min) and HbA_{1c} (–0.4%), in diabetic obese patients (24). Another study found that brief sprint interval training (4 – 6 × 30 s 'all-out' sprints on cycle ergometers, total session duration: 30

min) performed thrice weekly over 6 weeks led to significant improvements in intrahepatic triglyceride levels (–12.4%), visceral adipose tissue (–16.9%) and VO_{2max} (+13.6%) in obese men (25). More recently, Sabag *et al.* (26) reported that a low-volume HIIT protocol involving 3 weekly exercise sessions of 19 min duration (total time effort per week: 57 min) induced a similar mean reduction in liver fat (–1.7% vs. –0.9%) and HbA_{1c} (both 0.3%) compared to a MICT protocol (mean total session duration: 55 min) in inactive adults with obesity and type 2 diabetes despite requiring significantly less time commitment.

Given remaining concerns regarding intense exercise in obese individuals with clustering of cardiometabolic disorders, it is to note that neither we nor the previous studies have observed serious adverse events during exercise, indicating that HIIT seems to be well tolerated by patients at increased cardiometabolic risk. Nevertheless, we point out that previously sedentary individuals, particularly those with preexisting health conditions, should obtain medical clearance before engaging in HIIT, as generally recommended before starting an exercise program.

Regarding total time-effort, it is apparent, however, that with the exception of the study of Oh *et al.* (22) (weekly time-effort: 54 min), the differences in the duration of the protocols applied in previous studies (ranging from 90 – 222 min/week) are relatively small in comparison to traditional exercise programs. Thus, adherence to these protocols may potentially wear off with time. This assumption is enforced by a recent meta-analysis showing that longer session time and higher weekly time-effort are significant predictors for premature dropout in HIIT interventions with untrained cohorts (47). In this context, it is important to highlight that time-effort for our protocol (28 min/week) was ~50 – 90% lower than in previous studies (21-26). Despite this extremely low exercise volume, equivalent to less than a fifth of the common physical activity recommendations (7), our protocol yielded a significant improvement in NFS (–0.5 units), that is comparable to results reported after bariatric surgery (48, 49) or pharmacological treatment (50). More specifically, NFS reductions by 0.3 units have been observed following 6 months (49) and 1 year (48), respectively, of bariatric surgery. Similarly, a 0.3 point reduction of NFS has been observed in obese individuals receiving Silymarin (700 mg, 3 times/day) for 48 weeks (50). Consequently, our data indicate that low-volume HIIT can be regarded as an effective, non-invasive and side-effect free treatment to improve indices associated with NAFLD and that these improvements are similar to those achieved through bariatric surgery or pharmacological treatment.

Furthermore, we found significant improvements in MetS severity, glucose control and CRF. Metabolic syndrome severity was classified using the MetS z-score, suggested as better encompassing the spectrum of cardiometabolic risk status in comparison with the presence of a pre-determined number of clustered cardiometabolic risk factors with specific cut-off values (39). The average MetS z-score reduction by –2.0 units is in line with the observation of Ramos *et al.* (51), who found a mean score reduction by –1.6 units in obese patients with MetS after 16 weeks of low-volume HIIT (time-effort/week: 51 min) and data from our group demonstrating a –1.3 unit decrease in obese MetS patients following 12 weeks of low-volume, single-set resistance training (average time-effort: ~32 min/week) (52) and –1.2 unit reduction in female obese MetS patients performing whole-body electromyostimulation exercise for 12 weeks (time-effort per week: 40 min) (53), respectively. Together, these results indicate that specific low-volume exercise programs may evoke beneficial changes in cardiometabolic outcomes in high-risk groups and that these effects can be similar to those achieved with higher-volume, moderate-intensity PA-programs, respectively (39).

Considering the single MetS components, it is very likely that our protocol evoked clinically meaningful benefits on BP (–11 mmHg systolic, –7 mmHg diastolic) and WC (–5%). It has been demonstrated that each BP reduction of 10 mmHg systolic and 5 mmHg diastolic is linked to a lower risk of developing CVD and stroke by 22% and 41% (54), respectively, and a 10% reduction in WC has been associated with a ~1.5 times lower risk of mortality (55). Additionally, the significant reduction in HbA_{1c} by 0.2% achieved in the present study has been suggested to lower mortality by 10% according to extrapolated data (56). Moreover, and perhaps even more important, HIIT group patients increased VO_{2max} by an average of 3.1. mL/kg/min. It has been suggested that the degree of CRF is an independent predictor of cardiovascular disorders and premature death, and much more powerful than other common risk outcomes, including diabetes, elevated blood pressure or smoking, in both normal-weight and overweight/obese populations (14). It has been estimated that each 1 mL/kg/min increase in VO_{2max} is associated with reduction of CVD-related mortality by 9% (57). Therefore, the observed improvement in VO_{2max}, together with significant increases in VT-performance (a submaximal measure of CRF and more sensitive to evaluate the ability of performing daily living physical activities) (38), can most likely considered to be highly clinically meaningful.

The achieved weight loss of ~3% in the present study was in line with the mean weight reduction values reported in most obesity exercise treatment programs (58). Interestingly, although the relative extent of weight loss was not significantly different between both groups, patients in the CON group did not show any improvements in NFS, MetS z-score and CRF. Instead, we observed a decline in VO_{2max} in the CON group, which may be linked to unfavorable changes in the cardiovascular system induced by caloric restriction, as previously observed in overweight individuals who reduced body weight through fasting without concomitant exercise (59). These findings underscore the large range of health benefits of exercise mediated through several physiological pathways that far exceed simple body weight loss achieved through a reduction in caloric intake alone (60). Moreover, although a weight loss of at least 5% is commonly regarded as a necessary threshold to achieve meaningful reductions in cardiometabolic risk factors and advocated as a criterion to define a ‘successful’ obesity treatment (61), our data support previous research (60), indicating that exercise, even with little or no weight loss, can provide significant health benefits.

There are limitations of the present investigation that should be considered. First, it is to note that the sample of this sub-study was retrospectively selected based on their NFS (which also resulted in unequal group sizes). Although it has been demonstrated that NFS is a valid and accurate tool to detect patients with/without advanced fibrosis in various populations including cohorts with obesity and metabolic abnormalities (33, 34, 36-38), liver biopsy remains the gold standard for NAFLD diagnosis. However, liver biopsies are expensive, invasive and pose risks for complications, rendering them unsuitable for routine use. Nevertheless, future studies may wish to use imaging diagnostic methods, such as ultrasound or computed tomography, to provide a more comprehensive picture of the impact of very low-volume HIIT on hepatic health. Second, the additional impact of nutritional counseling provided to our study patients on the outcomes of interest can potentially be considered as a confounding variable limiting our results. However, our results are well in line with previous research demonstrating that exercise *per se* provides numerous beneficial health effects that go well beyond weight loss through dietary modifications alone (59, 60). Moreover, the inclusion of an additional group of patients and withholding of an established

standard care treatment (nutritional counseling to promote body weight reduction) to investigate the separate effects of dietary modification and our exercise protocol would not have been ethically justifiable. Third, it must be taken into account that the exercise intervention was carried out in a well-controlled environment under close supervision. Further research is required to determine the impact of the applied HIIT protocol in real-world settings. Finally, some of our results and corresponding conclusions are based on questionnaire-derived data (dietary intakes and assessment of physical activity). In this context, it should be considered that patient-reported measures may potentially be linked to some bias, such as lack of honesty, conscientious disclosures or insufficient memory. Consequently, it cannot be excluded that the patient-reported food and physical activity record data were biased by social desirability to some extent. Although we suspect that the thorough instruction and monitoring throughout the recording periods should have significantly reduced the magnitude of potential bias, we acknowledge that the use of activity trackers, for example, would have allowed for a more accurate assessment of patients' physical activity.

In conclusion, this is the first investigation to examine the impact of very low-volume HIIT on hepatic and cardiometabolic risk indices in obese MetS patients with increased NFS. Our data demonstrate that as little as two 14 min sessions/week of HIIT - which represents, to our knowledge, the smallest weekly volume of HIIT yet employed in a clinical collective - may produce significant improvements in NFS, MetS severity and CRF in this high-risk patient group. Consequently, our very low-volume HIIT protocol can be deemed as a viable exercise modality for obese individuals at increased cardiometabolic risk who are not physically able or unwilling to participate in higher-volume exercise programs. Fitness and health professionals working with such high-risk groups of patients can be encouraged to incorporate very low-volume HIIT into treatment programs and/or to implement it as a preparatory exercise modality before prescribing higher-volume exercise regimes.

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