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ORIGINAL ARTICLE

Low cardiopulmonary fitness is associated with higher liver fat content and higher gamma-glutamyltransferase concentrations in the general population – "The Sedentary's Liver"

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Abstract

Background: We investigated the association between low cardiorespiratory fitness and liver fat content (LFC) in the general population.

Materials and Methods: We evaluated data from 2151 adults (51.1% women) from two population-based cohorts of the Study of Health in Pomerania (SHIP-2 and SHIP-TREND-0). We analysed the cross-sectional associations of peak oxygen uptake (VO_{2peak}) with LFC, assessed by magnetic resonance imaging proton density fat fraction, as well as serum gamma-glutamyltransferase (GGT) and aminotransferase concentrations by multivariable regression models.

Results: We observed significant inverse associations of VO_{2peak} with LFC and serum GGT, but not with serum aminotransferase levels. Specifically, a 1 L/min lower VO_{2peak} was associated with a 1.09% (95% confidence interval [CI]: 0.45-1.73; $P = .002$) higher LFC and a 0.18 μkatal/L (95% CI: 0.09-0.26; $P < .001$) higher GGT levels. The adjusted odds ratio (OR) for the risk of prevalent hepatic steatosis (HS) by a 1 L/min decrease

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CRF, cardiorespiratory fitness; CV, cardiovascular; CVD, cardiovascular diseases; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HS, hepatic steatosis; LDL, low-density lipoprotein; LFC, liver fat content; MRI, magnetic resonance imaging; OR, odds ratio; PDFF, proton density fat fraction; SHIP, Study of Health in Pomerania; T2DM, type 2 diabetes mellitus; VO_{2peak}, peak oxygen uptake.

The Study of Health in Pomerania (SHIP)

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in VO_{2peak} was 1.61 (95% CI: 1.22-2.13; $P = .001$). Compared to subjects with high VO_{2peak}, obese and overweight individuals with low VO_{2peak} had 1.78% (95% CI: 0.32-3.25; $P = .017$) and 0.94% (95% CI: 0.15-1.74; $P = .021$) higher mean LFC, respectively. Compared to those with high VO_{2peak}, low VO_{2peak} was independently associated with a higher risk of prevalent HS in the obese (adjusted-OR 2.29, 95% CI=1.48-3.56; $P < .001$) and overweight (adjusted OR 1.57, 95% CI=1.16-2.14; $P = .04$) groups.

Conclusions: Lower VO_{2peak} was significantly associated with greater LFC and higher serum GGT levels in a population-based cohort of adult individuals. Our results suggest that low VO_{2peak} might be a risk factor for HS.

KEYWORDS
fat-free mass, liver fat content, MRI-proton-density-fat-fraction (PDFF), peak oxygen uptake, sedentarism, cardiorespiratory fitness

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the general population. It consists from simple hepatic steatosis (HS) to progressive nonalcoholic steatohepatitis (NASH) with/without cirrhosis and hepatocellular carcinoma (HCC).[1] NAFLD is present in up to nearly 70-90% of individuals with severe obesity or type 2 diabetes mellitus (T2DM).[2,3] Moreover, NAFLD is associated with metabolic disorders, including atherogenic dyslipidaemia, hypertension and insulin resistance.[4] Growing evidence suggests that NAFLD is not simply a marker of increased CVD risk but it may also directly contribute to CVD morbidity and mortality.[2,5,6]

Previous studies showed that reduced physical activity is closely associated with obesity, T2DM and HS, the most common manifestation of NAFLD.[1,7,8] Cardiorespiratory fitness (CRF) is considered a quantitatively objective marker of physical activity[8] and it is better determined by the measurement of peak oxygen uptake (VO_{2peak})[9] during a cardiopulmonary exercise test. Importantly, while some previous studies reported a significant inverse association between levels of physical activity and HS,[10,11] a sedentary lifestyle is the predominant characteristic of the general adult population[12] in Western countries and it will most probably increase in the next decades.[8] Moreover, a sedentary lifestyle and its accompanying pathophysiological mechanisms are not necessarily the reverse of those related with vigorous physical activity and exercise training.[13] Recent studies published by our group,[14-17] using echocardiographic or magnetic resonance imaging (MRI) examination, showed that low levels of VO_{2peak} or handgrip strength (a marker of muscular fitness) were significantly associated with a smaller and stiffer heart, which we have called "the sedentary's heart". In a similar way, we aimed to analyse the effects of a sedentary lifestyle on other organs such as the liver. A previous study[18] showed that prolonged sitting time and decreased physical activity were associated with the presence of HS on ultrasound. Another study[19] with 463 individuals (aged 30–47 years) showed that lower CRF was also associated with ultrasound-detected HS. Therefore, the aim of our cross-sectional study was to contribute to the understanding of the impact of low CRF on metabolic health by investigating the associations of VO_{2peak} with serum liver enzyme levels and liver fat content (LFC), as assessed by magnetic resonance imaging, in a population-based cohort of German adults. Moreover, we also aimed to analyse whether obesity modified these associations.

2 | MATERIALS AND METHODS

2.1 | Study population

The data were obtained from the population-based Study of Health in Pomerania (SHIP) using SHIP-2 (2008-2012) and SHIP-TREND-0 (2008-2012) databases.[20,21] The study design and recruitment strategy have been described elsewhere.[20,21] We performed cross-sectional analyses using pooled data from the SHIP-2 and SHIP-TREND-0 (n = 6753 participants; 3510 women [52.0%]).

From these 6753 participants, we used a subsample of 2788 subjects who were eligible and underwent a whole-body MRI. From this subsample, we excluded participants with missing values for cardiopulmonary exercise test (n = 574) or other covariates included in the analysis (n = 54). We also excluded individuals with previous self-reported cirrhosis or chronic hepatitis (n = 9). The final sample comprised 2151 subjects (1100 women; 51.1%) aged 21–90 years (Figure S1).

All participants gave written informed consent. The study was approved by the ethics committee of the University of Greifswald[21] and complies with the Declaration of Helsinki.

2.2 | Cardiopulmonary exercise testing and gas exchange variables

A symptom-limited exercise test using a calibrated electromagnetically braked cycle ergometer (Ergoselect 100, Ergoline, Germany) was performed according to a modified Jones protocol.[22] Oxygen uptake (VO_2) was analysed breath-by-breath averaged over 10-s intervals using the Oxycon Pro system (Jaeger/Viasys Healthcare; Hoechberg, Germany), together with a Rudolph's mask, which was recalibrated

before each test. VO_{2peak} in L/min was defined as the highest 10-sec average of absolute VO_2 during late exercise or early recovery.[22]

2.3 | Bioelectrical impedance analysis

Fat-free mass and fat mass were measured by bioelectrical impedance analysis using a multifrequency Nutriguard-M device (Data Input, Pöcking, Germany) and the NUTRI4 software (Data Input, Pöcking, Germany).[23,24]

2.4 | Magnetic resonance imaging

Liver MRI was performed by using a 1.5-Tesla MRI system (Magnetom Avanto, software version VB15; Siemens Healthineers Erlangen, Germany) with a 12-channel phased-array surface coil.[25] The LFC was determined by offline reconstructions of a proton density fat fraction (PDFF) map. Mean PDFF values were determined at operator-defined regions of interest placed at the centre of the liver, by using Osirix (v3.8.1; Pixmec Sarl, Bernex, Switzerland). Presence of HS was defined as MRI-PDFF ≥5.1%.[26]

2.5 | Statistical analysis

Descriptive data were reported as absolute numbers (percentages) for categorical variables and as medians (25th and 75th percentiles) for continuous variables, stratified by high and low VO_{2peak} values. High and low VO_{2peak} values were defined according to values above and below the fat-free mass-specific median. Due to tissue-specific oxygen requirements, the influence of body composition has to be considered when comparing VO_{2peak} values in a heterogeneous population. Thus, it is advised to standardize VO_{2peak} in order to determine unbiased values. In our study, we standardized VO_{2peak} to fat-free mass since recent studies considered fat-free mass to be the more reliable body composition variable to standardize, especially when compared to the habitually used total body weight.[24,27] For this, we conducted a median regression with VO_{2peak} as outcome and fat-free mass as independent variable. From this regression model, we derived a VO_{2peak} cut-off for each specific level of fat-free mass. This is done to derive for each fat-free mass value in the population a specific median of VO_{2peak} to categorize probands into low (i.e. equal to or below the median) and high (above the median) VO_{2peak} groups.

The associations of VO_{2peak} with LFC and serum liver enzyme levels (aspartate aminotransferase [AST], alanine aminotransferase [ALT] and gamma-glutamyltransferase [GGT]) were analysed by multivariable linear regression models adjusted for age, sex, fat-free mass, fat mass, pre-existing T2DM, hypertension, smoking status, alcohol consumption and use of lipid-lowering medications. The associations of low vs. high VO_{2peak} with hepatic parameters in different groups of body mass index (BMI), were analysed by linear regression models adjusted for age, sex, pre-existing T2DM, hypertension, smoking status, alcohol consumption and use of lipid-lowering medications (when stratified by increasing BMI levels, no adjustment was made for fat-free mass and fat mass). The odds ratio (OR) for the association of VO_{2peak} with the risk of prevalent HS in BMI groups was calculated by logistic regression models adjusted for age, sex, pre-existing T2DM, hypertension, smoking status, alcohol consumption and use of lipid-lowering medications.

A *P*-value < .05 was considered as statistically significant. STATA 16.1 (Stata Corporation, College Station, TX, USA) was used to perform statistical analyses.

Please see the online data supplement for a more detailed description.

3 | RESULTS

3.1 | Characteristics of the study population stratified by high and low VO_{2peak}

The study population consisted of 2151 German adults, who were stratified by high and low fat-free mass-specific median of VO_{2peak}. The overall prevalence of HS (as assessed by MRI-PDFF) was 41%. HS was more prevalent in the low VO_{2peak} group compared to the high VO_{2peak} group (53% vs 28%). Individuals with low VO_{2peak} levels were more often men and were on average 13 years older. Additionally, while individuals with low VO_{2peak} had more previous CVDs and cardiometabolic risk factors, such as increased fat mass, hypertension, T2DM, hypercholesterolaemia and sedentary lifestyle compared to individuals with high VO_{2peak}, they consumed less amount of alcohol per day. The two groups of individuals did not significantly differ in terms of smoking status (Table 1).

3.2 | Reversion of the x-axis scale to run from maximum VO_{2peak} value to minimum

Figure 1 shows the associations from univariable linear regression models of age with either VO_{2peak} (A) or LFC (B). Older age was associated with lower values of VO_{2peak} and higher values of LFC. Since the centre of our primary analyses was the outcome of sequentially lower values of VO_{2peak} on both LFC and serum liver enzyme levels (after adjustment for age and other potential confounding factors), we presented all figures showing the associations between VO_{2peak} and the aforementioned liver parameters with a reverse x-axis to allow a more intuitive interpretation of the results.

3.3 | Associations of VO_{2peak} values with LFC and serum liver enzymes

After multivariable-adjusted regression analyses, we found significant inverse associations of VO_{2peak} with LFC and serum GGT levels (Figures 2A and D and Table 2). Specifically, a 1 L/min lower VO_{2peak}

TABLE 1 Characteristics of the study population stratified by high and low fat-free mass-specific median* of peak oxygen uptake (VO_{2peak}) (n = 2151)

Parameter	High VO_{2peak}	Low VO_{2peak}	*P*
N	1066	1085	
VO_{2peak} (L/min)	2.30 (1.81, 2.80)	1.55 (1.29, 1.92)	<.001
Age (years)	47 (39, 56)	60 (50, 68)	<.001
Women (%)	46.3	55.9	<.001
Fat-free mass (kg)	56.6 (46.9, 65.9)	54.2 (47.0, 66.7)	<.001
Fat mass (kg)	20.0 (16.2, 25.4)	24.5 (19.0, 30.5)	.004
Body mass index (kg/m^2)	26.0 (23.7, 28.8)	28.8 (25.9, 31.6)	<.001
Normal-weight (%)	37.8	19.1	<.001
Overweight (%)	44.2	42.0	
Obese (%)	18.0	38.9	
Waist circumference (cm)	86 (78, 94)	94 (84, 103)	<.001
Systolic blood pressure (mm Hg)	124 (113, 135)	131 (118, 143)	<.001
Diastolic blood pressure (mm Hg)	76.5 (70.5, 83.0)	78.0 (71.0, 84.5)	.056
Hypertension (%)	32.1	59.2	<.001
Antihypertensive medications (%)	19.8	46.8	<.001
Glycated haemoglobin (%)	5.2 (4.9, 5.5)	5.4 (5.0, 5.8)	<.001
Diabetes mellitus (%)	3.00	12.0	<.001
Hypoglycaemic medications (%)	1.50	8.39	<.001
Total cholesterol (mmol/l)	5.40 (4.70, 6.10)	5.50 (4.80, 6.30)	.040
LDL-cholesterol (mmol/l)	3.32 (2.69, 3.90)	3.40 (2.78, 4.03)	.142
HDL-cholesterol (mmol/l)	1.47 (1.22, 1.73)	1.38 (1.15, 1.67)	<.001
Lipid-lowering medications (%)	5.72	17.0	<.001
Estimated glomerular filtration rate ($mL/min/1.73\ m^2$)	85.6 (75.3, 97.6)	77.0 (67.4, 88.0)	<.001
Smoking (%)			
Never	38.2	39.7	.360
Current	22.8	20.3	
Former	39.0	40.0	
Alcohol consumption (g/day)	5.52 (1.77, 13.4)	3.42 (0.73, 9.87)	<.001
Sedentary lifestyle (%)	23.5	31.7	<.001
Previous cardiovascular disease (%)	1.03	3.60	<.001

The low and high fat-free mass-specific median of peak oxygen uptake (VO_{2peak}) were calculated as a VO_{2peak} cut-off for each specific level of fat-free mass.

Data are expressed as medians (25th and 75th percentile) or percentages.

P values derived from the Wilcoxon-Mann-Whitney test (for continuous data) or the chi-squared test (for categorical data).

was associated with a 1.09 % (95% CI: 0.45 to 1.73; $P = .002$) higher LFC and a 0.18 μkatal/L (95% CI: 0.09 to 0.26; $P < .001$) higher serum GGT concentration. Conversely, there were no associations of VO_{2peak} with serum ALT or AST concentrations (Figures 2B and C and Table 2). The adjusted-odds ratio for the risk of prevalent HS by a 1 L/min decrease in VO_{2peak} was 1.61 (95% CI: 1.22 to 2.13; $P = .001$) (Figure 3).

In sensitivity analyses, in which we repeated the above-mentioned regression models stratified by sex and menopausal status, we found similar results for both men and women with inverse associations of VO_{2peak} with LFC and GGT levels. In post-menopausal women, we found a significant inverse association of VO_{2peak} values with serum GGT concentrations, but not with LFC ($P = .081$). On the other hand, in premenopausal women, we did not find any association between VO_{2peak} and LFC and GGT levels, although these findings might be related to a lower statistical power due to the small sample size (Table S1).

3.4 | Associations of VO_{2peak} values with LFC stratified by BMI groups

We observed a significant interaction between VO_{2peak} and BMI on LFC ($P = .016$). Our analyses showed that in obese and overweight individuals there was a significant different association of high and low VO_{2peak} with LFC. On the other hand, this difference was not observed in normal-weight individuals (Figure 4). In the obese group, subjects

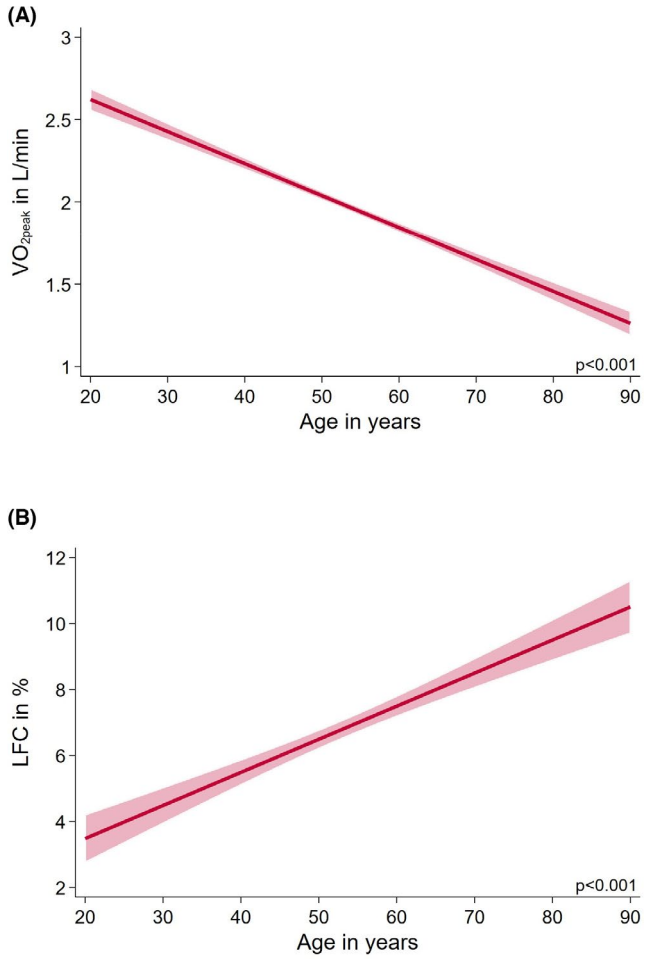

FIGURE 1 Univariable linear regression line (95% CI) showing the association of age with (A) peak oxygen uptake (VO_{2peak}) and (B) liver fat content (LFC as measured by MRI-PDFF) (n = 2151)

with low VO_{2peak} had in mean 1.78% (95% CI: 0.32 to 3.25; $P = .017$) greater LFC than those with high VO_{2peak}. Moreover, in the overweight group, subjects with low VO_{2peak} had also a greater mean LFC than those with high VO_{2peak} (difference = 0.94% [95% CI: 0.15 to 1.74; $P = .021$]). Conversely, there was no difference in mean LFC between individuals with high and low VO_{2peak} in the normal-weight group.

Compared to individuals with high VO_{2peak}, low VO_{2peak} was significantly associated with a higher risk of prevalent HS in individuals who were obese (adjusted-OR 2.29, CI = 1.48 to 3.56; $P < .001$) or overweight (adjusted-OR 1.57, CI = 1.16 to 2.14; $P = .04$). Conversely, the risk of prevalent HS was not significantly different between normal-weight subjects with high or low VO_{2peak} values.

Low VO_{2peak} was not significantly associated with serum ALT, AST or GGT levels in any of the three BMI groups of individuals.

4 | DISCUSSION

In this large community-based sample of German middle-aged individuals, we found that lower levels of VO_{2peak} were significantly associated with greater MRI-measured LFC and higher serum GGT concentrations even after adjustment for age, sex, fat-free mass, fat mass, pre-existing T2DM, hypertension, smoking status, alcohol consumption and use of lipid-lowering medications. Lower levels of VO_{2peak} were also independently associated with a higher risk of prevalent HS (defined as MRI-PDFF ≥5.1%). Importantly, our study showed that especially in obese and overweight subjects, and contrary to normal-weight subjects, lower VO_{2peak} may have an adverse impact on hepatic health, making these subjects most affected by a sedentary lifestyle. According to our analyses, obese subjects with low VO_{2peak} were more than twice (+129%) as likely to have HS as obese subjects with high VO_{2peak}. Moreover, overweight subjects with low VO_{2peak} had 54% more chances to develop HS as overweight subjects with high VO_{2peak}. On the other hand, we did not find any association between VO_{2peak} levels and serum aminotransferase levels.

4.1 | In the context of the published literature

While there are previous studies that analysed the possible effects of exercise and higher physical activity on HS, to the best of our knowledge, this is the first population-based study that evaluated the relationship between low levels of CRF, determined by VO_{2peak}, and LFC, as measured by MRI-PDFF.

A cross-sectional analysis of the Kangbuk Samsung Health Study,[18] a large population study involving nearly 139 000 South Korean individuals showed that physical inactivity and sedentary time (determined by a questionnaire) were significantly associated with ultrasound-detected HS. Interestingly, in line with our results, the observed associations of such study remained statistically significant even after adjustment for BMI and the homeostatic model assessment–insulin resistance (HOMA-IR). However, contrary to our findings, these associations were no longer significant after further adjustment for fat-free mass and/or fat mass. While our sample was not as large as the aforementioned Korean analyses, in our study we measured the peak oxygen uptake, which is a more precise method for CRF characterization than a simple questionnaire.[28] Moreover, we used MRI-PDFF for the diagnosis of HS, which is considered to be a more accurate method for quantifying LFC when compared to ultrasonography.[29-31]

The Cardiovascular Risk in Young Finns Study[19] including 463 participants aged 30–47 years showed that higher CRF was associated with lower prevalence of HS (assessed by ultrasound). Furthermore, similar to our results, this study[19] also showed that VO_{2peak} was inversely related to LFC in a BMI-dependent manner. While low VO_{2peak} was not associated with a negative effect on the risk for prevalent HS in normal-weight individuals, it was statistically significant in obese individuals.

In agreement with our findings, a cross-sectional analysis of the INSYTE trial (Investigation of SYnbiotic TreatmEnt in NAFLD)[32] involving 97 UK patients with NAFLD showed that higher serum GGT levels were significantly associated with lower VO_{2peak}, explaining 24% of the total variance in the CRF. In this analysis, serum AST and ALT levels were not associated with VO_{2peak}. On the other hand, contrary to our results, a small cross-sectional study[33] of 84 Japanese

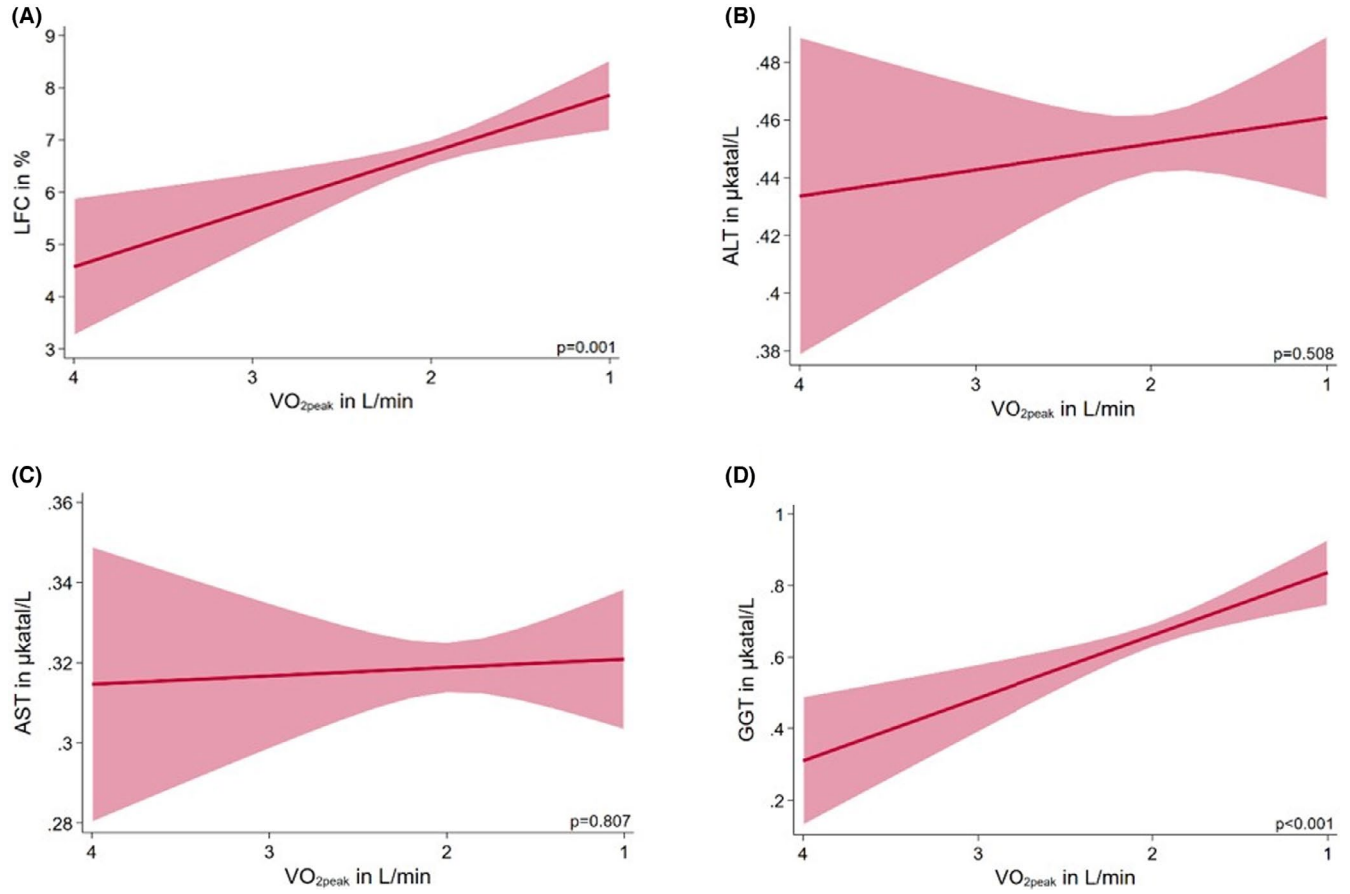

FIGURE 2 Adjusted[#] regression line (95% CI) showing the association of peak oxygen uptake (VO_{2peak}) with (A) liver fat content (LFC), (B) alanine aminotransferase (ALT), (C) aspartate aminotransferase (AST) and (D) gamma-glutamyltransferase (GGT) (n = 2151). [#]Linear regression was adjusted for age, sex, fat-free mass, fat mass, pre-existing T2DM, hypertension, smoking status, alcohol consumption and use of lipid-lowering medications

TABLE 2 Adjusted[*] β-coefficient (95% CI) of the associations of peak oxygen uptake (VO_{2peak}) with MRI measured liver fat content, serum alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltransferase concentrations (n = 2151)

Parameter	β-coefficient (95% CI), *P*-value
Liver fat content (%)	−1.09 (−1.73 to −0.45), *P* = .001
Alanine aminotransferase (µkatal/L)	−0.01 (−0.04 to 0.02), .508
Aspartate aminotransferase (µkatal/L)	−0.00 (−0.02 to 0.01), .807
Gamma-glutamyltransferase (µkatal/L)	−0.18 (−0.26 to -0.09), *P* < .001

*Linear regression model was adjusted for age, sex, fat-free mass, fat mass, pre-existing T2DM, hypertension, smoking status, alcohol consumption and use of lipid-lowering medications. Data were weighted according to dropouts from baseline to follow-up examination (SHIP-0 to SHIP-2) and individuals who did not take part in the MRI examinations (SHIP-2 and SHIP-TREND-0).

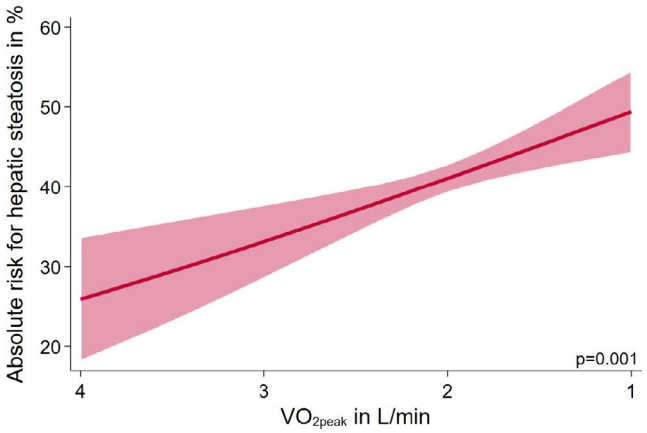

FIGURE 3 Adjusted[#] regression line (95% CI) showing the association of peak oxygen uptake (VO_{2peak}) with the odds ratio for the risk of prevalent hepatic steatosis (HS) (n = 2151). [#]Linear regression was adjusted for age, sex, fat-free mass, fat mass, pre-existing T2DM, hypertension, smoking status, alcohol consumption and use of lipid-lowering medications

adults with prediabetes or T2DM found that, after multiple adjustment for potential confounders, VO_{2peak} was inversely associated only with serum AST levels, but not with ALT, GGT levels and LFC determined by computed tomography.

A recent review[34] of 30 randomized controlled trials studying different levels of physical activity showed that, overall, exercise was associated with significant decreases in imaging-detected LFC and serum liver enzyme levels. The authors also found a significant association between physical inactivity and greater severity of fatty liver disease, independent of body weight.[34] Collectively, the results

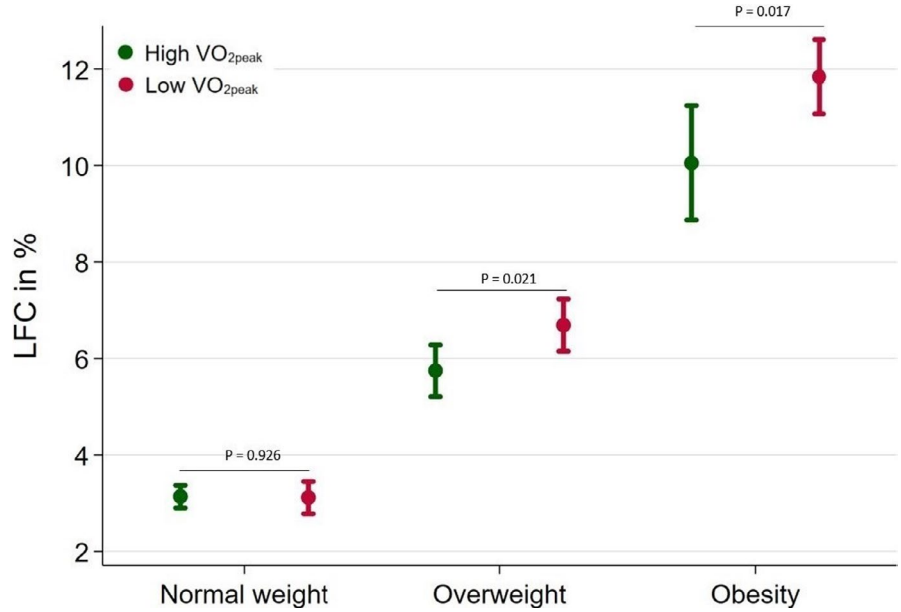

FIGURE 4 Adjusted[#] mean (95% CI) of the association of high and low peak oxygen uptake (VO_{2peak}) defined by the fat-free mass-specific median* with liver fat content (LFC), stratified by body mass index groups (normal-weight, overweight or obesity). [#]Linear regression adjusted for age, sex, pre-existing T2DM, hypertension, smoking status, alcohol consumption and use of lipid-lowering medications. *The high and low fat-free mass-specific median* of peak oxygen uptake (VO_{2peak}) were calculated as a VO_{2peak} cut-off for each specific level of fat-free mass. Central Figure: Ageing-related decline of fitness and its effects on liver. Adapted from Willis et al., New Engl J Med (2013)[43]

of this review are in line with our findings using MRI-PDFF and VO_{2peak} measurement.

4.2 | Potential mechanisms for the observed associations

Moderate-to-vigorous physical activity is directly associated with favourable cardiovascular health through various mechanisms, such as reductions in body weight and blood pressure and favourable influences on plasma lipid profile.[35] In contrast, sedentary activities and lifestyles (characterized by levels of physical activity below the recommended guidelines[36] to promote health benefits) are associated with more atherogenic risk profiles and adverse CVD outcomes.[7] This has already been reported in the early 1950s by Morris and colleagues,[37] who showed that the incidence of coronary heart disease in both bus drivers and postal office workers was higher than that in more active personnel, like bus conductors and postal carriers. On the other hand, at present, the underlying mechanisms that might explain the association between sedentary lifestyles and greater LFC or HS are poorly known. A current misconception is that the mechanisms involved with a sedentary behaviour are just the exact inverse of the ones related to physical exercise. Actually, previous studies showed that the molecular effects of a sedentary lifestyle are the result of down- and up-regulated pathways that are quite different from the ones related to exercise.[38,39]

Low muscle activity may have a direct effect on the normal body physiology, contributing to obesity and coexisting insulin resistance, dysglycaemia and atherogenic dyslipidaemia,[38] all of which will have a deleterious influence on LFC. On the other hand, our statistical regression models considered the influences of fat-free mass, fat mass and plasma glucose levels suggesting a possible direct effect of low levels of physical activity on HS.

An experimental study[40] using Otsuka Long-Evans Tokushima Fatty (OLETF) rats showed that after an abrupt beginning of physical inactivity for 173 hours (which is equivalent to an interruption of daily running for around 7 days) these rats exhibited decreased hepatic mitochondrial oxidative capacity, increased hepatic expression of *de novo* lipogenesis proteins, and increased hepatic malonyl-CoA levels; all of them molecular mechanisms that might contribute to the development of HS. Another experimental study[41] with OLETF rats reported several peripheral and hepatic changes associated with the development and progression of HS after 4 weeks of physical inactivity in these rats. Furthermore, these rats developed increased hepatic fat accumulation and had elevated serum free fatty acids, glucose, leptin and insulin levels. However, there was a decrease in hepatic mitochondrial function and fatty acid oxidation capacity, which are two conditions that might also contribute to the progression of the liver disease.

Finally, it is possible to hypothesize that the increase in serum GGT levels we observed in our study might be the result of the increased oxidative stress that results from the progression of HS. This increase in serum GGT occurs as a metabolic reaction to the rise in de novo production of intracellular reduced glutathione that is a major anti-oxidant molecule in hepatocytes.[32] A previous elegant study[42] found that higher serum GGT levels were a predictor of greater insulin resistance, irrespective of the aetiology of HS, such as NAFLD, familial heterozygous hypobetalipoproteinaemia or hepatitis C virus infection. This latter finding suggests that among subjects

with HS, particularly those with elevated GGT levels are more insulin resistant and more likely to develop T2DM over time.

4.3 | Study limitations

An important limitation of our study is its cross-sectional design, which does not permit to establish temporality and causality; we intend to conduct longitudinal analyses in the near future. Also, our study sample incorporates only Caucasian individuals from a very specific population in Northeast Germany; therefore, it may not be representative of other ethnicities. We only had self-reported information on whether individuals did sports more than two hours a week or not. A more precise information on physical activity level might have helped us to adjust more accurately for this potential confounding factor. For defining the dichotomous VO_{2peak} variable, we took fat-free mass-specific median values of VO_{2peak} to account for differences in fitness levels. Although important confounding factors were included in our multivariable regression models, unmeasured (or unknown) remaining confounders cannot be precluded. Finally, we did not perform liver biopsy that is the reference method for diagnosing and staging HS. However, this invasive diagnostic method would not be ethically feasible in persons of the general population with fairly normal serum liver enzyme levels.

The major strengths of our study include the large number of individuals (n = 2151) with a wide age range (21-90 years), the standardized assessment of VO_{2peak} by cardiopulmonary exercise testing. In addition, we used MRI-PDFF for the assessment of LFC, which is considered the most accurate imaging technique to non-invasively diagnose and quantify HS, with a sensitivity of 96.6% and a specificity of 74.8%.[31]

5 | CONCLUSIONS

In our population-based study of German adults, we found a strong association between lower VO_{2peak} and higher MRI-measured LFC, especially in obese and overweight individuals. These results may reflect the adverse impact of sedentary lifestyles on the liver.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All study participants provided written informed consent. The study was approved by the ethics committee of the University of Greifswald and complies with the Declaration of Helsinki.

CONSENT FOR PUBLICATION

All study participants gave written informed consent to participate in this study, and having their results published as part of this study.

ANIMAL RESEARCH (ETHICS)

Not applicable.

PLANT REPRODUCIBILITY

Not applicable.

CLINICAL TRIALS REGISTRATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analysed during the current study are not publicly available due to data protection aspects but are available in an anonymized form from the corresponding author on reasonable request.

COMPETING INTERESTS

All other authors have no conflicts of interest to disclose related to this manuscript.

DISCLOSURES

None.

AUTHORS' CONTRIBUTIONS

T.I. analyzed the data and is the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. I.Z. wrote the manuscript. M.R.P.M. have contributed to conception and design of the manuscript. T.I, S.S., S.G., H.A., S.K., R.E., R.B., J.P.K., M.M.L., H.V., S.B.F., M.B., G.T., M.D. and M.R.P.M. have contributed with substantial interpretation of the data and have critically revised the manuscript for important intellectual content. All authors gave final approval for the manuscript.

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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 may be found in the online version of the article at the publisher's website.

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