Factors independently associated with cardiorespiratory fitness in patients with non-alcoholic fatty liver disease

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Abstract
Low cardiorespiratory fitness (CRF) is associated with non-alcoholic fatty liver disease (NAFLD) and low CRF is an important risk factor for cardiovascular disease. The factors that influence CRF in NAFLD are poorly understood and it has been suggested that reduced hepatic mitochondrial function (HMF) may be linked to low CRF. Therefore, our aim was to determine the factors associated with CRF in NAFLD.

Methods: Ninety-seven patients with NAFLD were studied. CRF was assessed by treadmill testing and expressed as maximal O₂ consumption (VO₂ peak) per lean body mass. HMF was assessed by the ¹³C-ketoisocaproate breath test. Multivariable linear regression modelling was undertaken to test the independence of associations with CRF.

Results: Mean (SD) age was 51 (13) years and 61% were men. With CRF as the outcome, age (B coefficient −0.3, 95%CI −0.4, −0.2, P < .0001), total body fat mass (B coefficient −0.2, 95%CI −0.3, −0.05, P = .01), type 2 diabetes mellitus (T2DM) (B coefficient −3.6, 95%CI −1.1, −6.1, P = .005), smoking status (B coefficient −5.7, 95%CI −1.9, −9.5, P = .004), serum γ-glutamyl transferase (GGT) (B coefficient −0.04, 95%CI −0.05, −0.02, P < .0001), HMF (B coefficient −0.5, 95%CI −0.8, −0.1, P = .01) and diastolic function (B coefficient 0.1, 95%CI 0.05, 0.13, P < .0001) were independently associated with CRF. This model explained 60% of the total variance in CRF (R² = 0.6, P < .0001); and this model with GGT alone explained 24% of the variance in CRF.

Conclusions: In patients with NAFLD, HMF is independently associated with CRF and a model with GGT alone explained most of the variance in CRF.

KEYWORDS
cardiorespiratory fitness, cardiovascular disease, hepatic mitochondrial function, non-alcoholic fatty liver disease, serum γ-glutamyl transferase, type 2 diabetes

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a wide spectrum of liver disease from simple steatosis, to steatohepatitis (both with and without liver fibrosis), cirrhosis and end-stage liver failure that occurs in the absence of significant alcohol consumption. Epidemiological studies have shown that patients with NAFLD often have low cardiorespiratory fitness (CRF),² which is a very important cardiovascular risk factor.⁴ Since patients with NAFLD die as frequently from CVD events as from end-stage liver disease,⁵⁻⁸ it is important to understand the factors that contribute to low CRF because low levels of fitness might explain (at least in part) the increased risk of CVD in such
patients. It has been suggested that reduced hepatic mitochondrial function may be linked to low CRF as hepatic mitochondrial dysfunction is an important feature of NAFLD. However, there are no studies to date that have examined the relationship between CRF and hepatic mitochondrial function in patients with NAFLD. Furthermore, it is important to elucidate the key factors that contribute to CRF in NAFLD, since a better understanding of these factors would provide a better insight into the potential for improving CRF, to reduce CVD risk.

The aim of this study was to determine the factors independently associated with CRF in patients with NAFLD and to determine the proportion of the variance in CRF that could be explained by these factors. We tested the hypothesis that reduced hepatic mitochondrial function is associated with low CRF in patients with NAFLD while adjusting for potential confounders such as age, sex, increased adiposity and physical inactivity.

2 | MATERIALS AND METHODS

Ninety-seven patients with NAFLD were studied; they were recruited as part of the INSYTE trial (Investigation of SYmbiotic Treatment in NAFLD) (www.clinicaltrials.gov registration number NCT01680640) that is described in detail elsewhere. This trial was approved by the Southampton and South West Hampshire local research ethics committee (12/SC/0614).

2.1 | Inclusion and exclusion criteria for recruitment of patients with NAFLD

The inclusion and exclusion for participation in the INSYTE trial have been described in detail in the protocol paper.

2.2 | Assessment of liver fat percentage by Magnetic Resonance Spectroscopy (MRS)

Patients with confirmed NAFLD underwent MRS of the liver to measure the quantity of liver fat accumulated in three discrete liver zones. Three $20 \times 20 \times 20$ mm$^3$ spectroscopic volumes of interest (VOI) were positioned within segments 3 (inferior sub-segment of the lateral segment), 5 (inferior sub-segment of the anterior segment) and 8 (superior sub-segment of the anterior segment) of the liver, avoiding major blood vessels, intrahepatic bile ducts, and the lateral margins of the liver. The values for the lipid and water peak integrals were produced for each VOI and recorded for each subject. Liver fat percentage was estimated as the mean value of the liver fat percentages in the three liver regions.

2.3 | Assessment of cardiorespiratory fitness

Cardiorespiratory fitness (CRF) was measured in terms of maximal oxygen uptake ($\text{VO}_2$ peak) and determined from breath-by-breath analysis of $\text{O}_2$ consumption and $\text{CO}_2$ production using a Cortex metalyser 3B instrument (Cortex Biophysik, Germany) during maximal treadmill exercise (Woodway P55 treadmill) with 12-lead ECG monitoring throughout the test. Participants were advised to avoid strenuous exercise for 24 hours and alcohol on the day prior to testing. The treadmill exercise was undertaken in the morning and beforehand participants were asked not to take any medications such as beta-blockers for the duration of the study activity and had a standardized breakfast without tea or coffee. Participants were fitted with an air-tight facemask, which allowed the analysis of expired air. To allow participants to become acclimated to the facemask and to determine resting energy expenditure, resting measurements were taken for 3 minutes prior to commencement of activity-induced measurements. Participants were encouraged to continue until the respiratory exchange ratio was $>1.1$ and they reached at least 90% of their predicted maximum heart rate (as determined by 220-age in a previously study) unless they experienced chest pain or felt unwell. CRF was then measured by peak VO$_2$ adjusted for lean body mass.

We chose to express CRF relative to lean body mass instead of total body weight as we wanted to assess the relationship between CRF and other metabolic parameters of NAFLD independently of adiposity. Furthermore, recent studies have suggested that the expression of CRF relative to total body weight can introduce confounding by body adiposity when assessing the relationship between CRF and other metabolic parameters of NAFLD. We undertook an ECG before and after the treadmill test.

2.4 | Assessment of physical activity

Physical activity was assessed using an activity monitor (Sense Wear Pro2 Armband, Bodymedia Inc, Pittsburgh, PA, USA). The SenseWear Pro2 armband is a compact and lightweight 82 g device.
worn around the upper arm that is well tolerated and contains accelerometers that sense movement in two planes, a galvanic skin sensor, a temperature sensor, and a near-patient temperature sensor. The SenseWear Pro2 armband allows reliable measurement of physical activity levels and subjects were asked to monitor for 7-10 days to gain a reliable estimate of mean metabolic equivalents of tasks (METS) and time spent in physical activity, lying down and sleeping for a 24-h period while the device was worn. All subjects were instructed that the armband was to be worn at all possible times and to remove the armband only for bathing/showering purposes or any water-based activity. The inclusion criterion for accepting a subject’s armband data was ≥80% wear time. Data collected from the armband included: time spent in physical activity, lying down and sleeping at sedentary levels (<1.5 METS) using the software developed by the manufacturer (Innerview Professional Research Software V.5.1, BodyMedia).

### 2.5 | Assessment of body fat and lean body mass quantitation

Dual-energy X-ray absorptiometry (DEXA) using a Delfia W 4500 instrument (Hologic, Bedford, MA; coefficient of variation = 0.68%) was used to measure lean body mass (DEXA lean body mass) and total fat mass (DEXA total fat mass).

### 2.6 | Assessment of pulse wave analysis-derived measures of wave reflection and diastolic function

Participants underwent an overnight fast were asked not to take any alcohol during the fasted period. Furthermore, participants were asked not to take any medications that may affect the results of pulse wave analysis (PWA) for the duration of the study activity. The PWA was undertaken by a single observer by radial artery application tonometry (SphygmoCor, version 7, Atcor, Sydney, Australia) to obtain measures of wave reflection [augmentation index at 75 beats/min heart rate (AIx@HR75)], which is a measure of arterial stiffness and peripheral arteriolar resistance, and measures of diastolic function/myocardial perfusion (subendocardial viability ratio—SEVR%). Waveforms were processed using specialized software to calculate an averaged radial artery waveform and to derive a corresponding central aortic pressure waveform using a previously validated generalized transfer function. PWA has been validated as a non-invasive technique for estimating diastolic cardiac function, and myocardial perfusion relative to the left ventricular workload can be estimated by the SEVR. As the invasive nature of cardiac catheterization restricts its use in research on healthy volunteers, arterial tonometry has gained popularity in research studies. The wave format at the radial artery is easily and reproducibly measured at the wrist, and this non-invasive technique allows the estimation of diastolic function (SEVR%) and wave reflection [augmentation index at 75 beats/min (AIx@HR75)] as a proxy for arterial stiffness.

Although SEVR% does not take into account the left ventricular end-diastolic pressure, SEVR% is a good estimate of the subendocardial viability index (derived from cardiac catheterization) in individuals without evidence of ischaemic heart disease in whom the left ventricular end-diastolic pressure is normal. SEVR% is not related to aortic pulse pressure but, rather, solely to the diastolic time: systolic time in middle-aged individuals and is, therefore, a measure of diastolic function.

### 2.7 | Assessment of liver fibrosis by transient elastography

The presence of liver fibrosis was ascertained by use of transient elastography (FibroScan, Echosens, Paris, France). The liver stiffness measure (kPa) was assessed as a proxy measure of liver fibrosis. Details of the technical description and examination procedure have been described previously. Results are expressed as the median value in kilopascals (kPa).

### 2.8 | Anthropometric and biochemical measurements

Body mass index (BMI), hip and waist circumferences were recorded. Haemoglobin A1c (HbA1c), total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides (TAG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were measured in fasting serum using established procedures.

### 2.9 | Assessment of hepatic mitochondrial function by 13C-ketoisocaproate breath test

The 13C-ketoisocaproate breath test (13C-KICA BT) was used as a validated test of hepatic mitochondrial function. Subjects refrained from alcohol and had fasted overnight for at least 12 hours prior to each test. All subjects were at rest for the duration of the study and remained fasted throughout. On the morning of the study, to standardize CO2 production, subjects were asked to lie down on a bed and carbon dioxide produced (VCO2) at rest was measured by indirect calorimetry (GEM Nutrition, UK) for 25 minutes prior to the start of the 13C-KICA BT. Paired breath samples for baseline measurement of isotopic abundance were collected from each subject exhaling directly into 12-ml Exetainer breath tubes (LABCO Ltd, High Wycombe, UK) via straws. Each subject then ingested a solution containing 1 mg/kg body weight of 2-keto-[13C]-isocaproic acid (99% 13C; Cambridge Isotopes, USA) along with 20 mg/kg body weight of L-leucine (Sigma) in 200 ml of water. Further paired breath samples were then collected every 10 min for 60 min. The enrichment of 13C (atom % excess) in CO2 in breath at time (t) was calculated from the 13C
abundance measurements by Continuous Flow Isotope Ratio Mass Spectrometry (CF-IRMS, ABCA System, SERCON, Crewe, UK) and used with a measure of the total CO₂ production to calculate the cumulative per cent ¹³C-dose recovered over 1hr (cPDR over 1hr). The cPDR over 1 hour has been previously validated as a measure of hepatic mitochondrial function. ²⁸

2.10 Statistical analysis

Data were tested for normality using the Kolmogorov-Smirnov test. Data are presented as means ± SDs for normally distributed data and medians and interquartile ranges (IQRs) for non-normally distributed data. Data were analysed using Statistical Package for the Social Sciences (SPSS) Version 26 (IBM, New York, USA). Comparison of continuous variables between groups was performed with Mann-Whitney U-tests for non-normally distributed data or Student’s t-tests for normally distributed data, and differences in proportions were analysed using the Chi-squared test. Univariate associations between variables were investigated using Spearman’s rank correlation for non-normally distributed data or Pearson’s correlation for normally distributed data whereby correlation coefficients are indicated by r. Based on a previous study using a rule of thumb for interpreting the strength of correlation coefficients, values of correlation coefficients between 0 and 0.3 (0 and −0.3) indicate weak, 0.3 and 0.7 (−0.3 and −0.7) indicate moderate, values between 0.7 and 1.0 (−0.7 and 1.0) indicate strong positive (negative) associations. ²⁹ Pearson’s partial correlation coefficients between variables were calculated with adjustment for potential confounding factors in order to adjust for the effects of these variables.

To test for the independence of associations between explanatory factors and VO₂ peak relative to lean body mass, factors were entered into a multiple linear regression model with VO₂ peak relative to lean body mass as the outcome variable. Unstandardized B coefficients represent the amount of change in the dependent variable because of a change of 1 unit in the independent variable. Models were run with all the explanatory factors entered simultaneously and also stepwise, in order to investigate the proportion of the variance in VO₂ peak relative to lean body mass that could be explained by each of the independent factors in the model. The independent variables entered into final regression model were age, sex, DEXA total body fat mass, type 2 diabetes status, smoking status, GGT, hepatic mitochondrial function, SEVR% and Alx@HR75. The same model was then re-run stepwise to assess the contribution of each variable to the overall variance in VO₂ peak and five predictive models were generated: model 1 (GGT alone), model 2 (GGT and SEVR), model 3 (GGT, SEVR and age), model 4 (GGT, SEVR, age and smoking status) and model 5 (GGT, SEVR, age and smoking status type 2 diabetes status). A P-value < .05 was considered statistically significant. Data are expressed as mean ± SD unless otherwise stated.

3 RESULTS

3.1 Characteristics of the study participants

Table 1 shows the baseline characteristics of patients with NAFLD stratified by sex. There were significant differences between men and women for age, body weight, BMI, lean body mass, total fat mass, ALP, HDL cholesterol, augmentation index (Alx@HR75), diastolic function (SEVR), duration of physical activity and VO₂ peak relative to lean body mass.

Patients were then stratified by tertile of VO₂ peak relative to lean body mass (Table 2). Age, DEXA total body fat mass, HbA1c, hepatic mitochondrial function, Alx@HR75 and SEVR all showed significant differences between tertiles (Table 2). There were no significant differences in smoking status between the tertiles or differences in VO₂ peak relative to lean body mass between smokers and non-smokers (29.8 ± 6.0 ml/kg/min vs 32.7 ± 8.1 ml/kg/min, P = .2). The relationship between VO₂ peak relative to lean body mass and GGT was further examined and, although no significant difference in GGT was observed between tertiles, a significant linear trend was observed across tertiles (P = .01).

Univariate associations between VO₂ peak relative to lean body mass and anthropometric and metabolic parameters and measures of cardiovascular function in patients with NAFLD are shown in Table 3. VO₂ peak relative to lean body mass showed a significant moderate positive association with log GGT and Alx@75 along with a significant moderate positive association with SEVR. Furthermore, VO₂ peak relative to lean body mass also showed a significant moderate negative association with age and HbA1c and a significant weak positive association with hepatic mitochondrial function and the duration of physical activity. Figure 1 shows the significant association between VO₂ peak relative to lean body mass and GGT.

Partial correlation coefficients were analysed to further assess the relationship between VO₂ peak relative to lean body mass and hepatic mitochondrial function while controlling for age and DEXA total body fat mass. After controlling for age and DEXA total body fat mass, there was a non-significant partial correlation between VO₂ peak relative to lean body mass and hepatic mitochondrial function (Pearson correlation coefficient r = -.1; P = .5) and (Pearson correlation coefficient r = .12; P = .3) respectively.

Multiple linear regression modelling was undertaken to test the independence of associations between significant factors selected from univariate associations and the VO₂ peak relative to lean body mass as the outcome (Table 4). Smoking status was also included in this model as there was a trend towards higher VO₂ peak relative to lean body mass in non-smokers (32.7 ± 8.1 ml/kg/min) compared with smokers 29.8 ± 6.0 ml/kg/min (P = .2). The regression model was then re-run stepwise (Table 5) to estimate how much of the variance in VO₂ peak relative to lean body mass could be explained by each of the factors in the model.

The key explanatory factors in the model were age, sex, DEXA total body fat mass, T2DM, smoking status, GGT, hepatic mitochondrial function, SEVR% and Alx@HR75. The same model was then re-run stepwise (Table 5) to estimate how much of the variance in VO₂ peak relative to lean body mass could be explained by each of the factors in the model.
function, SEVR and Alx@HR75. Of these factors, only age (unstandardized B coefficient = −0.3; \( P < .0001 \)), DEXA total body fat mass (unstandardized B coefficient = −0.2; \( P = .01 \)), T2DM (unstandardized B coefficient = −3.6; \( P = .005 \)), smoking status (unstandardized B coefficient = −5.7; \( P = .004 \)), GGT (unstandardized B coefficient = −0.04; \( P < .0001 \)), hepatic mitochondrial function (unstandardized B coefficient = −0.5; \( P = .01 \)) and SEVR (unstandardized B coefficient = 0.1; \( P < .0001 \)) were independently associated with VO\(_2\) peak relative to lean body mass. The full predictive model of VO\(_2\) peak relative to lean body mass containing the explanatory variables was statistically significant (model fit \( R^2 = 0.60, P < .0001 \)) and identified 60% of the total variance in VO\(_2\) peak relative to lean body mass.

In the stepwise multiple linear regression analysis (Table 5), GGT alone (model 1) explained 24% of the variance in VO\(_2\) peak relative to lean body mass. The addition of SEVR to the model (model 2) led to a statistically significant increase in \( R^2 \) of 0.13, \( P < .0001 \). Addition of age (model 3) led to a further increase in \( R^2 \) of 0.11, \( P < .0001 \); addition of smoking status (model 4) led to a further increase in \( R^2 \) of 0.052, \( P = .002 \) and T2DM (model 5) a further increase in \( R^2 \) of 0.023, \( P = .03 \). Thus adding each of these factors in turn led to a statistically significant increase in \( R^2 \); and models 2, 3, 4 and 5 explained a further variance of 13%, 11%, 5% and 2.3%, compared to the 24% of the variance explained by GGT alone (as shown in model 1) (Table 5).

### TABLE 1
Baseline characteristics of patients with NAFLD

| Variables                               | Men (n = 59) | Women (n = 38) | \( P \)-value |
|-----------------------------------------|-------------|---------------|--------------|
| Age (yr)                                | 47.7 ± 12.9 | 55.0 ± 12     | .005         |
| Body weight (kg)                        | 102.5 ± 16  | 93.5 ± 16.5   | .01          |
| BMI (kg/m\(^2\))                       | 32.2 ± 4.5  | 35.0 ± 5.5    | .01          |
| DEXA lean body mass (kg)                | 70 ± 9.3    | 54 ± 9.0      | <.0001       |
| DEXA total body fat mass (kg)           | 32.0 ± 8.4  | 39.5 ± 9.3    | <.0001       |
| HbA1c (mmol/mol)                        | 40.0(24.2)  | 39.0(21.2)    | .1           |
| Type 2 diabetes\(^a\) (Yes) (n, %)      | 18.0(30.5%) | 18.0(47.4%)   | .1           |
| Smoking status\(^a\) (No) (n, %)        | 55.0(93.2%) | 30.0(81.1%)   | .1           |
| Beta-blockers use\(^a\) (Yes) (n, %)    | 3.0(5.1%)   | 3.0(7.9%)     | .6           |
| Antihypertensive drug use (Yes) (n, %)  | 23.0(30.9%) | 19.0(50.0%)   | .3           |
| Metformin\(^a\) (Yes) (n, %)            | 17.0(29.0%) | 11.0(30.0%)   | 1.0          |
| Statins\(^a\) (Yes) (n, %)              | 14.0(23.7%) | 13.0(34.2%)   | .3           |
| AST (lu/L)                              | 35.5(19.7)  | 37.5(18.0)    | .4           |
| ALT (lu/L)                              | 57.0(33.5)  | 49.0(40.0)    | .1           |
| ALP (lu/L)                              | 73.0(23.5)  | 95.0(40.5)    | .0004        |
| GGT (U/L)                               | 49.0(51.5)  | 50.0(78.0)    | .9           |
| TAG (mmol/L)                            | 2.0 ± 0.9   | 2.6 ± 4.1     | .2           |
| Cholesterol (mmol/L)                    |              |               |              |
| Total (mmol/L)                          | 4.8 ± 1.1   | 5.2 ± 1.3     | .1           |
| LDL (mmol/L)                            | 2.6 ± 0.9   | 3.0 ± 1.0     | .1           |
| HDL (mmol/L)                            | 1.1 ± 0.3   | 1.3 ± 0.2     | .01          |
| Liver stiffness (kPa)                   | 6.4 (5.0)   | 7.9 (4.0)     | .1           |
| Hepatic mitochondrial function (%)      | 14.0 ± 4.0  | 12.7 ± 3.0    | .1           |
| Magnetic resonance spectroscopy (MRS) liver fat (%) | 25.6(32.0) | 26.0(42.0)    | .3           |
| Augmentation index at 75 mins (Alx@HR75) (%) | 19.0(11.0) | 24.5(10.0)    | <.0001       |
| Subendocardial viability ratio (SEVR) (%) | 163.5(45.0) | 133.5(30.0) | .02          |
| Physical activity duration (hrday\(^{-1}\)) | 1.2(1.3)  | 0.7(0.8)     | .001         |
| Lying duration (hrday\(^{-1}\))        | 8.4(1.7)   | 8.8(2.9)     | .5           |
| Sleep duration (hrday\(^{-1}\))        | 6.6(1.4)   | 6.8(1.9)     | .1           |
| Predicted maximal heart rate (%)        | 77.5 ± 11.4 | 79.5 ± 14.1  | .6           |
| VO\(_2\)peak (ml/min/kg lean body mass) | 34.4 ± 7.3  | 29.0 ± 7.6    | .001         |

Note: Mean ± SD or Median (inter-quartile range).
\(^a\)Cross-tab Pearson chi-squared.
As duration of physical activity was also significantly associated with VO₂peak relative to lean body mass (Table 3), we also added duration of physical activity to the final regression model as shown in Table 4. The addition of duration of physical activity and the use of other medications such as statins, beta-blockers, antihypertensive drugs and metformin did not affect the associations between VO₂peak relative to lean body mass and the independent factors in the final regression model.

### DISCUSSION

The novel findings of our study are that there are independent associations between CRF and GGT, diastolic function, age, total body fat mass, smoking status, type 2 diabetes mellitus (T2DM) and hepatic mitochondrial function; but there was not an independent association between CRF and a measure of liver fibrosis as a marker of severity of NAFLD. Our study also shows that there is an independent

| Variables | Maximal oxygen uptake (VO₂peak) (ml/min/kg lean body mass) | P-value | Differences between the groups | Linear Trend |
|-----------|-----------------------------------------------------------|---------|-------------------------------|-------------|
| Low tertile (5.6-30.0) (n = 32) | Middle tertile (30.0-35.5) (n = 32) | High tertile (35.5-54.5) (n = 33) | | |
| Age (yr) | 56.2 ± 12.4 | 51.6 ± 12.5 | 44.1 ± 11.3 | .0005 | .0001 |
| Female/male (n) | 18/14 | 12/20 | 8/25 | .03 | N/A |
| Body weight (kg) | 97.0 ± 16.4 | 102.8 ± 16.5 | 97.2 ± 16.5 | .3 | .1 |
| BMI (kg/m²) | 33.8 ± 5.1 | 34.4 ± 3.3 | 31.7 ± 5.1 | .1 | .1 |
| DEXA lean mass (kg) | 61.0 ± 12 | 66.1 ± 12 | 64.1 ± 12 | .2 | .3 |
| DEXA total body fat mass (kg) | 36.0 ± 10.3 | 36.5 ± 9.6 | 32.5 ± 8.2 | .2 | .1 |
| HbA1c (mmol/mol) | 52.5(35) | 41.0(17.5) | 36.0(9.0) | .001 | <.0001 |
| Type 2 diabetes (Yes) (n,%) | 20(62.5%) | 10(31.3%) | 6(18.2%) | .001 | N/A |
| Smoking status (No) (n,%) | 26(84%) | 29(91%) | 30(91%) | .6 | N/A |
| AST (lu/L) | 42.5(38) | 35.0(23.5) | 33.5(25) | .8 | .2 |
| ALT (lu/L) | 61.0(49) | 51.0(44.5) | 59.5(39) | .8 | .5 |
| ALP (lu/L) | 85.5(71.0) | 93.0(33.0) | 76.0(26.5) | .1 | .01 |
| GGT (U/L) | 62.5(78.5) | 58.0(54.0) | 51.0(34.0) | .4 | .01 |
| TAG (mmol/L) | 2.9 ± 4.5 | 1.9 ± 0.6 | 1.7 ± 0.8 | .1 | .1 |
| Cholesterol (mmol/L) | | | | | |
| Total (mmol/L) | 5.1 ± 1.5 | 4.8 ± 1.1 | 5.0 ± 1.0 | .7 | .8 |
| LDL (mmol/L) | 2.6 ± 1.0 | 2.8 ± 1.0 | 2.9 ± 1.0 | .7 | .5 |
| HDL (mmol/L) | 1.3 ± 0.3 | 1.2 ± 0.2 | 1.2 ± 0.2 | .3 | .3 |
| Liver stiffness (kPa) | 6.9(4.7) | 7.0(5.3) | 5.8(2.2) | .1 | .05 |
| Hepatic mitochondrial function (%) | 13.0 ± 3.3 | 12.7 ± 3.6 | 14.8 ± 3.7 | .05 | .05 |
| Magnetic resonance spectroscopy (MRS) liver fat % | 18.5(29) | 26.0(25) | 32.0(38) | .4 | .5 |
| AxoHR75 (%) | 23.5(8.0) | 19.0(15.0) | 18.0(16.0) | .02 | .02 |
| SEVR (%) | 135.5(44.0) | 148.0(34.0) | 157.0(36.0) | .01 | .001 |
| Physical activity duration (hr/day) | 0.8(0.9) | 0.9(1.1) | 1.0(0.9) | .3 | .1 |
| Lying duration (hr/day) | 8.1(2.0) | 8.6(3.6) | 8.4(1.8) | .9 | .8 |
| Sleep duration (hr/day) | 6.7(1.5) | 6.6(2.8) | 6.7(0.9) | .7 | .5 |

Note: Cardiorespiratory fitness maximal oxygen uptake (VO₂peak) relative to lean body mass. Mean ± SD or Median (inter-quartile range); N/A - Not applicable.

| Cross-tab Pearson chi-squared; Significant differences between variables and across the groups were determined by either the one-way ANOVA or Kruskal-Wallis tests.
| Significant differences between low vs middle tertile.
| Significant differences between low vs high tertile.
| Significant differences between middle vs high tertile.

TABLE 2 Anthropometric and biochemical characteristics of patients with NAFLD stratified by tertiles of cardiorespiratory fitness (VO₂peak relative to lean body mass)
but weak negative association between hepatic mitochondrial function and CRF. To the best of our knowledge, this is the first study that has shown such an association between CRF and hepatic mitochondrial function in patients with NAFLD.

In the current study, we also showed for the first time that GGT was inversely associated with CRF and GGT alone explained most of the variance (ie 24%) in CRF (as shown in Table 5). These findings support observations from recent epidemiological studies showing that lower CRF is associated with elevated GGT.\(^3\,\text{30}\) That said, the biological mechanisms involved in the relationship between CRF, GGT and NAFLD are unclear. One possible mechanism that could explain the relationship between GGT and CRF is the key role that oxidative stress plays during the progression of NAFLD. Studies have shown that GGT is elevated during oxidative stress\(^3\,\text{1-33}\) and it has been suggested that the increase in serum GGT may be a metabolic adaptive response to increase the de-novo synthesis of intracellular reduced glutathione (GSH) which is major anti-oxidant in skeletal muscle cells and hepatocytes.\(^3\,\text{4-36}\) Our findings suggest that low CRF in patients with NAFLD could lead to an increase in oxidative stress and therefore an increase in GGT activity may be a metabolic adaptive response to the resulting oxidative stress.

Univariate correlation analysis showed that there was a weak but significant positive association between CRF and hepatic mitochondrial function. However, as many previous studies have suggested that CRF and hepatic mitochondrial function may be confounded by age and adiposity,\(^3\,\text{28,37}\) we therefore performed a partial correlation

### TABLE 3

Univariate associations between cardiorespiratory fitness (VO\(_{\text{peak}}\) relative to lean body) and measures of adiposity and cardio-metabolic markers in patients with NAFLD

| Parameters                      | VO\(_{\text{peak}}\) (ml/min/kg lean body mass) | Correlation coefficient \((r)\) | \(P\)-value |
|---------------------------------|-----------------------------------------------|---------------------------------|------------|
| Age (yr)                        | −0.41                                         | <.0001                          |            |
| DEXA total body fat mass (kg)   | −0.19                                         | .05                             |            |
| HbA1c (mmol/mol)                | −0.39                                         | <.0001                          |            |
| TAG (mmol/L)                    | −0.19                                         | .06                             |            |
| HDL-cholesterol (mmol/L)        | −0.07                                         | .47                             |            |
| AST (iu/L)                      | −0.09                                         | .39                             |            |
| ALT (iu/L)                      | 0.05                                          | .63                             |            |
| ALP (iu/L)                      | −0.19                                         | .07                             |            |
| log GGT (iu/L)\(^a\)            | −0.32                                         | .001                            |            |
| Liver stiffness (kPa)           | −0.19                                         | .07                             |            |
| Hepatic mitochondrial function (%) | 0.21                                         | .04                             |            |
| MRS liver fat (%)               | 0.09                                          | .34                             |            |
| Alx@HR75 (%)                    | −0.28                                         | .01                             |            |
| SEVR (%)                        | 0.39                                          | <.0001                          |            |
| Physical activity duration (hr/day) | 0.23                                         | .03                             |            |
| Lying duration (hr/day)         | 0.01                                          | .94                             |            |
| Sleep duration (hr/day)         | −0.03                                         | .75                             |            |

**Abbreviations:** ALP, alkaline phosphatase; ALT, alanine aminotransferase; Alx@HR75, augmentation index at 75mins; AST, aspartate aminotransferase; BMI, Body mass index; GGT, gamma glutaryl transferase. MRS, magnetic resonance spectroscopy; HbA1c, haemoglobin A1c; HDL, high density lipoprotein; METS, metabolic equivalents. \((r) = \) Spearman correlation coefficients; MRS, Magnetic resonance spectroscopy; PAEE, physical activity energy expenditure; SEVR, subendocardial viability ratio; TAG, triacylglycerides. \(^a(r) = \) Pearson correlation coefficient.

### FIGURE 1

The relationship between VO\(_{\text{peak}}\) relative to lean body mass and gamma-glutamyl transferase (GGT)

### TABLE 4

Associations between VO\(_{\text{peak}}\) relative to lean body mass and anthropometric, cardiovascular and metabolic markers in patients with NAFLD

| Independent variables | Unstandardised coefficient \(B\) | 95% CI          | \(P\)-value |
|-----------------------|----------------------------------|-----------------|------------|
| Age (years)           | −0.29                            | −0.40, −0.19    | <.0001     |
| Sex (F)               | −0.71                            | −2.07, 3.49     | .61        |
| DEXA total body fat mass (kg) | −0.18                          | −0.32, −0.05    | .01        |
| Type 2 diabetes       | −3.64                            | −1.14, −6.13    | .005       |
| Smoking status        | −5.68                            | −1.89, −9.48    | .004       |
| GGT (IU/L)            | −0.04                            | −0.05, −0.02    | <.0001     |
| Hepatic mitochondrial function (%) | −0.49                          | −0.84, −0.12    | .01        |
| SEVR (%)              | 0.09                             | 0.05, 0.13      | <.0001     |
| Alx@HR75 (%)          | 0.05                             | −0.07, 0.17     | .44        |

**Abbreviations:** Alx@HR75, augmentation index at 75 mins. \(R^2 = 0.60, P < .0001; F, female; GGT, gamma-glutamyltransferase; SE; SEVR, subendocardial viability ratio; standard error.**
analysis to further examine the relationship between CRF and hepatic mitochondrial function. Our results show that age and adiposity have potential confounding effects on the association between CRF and hepatic mitochondrial function. Furthermore, our study also showed that when the association between CRF and hepatic mitochondrial function was controlled for other potential confounding factors in regression analysis, such as type 2 diabetes, GGT and diastolic function, in addition to age and adiposity; there was an independent and significant albeit weak negative association between CRF and hepatic mitochondrial function. This observation is in contrast with the association suggested in previous studies.\textsuperscript{38,39} One possible explanation for our observation is that there is an increased metabolic adaptive response of the hepatic mitochondria, with increased mitochondrial function, in patients with NAFLD who have a low CRF. However, the precise underlying mechanisms involved in the association between CRF and hepatic mitochondrial function in patients with NAFLD need further investigation.

In our cohort only two patients had evidence of prior CVD and in both these patients their condition was stable. Our findings provide further evidence to highlight the role of diastolic function in CRF and importantly the association between CRF and diastolic function that we observed was not confounded by duration of physical activity at sedentary levels as observed in a previous study testing the relationship between SEVR and CRF in subjects with central obesity.\textsuperscript{40}

Overall, recent literature reviews have suggested that using exercise to increase CRF could be a therapeutic tool for NAFLD as well as T2DM.\textsuperscript{41,42} We suggest that the independent associations we observed between age, T2DM status, smoking status, GGT, hepatic mitochondrial function and diastolic function with CRF, could plausibly reflect their roles as cardiovascular risk factors in patients with NAFLD. From examining the unstandardized B coefficients in Table 4 for each of the explanatory factors independently associated with CRF; the presence of T2DM (~3.6 ml/min/kg lean body mass) and smoking status (~5.7 ml/min/kg lean body mass) are clinically meaningful, since a decrease in CRF of only 1 ml/min/kg has been shown to be associated with a significant 9% increase in all-cause mortality and cardiovascular mortality.\textsuperscript{43,44} As can be seen from the data in Tables 1 and 2, the IQRs for GGT levels in each group are large, reflecting the wide range of GGT concentrations that we observed in the INSYTE participants. From the data in Figure 1 (where GGT was log\textsubscript{10} transformed to show the linear relationship between log\textsubscript{10} GGT and CRF), there was a 1.5 log\textsubscript{10} difference between the minimum and maximum GGT values (16IUL to 529 IU/L) in participants in the cohort. Using the unstandardized B coefficient from Table 4 (~0.04), this range (513 IU/L) in GGT is therefore associated with a 513 x 0.04 = 20.5 ml/min/kg decrease in VO\textsubscript{2} peak. We reason that a decrease of 20.5 ml/min/kg VO\textsubscript{2} peak could also be highly clinically relevant.\textsuperscript{43,44}

The present study has some limitations that should be considered. We estimated diastolic function and arterial stiffness indirectly using a non-invasive device (SphymoCor), which has been used in a sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial in the Conduit Artery Function Evaluation study,\textsuperscript{45} and was approved by the US Food and Drug Administration in 2001. However, a potential weakness of this technology is that the calibration of central aortic pressures depends on the accuracy of the brachial pressure measurements.\textsuperscript{46} Other limitations of our study were that we used transient elastography instead of liver biopsy to characterize liver fibrosis and we did not have a healthy control group to compare measurements of CRF or other metabolic markers with those of patients with NAFLD. In addition, our relatively small study was undertaking in a predominantly white ethnic group and our results may not be applicable to other ethnic groups.

In conclusion, our study shows that HMF and GGT, together with diastolic function, age, total body fat mass, smoking status and T2DM were all independently associated with CRF. These factors together explained 60% of the total variance in CRF. Of these factors in the model, most of the variance was explained by GGT alone (24%); followed by the measure of diastolic function (SEVR), which explained a further 13%; age, which explained a further 11%; smoking status, which explained a further 5%; and type 2 diabetes, which explained a further 2% of the total variance in CRF.

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### Table 5 Stepwise multivariable linear regression models explaining variance in VO\textsubscript{2} peak relative to lean body mass

| Independent variables | R^2 change | P-value |
|-----------------------|------------|---------|
| Model 1  
GGT                  | 0.240      | 0.240   | <.0001 |
| Model 2  
GGT and SEVR.       | 0.366      | 0.126   | <.0001 |
| Model 3  
GGT, SEVR and Age.  | 0.477      | 0.111   | <.0001 |
| Model 4  
GGT, SEVR, Age and Smoking status. | 0.530 | 0.052 | .002 |
| Model 5  
GGT, SEVR, Age, Smoking status and Type 2 diabetes. | 0.553 | 0.023 | .03 |

Abbreviations: GGT, gamma-glutamyltransferase; SEVR, subendocardial viability ratio.
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CONFLICT OF INTEREST
None to declare.

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