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Non-alcoholic fatty liver disease: Prevalence and all-cause mortality according to sedentary behaviour and cardiorespiratory fitness. The HUNT Study☆

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ABSTRACT

Purpose: Sedentary behaviour (SB) and low physical activity (PA) are independently associated with non-alcoholic fatty liver disease (NAFLD). Compared to PA, high cardiorespiratory fitness (CRF) has been associated with a higher protection against all-cause mortality and a number of specific diseases. However, this relationship has not been investigated in NAFLD. This study examined the roles of SB and CRF on: i) the likelihood of having NAFLD in the general population, and ii) the risk of mortality over 9 years within individuals having NAFLD.
Methods: A cross-sectional analysis of 15,781 adults (52% female; age range 19–95 years) was conducted. Self-reported SB was divided into tertiles. CRF was estimated using validated non-exercise models, and the presence of NAFLD from the Fatty Liver Index. Adjusted Odds Ratios and 95% Confidence Intervals for NAFLD were estimated using logistic regression analyses. Hazard Ratios for all-cause mortality were estimated using Cox proportional hazard regression in individuals with NAFLD.
Results: For each additional 1 h/d of SB, the likelihood of having NAFLD was significantly increased by 4% (CI, 3–6%). In combined analyses, compared with the reference group [high CRF and low (≤4 h/d) SB], individuals with low CRF had a markedly higher likelihood of having NAFLD (OR, 16.9; CI 12.9–22.3), even if they had SB ≤ 4 h/d. High CRF attenuated the negative role of SB up to 7 h/d on NAFLD. Over 9.4 ± 1.3 years of follow-up, individuals with NAFLD and low CRF had the risk of mortality increased by 52% (CI, 10–106%) compared to those with high CRF, regardless of SB or meeting PA guidelines.
Conclusions: Low CRF increases the risk of premature death in individuals with NAFLD, and is strongly associated with higher likelihood of having NAFLD, outweighing the influence of SB.

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Abbreviations and acronyms: ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; CRF, cardiorespiratory fitness; GGT, gamma glutamyl transferase; HUNT3, Nord-Trøndelag Health Study (3rd wave); NAFLD, non-alcoholic fatty liver disease; MET, metabolic equivalent; PA, physical activity; SB, sedentary behavior; VO_{2peak}, peak oxygen consumption.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in industrialised countries, affecting up to 30% of the adult population; over 65% of obese individuals and the majority of patients with type 2 diabetes.[1] The term NAFLD is used to describe a wide range of liver damage ranging from simple steatosis to non-alcoholic steatohepatitis, fibrosis and cirrhosis, that occur in the absence of hazardous alcohol consumption. NAFLD is linked with obesity, visceral adiposity, lifestyle factors, insulin resistance, and genetic predisposition[2,3]; and contributes to the development of comorbid type 2 diabetes and cardiovascular disease. Indeed, cardiovascular disease is the leading cause of death in patients with NAFLD.[4]

While formal diagnosis of NAFLD requires evidence of hepatic steatosis on imaging or histology, individuals with high likelihood of having NAFLD can also be accurately identified using the Fatty Liver Index, an algorithm based on anthropometric data and standard laboratory measures.[5,6] The Fatty Liver Index, along with elevated serum alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) predict the incidence of type 2 diabetes[5,7–9] and cardiovascular disease risk.[10–14] Elevated GGT and NAFLD identified by the Fatty Liver Index are also associated with the risk of incident hypertension.[15]

Currently, there are no approved medications for the long-term management of NAFLD. Guidelines for the prevention and management of NAFLD focus heavily on diet and weight loss,[2,3] while they are vague on other lifestyle components such as physical activity (PA) (with no detail on target intensity), and omit other factors strongly associated with cardio-metabolic health such as sedentary behaviour (SB) and cardiorespiratory fitness (CRF).[16,17]

Time spent in SB is an independent risk factor for all-cause mortality,[18] and is a risk factor for cardiovascular disease,[19] independent of meeting PA guidelines[16] (i.e. 150 min/week of moderate intensity or 75 min/week of vigorous PA). Therefore, many countries have included generic, non-quantitative recommendations to reduce sedentary behavior in their public health guidelines.[20] SB also impacts on liver health, with recent data showing that SB is an independent predictor of NAFLD and elevated liver enzymes, independent of PA.[21,22] However, to date, much of the data on the associations between NAFLD, PA and SB at the population level comes from a Korean cohort, and therefore may not be generalisable to Caucasian populations.[22] Crucially, CRF, independent of PA, is a strong predictor of cardiovascular morbidity and mortality from all causes.[17,23–25] Recent studies have shown that high CRF attenuates the negative association between cardiovascular risk factors and SB, independent of physical inactivity.[19,26] However, whether high CRF has a similar protective effect on NAFLD, independent of SB and PA is not known. This is because the combined roles of SB and CRF on NAFLD at the population level have never been investigated. To date, only one smaller population study ($n = 463$) has explored the relationship between fatty liver and CRF.[27] It reported a negative association between NAFLD and CRF, however assessments of NAFLD and CRF were performed 3 years apart, and SB was not examined.

NAFLD, in addition to being associated with higher morbidity compared with the general population, is associated with increased all-cause and liver-related mortality.[28] In other populations, high SB,[16] low PA[29] and low CRF have all been shown to be associated with all-cause mortality.[23] Of these, the association between CRF and mortality appears to be the strongest, and to be independent of PA and SB.[23] In patients with NAFLD, the associations between SB, CRF and mortality are unknown.

The aims of our study were to examine the roles of SB and CRF on: i) the likelihood of having NAFLD in a large population-based cohort of adult men and women; ii) the risk of all-cause mortality over nine years within individuals having NAFLD. We hypothesized that, irrespective of SB and meeting PA guidelines, having a high age and sex-specific CRF is associated with i) lower likelihood of NAFLD in the general population, and ii) lower risk of mortality from all causes in individuals with NAFLD.

Materials and methods

Study population

The third wave of the Nord-Trøndelag Health Study (the HUNT3 study) in Norway was carried out between October 2006 and June 2008. A detailed account of the study has been previously described.[30] Briefly, all inhabitants of the Nord-Trøndelag county 20 year and older ($n = 94,194$) were invited, and 50,811 individuals (54%) accepted the invitation. Respondents filled in a questionnaire that was included in the invitation and later attended a clinical examination conducted by trained health professionals. All participants provided written informed consent before volunteering to participate. The present study was approved by the Regional Committee for Medical Research Ethics (2017/2055/REK midt). The flow of participants through the study is presented in Fig 1. Of the 50,811 participants, 28,662 were excluded due to GGT (which is necessary to calculate the Fatty Liver Index) not being measured, and 1949 due to alcohol consumption above the gender-specific thresholds defined by the European Association for the study of the Liver (≥30 g/day for males, ≥20 g/day for females),[2] and 3560 due to other reasons. There were no differences in participant's characteristics of individuals who had GGT assessed and those who did not. A total of 15,781 participants were included in the current study.

Clinical measurements and questionnaires

Trained health professionals conducted a clinical examination which consisted of standardized measurements of height, weight, blood pressure, and resting heart rate.[30,31] Hypertension was defined as ≥140/90 mmHg, or current use of antihypertensive medication. All clinical

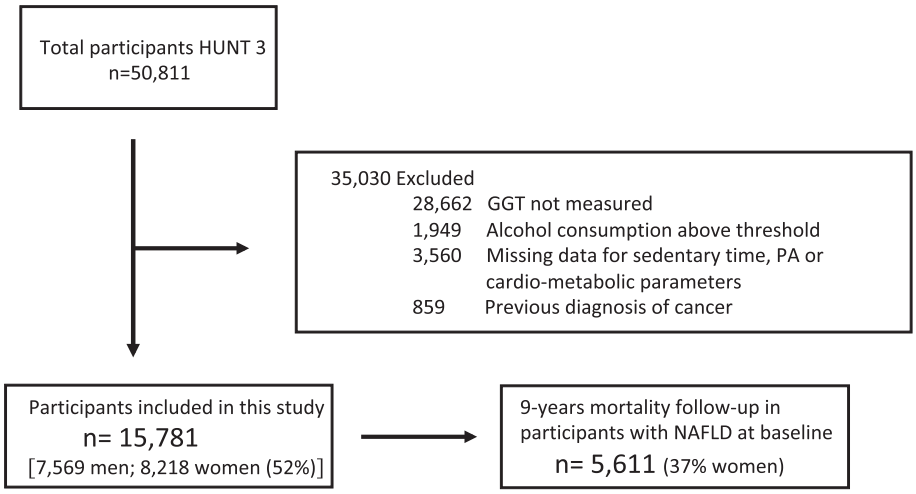

Fig 1. Flow of participants in this cohort study.

and biochemical analyses (including ALT and GGT) were performed at Levanger Hospital, Norway and have been described in detail elsewhere.[30] Self-administered questionnaires were completed, providing information about habitual PA, smoking habits, alcohol consumption, health status, and family history of disease, as previously described.[30]

Sedentary behaviour

SB refers to activities during sitting or lying while awake in which the energy expenditure is lower than 1.5 metabolic equivalent (MET) s, and is distinct from physical inactivity, which is defined as the lack of PA of ≥3 METs.[20] In the present study, information on SB was based on self-reported data. The main exposure variable, sedentary behaviour, was assessed with the question: "On a regular day, how many hours do you spend sitting?". This question is similar to the sitting measure of the commonly used International Physical Activity Questionnaire, which has shown acceptable reliability and validity[32] against objective measures of SB.

Physical activity

PA was assessed based on the responses to a self-administered questionnaire,[33] which included questions relating to PA frequency, intensity, and duration. Answers were used to calculate a previously published PA index score.[33,34] In line with international guidelines, participants were categorized as "meeting PA guidelines" if they exercised at high intensity for 30 min or more for at least two to three times per week, and/or exercised at moderate intensity for 30 min or more almost every day. If not, they were categorized into "not meeting PA guidelines".

Determination of Fatty Liver Index (FLI)

A surrogate marker of NAFLD, the Fatty Liver Index was calculated from body mass index (BMI), waist circumference, triglycerides and GGT, using the following validated equation[5]:

$$\text{Fatty Liver Index} = (e^{0.953\times\log e(\text{triglycerides})+0.139\times\text{BMI}+0.718\times\log e(\text{GGT})+0.053\times\text{waistcircumference}-15.745})/(1+e^{0.953\times\log e(\text{triglycerides})+0.139\times\text{BMI}+0.718\times\log e(\text{GGT})+0.053\times\text{waistcircumference}-15.745})\times 100$$

The Fatty Liver Index has been validated in the general population and has been shown to be accurate in detecting fatty liver.[5] A Fatty Liver Index ≥60 was used to define those who are likely to have NAFLD.[5,15,35]

Estimated CRF

To estimate each individual's CRF (peak oxygen consumption), a non-exercise prediction model was used.[36,37] The prediction models were derived and cross-validated in a subsample of participants from the current study (HUNT3); and their use has been recommended by the American Heart Association.[17] These algorithms are sex-specific and include age, waist, a published PA index score (PA-I),[33,34] and resting heart rate.

Female estimated peak oxygen consumption in ml/kg/min (R^2 0.56, SEE 5.1):

$$74.74-(0.247\ \text{age})-(0.259\ \text{waist})-(0.114\ \text{resting heart rate})+(0.198\ \text{PA}-\text{I})$$

Male estimated peak oxygen consumption in ml/kg/min (R^2 0.61, SEE 5.7):

$$100.27-(0.296\ \text{age})-(0.369\ \text{waist})-(0.155\ \text{resting heart rate})+(0.226\ \text{PA}-\text{I})$$

Measured CRF

A sensitivity analysis was performed in the 594 subjects (aged 20–90) who met the inclusion criteria for this liver sub-study and who also had participated in a sub-study that directly measured peak oxygen consumption during exercise (VO_{2peak}) via gas analysis (the HUNT Fitness Study[34]).

Mortality

Data on the cause and date of death was obtained from the Norwegian Cause of Death Registry, and matched to participants through their personal identification number. This study has a virtually complete follow-up of participants, since registration is mandatory in Norway. Only participants who emigrated from the country are missing in the analyses (<1%). The participants were followed from their participation date in HUNT3 until their date of death or end of follow-up on 31st December 2016 (over 9 years follow up).

Statistical analyses

Descriptive data are presented as mean (standard deviation, SD) for continuous variables, and number (percentage) for categorical variables. All variables presented normal distribution as assessed by the Shapiro-Wilk test and standard visual inspection (normal Q-Q plots). The following binary group categorisations were performed for markers of liver disease. NAFLD status was defined according to Fatty Liver index (≥60: NAFLD, <60: no NAFLD).[5,15,35] Elevated ALT was defined as the highest sex specific tertile (cut-offs were 35 U/L for men and 24 U/L for women). Elevated GGT was defined as the highest age and sex specific tertile. In individuals ≥40 years, cut-offs were 34 U/L for men and 24 U/L for women; while in individuals <40 years, cut-offs were 30 U/L for men and 17 U/L for women, which is in line with values defined by the European Association for the Study of the Liver.[38]

We classified estimated CRF into three sex and age-specific categories, as previously suggested[23]: low CRF was defined as the 20% least fit participants, moderate CRF as the middle 40%, and high CRF as the most fit 40%. As an example, estimated CRF values were < 37.8 ml/kg/min for low, 37.8–43.1 ml/kg/min for moderate, and >43.1 ml/kg/min for high fitness levels in men aged 49–59 years, and <30.4 ml/kg/min for low, 30.4–34.8 ml/kg/min for moderate, and >34.8 ml/kg/min for high fitness levels in women aged 49–59 years. Self-reported SB during a regular day was divided into sex-specific tertiles (cut-offs for both genders: ≤4, 5- < 7 and ≥ 7 h/d).

To address the first aim of this study, logistic regression analyses were used to estimate the association of NAFLD with: i) SB, ii) SB and CRF (combined association), iii) SB, PA and CRF (combined association). Participants with high CRF, low SB and meeting PA guidelines were used as the referent. Next, we conducted a sub-analysis (stratification by CRF) to separately investigate the association between SB and NAFLD within each CRF group (low, moderate and high). The same analyses were then performed using other markers of liver disease (i.e. elevated ALT and elevated GGT) as dependent variables. There were no significant interactions between sex and NAFLD (all $p > 0.05$), therefore, men and women were pooled. Results are expressed as odds ratios (OR), and precision of the estimates was assessed by 95% confidence intervals (CI). Assumptions of the statistical techniques used were met.

Sensitivity analyses to assess the robustness of our findings were also performed. These included i) an analysis stratifying individuals for BMI (normal weight, overweight and obese), and ii) a separate analysis on a subgroup of individuals who participated to the HUNT3 fitness study[37] and had CRF directly assessed as VO_{2peak} via gas analysis.

To address the second aim of this study, for individuals having NAFLD (36% of the overall sample; $n = 5611$), the risk of all-cause mortality were assessed using Cox proportional hazard regression models for all-cause mortality associated with SB and CRF and presented as hazard ratios (HR) with 95% CI.

Models were adjusted for potential confounders such as age, smoking (current smoker, non-smoker), BMI (<25 kg/m^2: normal- or underweight, 25–29.9 kg/m^2: overweight and ≥30 kg/m^2: obese), hypertension (normotensive, blood pressure ≥140/90 mmHg and/or antihypertensive medication), alcohol consumption (no consumption, <3 g/day, 3- < 9 g/day and >9 g/day), diabetes (normal, diabetic and/or pharmacological treatment) and self-reported history of cardiovascular disease. Further models, controlling for all confounders listed above, plus PA guidelines, levels of SB and CRF, were calculated. No evidence for violation of the proportional hazards assumption examined by Schoenfeld residuals was found. For all statistical analyses, tests were two sided and the level of significance was set at $p < 0.05$.

Table 1
Demographic, anthropometric, and laboratory characteristics of the study cohort according to levels of sedentary behaviour ($n = 15,781$).

	Sedentary behaviour (hours/day)[a]		
	≤4 (n = 5893)	5- < 7 (n = 5365)	≥7 (n = 4523)
Women, n (%)	3210 (54.5)	2609 (48.6)	2393 (52.9)
Age (years), mean (range)	50.9 (19–94)	52.8 (19–93)	49.2 (19–95)
NAFLD, n (% yes)	1868 (31.7)	2040 (38.0)	1703 (37.7)
Fatty Liver Index, mean (SD)	43.6 (28.7)	48.3 (29.1)	45.5 (30.3)
Alanine aminotransferase(U/L), mean (SD)	28.1 (21.4)	29.0 (17.7)	29.3 (19.6)
Elevated ALT (n, %)	2033 (34.5)	1887 (35.2)	1707 (37.7)
Gamma-glutamyl transferase (U/L), mean (SD)	27.8 (36.4)	30.7 (35.2)	30.9 (32.1)
Elevated GGT (n, %)	1845 (31.3)	1888 (35.2)	1734 (38.3)
Aspartate aminotransferase (U/L), mean (SD)	23.0 (16.5)	22.9 (9.3)	22.6 (11.0)
BMI (kg/m^2), mean (SD)	26.9 (4.2)	27.3 (4.3)	27.2 (4.6)
Overweight (BMI 25.0–29.9), n (%)	3811 (64.7)	3705 (69.1)	3044 (67.3)
Obesity status (BMI ≥ 30.0), n (%)	1194 (20.3)	1257 (23.4)	1063 (23.5)
Waist circumference (cm), mean (SD)	92.6 (11.7)	94.4 (11.9)	94.0 (12.9)
Systolic Blood Pressure (mmHg), mean (SD)	128.9 (18.0)	131.3 (18.2)	128.8 (17.7)
Diastolic Blood Pressure (mmHg), mean (SD)	72.5 (11.0)	73.7 (11.2)	73.5 (11.1)
Triglycerides (mmol/L), mean (SD)	1.55 (0.95)	1.63 (0.97)	1.63 (1.01)
Total cholesterol (mmol/L), mean (SD)	5.48 (1.10)	5.52 (1.10)	5.44 (1.08)
HDL cholesterol (mmol/L), mean (SD)	1.36 (0.35)	1.33 (0.35)	1.31 (0.34)
Glucose (mmol/L), mean (SD)	5.49 (1.36)	5.59 (1.50)	5.60 (1.62)
Alcohol consumption (g/day) mean (SD),	2 (2)	3 (3)	3 (3)
Current smokers, n (%)	1008 (17.1)	839 (15.6)	709 (15.7)
Diabetes, n (%)	174 (3.0)	227 (4.2)	191 (4.2)
History of CVD, n (%)	470 (8.0)	570 (10.6)	406 (9.0)
PA recommendations, n (%)			
No	3378 (57.3)	3203 (57.8)	2615 (58.3)
Yes	2515 (42.7)	1522 (39.8)	2548 (41.7)
CRF[b], n (%)			
Low	968 (16.4)	1087 (20.3)	945 (20.9)
Moderate	2337 (39.7)	2154 (40.2)	1733 (38.3)
High	2587 (43.9)	2123 (39.6)	1845 (40.8)

[a] Sample-specific tertiles of sedentary behaviour.
[b] Low, moderate and high cardiorespiratory fitness corresponded to the lowest 20th, intermediate (next 40th) and highest 40th percentiles, respectively (age and sex specific). ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; BMI, body mass index; HDL, high density lipoprotein; CVD, cardiovascular disease; PA, physical activity; CRF, cardiorespiratory fitness.

Table 2
Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the prevalence of NAFLD stratified according to sedentary behaviour among 15,781 adults.

Sedentary behaviour (h/day)[a]	NAFLD		OR (95% CI)	OR (95% CI)	OR (95% CI)
	Yes	No	Model 1	Model 2	Model 3
≤4	1868	4025	Reference (1.00)	Reference (1.00)	Reference (1.00)
5- < 7	2040	3325	1.17 (1.05–1.30)	1.16 (1.04–1.30)	1.12 (0.99–1.26)
≥7	1703	2820	1.34 (1.19–1.50)	1.33 (1.19–1.50)	1.31 (1.15–1.48)
p for trend			<0.001	<0.001	<0.001
Per hour increase			1.04 (1.03–1.06)	1.04 (1.03–1.06)	1.04 (1.02–1.06)

[a] Sex-specific tertiles of sedentary behaviour. Model 1: Adjusted for age, sex, smoking status, body mass index, hypertension, diabetes, history of cardiovascular disease, and alcohol consumption. Model 2: Same adjustments as Model 1, plus physical activity guidelines. Model 3: Same adjustments as Model 2, plus cardiorespiratory fitness.

Results

The characteristics of the study cohort are presented in Table 1. Overall, 15,781 participants were included (52% were women). Twenty nine percent reported SB ≥7 h/d and 42% were meeting PA guidelines. Sixty-six percent were overweight, 22% were obese and 36% percent had NAFLD. Mean (SD) estimated CRF was 39.2 (7.2) ml/kg/min and 31.8 (5.9) ml/kg/min, and mean (SD) SB was 5.9 (2.8) h/d and 5.6 (2.6) h/d for women and men, respectively. Characteristics of the cohort of 5611 participants with NAFLD (used for survival analyses) are presented as Supplementary Table 1.

SB and NAFLD

OR of NAFLD according to SB tertiles are presented in Table 2 (three adjusted models). Every additional 1 h of SB per day was associated with 4% higher likelihood of having NAFLD (OR, 1.04; 95% CI, 1.03–1.06); while the likelihood of having elevated liver enzymes ALT and GGT was significantly greater by 2% (OR, 1.02; 95% CI, 1.00–1.03) and 4% (OR, 1.04; 95% CI, 1.03–1.05), respectively (Supplementary Table 2). Compared with the reference group (SB ≤4 h/d), individuals reporting SB ≥7 h/d had 34% higher likelihood of having NAFLD (OR 1.34; 95% CI, 1.19–1.50).

Modifying role of estimated CRF on SB and NAFLD

Fig 2 presents adjusted OR of NAFLD according to estimated CRF and SB. The likelihood of having NAFLD, as well as elevated ALT and GGT (Supplementary Table 3 and Supplementary Fig 1), was markedly greater as CRF went from high to moderate and low. Compared with the reference group (individuals having high CRF and SB ≤4 h/d), individuals with low CRF were 17 times more likely to have NAFLD (OR, 16.9; 95% CI, 12.9–22.3), even if they had low SB (≤4 h/d). A similar pattern of results as for NAFLD was observed for the presence of elevated liver enzymes ALT and GGT, however the magnitude of the OR was different (Supplementary Fig 1). Compared with the reference group (individuals having high CRF and SB ≤4 h/d), individuals with low CRF were more likely to have elevated ALT (OR, 1.74; 95% CI, 1.46–2.08) and elevated GGT (OR, 2.62; 95% CI, 2.20–3.13), even if they had SB ≤ 4 h/d.

The adjusted OR between SB and NAFLD within each estimated CRF group (low, moderate and high CRF) are presented in Supplementary Table 4. In individuals with high CRF, the adverse effects of SB on NAFLD were attenuated in those with SB 5- < 7 h/d (OR, 1.00; 95% CI, 0.79–1.27) but persisted in those with SB ≥ 7 h/d (OR, 1.54; 95% CI, 1.20–1.97). In individuals with low CRF, the likelihood of having NAFLD was not significantly different between those with SB ≤ 4 h/d and those with SB ≥ 7 h/d (OR, 1.06; 95% CI, 0.77–1.46). Results of this analysis for GGT mirror those of NAFLD (Supplementary Table 4).

A further analysis stratifying individuals according to SB, estimated CRF and whether they met PA guidelines or not (18 groups in total) is presented in Fig 3 and Supplementary Table 5. A similar pattern of results was observed for individuals meeting PA guidelines and those who did not. Even when PA guidelines were being met, low CRF was strongly associated with higher NAFLD prevalence.

Sensitivity analyses

A separate analysis stratifying individuals for BMI (normal weight, overweight, obese), confirmed the outcomes seen in the overall population (Supplementary Fig 2). In addition, a sensitivity analysis using directly measured CRF data ($n = 594$; Supplementary Fig 3) confirmed the results obtained using estimated CRF, showing that fitness had a stronger association with NAFLD compared with PA and SB.

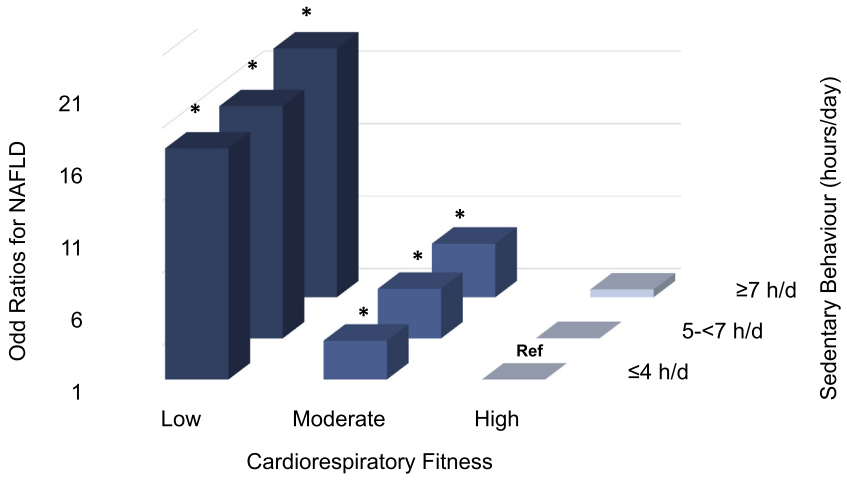

Fig 2. Odd Ratios of NAFLD according to estimated cardiorespiratory fitness and sedentary behaviour. $N = 15{,}781$. Adjusted for age, sex, smoking status, body mass index, hypertension, diabetes, history of cardiovascular disease and alcohol consumption. Low, moderate and high CRF corresponded to the lowest 20th, intermediate (next 40th) and highest 40th percentiles, respectively (age and sex specific). Ref, reference category. * Significantly different from the reference category ($p < 0.05$).

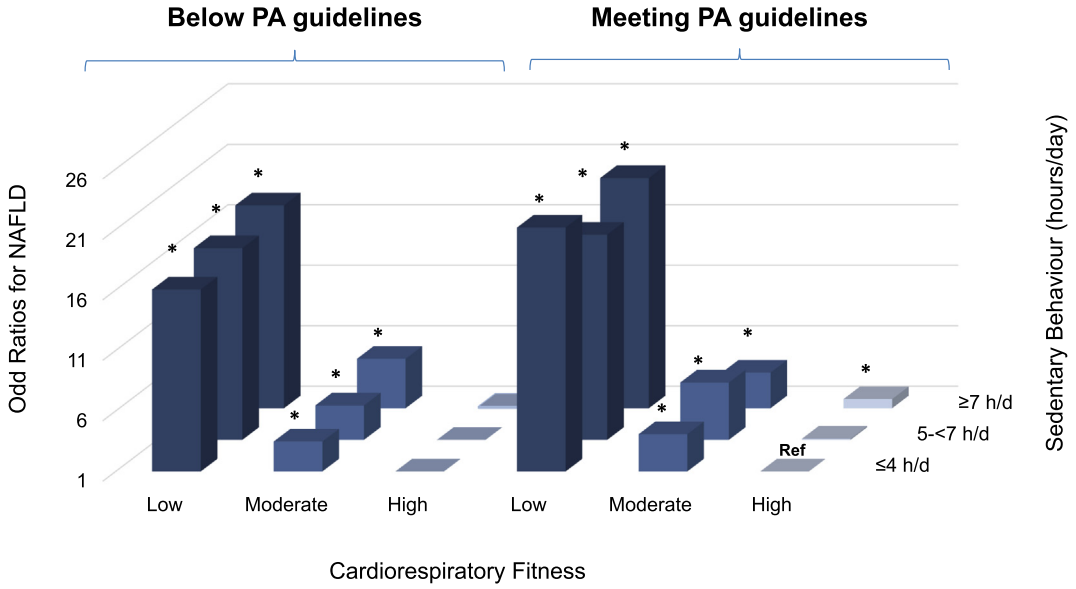

Fig 3. Odd ratios for the prevalence of NAFLD stratified according to sedentary behaviour, physical activity guidelines and estimated cardiorespiratory fitness. N = 15,781. Adjusted for age, sex, smoking status, body mass index, hypertension, diabetes, history of cardiovascular disease and alcohol consumption. Low, moderate and high CRF corresponded to the lowest 20th, intermediate (next 40th) and highest 40th percentiles, respectively (age and sex specific). Ref, reference category. * Significantly different from the reference category ($p < 0.05$).

All-cause mortality in individuals with NAFLD according to SB, PA and estimated CRF

Within the cohort of individuals having NAFLD ($n = 5611$; 3535 men and 2076 female), after 52,897 person-years of observations and a mean follow-up of 9.4 (SD 1.3) years, there were 353 deaths from all-causes (253 men and 100 women). Individuals with high SB (≥7 h/d) did not have a significantly increased risk of all-cause mortality compared with those with SB ≤ 4 h (HR, 1.21; 95% CI, 0.92–1.59; Table 3, Model 3). On the other hand, estimated CRF levels at baseline significantly influenced survival. Individuals with low CRF had 52% (HR, 1.52; 95% CI, 1.10–2.06) increased risk of all-cause mortality compared with those with high CRF, even after correction for SB and PA (Table 3, Model 3).

Discussion

The main findings of the present study were that: i) in the general population, low CRF is an independent predictor of NAFLD and elevated liver enzymes, with a stronger role than that of high SB and low PA; and that ii) within individuals having NAFLD, low age and sex-specific estimated CRF were associated with higher mortality rates, independent of SB and meeting PA guidelines.

This is the first study to demonstrate that high CRF provides survival benefits in individuals with NAFLD, independent of PA and SB. This is in agreement with previous findings in the general population and other cardiometabolic diseases, where CRF is a strong independent predictor of all-cause mortality.[17,23–25] The increased risk of death was observed only in the group with low CRF at baseline (below 35.0 ml/kg/min in male aged 49–59 years), but not in the moderate CRF group (between 35.0 and 39.7 ml/kg/min in male aged 49–59 years). This is consistent with observations in the general population and other disease states[23,25] and has important clinical implications; it suggests that the greatest benefit for the reduction in all-cause mortality in individuals with NAFLD occurs when those in the lowest fitness group move into the moderate fitness category. This can be achieved with relatively small improvements in CRF, that can be seen with traditional exercise programs lasting as little as 6–8 weeks in duration.[17] Moreover, an improvement in CRF of 1 MET (3.5 ml/kg/min) has been shown to reduce the risk of mortality by 13% in healthy men and women, which consists of a risk reduction comparable to that achieved with a decrease in waist circumference of 7 cm, a decrease in plasma glucose of 1 mmol/L, and a decrease in systolic blood pressure of 5 mmHg.[24] These benefits

Table 3
Adjusted hazard ratios (HR) and 95% confidence intervals (CI) for all-cause mortality in individuals with NAFLD according to sedentary behaviour and cardiorespiratory fitness.

	Person-years	Deaths	Rate	HR (95% CI)	HR (95% CI)	HR (95% CI)
				Model 1	Model 2	Model 3
Sedentary behaviour						
≤4 (h/d)	17,649	108	6.1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
5- < 7 (h/d)	19,212	140	7.3	1.00 (0.78–1.29)	1.00 (0.78–1.29)	0.97 (0.76–1.25)
≥7 (h/d)	16,038	105	6.5	1.26 (0.96–1.65)	1 1.26 (0.96–1.65)	1.21 (0.92–1.59)
p for trend				0.15	1 0.14	0.21
CRF						
High	22,431	120	5.3	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Moderate	21,053	127	6.0	0.99 (0.76–1.28)	0.99 (0.77–1.30)	1.00 (0.77–1.30)
Low	9413	106	11.3	1.51 (1.12–2.05)	1.54 (1.13–2.11)	1.52 (1.10–2.06)
p for trend				0.004	0.005	0.009

$n = 5611$. Model 1: Adjusted for age, sex, smoking status, body mass index, hypertension, diabetes, history of cardiovascular disease, and alcohol consumption. Model 2: Model 1 plus PA guidelines. Model 3: Model 2, plus mutual adjustments (i.e., SB adjusted for PA CRF; CRF adjusted for SB). Low, moderate and high CRF corresponded to the lowest 20th, intermediate (next 40th) and highest 40th percentiles, respectively, within this NAFLD cohort (age and sex specific). NAFLD specific CRF cut-off values were <35.0 for low, 35.0–39.7 for moderate, and >39.7 ml/kg/min for high CRF levels in men aged 49–59 years, and <26.1 for low, 26.1–29.6 for moderate, and >29.6 ml/kg/min for high CRF levels in women aged 49–59 years.

highlight the importance of advocating exercise aimed at improving CRF in NAFLD, independent of an effect on liver disease.

The other key finding from this study was that in the general population, estimated CRF is strongly negatively associated with the likelihood of having NAFLD, outweighing the influence of high SB and not meeting PA guidelines (Figs 2 and 3). The association between SB and NAFLD observed in this study is in agreement with findings from Ryu et al.,[22] which reported a positive relationship between sitting time and NAFLD independent of PA levels. Our study considerably extends previous findings by also exploring the role of CRF, or maximal aerobic capacity, which is a measure of the whole body's ability to deliver and utilize oxygen during "maximal intensity" exercise. High CRF has been shown to attenuate the negative association between cardiovascular risk factors and SB, independent of meeting PA guidelines.[26] It appeared plausible to expect a similar attenuation of the negative effects of SB by CRF on NAFLD and elevated liver enzymes, however this was seen only in part. In individuals with high CRF, the negative effects of SB on NAFLD and elevated GGT were attenuated when individuals had <7 h/d of SB, but persisted in those with SB ≥7 h/d. This is in line with previous research showing that the adverse effect of prolonged SB (≥10 h/d) on NAFLD was observed even among individuals who performed health-enhancing PA.[22] On the other hand, in individuals with low CRF, the likelihood of having NAFLD (and elevated liver enzymes) was not significantly different between those with high and low SB. This suggests that if someone has low fitness, reducing SB is not sufficient to reduce their likelihood of having NAFLD. Overall, while high SB and low CRF both appear to be independently associated with NAFLD and elevated GGT, the results of this study implicates that efforts towards improving CRF should be prioritized over reducing SB, particularly in those with low CRF. Indeed, across SB categories, individuals with low CRF have markedly higher likelihood of having NAFLD compared to individuals with high CRF.

It might seem counterintuitive that the benefits of high CRF on NAFLD morbidity and mortality are independent of meeting PA guidelines. However, although increases in PA are associated with concomitant increases in CRF,[39] the association between CRF and PA at the population level is modest, explaining at most 36% of the variance.[40,41] Further, CRF response to PA depends on the type of activity, is highly variable between individuals, and is in part influenced by genetics.[17] Indeed, meeting the PA guidelines (150 min/week at 60% of the predicted maximal heart rate) is insufficient to improve CRF in 40% of individuals.[42] The intensity of PA/exercise is a much stronger determinant of CRF than duration.[43] Therefore, promotion of regular, structured exercise (ideally high-intensity aerobic exercise), as well as implementation of PA guidelines better suited for improving CRF (engaging in higher intensity habitual-PA), seem important in the prevention of NAFLD and management of patients with NAFLD.

In addition to investigating the combined roles of SB, PA and estimated CRF on NAFLD, we also examined the influences of these factors on the likelihood of having elevated ALT and elevated GGT (presented in supplementary material). This is because ALT and GGT are markers of hepatocellular injury, and are used in clinical decision making.[2] Compared with the results observed for NAFLD, the magnitude of the OR was markedly lower; however, the main trend of the results were consistent with those found with NAFLD. This is likely because liver enzymes are not particularly sensitive and are within the normal range in a proportion of individuals with NAFLD.[44] Overall, our findings support the argument that high CRF has a direct benefit on the liver.

All results presented in this manuscript were independent of BMI, since BMI was included as a covariate in the models adopted. In addition, to further elucidate its role, analyses were performed stratifying individuals according to BMI (Supplementary Fig 2). These analyses revealed that in all BMI strata (normal weight, overweight and obese) the role of low CRF on the likelihood of having NAFLD exceeded that of low PA and high SB.

The main strengths of the present study include the ability to study the association between CRF and survival in NAFLD (achieved by linking data to death registries which ensure almost complete follow-up); and the large sample size of representative adult men and women with comprehensive biochemical assessment (including liver enzymes), extensive qualitative data, and CRF data. Limitations of the current study include the absence of a direct measure of intrahepatic fat content, and therefore of a clinical diagnosis of NAFLD. However, the Fatty Liver Index has been repeatedly validated against ultrasound,[5,6] and has shown good accuracy in predicting presence of intrahepatic fat compared with magnetic resonance spectroscopy, the gold standard imaging technique. Further, the Fatty Liver Index is commonly used in epidemiological studies,[15,45] as recommended by the European Association for the study of the Liver[2,38] and studies that have employed both Fatty Liver Index and ultrasound assessments of NAFLD[22,27] have observed consistent results with both methods. Another limitation was that SB was self-reported, which can lead to over- or underreporting due to recall bias and/or social desirability bias. Objective monitoring of SB through accelerometers would provide more reliable data, however this is not generally undertaken in large epidemiological studies. Further, the SB questionnaire adopted was similar to the sitting measure of the commonly used International Physical Activity Questionnaire, which has shown acceptable reliability and validity.[32] Furthermore, CRF was estimated through a non-exercise model and not directly measured; however, similar results were observed in a subgroup that underwent direct CRF measurements ($n = 594$). In addition, the CRF algorithm adopted in the current study has been shown to predict long-term risk of mortality from all-causes with a similar accuracy to direct measurements of CRF.[36] Future population studies adopting objective measurement of CRF and NAFLD are warranted. Additionally, future research on the role of SB and CRF on liver specific mortality (mortality primarily caused by liver disease) is needed. In line with normative data from Norway and Europe,[46] liver-specific mortality in this HUNT3 study cohort accounted for only 4% of total deaths, hence not permitting to investigate these aspects.

In summary, this study is the first to investigate the association between CRF and the prevalence of NAFLD at this scale; and the first to examine the roles of SB and CRF on mortality in individuals with NAFLD. It provided evidence that low CRF is strongly associated with a higher prevalence of NAFLD and of elevated liver enzymes in the general population, with a role that tends to outweigh that of SB. Further, it demonstrated that low CRF is associated with increased risk of all-cause mortality in individuals with NAFLD, independent of SB and PA. Based on our data, in the management and prevention of NAFLD, more emphasis should be placed on improving CRF. To this end, interventions should focus on encouraging individuals to engage in regular structured exercise and/or to increase habitual higher intensity PA. Results from the present study add weight to the argument of incorporating recommendations to improve CRF in the guidelines for the prevention and management of NAFLD. Implementation of such recommendations is likely to reduce the prevalence of NAFLD, which would also reduce the burden of cardiovascular disease at the population level.

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Author contributions

IC: writing the manuscript; study design; data analysis; interpretation of data. JSC, SBS, SEK, JN, GAM, UW: study design; interpretation of data; critical revision of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pcad.2019.01.005.

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