

BMJ Open Dose–response association between physical activity and non-alcoholic fatty liver disease: a case–control study in a Chinese population

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ABSTRACT

Aim Physical activity plays an important role in the development of non-alcoholic fatty liver disease (NAFLD). However, the optimal intensity and dose of physical activity for the treatment of NAFLD have yet to be found. In the present study, we aimed to provide a dose–response association between physical activity and NAFLD in a Chinese population.

Methods We recruited 543 patients with NAFLD diagnosed by abdominal ultrasonography, and 543 age-matched and sex-matched controls. The amount of physical activity, sedentary time and energy intake was collected through a structured questionnaire. Logistic regression analyses were performed to investigate the association between physical activity and NAFLD.

Results After adjusting for hypertension, diabetes, body mass index, fasting blood glucose, energy intake and sedentary time, the total amount of physical activity was found to be inversely associated with NAFLD in a dose-dependent manner in men (>3180 metabolic equivalent of energy [MET]-min/week vs ≤1440 MET-min/week: OR 0.60, 95% CI 0.40 to 0.91, p for trend=0.01). In addition, both moderate-intensity and vigorous-intensity physical activity were effective in reducing the risk of NAFLD, independent of confounding variables in men (moderate-intensity physical activity: >684 MET-min/week vs none: OR 0.58, 95% CI 0.40 to 0.86, p for trend=0.01; vigorous-intensity physical activity: >960 MET-min/week vs none: OR 0.63, 95% CI 0.41 to 0.95, p for trend=0.02).

Conclusions Physical activity was inversely associated with risk of NAFLD in a dose-dependent manner in men. Vigorous-intensity and moderate-intensity physical activity were both beneficial to NAFLD, independent of sedentary time and energy intake.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as fat accumulation in more than 5% of hepatocytes, without competing liver disease such as viral hepatitis or autoimmune hepatitis.¹ It encompasses a broad spectrum of hepatic dysfunction ranging from simple hepatic lipid accumulation (steatosis) to non-alcoholic steatohepatitis, fibrosis,

Strengths and limitations of this study

- This study had a considerable sample size and several potential confounding variables, such as energy intake and sedentary time, were taken into account.
- The intensity of physical activity was measured in terms of metabolic equivalent of energy (MET) and dose of physical activity was presented in the form of MET-min/week
- This study was a case–control design; thus, the causal association between physical activity and non-alcoholic fatty liver disease could not be precisely identified.
- This study was a case–control study, recall bias was inevitable and randomised controlled trial studies are therefore required for more accurate results.

cirrhosis and, finally, hepatocellular carcinoma.² A meta-analysis indicated that 25.24% of global population have NAFLD,³ similar to the prevalence rate in China of 20%.⁴ Observation studies showed that patients with NAFLD have a higher risk of developing extrahepatic complications such as cardiovascular disease, diabetes and metabolic syndrome.^{5–7} Therefore, NAFLD is recognised as a global health burden and it is crucial to explore effective prevention and treatment strategies.

Physical activity as a lifestyle modification plays an important role in the development of NAFLD. Previous studies found an inverse relationship between physical activity and the risk of NAFLD,^{8,9} and randomised controlled trials also demonstrated that physical activity improved liver enzyme function and reduced fat accumulation.^{10–13} A meta-analysis of 20 randomised controlled trials (RCTs) showed that levels of alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (GGT), aspartate aminotransferase (AST) and intrahepatic fat of the intervention group were significantly better than the control group.¹⁴ However, physical

activity is a complex concept and includes type, intensity, frequency and duration. Many studies only consider the frequency of physical activity, and this does not reflect the dose. In addition, most studies had a limited sample size and the data on physical activity were retrieved from populations with diverse demographic characteristics. Therefore, the optimal intensity and dose of physical activity for the treatment of NAFLD have yet to be found. For example, a report from the Korean suggested that exercising more than twice a week and for more than 30 min can decrease the risk of hepatic steatosis.¹⁵ Another study, from USA found that moderate-intensity exercise might reduce the risk of hepatic steatosis, but did not make a specific recommendation about the desired.¹⁶

In the present study, metabolic equivalent of energy (MET) was used as a measure of physical activity. We aimed to explore the dose–response relationship between physical activity and NAFLD in a Chinese population, taking into consideration confounding variables such as energy intake and sedentary time.

METHODS

Patient and public involvement

This study is a case–control design focused on a Chinese Han population between 18 and 70 years old. Subjects were recruited from a health examination centre of Nanping First Affiliated Hospital of Fujian Medical University from October 2015 to September 2017. All subjects underwent abdominal ultrasound and blood biochemical tests. Once cases and controls have been linked to the NAFLD, a letter of invitation and information about the study will be sent to each potential case and control to obtain consent. Eligible subjects will be interviewed face-to-face by investigators to collect data. In addition, all methods were performed in accordance with the relevant guidelines and regulations.

Sample size calculation

This study is a case–control design; thus, we estimate the sample size based on the case–control study formula for 1:1 frequency matching. By consulting the literature,¹⁷ we estimate OR 0.7, $p_0=0.6$. The calculated sample size was $N_{\text{case}}=N_{\text{control}}=508$. Finally, 1086 subjects (543 cases and 543 controls) were recruited in this study.

Outcome: eligibility of NAFLD cases and controls

NAFLD was diagnosed by the presence of at least two of the following three abnormal findings on abdominal ultrasonography¹⁸: (1) increased echogenicity of the liver near-field region with deep attenuation of the ultrasound signal; (2) hyperechogenicity of liver tissue ('bright liver'), as often compared with hypoechogenicity of the kidney cortex; and (3) vascular blurring. Exclusion criteria were as follows: (1) alcohol consumption >140 g/week for men and >70 g/week for women; (2) presence of hepatitis B surface antigen or hepatitis C antibodies; (3) use of hepatotoxic drugs (such as tamoxifen, amiodarone, valproate and methotrexate)¹⁹

which can induce hepatic fat accumulation; (4) hepatic disease which can induce hepatic fat accumulation; (5) hepatic disease such as Wilson's disease, autoimmune hepatitis and haemochromatosis. A total of 543 newly diagnosed patients with NAFLD were enrolled; and 543 controls were selected by frequency matching according to age (± 5 years) and gender from a healthy population who underwent abdominal ultrasonography examination during the same period.

Exposure: physical activity measurements

Physical activity during the past 7 days was quantified through a questionnaire based on the International Physical Activity Questionnaire, adapted to the characteristics of Nanping residents.²⁰ It includes four domains (transportation-related, work-related, household-related and leisure time-related). Each domain includes specific activities which correspond to various intensities of exercise (light, moderate and vigorous intensity). Participants were asked to estimate information on the frequency and duration spent in specific activities during the past 7 days. Sedentary time was measured by the single question, 'During the past seven days, how much time did you usually spend sitting on a day?'

The intensity of physical activity was defined in terms of MET. According to a standard reference, each kind of activity was assigned a specific MET value: low-intensity physical activities were defined as <3 METs, moderate-intensity activities defined as 3–6 METs and vigorous-intensity activities defined as >6 METs.²¹ The dose of specific physical activity was quantified by the frequency and duration and presented in the form of MET-min per week ($\text{MET-min/week}=\text{duration} \times \text{frequency per week} \times \text{MET value}$). The total dose of physical activity equals the sum of the doses for each specific activity.

Potential confounders

Face-to-face investigation was performed by uniformly trained investigators. Data were collected in the following four categories, using a structured questionnaire for the first two:

1. Demographic characteristics including age, gender, education, income, marriage status and history of diabetes.
2. Health-related behaviours including smoking status, alcohol drinking, tea consumption, total energy intake.

Total energy intake was assessed by semiquantitative food frequency questionnaire,²² which had been specifically developed and validated for the southern Chinese population.²³ Participants were asked to estimate information on the average frequency of consumption of selected foods and the estimated portion size over the previous year, ignoring any recent changes. Intakes of food were converted into gram per day. Each food item was assigned a specific energy according to Food Nutrition Facts Table and total energy intake was the sum of the energy of various foods ingested in a day.²⁴

Smokers were defined as those who had smoked at least one cigarette per day during the previous 6 months. Tea consumption was defined as drinking one or more cups of tea per day during the previous 6 months.

3. Anthropometric assessment including height, body weight and blood pressure.

Body mass index (BMI) was calculated as body weight (kg)/height² (m²), and classified into four categories: lean ≤ 18.5 kg/m², normal 18.6–23.9 kg/m², overweight 24.0–27.9 kg/m², obese ≥ 28.0 kg/m².²⁵

For blood pressure measurement, participants were first asked to rest for 10 min. Then, the trained investigators measured blood pressure twice on seated participants using a standard mercury sphygmomanometer, and the mean of the two measurements was considered as the participant's blood pressure. Hypertension was defined as systolic arterial blood pressure ≥ 140 mm Hg or diastolic arterial blood pressure ≥ 90 mm Hg.²⁶

4. Biochemical examinations after a 12-hour overnight fast

Biochemical parameters included serum AST, ALT, GGT, serum fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

Blood samples were collected between 08:00 and 10:00 after fasting overnight (12 hours). Blood biochemical analysis was carried out by the medical laboratory department of Nanping First Affiliated Hospital of Fujian Medical University.

Statistical analysis

The χ^2 test was used to assess categorical variables and the Mann-Whitney U-test was used for continuous variables. An unconditional logistic regression model was employed to progressively reduce the confounding effect of the relationship between physical activity and NAFLD risk. The bivariate spearman correlation was conducted to explore the association between physical activity and biochemical parameters. All statistical analyses were conducted using SPSS V.23.0. The p value was defined as two-tailed and set at <0.05 .

RESULTS

A total of 1086 subjects (543 cases and 543 controls) were recruited. Seven hundred and forty-two (68.3%) were men, 344 (3.7%) were women. Baseline characteristics are shown in [table 1](#). The prevalence of hypertension (30.0%), overweight or obesity (66.5%) and diabetes (4.8%) were higher in subjects with NAFLD (each $p < 0.05$). And they tend to have tea consumption ($p = 0.04$). Serum levels of GGT, ALT, AST, TC, TG and FBG were also higher than in the control population (each $p < 0.05$). Whereas HDL were lower in the cases ($p < 0.05$). There was no difference in age, gender, income, marriage status, smoking status, education level, sedentary time or serum level of LDL between the two groups.

In total population, there is no significant dose-response association between physical activity and

NAFLD after adjusting for BMI, hypertension, diabetes, fasting blood glucose, energy intake and sedentary time (see online supplementary table S1). Because the prevalence of NAFLD differed between men and women, we then used a gender-specific model in the further analysis.

In men, after adjusting for BMI, hypertension, diabetes, fasting blood glucose and sedentary time in multivariate logistic model 3, physical activity was associated with the risk of NAFLD in a dose-dependent manner (>3180 MET-min/week vs ≤ 1440 MET-min/week: OR 0.61, 95% CI 0.41 to 0.92, p for trend=0.02). After further adjusting for energy intake, this association was maintained (>3180 MET-min/week vs ≤ 1440 MET-min/week: OR 0.60, 95% CI 0.40 to 0.91, p for trend=0.01, [table 2](#)). In women, there exists no relationship between physical activity and NAFLD (see online supplementary table S2).

We also calculated the distribution of the three energy nutrients (carbohydrate, fat and protein) in cases and controls stratified by gender (see online supplementary table S3 and S4). After adjusting for the carbohydrate, total fat and protein, the association between physical activity and NAFLD was maintained in men (see online supplementary table S5 and S6). However, daily diets contain a variety of foods, not individual nutrients or individual foods, and there are complex interactions between different nutrients or foods. Based on individual food or nutrient studies, the association between diet and NAFLD cannot be accurately assessed. Thus, we finally analysed only total energy intake in the final multivariate logistic model.

Then, we further analysed the association between various intensities of physical activity and the risk of NAFLD. In men, the moderate-intensity and vigorous-intensity levels were inversely associated with the risk of NAFLD, independent of the confounding variables: (moderate-intensity physical activity: >684 MET-min/week vs none: OR 0.58, 95% CI 0.40 to 0.86, p for trend=0.01; vigorous-intensity physical activity: >960 MET-min/week vs none: OR 0.63, 95% CI 0.41 to 0.95, p for trend=0.02, [table 2](#)). In women, there is no association between various intensity of physical activity and NAFLD (see online supplementary table S3).

According to the Physical Activity Guidelines for Americans (PAGA) released by the US Department of health and human service (USDHHS),²⁷ more than 150 min of moderate-intensity physical activity per week or 75 min of vigorous-intensity physical activity per week is beneficial to health; we divided physical activity into different levels. The dose-response association was shown: men who underwent moderate-intensity or vigorous-intensity physical activity had a significantly lower risk of NAFLD (moderate-intensity physical activity ≥ 2.5 hours vs none: OR 0.63, 95% CI 0.43 to 0.92; p for trend=0.01; vigorous-intensity physical activity ≥ 1.25 hours vs none: OR 0.66, 95% CI 0.45 to 0.96; p for trend=0.03, [table 3](#)).

We explored the association between physical activity and biochemical indicators. In patients with NAFLD, subjects who undergo a higher total amount of physical activity tend to have significantly lower levels of GGT

Table 1 Baseline characteristics of cases and controls

Variable	Case	Control	Z/ χ^2	P value
	No (%) or median (quartiles)	No (%) or median (quartiles)		
Age (years)	48 (39–54)	48 (39–54)	–0.03	0.97
Gender			<0.01	1
Male	371 (68.3)	371 (68.3)		
Female	172 (31.7)	172 (31.7)		
Blood pressure (mm Hg)			20.60	<0.001*
<140/90	380 (70.0)	444 (81.8)		
≥140/90	163 (30.0)	99 (18.2)		
BMI (kg/m ²)			208.51	<0.001*
≤18.5	3 (0.6)	20 (3.7)		
18.6–23.9	179 (33.0)	388 (71.5)		
24.0–27.9	284 (52.3)	129 (23.8)		
≥28.0	77 (14.2)	6 (1.1)		
Diabetes			5.35	0.02*
No	517 (95.2)	531 (97.8)		
Yes	26 (4.8)	12 (2.2)		
Education level			5.52	0.06
Primary education	274 (50.5)	286 (52.7)		
Secondary education	158 (29.1)	126 (23.2)		
Bachelor degree	111 (20.4)	131 (24.1)		
Income (¥)			1.44	0.49
<1000	33 (6.1)	35 (6.4)		
1000–2000	161 (29.7)	178 (32.8)		
≥2000	349 (64.3)	330 (60.8)		
Tea consumption			4.40	0.04*
No	338 (62.2)	239 (44.0)		
Yes	205 (37.8)	304 (56.0)		
Smoking habit			0.24	0.62
No	140 (25.8)	131 (24.1)		
Yes	403 (74.2)	412 (75.9)		
Marital status			2.65	0.10
Single or divorced	53 (9.8)	70 (12.9)		
Married	490 (90.2)	473 (87.1)		
Sedentary time (hours/day)			2.98	0.23
<4	167 (30.8)	184 (33.9)		
4–8	250 (46.0)	255 (47.0)		
≥8	126 (23.2)	104 (19.2)		
Energy intake (kJ)	2227.34 (1778.78–2664.85)	2106.85 (1696.41–2600.52)	–2.32	0.02*
GGT (IU/L)	32 (23.00–45.00)	23 (17.00–32.00)	–10.18	<0.001*
ALT (IU/L)	27 (20.00–38.00)	20 (15.00–25.00)	–11.47	<0.001*
AST (IU/L)	24 (20.00–28.00)	22 (18.00–25.00)	–5.69	<0.001*
TC (mmol/L)	5.19 (4.64–5.77)	5.03 (4.53–5.53)	–2.76	0.06
TG (mmol/L)	1.85 (1.29–2.54)	1.18 (0.87–1.59)	–13.48	<0.001*
FBG (mmol/L)	5.37 (5.03–5.84)	5.20 (4.90–5.53)	–6.16	<0.001*

Continued

Table 1 Continued

Variable	Case	Control	Z/ χ^2	P value
	No (%) or median (quartiles)	No (%) or median (quartiles)		
HDL (mmol/L)	1.21 (1.06–1.37)	1.34 (1.18–1.48)	–9.16	<0.001*
LDL (mmol/L)	3.27 (2.63–3.79)	3.17 (2.68–3.74)	–0.61	0.54

ALT, alanine aminotransferase; AST, serum aspartate aminotransferase; BMI, body mass index; FBG, serum fasting blood glucose; GGT, gamma-glutamyltranspeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

*P < 0.05.

($p=0.02$). In the control population, greater physical activity was significantly associated with greater AST ($p=0.001$) (table 4).

DISCUSSION

Physical activity is a complex concept including type, intensity, frequency and duration. The parameters used to define the intensity of physical activity fall into two categories: absolute or relative. Absolute intensity refers to the

rate of energy expenditure during physical activity and is usually presented as MET. MET is a widely used physiological concept defined as the ratio of work metabolic rate to a standard resting metabolic rate of 1 kcal/kg/hour (1 MET=3.5 mL O₂/kg/min=1 kcal/kg/hour).²⁸ Moderate-intensity physical activity corresponds to 40%–60% of VO₂ max or 4–6 METs. Vigorous-intensity physical activity corresponds to ≥60% of VO₂ max or >6 METs.²⁹ Since different methods are used to assess physical activity

Table 2 Association between physical activity and NAFLD in male

Variable (MET-min/week)	Case No (%)	Control No (%)	Univariate model OR (95% CI)	Multivariate model 1 aOR (95% CI)	Multivariate model 2 aOR (95% CI)	Multivariate model 3 aOR (95% CI)
Total amount of physical activity						
≤1440	153 (41.2)	124 (33.4)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1440–3180	104 (28.0)	124 (33.4)	0.68 (0.48 to 0.97)	0.62 (0.41 to 0.93)*	0.62 (0.41 to 0.93)*	0.62 (0.41 to 0.92)*
>3180	114 (30.7)	123 (33.2)	0.75 (0.53 to 1.06)	0.62 (0.41 to 0.92)*	0.61 (0.41 to 0.92)*	0.60 (0.40 to 0.91)*
P value for trend			0.09	0.02*	0.02*	0.01*
Light-intensity physical activity						
≤525	121 (32.6)	125 (33.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
525–1500	127 (34.2)	125 (33.7)	1.05 (0.74 to 1.49)	1.00 (0.67 to 1.49)	1.00 (0.67 to 1.50)	1.03 (0.69 to 1.55)
>1500	123 (33.2)	121 (32.6)	1.05 (0.74 to 1.50)	0.96 (0.64 to 1.43)	0.96 (0.64 to 1.44)	0.95 (0.63 to 1.44)
P value for trend			0.79	0.83	0.84	0.82
Moderate-intensity physical activity						
None	204 (55)	170 (45.8)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤684	74 (19.9)	79 (21.3)	0.78 (0.54 to 1.14)	0.79 (0.52 to 1.21)	0.79 (0.51 to 1.21)	0.78 (0.51 to 1.20)
>684	93 (25.1)	122 (32.9)	0.64 (0.45 to 0.89)*	0.59 (0.40 to 0.86)*	0.58 (0.39 to 0.86)*	0.58 (0.40 to 0.86)*
P value for trend			0.01*	0.01*	0.01*	0.01*
Vigorous-intensity physical activity						
None	272 (73.3)	251 (67.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤960	28 (7.5)	35 (9.4)	0.74 (0.44 to 1.25)	0.77 (0.42 to 1.42)	0.77 (0.42 to 1.42)	0.77 (0.42 to 1.41)
>960	71 (19.1)	85 (22.9)	0.77 (0.54 to 1.10)	0.65 (0.43 to 0.98)*	0.65 (0.43 to 0.98)*	0.63 (0.41 to 0.95)*
P value for trend			0.12	0.03*	0.03*	0.02*

Multivariate model 1: adjusted for BMI, hypertension, diabetes and fasting blood glucose.

Multivariate model 2: adjusted for BMI, hypertension, diabetes, fasting blood glucose and sedentary time.

Multivariate model 3: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and energy intake.

aOR, adjusted OR; BMI, body mass index; MET, metabolic equivalent of energy; NAFLD, non-alcoholic fatty liver disease.

*P < 0.05.

Table 3 Association between moderate-intensity or vigorous-intensity physical activity and NAFLD in men

Variable	Case	Control	Univariate model	Multivariate model 1	Multivariate model 2	Multivariate model 3
(MET-min/week)	No (%)	No (%)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Moderate-intensity physical activity						
None	204 (55.0)	170 (45.8)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
~2.5 hours	67 (18.1)	75 (20.2)	0.74 (0.51 to 1.10)	0.73 (0.47 to 1.13)	0.72 (0.47 to 1.12)	0.72 (0.46 to 1.12)
≥2.5 hours	100 (27.0)	126 (34.0)	0.66 (0.47 to 0.92)*	0.63 (0.43 to 0.91)*	0.62 (0.43 to 0.91)*	0.63 (0.43 to 0.92)*
P value for trend			0.01*	0.01*	0.01*	0.01*
Vigorous-intensity physical activity						
None	272 (73.3)	251 (67.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
~1.25 hours	9 (2.4)	12 (3.2)	0.69 (0.29 to 1.67)	0.73 (0.26 to 2.07)	0.73 (0.26 to 2.07)	0.74 (0.26 to 2.08)
≥1.25 hours	90 (24.3)	108 (29.1)	0.77 (0.55 to 1.07)	0.68 (0.47 to 0.98)*	0.68 (0.46 to 0.98)*	0.66 (0.45 to 0.96)
P value for trend			0.10	0.04*	0.04*	0.03*

Multivariate model 1: adjusted for BMI, hypertension, diabetes and fasting blood glucose.

Multivariate model 2: adjusted for BMI, hypertension, diabetes, fasting blood glucose and sedentary time.

Multivariate model 3: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and energy intake.

aOR, adjusted OR; MET, metabolic equivalent of energy; NAFLD, non-alcoholic fatty liver disease.

* P<0.05.

in the literature, the optimal intensity and dose of physical activity for the treatment of NAFLD have yet to be determined.

In the present study, the intensity of physical activity was measured in terms of MET; and dose of physical activity was presented in the form of MET-min/week. We observed an inverse dose–response association between physical activity and the risk of NAFLD, independent of potential confounding variables. In men, patients with more than 3180 MET-min/week total physical activity had

a 40% lower risk of NAFLD compared with those with less than 1440 MET-min/week. In addition, we also found that moderate-intensity and vigorous-intensity physical activity were beneficial to NAFLD in men. When the dose of physical activity was divided according to the PAGA released by the USDHHS²⁷ (more than 150 min of moderate-intensity physical activity per week or 75 min of vigorous-intensity physical activity per week is beneficial to health), the dose–response association was maintained. However, the relationship between physical activity and NAFLD has not been observed in women. The gender difference observed in this study may be explained by sex hormones. Mechanism research have found that sex hormones can upregulate insulin receptor expression and increase receptor phosphorylation protein kinase levels, thereby enhancing insulin signalling and preventing NAFLD.^{30 31} Furthermore, some sex hormones and their derivatives are strong endogenous antioxidants, which can inhibit the production of lipid peroxides in the liver and reduce its concentration, and play a protective role in the liver.³² Several studies using maximal heart rate or percentage of VO₂ max to define the intensity of physical activity indirectly supported our findings. One other cross-sectional study has also found a dose–response association between physical activity and NAFLD risk in terms of MET.³³ This study suggested that men with a dose of more than 5760 MET-min/week had a 31% lower risk of NAFLD compared with those with less than 498 MET-min/week. In women, the association was weaker. However, the study population was heterogeneous, meaning that the results should be interpreted with caution and that optimal dose of physical activity should be tailored to the patient's clinical characteristics, fitness status and preferences.

The mechanism by which physical activity improves NAFLD is unclear, although several potential mechanisms

Table 4 Association between total amount of physical activity and biochemical indicators in men

Variable	Case		Control	
	Physical activity (MET-min/week)	P value	Physical activity (MET-min/week)	P value
GGT (IU/L)	-0.13	0.02*	0.05	0.33
ALT (IU/L)	-0.09	0.09	0.10	0.05
AST (IU/L)	0.01	0.80	0.17	0.001*
TC (mmol/L)	0.01	0.87	0.01	0.80
TG (mmol/L)	-0.04	0.47	-0.04	0.40
HDL (mmol/L)	0.05	0.35	0.03	0.53
LDL (mmol/L)	-0.03	0.59	0.02	0.76

ALT, alanine aminotransferase; AST, serum aspartate aminotransferase; BMI, body mass index; FBG, serum fasting blood glucose; GGT, gamma-glutamyltranspeptidase; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; TG, triglycerides.
P<0.05.

have been suggested. First, insulin sensitivity is a plausible explanation,³⁴ via increasing expression of glucose transport protein and synthase activity of muscle glycogen, and decreasing the accumulation of serum triglyceride. Second, physical activity decreases visceral adiposity, which in turn decreases free fatty acid influx to the liver. Third, physical activity is known to upregulate the intake of glucose and lipid oxidation in skeletal muscle, which in turn depletes the accumulation of fatty acid in the liver.³⁵ In the present study, we observed that increased physical activity was associated with decreased GGT levels in men with NAFLD, and men with higher physical activity tend to have higher AST levels in controls. Nevertheless, more studies are still needed to confirm the association between physical activity and NAFLD and potential mechanisms should be explored.

Strengths and limitations

There were several advantages to the current study. First, several potential confounding variables, including energy intake and sedentary time, were taken into account. With the development of technology and a better economy, people tend to spend more time in sedentary activities: one study showed that sitting time was positively associated with risk of NAFLD, even in subjects with a high level of physical activity.³⁶ Similarly, another study indicated that regular participation in high levels of physical activity does not fully protect against the risks associated with prolonged bouts of sedentary behaviours.³⁷ Other known risk factors of NAFLD are energy intake and BMI. Several previous studies have found that patients with NAFLD tend to have higher energy intake, and a energy-restricted diet was found to have great benefits for weight loss and improving BMI.^{38–41} However, few studies have considered sedentary time and energy intake at the same time when investigating the association between physical activity and NAFLD. The potential confounding effect of these factors may reduce the power to detect associations between physical activity and the risk of NAFLD.

A second advantage to our study was that we used the well-known parameter MET to quantify the intensity of physical activity; and also quantified dose of physical activity as frequency and duration. We found a dose-response association between physical activity and risk of NAFLD, which could provide evidence for a clinical treatment guideline for NAFLD.

A third advantage was that this study had a considerable sample size and could thus provide substantial statistical power to assess the effect of physical activity on NAFLD.

However, several limitations should be considered. First, this study was a case-control design; thus, the causal association between physical activity and NAFLD could not be precisely identified. Second, the level of physical activity was self-reported: subjects often have difficulty in recalling physical activity undertaken in the past 7 days and tend to underestimate the time spent in specific activities. Therefore, misclassification bias was inevitable and could have affected the calculated association between physical activity and NAFLD. RCT studies are therefore required for more

accurate results. Third, liver biopsy is the gold standard for quantitative diagnosis of NAFLD. However, it is an invasive examination; there exists the possibility of postoperative blood and bile leakage, and there are sampling errors; therefore, it does not apply to routine screening. In the current study, NAFLD was diagnosed by abdominal ultrasonography. Ultrasound examination currently is the preferred method for the initial screening of NAFLD with its advantages of no scratching, no radiation damage, reproducibility and low price. It is based on the enhancement or attenuation of intrahepatic echo and the progression of intravascular blood vessels. In moderate to severe steatosis, the sensitivity and specificity of ultrasound diagnosis are high (78.4%–90.8% and 76.9%–90.9%, respectively).⁴² However, ultrasound diagnosis is susceptible to individual differences, checking instrument performance and parameter selection, operating experience and many other factors, so ultrasound quantitative diagnosis of fatty liver still has limitations. This diagnosis mainly depends on the subjective judgement of the operator, and there is no objective and unified quantitative index. Also, it is difficult to identify liver fibrosis and liver fat. Each method has its own advantages and disadvantages. It is hoped that with the advancement of science and technology, better non-invasive diagnostic methods will emerge.

CONCLUSIONS

The present study found that high physical activity was inversely associated with the risk of NAFLD in a dose-dependent manner in men, with moderate-intensity and vigorous-intensity physical activity having the greatest effect on reducing risk.

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