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Complete List of Authors:	li, yangfan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China He, Fei; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China he, yun; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, Chin pan, xinting; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China hu, zhijian; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China lin, xu; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China xu, shanghua; Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China Peng, Xian-E; Fujian Medical University, Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China
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The dose-response association between physical activity and non-alcoholic fatty liver disease: a case-control study in a Chinese population

YangFan Li[1¶], Fei He[1¶], Yun He[2], XinTing Pan [1], ZhiJian Hu [1], Xu Lin[2],

ShangHua Xu [3 *] and XianE Peng [1,2*]

[1] Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China;

[2] Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China;

[3] Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China

¶ Both authors contributed equally to this work.

* Correspondence: fmuxe@163.com (X.E.P.) or 13365998866@163.com(S.H.X.);

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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- 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 age, gender, blood pressure, body mass index (BMI), energy intake and sedentary time, the total amount of physical activity was found to be inversely associated with NAFLD in a dose-dependent manner (>3943.8 MET-min/week vs. ≤1620 MET-min/week: OR=0.67, 95% CI=0.47-0.96, *P* for trend=0.03). In addition, both moderate- and vigorous-intensity physical activity were effective in reducing the risk of NAFLD, independent of confounding variables (Moderate-intensity physical activity: >840 MET-min/week vs. none: OR=0.69, 95%CI= 0.48-0.98, *P* for trend=0.04; Vigorous-intensity physical activity: >960 MET-min/week vs. none: OR=0.65, 95%CI=0.44-0.94, *P* for trend=0.02).

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

Key words: Energy intake; Metabolic Equivalent of Energy ; non-alcoholic fatty liver disease; physical activity

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 NAFLD could not be precisely identified.

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 encompass a broad spectrum of hepatic dysfunction ranging from simple hepatic lipid accumulation (steatosis) to non-alcoholic steatohepatitis (NASH), fibrosis, 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 recognized 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 randomized controlled trials also demonstrated that physical activity improved liver enzyme function and reduced fat accumulation.[10-13] A meta-analysis of 20 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 was 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 minutes can decrease the risk of hepatic steatosis. [15]Another study, from America, 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. All subjects were recruited from a health examination center of Nanping First Affiliated Hospital of Fujian Medical University from October 2015 to September 2017. All subjects who participated in this study provided written informed consent and the study was were approved by the local ethics committees of Fujian Medical University (ethics number 2014096). In addition, all methods were performed in accordance with the relevant guidelines and regulations.

Eligibility of NAFLD cases and controls

NAFLD was diagnosed by the presence of at least two of the following three abnormal findings on abdominal ultrasonography: [17] (1) increased echogenicity of the liver near-field region with deep attenuation of the ultrasound signal; (2) hyperechogenity of liver tissue ("bright liver"), as often compared to hypoechogenity 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) [18]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 hemochromatosis. A total of 543 newly-diagnosed NAFLD patients 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.

Survey content and variable definition

Data collection

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: demographic characteristics (age, gender, education, income and marriage status); health-related behaviors (smoking status, alcohol drinking, tea consumption, dietary habit, physical activity and sedentary time); anthropometric assessment (height, body weight and blood pressure) and biochemical examinations after a 12-hour overnight fast (AST, ALT, GGT, FBG, TC, TG, LDL and HDL).

Energy intake assessment

Total energy intake was assessed by semi-quantitative food frequency questionnaire (FFQ).[19] The FFQ was based on a similar questionnaire used in the 2010 China National Nutrition and Health Survey (CNNHS).[20] 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 g 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.[21]

Physical activity measurements

Physical activity during the past seven days was quantified through a questionnaire based on the International Physical Activity Questionnaire, adapted to the characteristics of Nanping

residents.[22] 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 seven 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 Metabolic Equivalent of Energy (MET). According to a standard reference, each kind of activity was assigned a specific MET value: low-intensity physical activities were defined as <3METs, moderate-intensity activities defined as 3~6 METs and vigorous-intensity activities defined as >6METs.[23] The dose of specific physical activity was quantified by the frequency and duration and presented in the form of MET-minutes per week (MET-min/week = duration X frequency per week X MET value). The total dose of physical activity equals the sum of the doses for each specific activity.

Definition of other variables

Smokers were defined as those who had smoked at least one cigarette per day during the previous six months. Tea consumption was defined as drinking one or more cups of tea per day during the previous six months. Body mass index (BMI) was classified into two categories: ≥24 kg/m^2 and <24 kg/m^2.[24] 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(SABP) ≥140 mmHg or diastolic arterial blood pressure(DABP) ≥90 mmHg.[25]

Blood sample collection

Blood samples were collected between 8:00 and 10:00 a.m. after fasting overnight (12 h). Blood biochemical analysis was carried out by the medical laboratory department of Nanping First Affiliated Hospital of Fujian Medical University.

Statistical analysis

The chi-square 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 Kruskal-Wallis H test was conducted to explore the association between physical activity and biochemical parameters. All statistical analyses were conducted using SPSS 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. Baseline characteristics are shown in Table 1. Subjects with NAFLD tend to have a higher BMI, blood pressure, energy intake and tea consumption (each $P<0.05$); 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.

Table 1. Baseline characteristics of cases and controls

Variable	case Number(%)or median(quartiles)	control Number(%)or median(quartiles)	*Z/□2*	*P*
Age (years)	48 (39-54)	48 (39-54)	-0.03	0.97
Gender			<	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/m2)			189.5	**< 0.001**
<24	182 (33.5)	408 (75.1)		
≥ 24.00	361 (66.5)	135 (24.9)		
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 (Kilojoule)	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)	-10.1	**< 0.001**
ALT (IU/L)	27(20.00-38.00)	20(15.00-25.00)	-11.4	**< 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)	-13.4	**< 0.001**
FBG (mmol/L)	5.37 (5.03-5.84)	5.20(4.90-5.53)	-6.16	**< 0.001**
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

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

Multivariate logistic model 1, adjusted for age, gender, blood pressure，BMI and sedentary time, showed a significant inversely dose-dependent association between physical activity and NAFLD (>3943.8 MET-min/week vs. ≤1620 MET-min/week: OR= 0.69, 95% CI=0.48-0.98, *P* for trend= 0.04). After further adjusting for energy intake, this association was maintained (>3943.8 MET-min/week vs. ≤1620 MET-min/week: OR=0.66, 95% CI=0.46-0.94, *P* for trend=0.02, Table 2).

Table 2. Association between physical activity and NAFLD

Variable	Case	Control	Univariate model	Multivariate model 1	Multivariate model 2	Multivariate model 3
(MET-minute/week)	number	number(%	OR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Total amount of						
≤1620	182 (33.5)	152 (28)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
~3943.8	180 (33.1)	200(36.8)	0.75(0.56-1.01)	**0.69 (0.49-0.96)**	**0.68(0.49-0.95)**	**0.69(0.49-0.96)**
>3943.8	181(33.3)	191(35.2)	0.79 (0.59-1.06)	**0.69 (0.48-0.98)**	**0.66(0.46-0.94)**	**0.67(0.47-0.96)**
P value for trend			0.13	**0.04**	**0.02**	**0.03**
Light intensity						
≤840	183 (33.7)	193 (35.5)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
~2100	185 (34.1)	196(36.1)	1.00 (0.75-1.32)	0.95(0.69-1.32)	0.94 (0.68-1.30)	0.95(0.68-1.31)
>2100	175 (32.2)	154 (28.4)	1.20 (0.89-1.61)	1.20(0.83-1.72)	1.15(0.80-1.65)	1.18(0.82-1.69)
P value for trend			0.24	0.35	0.46	0.39
Moderate intensity						
None	223 (41.1)	193 (35.5)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤840	163 (30.0)	163 (30.0)	0.87(0.65-1.16)	0.79 (0.57-1.10)	0.78 (0.56-1.08)	0.79(0.56-1.09)
>840	157 (28.9)	187 (34.4)	**0.73 (0.55-0.97)**	**0.69 (0.48-0.99)**	**0.68 (0.47-0.96)**	**0.69(0.48-0.98)**
P value for trend			**0.03**	**0.04**	**0.03**	**0.04**
Vigorous intensity						
none	432 (79.6)	400 (73.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤960	32 (5.9)	46(8.5)	0.64 (0.40-1.03)	0.68 (0.40-1.15)	0.68(0.40-1.15)	0.69(0.41-1.16)
>960	79 (14.5)	97 (17.9)	**0.75 (0.54-1.05)**	**0.67 (0.46-0.97)**	**0.64 (0.44-0.94)**	**0.65(0.44-0.94)**
P value for trend			**0.04**	**0.02**	**0.01**	**0.02**

NAFLD: Non-alcoholic fatty liver disease

Multivariate model 1: adjusted for age, gender, BMI, blood pressure and sedentary time

Multivariate model 2: adjusted for age, gender, BMI, blood pressure and energy intake.

Multivariate model 3: adjusted for age, gender, BMI, blood pressure, sedentary time and energy intake.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

We further analyzed the association between various intensities of physical activity and the

risk of NAFLD. The moderate- and vigorous-intensity levels were inversely associated with the risk of NAFLD, independent of the confounding variables: (Moderate-intensity physical activity: >840 MET-min/week vs. none: OR=0.69, 95% CI=0.48-0.98, *P* for trend=0.04; Vigorous-intensity physical activity: >960 MET-min/week vs. none: OR=0.65, 95% CI= 0.44-0.94, *P* for trend=0.02, Table 2).

When levels of activity were divided according to time, the dose-response association was shown: subjects who underwent moderate- or vigorous-intensity physical activity had a significantly lower risk of NAFLD (Moderate-intensity physical activity >3.5 hours vs. none: OR=0.67, 95% CI=0.47-0.96; *P* for trend=0.03; Vigorous-intensity physical activity>1.72 hours vs. none: OR=0.64, 95% CI= 0.45-0.90; *P* for trend=0.01, Table 3).

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

Variable	Case	Control	Univariate model	Multivariate	Multivariate model 2	Multivariate model 3
(MET-minute/week)	number	number(%)	OR (95%	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Moderate intensity						
None	223(41.1)	193 (35.5)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
~3.5 hours	166 (30.6)	164(30.2)	0.79 (0.41-1.52)	0.81 (0.58-1.12)	0.79 (0.57-1.10)	0.80(0.57-1.11)
>3.5 hours	154 (28.4)	186(34.3)	**0.71 (0.52-0.96)**	**0.67 (0.47-0.96)**	**0.66(0.46-0.94)**	**0.67(0.47-0.96)**
P value for trend			**0.02**	**0.03**	**0.02**	**0.03**
Vigorous intensity						
None	432 (76.6)	400 (73.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
~1.72 hours	17(3.4)	20 (3.7)	0.88 (0.66-1.17)	0.79 (0.38-1.64)	0.79 (0.38-1.65)	0.80(0.39-1.67)
>1.72 hours	94 (20)	123 (22.7)	**0.72 (0.54-0.96)**	**0.65 (0.46-0.92)**	**0.63 (0.45-0.90)**	**0.64(0.45-0.90)**
P value for trend			**0.02**	**0.02**	**0.01**	**0.01**

NAFLD: Non-alcoholic fatty liver disease

Multivariate model 1: adjusted for age, gender, BMI, blood pressure and sedentary time

Multivariate model 2: adjusted for age, gender, BMI, blood pressure and energy intake.

Multivariate model 3: adjusted for age, gender, BMI, blood pressure, sedentary time and energy intake.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

We explored the association between physical activity and biochemical indicators. In

NAFLD patients, subjects who undergo a higher total amount of physical activity tend to have significantly lower levels of GGT, ALT, FBG (P<0.05). In the control population, greater physical activity was significantly associated with greater HDL and LDL (P<0.05). (Table 4).

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

	Case median(quartiles)			
Physicalactivity(MET-minute/week) / biochemical indicators	≤1620	1620~3943.8	>3943.8	*P*
GGT (IU/L)	38.00(26.75-53)	30 (23.00-42.00)	28(20.00-33.50)	**< 0.001**
ALT (IU/L)	31(23.00-41.25)	27 (19.00-36.00)	25(18.00-34.00)	**< 0.001**
AST (IU/L)	24(20.00-30.00)	22 (19.00-27.00)	23(20.00-28.5)	0.15
TC (mmol/L)	5.16(4.44-5.74)	5.22 (4.67-5.84)	5.16(4.67-5.72)	0.42
TG (mmol/L)	1.92(1.41-2.69)	1.91(1.26-2.57)	1.77(1.22-2.36)	0.16
FBG (mmol/L)	5.55 (5.06-6.05)	5.36(5.02-5.80)	5.28(5.01-5.70)	**0.02**
HDL (mmol/L)	1.17(1.03-1.33)	1.21(1.06-1.37)	1.24(1.07-1.38)	0.09
LDL (mmol/L)	3.24 (2.53-3.70)	3.32(2.69-3.96)	3.31(2.58-3.78)	0.29
	Control median(quartiles)			
Physicalactivity(MET-minute/week) / biochemical indicators	≤1620	1620~3943.8	>3943.8	*P*
GGT (IU/L)	23 (18.00-32.00)	23(17.00-32.00)	21(15.00-30.00)	0. 12
ALT (IU/L)	20 (15.00-24.75)	19 (15.00-26.75)	20 (15.00-25.00)	0.97
AST (IU/L)	20.5 (18.00-23.00)	22(18.00-25.00)	22(18.00-25.00)	**0.04**
TC (mmol/L)	4.98 (4.46-5.33)	4.46(5.01-5.45)	5.11(4.64-5.84)	**0.02**
TG (mmol/L)	1.27 (0.91-1.54)	1.15(0. 89-1.66)	1.11(0.83-1.59)	0.45
FBG (mmol/L)	5.20 (4.89-5.54)	5.24(4.91-5.55)	5.18(4.89-5.46)	0.71
HDL (mmol/L)	1.32(1.14-1.45)	1.34(1.18-1.48)	1.39(1.21-1.48)	**0.02**
LDL (mmol/L)	3.09 (2.67-3.61)	3.14(2.60-3.70)	3.33(2.76-3.80)	**0.04**

AST = serum aspartate aminotransferase, ALT = alanine aminotransferase, GGT = gamma-glutamyltranspeptidase, FBG = serum fasting glucose,TC = total cholesterol, TG = triglycerides, LDL = low-density lipoprotein, HDL = high-density lipoprotein

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 Metabolic Equivalent of Energy (MET). MET is a widely-used

physiological concept defined as the ratio of work metabolic rate to a standard resting metabolic rate of 1Kcal/kg·h (1 MET=3.5ml O_2/kg·min=1Kcal/kg·h). [26] Moderate-intensity physical activity corresponds to 40%~60% of VO_2 max or 4~6 METs. Vigorous-intensity physical activity corresponds to ≥60% of VO_2 max or > 6METs.[27] Since different methods are used to assess physical activity 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. Subjects with more than 3943.8MET-min/week total physical activity had a 33% lower risk of NAFLD compared to those with less than 1620 MET-min/week. In addition, we also found that moderate- and vigorous-intensity physical activity were beneficial to NAFLD. When the dose of physical activity was divided according to time, the dose-response association was maintained. Several studies using maximal heart rate or percentage of VO_2 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.[28] This study suggested that males with a dose of more than 5760 MET-min/week had a 31% lower risk of NAFLD compared to those with less than 498 MET-min/week. In females, the association was weaker. The Physical Activity Guidelines for Americans (PAGA) released by the USDHHS[29]suggested that more than 150 minutes of moderate-intensity physical activity per week or 60 minutes of vigorous-intensity physical

activity per week is beneficial to health. 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 have been suggested. First, insulin sensitivity is a plausible explanation,[30] via increasing expression of glucose transport protein and synthase activity of muscle glycogen, and decreasing the accumulation of serum triglyceride. Secondly, physical activity decreases visceral adiposity, which in turn decreases free fatty acid influx to the liver. Thirdly, 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.[31] In the present study, we observed that increased physical activity was associated with decreased FBG levels in NAFLD patients, and subjects with higher physical activity tend to have higher HDL 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.[32] 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 behaviors.[33] Other known risk factors of NAFLD are energy intake and BMI. Several previous studies have found that NAFLD patients tend to have higher energy intake, and a restricted-energy diet was found to have great benefits for weight loss and improving BMI.[34-37] 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 seven 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. Randomized controlled trial studies are therefore required for more accurate results.

Conclusions

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

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Conflict of interests, and authorship

The authors declare that they have no conflicts of interest.

Data sharing

No additional data are available.

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Contributorship statement

All authors included on a paper fulfil the criteria of authorship. There is no one else who fulfils the criteria that has been excluded as an author.

YangFan Li: Writing original draft and Investigation

Fei He: Writing review & editing and Conceptualization

Yun He: Investigation and Supervision

XinTing Pan: Investigation and Supervision

ZhiJian Hu: Formal analysis and Software

Xu Lin: Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ShangHua Xu: Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

XianE Peng: Final approval of the version published. Substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data.

We thank all study participants for their cooperation. We also thank our staff for recruiting subjects and their technical assistance.

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-8
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	5
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14-15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Complete List of Authors:	li, yangfan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China He, Fei; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China he, yun; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, Chin pan, xinting; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China wu, yunli; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, Chin hu, zhijian; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China lin, xu; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China xu, shanghua; Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China Peng, Xian-E; Fujian Medical University, Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China
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The dose-response association between physical activity and non-alcoholic fatty liver disease: a case-control study in a Chinese population

YangFan Li[1¶], Fei He[1¶], Yun He[2],XinTing Pan [1], Yunli Wu[2], ZhiJian Hu [1], Xu Lin[2],

ShangHua Xu [3 *] and XianE Peng [1,2*]

[1] Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China;

[2] Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China;

[3] Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China

¶ Both authors contributed equally to this work.

* Correspondence: fmuxe@163.com (X.E.P.) or 13365998866@163.com(S.H.X.);

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Running title: Exercise and non-alcoholic fatty liver disease

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- 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 (BMI),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 males. (>3180 MET-min/week vs. ≤1440 MET-min/week: OR=0.60, 95% CI=0.40-0.91, *P* for trend=0.01). In addition, both moderate- and vigorous-intensity physical activity were effective in reducing the risk of NAFLD, independent of confounding variables in males (Moderate-intensity physical activity: >684 MET-min/week vs. none: OR=0.58, 95%CI= 0.40-0.86, *P* for trend=0.01; Vigorous-intensity physical activity: >960 MET-min/week vs. none: OR=0.63, 95%CI=0.41-0.95, *P* for trend=0.02).

Conclusions: Physical activity was inversely associated with risk of NAFLD in a

dose-dependent manner in males. Vigorous- and moderate-intensity physical activity were both beneficial to NAFLD, independent of sedentary time and energy intake.

Key words: Energy intake; Metabolic Equivalent of energy ; non-alcoholic fatty liver disease; physical activity

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 NAFLD could not be precisely identified.
- This study was a case-control study , recall bias was inevitable and randomized controlled trial studies are therefore required for more accurate results.

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 encompass a broad spectrum of hepatic dysfunction ranging from simple hepatic lipid accumulation (steatosis) to non-alcoholic steatohepatitis (NASH), fibrosis, 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 recognized 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 randomized controlled trials also demonstrated that physical activity improved liver enzyme function and reduced fat accumulation. [10-13]A meta-analysis of 20 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 was 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 minutes can decrease the risk of hepatic steatosis. [15]Another study, from America, 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 center 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 .The study was were approved by the local ethics committees of Fujian Medical University (ethics number 2014096).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_{case} =508 =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 ultrasonography: [18](1) increased echogenicity of the liver near-field

region with deep attenuation of the ultrasound signal; (2) hyperechogenity of liver tissue ("bright liver"), as often compared to hypoechogenity 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 hemochromatosis. A total of 543 newly-diagnosed NAFLD patients 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 seven 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 seven 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 Metabolic Equivalent of Energy (MET). According to a standard reference, each kind of activity was assigned a specific MET

value: low-intensity physical activities were defined as <3METs, moderate-intensity activities defined as 3~6 METs and vigorous-intensity activities defined as >6METs.[21] The dose of specific physical activity was quantified by the frequency and duration and presented in the form of MET-minutes per week (MET-min/week = duration X frequency per week X 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 behaviors including smoking status, alcohol drinking, tea consumption, total energy intake.

Total energy intake was assessed by semi-quantitative food frequency questionnaire (FFQ),[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 g 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 six months. Tea consumption was defined as drinking one or more cups of tea per day

during the previous six months.

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

Body mass index (BMI) was calculated as body weight (kg)/height^2 (m^2), and classified into four categories: lean≤18.5 kg/m^2, normal: 18.6–23.9 kg/m^2, overweight: 24.0–27.9 kg/m^2, obese: ≥28.0 kg/m^2. [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(SABP) ≥140 mmHg or diastolic arterial blood pressure(DABP) ≥90 mmHg.[26]

(4) Biochemical examinations after a 12-hour overnight fast

Biochemical parameters including serum aspartate aminotransferase (AST), alanine aminotransferase(ALT), gamma-glutamyltranspeptidase(GGT), serum fasting glucose(FBG), total cholesterol(TC), triglycerides(TG), low-density lipoprotein(LDL), high-density lipoprotein(HDL).

Blood samples were collected between 8:00 and 10:00 a.m. after fasting overnight (12 h). Blood biochemical analysis was carried out by the medical laboratory department of Nanping First Affiliated Hospital of Fujian Medical University.

Statistical analysis

The chi-square 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 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.742 (68.3%) were male, 344 (3.7%) were female. 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.

Table 1. Baseline characteristics of cases and controls

	case	control		
Variable	Number(%)or median (quartiles)	Number(%)or median (quartiles)	*Z/□2*	*P*
Age (years)	48 (39-54)	48 (39-54)	-0.03	0.97
Gender			<	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/m2)			208.5	**< 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 (Kilojoule)	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)	-10.1	**< 0.001**
ALT (IU/L)	27(20.00-38.00)	20(15.00-25.00)	-11.4	**< 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)	-13.4	**< 0.001**
FBG (mmol/L)	5.37 (5.03-5.84)	5.20(4.90-5.53)	-6.16	**< 0.001**
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

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

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 (table s1). Because the prevalence of NAFLD was differed between males and females, then we used gender-specific model in the further analysis.

In male, 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=0.41-0.92, *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=0.40-0.91, *P* for trend= 0.01, Table 2).In female, there exist no relationship between physical activity and NAFLD (table s2).

Then we further analyzed the association between various intensities of physical activity and the risk of NAFLD. In males, the moderate- and vigorous-intensity levels were inversely associated with the risk of NAFLD, independent of the confounding variables: (Moderate-intensity physical activity: >684MET-min/week vs. none: OR=0.58, 95% CI=0.40-0.86, *P* for trend=0.01; Vigorous-intensity physical activity: >960 MET-min/week vs. none: OR=0.63, 95% CI= 0.41-0.95, *P* for trend=0.02, Table 2).In female, there is no association between various intensity of physical activity and NAFLD (table s3).

Table 2. Association between physical activity and NAFLD in male

Variable	Case	Control	Univariate model	Multivariate model 1	Multivariate model 2	Multivariate model 3
(MET-minute/week)	number (%)	number(%)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Total amount of						
≤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(0.48-0.97)	**0.62 (0.41-0.93)**	**0.62 (0.41-0.93)**	**0.62 (0.41-0.92)**
>3180	114(30.7)	123(33.2)	0.75 (0.53-1.06)	**0.62 (0.41-0.92)**	**0.61 (0.41-0.92)**	**0.60(0.40-0.91)**
P value for trend			0.09	**0.02**	**0.02**	**0.01**
Light intensity						
≤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 (0.74-1.49)	1.00(0.67-1.49)	1.00 (0.67-1.50)	1.03(0.69-1.55)
>1500	123 (33.2)	121 (32.6)	1.05 (0.74-1.50)	0.96(0.64-1.43)	0.96(0.64-1.44)	0.95(0.63-1.44)
P value for trend			0.79	0.83	0.84	0.82
Moderate intensity						
None	204 (55)	170 (45.8)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤684	74 (19.9)	79 (21.3)	0.78 (0.54-1.14)	0.79 (0.52-1.21)	0.79 (0.51-1.21)	0.78(0.51-1.20)
>684	93 (25.1)	122 (32.9)	**0.64 (0.45-0.89)**	**0.59 (0.40-0.86)**	**0.58(0.39-0.86)**	**0.58(0.40-0.86)**
P value for trend			**0.01**	**0.01**	**0.01**	**0.01**
Vigorous intensity						
none	272 (73.3)	251 (67.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤960	28 (7.5)	35(9.4)	0.74 (0.44-1.25)	0.77 (0.42-1.42)	0.77(0.42-1.42)	0.77(0.42-1.41)
>960	71 (19.1)	85 (22.9)	0.77 (0.54-1.10)	**0.65 (0.43-0.98)**	**0.65 (0.43-0.98)**	**0.63(0.41-0.95)**
P value for trend			0.12	**0.03**	**0.03**	**0.02**

NAFLD: Non-alcoholic fatty liver disease

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.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

According to the Physical Activity Guidelines for Americans (PAGA) released by the USDHHS[27]: more than 150 minutes of moderate-intensity physical activity per week or 75 minutes of vigorous-intensity physical activity per week is beneficial to health, we divided physical activity into different levels. The dose-response association was shown: males who underwent moderate- 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=0.43-0.92; *P* for trend=0.01; Vigorous-intensity physical activity≥1.25 hours vs. none: OR=0.66, 95% CI= 0.45-0.96; *P* for trend=0.03, Table 3).

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

Variable	Case	Control	Univariate model	Multivariate	Multivariate model 2	Multivariate model 3
(MET-minute/week	number (%)	number(%)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Moderate intensity						
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 (0.51-1.10)	0.73 (0.47-1.13)	0.72 (0.47-1.12)	0.72 (0.46-1.12)
≥2.5 hours	100 (27.0)	126(34.0)	**0.66 (0.47-0.92)**	**0.63 (0.43-0.91)**	**0.62(0.43-0.91)**	**0.63(0.43-0.92)**
P value for trend			**0.01**	**0. 01**	**0. 01**	**0. 01**
Vigorous intensity						
None	272(73.3)	251(67.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
~1.25hours	9(2.4)	12 (3.2)	0.69 (0.29-1.67)	0.73 (0.26-2.07)	0.73 (0.26-2.07)	0.74(0.26-2.08)
≥1.25 hours	90(24.3)	108 (29.1)	0.77 (0.55-1.07)	**0.68 (0.47-0.98)**	**0.68 (0.46-0.98)**	**0.66(0.45-0.96)**
P value for trend			0.10	**0.04**	**0.04**	**0.03**

NAFLD: Non-alcoholic fatty liver disease

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.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

We explored the association between physical activity and biochemical indicators. In NAFLD patients, subjects who undergo a higher total amount of physical activity tend to have significantly lower levels of GGT (*P*=0.02). In the control population, greater physical activity was significantly associated with greater AST (*P*=0.001) (Table 4).

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

Variable	case		control	
	Physical activity (MET-minute/week)		Physical activity (MET-minute/week)	
	Correlation	*p*	Correlation coefficient	*p*
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

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

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 Metabolic Equivalent of Energy (MET). MET is a widely-used physiological concept defined as the ratio of work metabolic rate to a standard resting metabolic rate of 1Kcal/kg·h (1 MET=3.5ml O_2/kg·min=1Kcal/kg·h).[28] Moderate-intensity physical activity corresponds to 40%~60% of VO_2 max or 4~6 METs. Vigorous-intensity physical activity corresponds to ≥60% of VO_2 max or > 6METs.[29] Since different methods are used to assess physical activity 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 males. Males with more than 3180 MET-min/week total physical activity had a 40% lower risk of NAFLD compared to those with less than 1440 MET-min/week. In addition, we also found that moderate- and vigorous-intensity physical activity were beneficial to NAFLD in males. When the dose of physical activity was divided according to the Physical Activity Guidelines for Americans (PAGA) released by the USDHHS[27] (more than 150 minutes of moderate-intensity physical activity per week or 75 minutes of vigorous-intensity physical activity per week is beneficial to health), the dose-response association was maintained. Several studies using maximal heart rate or percentage of VO_2 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.[30] This study suggested that males with a dose of more than 5760 MET-min/week had a 31% lower risk of NAFLD compared to those with less than 498 MET-min/week. In females, 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 have been suggested. First, insulin sensitivity is a plausible explanation,[31] via increasing expression of glucose transport protein and synthase activity of muscle glycogen,

and decreasing the accumulation of serum triglyceride. Secondly, physical activity decreases visceral adiposity, which in turn decreases free fatty acid influx to the liver. Thirdly, 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.[32] In the present study, we observed that increased physical activity was associated with decreased GGT levels in males with NAFLD, and males 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.[33] 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 behaviors.[34] Other known risk factors of NAFLD are energy intake and BMI. Several previous studies have found that NAFLD patients tend to have higher energy intake, and a restricted-energy diet was found to have great benefits for weight loss and improving BMI. [35-38] 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 seven 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. Randomized controlled trial 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 exist the possibility of postoperative blood and bile leakage, and there are sampling errors, therefore does not apply to routine screening. In 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). [39]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 judgment of the operator, and there is no objective and unified quantitative index. And 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 males, with moderate- and vigorous-intensity physical activity having the greatest effect on reducing risk.

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Conflict of interests

all authors have declare: no support from any organization for the submitted work; no financial relationships with any organization that might have an interest in the submitted work in the previous three years, no other relationships or activities that could appear to have influenced the submitted work.

Contributors

YFL and FH are joint first authors. XEP obtained funding. XEP, ZJH , XL and SHX designed the study. XTP and YFL collected the data. YLW and YH were involved in data cleaning and verification. YFL and XTP analyzed the data. YFL and FH drafted the manuscript. XEP and ZJH contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript.

Data sharing

No additional data are available.

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Table S1. Association between physical activity and NAFLD

Variable (MET-minute/week)	Case number	Control number(%	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						
≤1620	182 (33.5)	152 (28)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1620~3943.8	180 (33.1)	200(36.8)	0.75(0.56-1.01)	0.72 (0.52-1.00)	0.72(0.52-1.01)	0.74(0.53-1.03)
>3943.8	181(33.3)	191(35.2)	0.79 (0.59-1.06)	0.75 (0.54-1.05)	0.76(0.54-1.07)	0.79(0.56-1.11)
P value for trend			0.13	0.11	0.13	0.19
Light intensity						
≤840	183 (33.7)	193 (35.5)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
840~2100	185 (34.1)	196(36.1)	1.00 (0.75-1.32)	0.97(0.70-1.34)	0.98 (0.71-1.35)	1.00(0.73-1.39)
>2100	175 (32.2)	154 (28.4)	1.20 (0.89-1.61)	1.22(0.88-1.71)	1.26(0.90-1.77)	1.32(0.94-1.86)
P value for trend			0.24	0.24	0.19	0.12
Moderate intensity						
None	223 (41.1)	193 (35.5)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤840	163 (30.0)	163 (30.0)	0.87(0.65-1.16)	0.89 (0.64-1.23)	0.89 (0.64-1.24)	0.91(0.65-1.26)
>840	157 (28.9)	187 (34.4)	**0.73 (0.55-0.97)**	0.80 (0.58-1.10)	0.81(0.59-1.12)	0.85(0.61-1.19)
P value for trend			**0.03**	0.17	0.21	0.34
Vigorous intensity						
none	432 (79.6)	400 (73.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤960	32 (5.9)	46(8.5)	0.64 (0.40-1.03)	0.70 (0.41-1.20)	0.70(0.41-1.20)	0.69(0.40-1.18)
>960	79 (14.5)	97 (17.9)	0.75 (0.54-1.05)	**0.67 (0.47-0.97)**	**0.68 (0.47-0.98)**	**0.64(0.44-0.93)**
P value for trend			0.04	**0.02**	**0.02**	**0.06**

NAFLD: Non-alcoholic fatty liver disease

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.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

Table S2. Association between physical activity and NAFLD in female

Variable (MET-minute/week)	Case number	Control number(%	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						
≤3010.2	58 (33.7)	57 (33.1)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
3010.2~5833.8	65 (37.8)	58(33.7)	1.10(0.66-1.83)	0.72 (0.40-1.30)	0.73 (0.40-1.32)	0.73(0.40-1.31)
>5833.8	49(28.5)	57(33.1)	0.85 (0.50-1.43)	0.71 (0.39-1.30)	0.73 (0.40-1.36)	0.72(0.39-1.33)
P value for trend			0.55	0.27	0.32	0.29
Light intensity						
≤1575	57 (33.1)	60 (34.9)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1575~3150	66(38.4)	66(38.4)	1.05 (0.64-1.73)	0.85(0.48-1.50)	0.87 (0.49-1.55)	0.88(0.50-1.57)
>3150	49 (28.5)	46 (26.7)	1.12 (0.65-1.93)	0.75(0.40-1.41)	0.77(0.41-1.48)	0.76(0.40-1.46)
P value for trend			0.68	0.36	0.44	0.41
Moderate intensity						
≤840	72 (41.9)	67 (39)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
840~1984.8	50 (29.1)	48 (27.9)	0.97 (0.58-1.63)	0.81 (0.44-1.47)	0.82 (0.45-1.51)	0.82(0.45-1.50)
>1984.8	50 (29.1)	57 (33.1)	0.82 (0.49-1.35)	0.78 (0.44-1.38)	0.80 (0.45-1.42)	0.80(0.45-1.43)
P value for trend			0.44	0.38	0.43	0.44
Vigorous intensity						
none	160 (93)	149 (86.6)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
yes	12 (7)	23 (13.4)	0.49 (0.23-1.01)	0.77 (0.34-1.76)	0.77 (0.34-1.76)	0.77(0.34-1.74)

NAFLD: Non-alcoholic fatty liver disease

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.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

TableS3. the distribution of the three energy nutrients in the case and the control in males

Variable	case	control	z	*p*
Energy intake (Kilojoule)	2456.70 (2074.46-2856.50)	2364.34 (1933.76-2789.0)	**-2.42**	**0.02**
Carbohydrate(g)	349.64 (276.77-429.11)	349.45 (261.89-427.34)	-0.90	0.37
Fat(g)	79.37 (66.86-93.67)	71.97 (61.19-84.63)	**-4.87**	**<0.001**
Protein(g)	77.96 (65.05-93.07)	75.00 (62.38-91.73)	-2.00	0.05

TableS4. the distribution of the three energy nutrients in the case and the control in females

Variable	case	control	z	*p*
Energy intake (Kilojoule)	1755.38(1540.99-2065.81)	1669.87(1468.99-2014.09)	-1.71	0.09
Carbohydrate(g)	228.70(183.64-270.82)	233.72(194.89-278.61)	-1.19	0.23
Fat(g)	67.29(58.74-80.90)	57.90(49.91-67.89)	**-5.45**	**<0.001**
Protein(g)	57.44(49.57-68.92)	57.07(47.44-68.26)	-0.40	0.69

Table S5. Association between physical activity and NAFLD in male

Variable (MET-minute/week)	Case number (%)	Control number(%)	Multivariate model 1 aOR (95% CI)	Multivariate model 2 aOR (95% CI)	Multivariate model3 aOR (95% CI)	Multivariate model 4 aOR (95% CI)
Total amount of						
≤1440	153 (41.2)	124 (33.4)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1440~3180	104 (28.0)	124(33.4)	**0.62 (0.41-0.91)**	**0.62 (0.41-0.93)**	**0.60 (0.40-0.91)**	**0.61(0.41-0.91)**
>3180	114(30.7)	123(33.2)	**0.60(0.40-0.91)**	**0.61 (0.41-0.92)**	**0.58 (0.39-0.88)**	**0.60(0.40-0.89)**
P value for trend			**0.01**	**0.02**	**0.01**	**0.01**
Light intensity						
≤525	121 (32.6)	125 (33.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
525~1500	127(34.2)	125(33.7)	1.03(0.69-1.55)	1.01(0.68-1.52)	1.05 (0.70-1.57)	1.02(0.68-1.52)
>1500	123 (33.2)	121 (32.6)	0.95(0.63-1.44)	0.96(0.64-1.45)	0.94(0.62-1.41)	0.94(0.63-1.42)
P value for trend			0.82	0.85	0.75	0.79
Moderate intensity						
None	204 (55)	170 (45.8)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤684	74 (19.9)	79 (21.3)	0.78(0.51-1.20)	0.79 (0.51-1.20)	0.79 (0.51-1.22)	0.78(0.51-1.20)
>684	93 (25.1)	122 (32.9)	**0.58(0.40-0.86)**	**0.58 (0.39-0.86)**	**0.60(0.40-0.88)**	**0.58(0.40-0.86)**
P value for trend			**0.01**	**0.01**	**0.01**	**0.01**
Vigorous intensity						
none	272 (73.3)	251 (67.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤960	28 (7.5)	35(9.4)	0.77(0.42-1.41)	0.78(0.42-1.43)	0.77(0.39-1.30)	0.76(0.41-1.39)
>960	71 (19.1)	85 (22.9)	**0.63(0.41-0.95)**	**0.64 (0.43-0.97)**	**0.61(0.40-0.92)**	**0.62(0.41-0.93)**
P value for trend			**0.02**	**0.03**	**0.02**	**0.02**

NAFLD: Non-alcoholic fatty liver disease

Multivariate model 1: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and total energy intake

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

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

Multivariate model 4: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and protein

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

Table S6. Association between physical activity and NAFLD in female

Variable (MET-minute/week)	Case number	Control number(%	Multivariate model 1 aOR (95% CI)	Multivariate model 2 aOR (95% CI)	Multivariate model3 aOR (95% CI)	Multivariate model 4 aOR (95% CI)
Total amount of						
≤3010.2	58 (33.7)	57 (33.1)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
3010.2~5833.8	65 (37.8)	58(33.7)	0.73(0.40-1.31)	0.74 (0.41-1.35)	0.80 (0.44-1.48)	0.73(0.40-1.32)
>5833.8	49(28.5)	57(33.1)	0.72(0.39-1.33)	0.75 (0.41-1.40)	0.75 (0.40-1.40)	0.74(0.40-1.36)
P value for trend			0.29	0.38	0.37	0.32
Light intensity						
≤1575	57 (33.1)	60 (34.9)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1575~3150	66(38.4)	66(38.4)	0.88(0.50-1.57)	0.87(0.49-1.56)	1.02 (0.56-1.84)	0.86(0.48-1.54)
>3150	49 (28.5)	46 (26.7)	0.76(0.40-1.46)	0.80(0.42-1.52)	0.80(0.41-1.55)	0.77(0.41-1.48)
P value for trend			0.41	0.36	0.53	0.43
Moderate intensity						
≤840	72 (41.9)	67 (39)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
840~1984.8	50 (29.1)	48 (27.9)	0.82(0.45-1.50)	0.84 (0.46-1.53)	0.87(0.47-1.61)	0.79(0.44-1.41)
>1984.8	50 (29.1)	57 (33.1)	0.80(0.45-1.43)	0.80 (0.45-1.43)	0.87 (0.48-1.56)	1.00(0.99-1.01)
P value for trend			0.44	0.45	0.63	0.42
Vigorous intensity						
none	160 (93)	149 (86.6)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
yes	12 (7)	23 (13.4)	0.77(0.34-1.74)	0.77 (0.34-1.75)	0.72 (0.31-1.66)	0.78(0.34-1.79)

NAFLD: Non-alcoholic fatty liver disease

Multivariate model 1: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and total energy intake

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

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

Multivariate model 4: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and protein

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5-6
		(*b*) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	17
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	5
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	12-13

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-14
		(*b*) Report category boundaries when continuous variables were categorized	11-13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11-13
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Complete List of Authors:	li, yangfan; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China He, Fei; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China he, yun; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, Chin pan, xinting; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China wu, yunli; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, Chin hu, zhijian; Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China lin, xu; Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China xu, shanghua; Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China Peng, Xian-E; Fujian Medical University, Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China
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The dose-response association between physical activity and non-alcoholic fatty liver disease: a case-control study in a Chinese population

YangFan Li[1¶], Fei He[1¶], Yun He[2],XinTing Pan [1], Yunli Wu[2], ZhiJian Hu [1], Xu Lin[2],

ShangHua Xu [3 *] and XianE Peng [1,2*]

[1] Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China;

[2] Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fujian, China;

[3] Department of Cardiology, Affiliated Nanping First Hospital, Fujian Medical University, Nanping, China

¶ Both authors contributed equally to this work.

* Correspondence: fmuxe@163.com (X.E.P.) or 13365998866@163.com(S.H.X.);

Word count: 3109

Running title: Exercise and non-alcoholic fatty liver disease

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- 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 (BMI),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 males. (>3180 MET-min/week vs. ≤1440 MET-min/week: OR=0.60, 95% CI=0.40-0.91, *P* for trend=0.01). In addition, both moderate- and vigorous-intensity physical activity were effective in reducing the risk of NAFLD, independent of confounding variables in males (Moderate-intensity physical activity: >684 MET-min/week vs. none: OR=0.58, 95%CI= 0.40-0.86, *P* for trend=0.01; Vigorous-intensity physical activity: >960 MET-min/week vs. none: OR=0.63, 95%CI=0.41-0.95, *P* for trend=0.02).

Conclusions: Physical activity was inversely associated with risk of NAFLD in a

dose-dependent manner in males. Vigorous- and moderate-intensity physical activity were both beneficial to NAFLD, independent of sedentary time and energy intake.

Key words: Energy intake; Metabolic Equivalent of energy ; non-alcoholic fatty liver disease; physical activity

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 NAFLD could not be precisely identified.
- This study was a case-control study , recall bias was inevitable and randomized controlled trial studies are therefore required for more accurate results.

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 encompass a broad spectrum of hepatic dysfunction ranging from simple hepatic lipid accumulation (steatosis) to non-alcoholic steatohepatitis (NASH), fibrosis, 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 recognized 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 randomized controlled trials also demonstrated that physical activity improved liver enzyme function and reduced fat accumulation. [10-13]A meta-analysis of 20 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 was 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 minutes can decrease the risk of hepatic steatosis. [15]Another study, from America, 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 center 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 .The study was were approved by the local ethics committees of Fujian Medical University (ethics number 2014096).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_{case} =508 =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 ultrasonography: [18](1) increased echogenicity of the liver near-field

region with deep attenuation of the ultrasound signal; (2) hyperechogenity of liver tissue ("bright liver"), as often compared to hypoechogenity 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 hemochromatosis. A total of 543 newly-diagnosed NAFLD patients 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 seven 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 seven 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 Metabolic Equivalent of Energy (MET). According to a standard reference, each kind of activity was assigned a specific MET

value: low-intensity physical activities were defined as <3METs, moderate-intensity activities defined as 3~6 METs and vigorous-intensity activities defined as >6METs.[21] The dose of specific physical activity was quantified by the frequency and duration and presented in the form of MET-minutes per week (MET-min/week = duration X frequency per week X 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 behaviors including smoking status, alcohol drinking, tea consumption, total energy intake.

Total energy intake was assessed by semi-quantitative food frequency questionnaire (FFQ),[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 g 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 six months. Tea consumption was defined as drinking one or more cups of tea per day

during the previous six months.

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

Body mass index (BMI) was calculated as body weight (kg)/height^2 (m^2), and classified into four categories: lean≤18.5 kg/m^2, normal: 18.6–23.9 kg/m^2, overweight: 24.0–27.9 kg/m^2, obese: ≥28.0 kg/m^2. [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(SABP) ≥140 mmHg or diastolic arterial blood pressure(DABP) ≥90 mmHg.[26]

(4) Biochemical examinations after a 12-hour overnight fast

Biochemical parameters including serum aspartate aminotransferase (AST), alanine aminotransferase(ALT), gamma-glutamyltranspeptidase(GGT), serum fasting glucose(FBG), total cholesterol(TC), triglycerides(TG), low-density lipoprotein(LDL), high-density lipoprotein(HDL).

Blood samples were collected between 8:00 and 10:00 a.m. after fasting overnight (12 h). Blood biochemical analysis was carried out by the medical laboratory department of Nanping First Affiliated Hospital of Fujian Medical University.

Statistical analysis

The chi-square 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 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.742 (68.3%) were male, 344 (3.7%) were female. 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.

Table 1. Baseline characteristics of cases and controls

	case	control		
Variable	Number(%)or median (quartiles)	Number(%)or median (quartiles)	*Z/□2*	*P*
Age (years)	48 (39-54)	48 (39-54)	-0.03	0.97
Gender			<	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/m2)			208.5	**< 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 (Kilojoule)	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)	-10.1	**< 0.001**
ALT (IU/L)	27(20.00-38.00)	20(15.00-25.00)	-11.4	**< 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)	-13.4	**< 0.001**
FBG (mmol/L)	5.37 (5.03-5.84)	5.20(4.90-5.53)	-6.16	**< 0.001**
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

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

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 (table s1). Because the prevalence of NAFLD was differed between males and females, then we used gender-specific model in the further analysis.

In male, 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=0.41-0.92, *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=0.40-0.91, *P* for trend= 0.01, Table 2).In female, there exist no relationship between physical activity and NAFLD (table s2).

We also calculated the distribution of the three energy nutrients (carbohydrate, fat and protein) in cases and controls stratified by gender (tableS3 and tableS4). After adjusting for the carbohydrate, total fat and protein, the association between physical activity and NAFLD was maintained in males(tableS5, tableS6). 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 analyzed only total energy intake in the finally multivariate logistic model.

Then we further analyzed the association between various intensities of physical activity

and the risk of NAFLD. In males, the moderate- and vigorous-intensity levels were inversely associated with the risk of NAFLD, independent of the confounding variables: (Moderate-intensity physical activity: >684MET-min/week vs. none: OR=0.58, 95% CI=0.40-0.86, *P* for trend=0.01; Vigorous-intensity physical activity: >960 MET-min/week vs. none: OR=0.63, 95% CI= 0.41-0.95, *P* for trend=0.02, Table 2).In female, there is no association between various intensity of physical activity and NAFLD (table s3).

Table 2. Association between physical activity and NAFLD in male

Variable	Case	Control	Univariate model	Multivariate model 1	Multivariate model 2	Multivariate model 3
(MET-minute/week)	number (%)	number(%)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Total amount of						
≤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(0.48-0.97)	**0.62 (0.41-0.93)**	**0.62 (0.41-0.93)**	**0.62 (0.41-0.92)**
>3180	114(30.7)	123(33.2)	0.75 (0.53-1.06)	**0.62 (0.41-0.92)**	**0.61 (0.41-0.92)**	**0.60(0.40-0.91)**
P value for trend			0.09	**0.02**	**0.02**	**0.01**
Light intensity						
≤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 (0.74-1.49)	1.00(0.67-1.49)	1.00 (0.67-1.50)	1.03(0.69-1.55)
>1500	123 (33.2)	121 (32.6)	1.05 (0.74-1.50)	0.96(0.64-1.43)	0.96(0.64-1.44)	0.95(0.63-1.44)
P value for trend			0.79	0.83	0.84	0.82
Moderate intensity						
None	204 (55)	170 (45.8)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤684	74 (19.9)	79 (21.3)	0.78 (0.54-1.14)	0.79 (0.52-1.21)	0.79 (0.51-1.21)	0.78(0.51-1.20)
>684	93 (25.1)	122 (32.9)	**0.64 (0.45-0.89)**	**0.59 (0.40-0.86)**	**0.58(0.39-0.86)**	**0.58(0.40-0.86)**
P value for trend			**0.01**	**0.01**	**0.01**	**0.01**
Vigorous intensity						
none	272 (73.3)	251 (67.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤960	28 (7.5)	35(9.4)	0.74 (0.44-1.25)	0.77 (0.42-1.42)	0.77(0.42-1.42)	0.77(0.42-1.41)
>960	71 (19.1)	85 (22.9)	0.77 (0.54-1.10)	**0.65 (0.43-0.98)**	**0.65 (0.43-0.98)**	**0.63(0.41-0.95)**
P value for trend			0.12	**0.03**	**0.03**	**0.02**

NAFLD: Non-alcoholic fatty liver disease

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.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

According to the Physical Activity Guidelines for Americans (PAGA) released by the

USDHHS[27]: more than 150 minutes of moderate-intensity physical activity per week or 75 minutes of vigorous-intensity physical activity per week is beneficial to health, we divided physical activity into different levels. The dose-response association was shown: males who underwent moderate- 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=0.43-0.92; *P* for trend=0.01; Vigorous-intensity physical activity≥1.25 hours vs. none: OR=0.66, 95% CI= 0.45-0.96; *P* for trend=0.03, Table 3).

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

Variable	Case	Control	Univariate model	Multivariate	Multivariate model 2	Multivariate model 3
(MET-minute/week	number (%)	number(%)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Moderate intensity						
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 (0.51-1.10)	0.73 (0.47-1.13)	0.72 (0.47-1.12)	0.72 (0.46-1.12)
≥2.5 hours	100 (27.0)	126(34.0)	**0.66 (0.47-0.92)**	**0.63 (0.43-0.91)**	**0.62(0.43-0.91)**	**0.63(0.43-0.92)**
P value for trend			**0.01**	**0.01**	**0.01**	**0.01**
Vigorous intensity						
None	272(73.3)	251(67.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
~1.25hours	9(2.4)	12 (3.2)	0.69 (0.29-1.67)	0.73 (0.26-2.07)	0.73 (0.26-2.07)	0.74(0.26-2.08)
≥1.25 hours	90(24.3)	108 (29.1)	0.77 (0.55-1.07)	**0.68 (0.47-0.98)**	**0.68 (0.46-0.98)**	**0.66(0.45-0.96)**
P value for trend			0.10	**0.04**	**0.04**	**0.03**

NAFLD: Non-alcoholic fatty liver disease

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.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

We explored the association between physical activity and biochemical indicators. In NAFLD patients, subjects who undergo a higher total amount of physical activity tend to have significantly lower levels of GGT (*P*=0.02). In the control population, greater physical activity

was significantly associated with greater AST (*P*=0.001) (Table 4).

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

Variable	case		control	
	Physical activity (MET-minute/week)		Physical activity (MET-minute/week)	
	Correlation	*p*	Correlation coefficient	*p*
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

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

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 Metabolic Equivalent of Energy (MET). MET is a widely-used physiological concept defined as the ratio of work metabolic rate to a standard resting metabolic rate of 1Kcal/kg·h (1 MET=3.5ml O_2/kg·min=1Kcal/kg·h).[28] Moderate-intensity physical activity corresponds to 40%~60% of VO_2 max or 4~6 METs. Vigorous-intensity physical activity corresponds to ≥60% of VO_2 max or > 6METs.[29] Since different methods are used to assess physical activity 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 males, patients with more than 3180 MET-min/week total physical activity had a 40% lower risk of NAFLD compared to those with less than 1440 MET-min/week. In addition, we also found that moderate- and vigorous-intensity physical activity were beneficial to NAFLD in males. When the dose of physical activity was divided according to the Physical Activity Guidelines for Americans (PAGA) released by the USDHHS[27] (more than 150 minutes of moderate-intensity physical activity per week or 75 minutes of vigorous-intensity physical activity per week is beneficial to health), the dose-response association was maintained. However, the relationship between physical activity and nonalcoholic fatty liver disease has not been observed in females. The gender difference observed in this study may be explained by sex hormones. Mechanism research have found that sex hormones can up-regulate insulin receptor expression and increase receptor phosphorylation protein kinase levels, thereby enhancing insulin signaling 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 VO_2 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 males with a dose of more than 5760 MET-min/week had a 31% lower risk of NAFLD compared to those with less than 498 MET-min/week. In females, 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 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. Secondly, physical activity decreases visceral adiposity, which in turn decreases free fatty acid influx to the liver. Thirdly, 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 males with NAFLD, and males 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 behaviors.[37] Other known risk factors of NAFLD are energy intake and BMI. Several previous studies have found that NAFLD patients tend to have higher energy intake, and a restricted-energy 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 seven 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. Randomized controlled trial 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 exist the possibility of postoperative blood and bile leakage, and there are sampling errors, therefore does not apply to routine screening. In 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 judgment of the operator, and there is no objective and unified quantitative index. And 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 males, with moderate- and vigorous-intensity physical activity having the greatest effect on reducing risk.

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Conflict of interests

all authors have declare: no support from any organization for the submitted work; no financial relationships with any organization that might have an interest in the submitted work in the previous three years, no other relationships or activities that could appear to have influenced the submitted work.

Contributors

YFL and FH are joint first authors. XEP obtained funding. XEP, ZJH , XL and SHX designed the study. XTP and YFL collected the data. YLW and YH were involved in data cleaning and verification. YFL and XTP analyzed the data. YFL and FH drafted the manuscript. XEP and ZJH contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript.

Data sharing

Data is stored in Department of Epidemiology and Health Statistics, Fujian Provincial Key Laboratory of Environment Factors and Cancer, School of Public Health, Fujian Medical University, Fujian, China. Data are available upon request from XianE Peng; email address:Peng;fmuxe@163.com (X.E.P.)

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Table S1. Association between physical activity and NAFLD

Variable (MET-minute/week)	Case number	Control number(%	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						
≤1620	182 (33.5)	152 (28)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1620~3943.8	180 (33.1)	200(36.8)	0.75(0.56-1.01)	0.72 (0.52-1.00)	0.72(0.52-1.01)	0.74(0.53-1.03)
>3943.8	181(33.3)	191(35.2)	0.79 (0.59-1.06)	0.75 (0.54-1.05)	0.76(0.54-1.07)	0.79(0.56-1.11)
P value for trend			0.13	0.11	0.13	0.19
Light intensity						
≤840	183 (33.7)	193 (35.5)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
840~2100	185 (34.1)	196(36.1)	1.00 (0.75-1.32)	0.97(0.70-1.34)	0.98 (0.71-1.35)	1.00(0.73-1.39)
>2100	175 (32.2)	154 (28.4)	1.20 (0.89-1.61)	1.22(0.88-1.71)	1.26(0.90-1.77)	1.32(0.94-1.86)
P value for trend			0.24	0.24	0.19	0.12
Moderate intensity						
None	223 (41.1)	193 (35.5)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤840	163 (30.0)	163 (30.0)	0.87(0.65-1.16)	0.89 (0.64-1.23)	0.89 (0.64-1.24)	0.91(0.65-1.26)
>840	157 (28.9)	187 (34.4)	**0.73 (0.55-0.97)**	0.80 (0.58-1.10)	0.81(0.59-1.12)	0.85(0.61-1.19)
P value for trend			**0.03**	0.17	0.21	0.34
Vigorous intensity						
none	432 (79.6)	400 (73.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤960	32 (5.9)	46(8.5)	0.64 (0.40-1.03)	0.70 (0.41-1.20)	0.70(0.41-1.20)	0.69(0.40-1.18)
>960	79 (14.5)	97 (17.9)	0.75 (0.54-1.05)	**0.67 (0.47-0.97)**	**0.68 (0.47-0.98)**	**0.64(0.44-0.93)**
P value for trend			0.04	**0.02**	**0.02**	**0.06**

NAFLD: Non-alcoholic fatty liver disease

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.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

Table S2. Association between physical activity and NAFLD in female

Variable (MET-minute/week)	Case number	Control number(%	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						
≤3010.2	58 (33.7)	57 (33.1)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
3010.2~5833.8	65 (37.8)	58(33.7)	1.10(0.66-1.83)	0.72 (0.40-1.30)	0.73 (0.40-1.32)	0.73(0.40-1.31)
>5833.8	49(28.5)	57(33.1)	0.85 (0.50-1.43)	0.71 (0.39-1.30)	0.73 (0.40-1.36)	0.72(0.39-1.33)
P value for trend			0.55	0.27	0.32	0.29
Light intensity						
≤1575	57 (33.1)	60 (34.9)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1575~3150	66(38.4)	66(38.4)	1.05 (0.64-1.73)	0.85(0.48-1.50)	0.87 (0.49-1.55)	0.88(0.50-1.57)
>3150	49 (28.5)	46 (26.7)	1.12 (0.65-1.93)	0.75(0.40-1.41)	0.77(0.41-1.48)	0.76(0.40-1.46)
P value for trend			0.68	0.36	0.44	0.41
Moderate intensity						
≤840	72 (41.9)	67 (39)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
840~1984.8	50 (29.1)	48 (27.9)	0.97 (0.58-1.63)	0.81 (0.44-1.47)	0.82 (0.45-1.51)	0.82(0.45-1.50)
>1984.8	50 (29.1)	57 (33.1)	0.82 (0.49-1.35)	0.78 (0.44-1.38)	0.80 (0.45-1.42)	0.80(0.45-1.43)
P value for trend			0.44	0.38	0.43	0.44
Vigorous intensity						
none	160 (93)	149 (86.6)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
yes	12 (7)	23 (13.4)	0.49 (0.23-1.01)	0.77 (0.34-1.76)	0.77 (0.34-1.76)	0.77(0.34-1.74)

NAFLD: Non-alcoholic fatty liver disease

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.

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

TableS3. the distribution of the three energy nutrients in the case and the control in males

Variable	case	control	z	*p*
Energy intake (Kilojoule)	2456.70 (2074.46-2856.50)	2364.34 (1933.76-2789.0)	**-2.42**	**0.02**
Carbohydrate(g)	349.64 (276.77-429.11)	349.45 (261.89-427.34)	-0.90	0.37
Fat(g)	79.37 (66.86-93.67)	71.97 (61.19-84.63)	**-4.87**	**<0.001**
Protein(g)	77.96 (65.05-93.07)	75.00 (62.38-91.73)	-2.00	0.05

TableS4. the distribution of the three energy nutrients in the case and the control in females

Variable	case	control	z	*p*
Energy intake (Kilojoule)	1755.38(1540.99-2065.81)	1669.87(1468.99-2014.09)	-1.71	0.09
Carbohydrate(g)	228.70(183.64-270.82)	233.72(194.89-278.61)	-1.19	0.23
Fat(g)	67.29(58.74-80.90)	57.90(49.91-67.89)	**-5.45**	**<0.001**
Protein(g)	57.44(49.57-68.92)	57.07(47.44-68.26)	-0.40	0.69

Table S5. Association between physical activity and NAFLD in male

Variable (MET-minute/week)	Case number (%)	Control number(%)	Multivariate model 1 aOR (95% CI)	Multivariate model 2 aOR (95% CI)	Multivariate model3 aOR (95% CI)	Multivariate model 4 aOR (95% CI)
Total amount of						
≤1440	153 (41.2)	124 (33.4)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1440~3180	104 (28.0)	124(33.4)	**0.62 (0.41-0.91)**	**0.62 (0.41-0.93)**	**0.60 (0.40-0.91)**	**0.61(0.41-0.91)**
>3180	114(30.7)	123(33.2)	**0.60(0.40-0.91)**	**0.61 (0.41-0.92)**	**0.58 (0.39-0.88)**	**0.60(0.40-0.89)**
P value for trend			**0.01**	**0.02**	**0.01**	**0.01**
Light intensity						
≤525	121 (32.6)	125 (33.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
525~1500	127(34.2)	125(33.7)	1.03(0.69-1.55)	1.01(0.68-1.52)	1.05 (0.70-1.57)	1.02(0.68-1.52)
>1500	123 (33.2)	121 (32.6)	0.95(0.63-1.44)	0.96(0.64-1.45)	0.94(0.62-1.41)	0.94(0.63-1.42)
P value for trend			0.82	0.85	0.75	0.79
Moderate intensity						
None	204 (55)	170 (45.8)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤684	74 (19.9)	79 (21.3)	0.78(0.51-1.20)	0.79 (0.51-1.20)	0.79 (0.51-1.22)	0.78(0.51-1.20)
>684	93 (25.1)	122 (32.9)	**0.58(0.40-0.86)**	**0.58 (0.39-0.86)**	**0.60(0.40-0.88)**	**0.58(0.40-0.86)**
P value for trend			**0.01**	**0.01**	**0.01**	**0.01**
Vigorous intensity						
none	272 (73.3)	251 (67.7)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
≤960	28 (7.5)	35(9.4)	0.77(0.42-1.41)	0.78(0.42-1.43)	0.77(0.39-1.30)	0.76(0.41-1.39)
>960	71 (19.1)	85 (22.9)	**0.63(0.41-0.95)**	**0.64 (0.43-0.97)**	**0.61(0.40-0.92)**	**0.62(0.41-0.93)**
P value for trend			**0.02**	**0.03**	**0.02**	**0.02**

NAFLD: Non-alcoholic fatty liver disease

Multivariate model 1: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and total energy intake

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

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

Multivariate model 4: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and protein

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

Table S6. Association between physical activity and NAFLD in female

Variable (MET-minute/week)	Case number	Control number(%	Multivariate model 1 aOR (95% CI)	Multivariate model 2 aOR (95% CI)	Multivariate model3 aOR (95% CI)	Multivariate model 4 aOR (95% CI)
Total amount of						
≤3010.2	58 (33.7)	57 (33.1)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
3010.2~5833.8	65 (37.8)	58(33.7)	0.73(0.40-1.31)	0.74 (0.41-1.35)	0.80 (0.44-1.48)	0.73(0.40-1.32)
>5833.8	49(28.5)	57(33.1)	0.72(0.39-1.33)	0.75 (0.41-1.40)	0.75 (0.40-1.40)	0.74(0.40-1.36)
P value for trend			0.29	0.38	0.37	0.32
Light intensity						
≤1575	57 (33.1)	60 (34.9)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
1575~3150	66(38.4)	66(38.4)	0.88(0.50-1.57)	0.87(0.49-1.56)	1.02 (0.56-1.84)	0.86(0.48-1.54)
>3150	49 (28.5)	46 (26.7)	0.76(0.40-1.46)	0.80(0.42-1.52)	0.80(0.41-1.55)	0.77(0.41-1.48)
P value for trend			0.41	0.36	0.53	0.43
Moderate intensity						
≤840	72 (41.9)	67 (39)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
840~1984.8	50 (29.1)	48 (27.9)	0.82(0.45-1.50)	0.84 (0.46-1.53)	0.87(0.47-1.61)	0.79(0.44-1.41)
>1984.8	50 (29.1)	57 (33.1)	0.80(0.45-1.43)	0.80 (0.45-1.43)	0.87 (0.48-1.56)	1.00(0.99-1.01)
P value for trend			0.44	0.45	0.63	0.42
Vigorous intensity						
none	160 (93)	149 (86.6)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
yes	12 (7)	23 (13.4)	0.77(0.34-1.74)	0.77 (0.34-1.75)	0.72 (0.31-1.66)	0.78(0.34-1.79)

NAFLD: Non-alcoholic fatty liver disease

Multivariate model 1: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and total energy intake

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

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

Multivariate model 4: adjusted for BMI, hypertension, diabetes, fasting blood glucose, sedentary time and protein

ORs = odds ratios, aOR= adjusted odds ratios, CIs = confidence intervals

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5-6
		(*b*) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	17
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	5
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	12-13

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-14
		(*b*) Report category boundaries when continuous variables were categorized	11-13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11-13
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.