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The Preventive Effect of Sustained Physical Activity on Incident Nonalcoholic Fatty Liver Disease

Min-Sun Kwak[1], Donghee Kim[1,2], Goh Eun Chung[1], Won Kim[3], Joo Sung Kim[1]

[1]Department of Internal Medicine, Healthcare Research Institute, Healthcare System Gangnam Center, Seoul National University Hospital, Seoul, Korea.

[2]Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, CA, United States.

[3]Department of Internal Medicine, Seoul Metropolitan Government Seoul National University Boramae Medical Center, Seoul, Korea.

Correspondence: Donghee Kim, MD

Department of Internal Medicine, Healthcare System Gangnam Center, Seoul National University Hospital 39th FL. Gangnam Finance Center, 737 Yeoksam-dong, Gangnam-gu, 135-984, Seoul, Korea

Tel: 82-2-2112-5574; FAX: 82-2-2112-5635

E-mail: messmd@chol.com

Running title: Preventive effect of physical activity on incident NAFLD

Abbreviations

NAFLD, Nonalcoholic fatty liver disease; PA, physical activity; MET, metabolic equivalent; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment-estimated insulin resistance; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; CT, computed tomography; HR, hazard ratio; CI, confidence interval

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Abstract

Background & Aims: Physical activity (PA) is inversely associated with nonalcoholic fatty liver disease (NAFLD) prevalence. However, few studies evaluated the effect of PA on NAFLD incidence in regard to visceral adipose tissue (VAT) and insulin resistance (IR). We investigated whether PA at baseline and change in PA during follow-up have any effect on incident NAFLD.

Methods: We enrolled subjects who underwent health screenings between 2007 and 2008 and participated in voluntary follow-up between 2011 and 2013 (median 4.42 years). Incident NAFLD was defined as NAFLD absence at baseline and presence at follow-up by ultrasonography. PA was measured using a detailed questionnaire-based metabolic equivalent at baseline and follow-up; the difference during follow-up was calculated.

Results: Of the 1,373 subjects enrolled, 288 (21.0%) developed NAFLD. Both total and leisure-time PA at baseline were inversely associated with incident NAFLD (*p* for trend=0.005 and 0.003, respectively). Decreased PA at follow-up was associated with increased incident NAFLD risk after adjusting for age, gender, body mass index, smoking, hypertension, diabetes, and diet [hazard ratio(HR) 1.45, 95% confidence interval (CI) 1.04-2.02, 4^{th} (most decreased PA) vs. 1^{st} quartile (increased PA), *p*=0.028]. This relationship was attenuated but remained statistically significant after adjustment for VAT(HR 1.48, 95% CI 1.06-2.06, 4th vs. 1st quartile) and IR(HR 1.59, 95% CI 1.11-2.27, 4th vs. 1st quartile).

Conclusions: This study shows an independent protective effect of PA at baseline on incident NAFLD after 4-year follow-up. Furthermore, sustained or increased PA had a preventive effect on incident NAFLD independent of VAT and IR.

Keywords: hepatic steatosis; development; exercise; leisure time

Key points box

- Physical activity is known to be associated with NAFLD, however, there are few studies investigating whether sustained PA can prevent incident NAFLD in the population without NAFLD (primary prevention).

- Physical activity at baseline were inversely associated with incident NAFLD during 4-year follow-up period.

- Decreased PA at follow-up was associated with an increased risk for incident NAFLD.

- Sustained or increased PA had a preventive effect on incident NAFLD independent of VAT and insulin resistance.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent liver diseases, and its prevalence has increased to 20–30% in the general population [1, 2]. NAFLD is defined as fat accumulation in more than 5% of the hepatocytes in the liver [3] and includes a spectrum from nonalcoholic fatty liver to nonalcoholic steatohepatitis to end-stage liver disease [4]. NAFLD is closely associated with components of metabolic syndrome, including dyslipidemia, central obesity, and type 2 diabetes [5]. Additionally, as the major pathogenesis of NAFLD is insulin resistance, NAFLD is considered a hepatic manifestation of metabolic syndrome [6, 7].

There has been significant effort to reduce NAFLD, and weight reduction through lifestyle modification remains the most well-established intervention [8, 9]. As increased physical activity (PA) improves metabolic comorbidities, including cardiovascular disease, diabetes, metabolic syndrome, and dyslipidemia, independent of weight loss, PA may also exert independent benefits on NAFLD [10, 11]. Previous cross-sectional studies have shown an inverse relationship between NAFLD prevalence and the various types of PA after adjusting for known risk factors [12, 13]. Several randomized controlled trials have also consistently demonstrated that PA has an effect on NAFLD regression and improvement of liver enzymes [14-18]. However, the number of enrolled patients was small, and the change in PA over time, which is an important factor in the clinical setting, was not measured in these studies [14-18]. Furthermore, there are scarce data on whether sustained PA can prevent incident NAFLD in the population without NAFLD (primary prevention).

Therefore, the aim of this longitudinal cohort study was to evaluate whether PA at baseline and the change in PA during the follow-up period have effects on incident NAFLD.

Patients and methods

Subjects and Study Design

In this study, we analyzed data from a previously described cohort [12, 19, 20]. Briefly, 5100 subjects older than the age of 18 years who performed abdominal ultrasonography, abdominal fat CT scan, and blood samplings between March 2007 and December 2008 were initially included in this cohort. Among them, we excluded 1230 subjects with at least 1 potential cause of chronic liver disease; 880 subjects with significant

alcohol consumption (>30 g/day for men and >20 g/day for women measured by detailed questionnaire including alcohol amount, frequency), [21] 280 subjects with chronic hepatitis B (determined by the presence of hepatitis B surface antigen), 56 subjects with chronic hepatitis C (determined by the presence of hepatitis C antibodies), 14 subjects with a history of other type of hepatitis (Wilson's disease, hemochromatosis, autoimmune hepatitis, and primary biliary cirrhosis determined by a detailed medical history and a questionnaire), and 99 subjects who had taken drugs which is associated with NAFLD, including tamoxifen, amiodarone, glucosteroids, valproate, methotrexate, etc. Fifty three subjects who did not answer the questionnaire about physical activity were also excluded. A total of 3,718 subjects without liver disease and/or significant alcohol consumption were initially included in the Gangnam NAFLD cohort. We further excluded 1,701 subjects who did not attend any voluntary follow-up between 2011 and 2013. This follow-up cohort included 2,017 subjects from the initial cohort who participated in a 5-year voluntary follow-up health screening performed in 2011 and 2013. Of the 2017 subjects, we excluded 642 subjects who had NAFLD based on the baseline hepatic ultrasonography and 2 subjects who did not answer the PA questions at follow-up. Finally, 1,373 subjects from the initial cohort without NAFLD were included in the final analysis (Figure 1). The median follow-up duration was 4.42 years for these 1,373 subjects. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University Hospital (2015-047-647). The need for informed consent was waived by the Institutional Review Board of Seoul National University Hospital because the researchers only accessed de-identified databases for analytical purposes.

Hepatic Ultrasonographic Examinations

The methods employed in this study have been described in detail elsewhere [12, 19, 22]. Hepatic ultrasonography was performed by experienced radiologists who were blinded to the clinical and laboratory data at the time of the procedure. Fatty liver was diagnosed by ultrasonographic findings (Acuson, Sequoia 512, Siemens, Mountain View, CA, United States) based on liver brightness, echo contrast between the hepatic and renal parenchyma, vascular blurring, and deep attenuation [23]. NAFLD was defined as the presence of fatty liver by ultrasonography without the presence of the following other possible causes of chronic liver disease: (1) significant alcohol consumption (defined as >30 g/d for men and >20 g/d for women), (2) positivity for hepatitis B surface antigen or antibodies against the hepatitis C virus, (3) other known etiologies of chronic liver disease, and (4) the use of medications that can cause fatty liver [24]. The hepatic ultrasonography conducted during the follow-up evaluations followed the same procedures and protocols using the same equipment as in the baseline examination. Follow-up hepatic ultrasonography was also performed in a blinded manner to determine the incidence of NAFLD. The outcome variable, incident NAFLD, was defined as NAFLD absence at baseline and presence at follow-up based on the hepatic ultrasonographic findings. We used a previously described method for grade of severity of NAFLD [25]. Briefly, the degree of NAFLD was graded semi-quantitatively according to the criteria described by Saadeh et al.[26]. Moderate and severe NAFLD were combined into a moderate to severe category due to the small number of severe NAFLD.

Physical Activity Questionnaire

All subjects answered questionnaires about their daily PA according to a previously described method [12]. Briefly, PA was measured using a modified Korean version of the PA questionnaire from the National Health and Nutrition Examination Survey, which employs a previously well-established metabolic equivalent (MET) quantification of PA [27-32].

The detailed PA questionnaire assessed leisure-time PA and non-leisure-time PA, including domestic PA, work-related PA, and transport-related PA. For each type of PA, the questionnaire inquired about the type of PA, frequency per week, and duration (min). The total amount of PA was defined as the sum of the leisure-time, domestic, work-related, and transport-related PA. The amount of PA was quantified according to the well-known parameter of MET-minutes based on a standard reference [27-33]. One MET is the rate of energy expenditure while at rest, and each type of PA was assigned a MET value [29]. MET-minutes per week were calculated as follows: MET value x minutes spent per week in activity. Because PA notably differed by gender, the subjects were categorized separately by gender quartiles based on the amount of PA performed. The follow-up questionnaire about daily PA was also answered by subjects, and the amount of PA was calculated as MET-minutes in the same manner. The difference in total PA was calculated as follows: difference in total PA = total PA (MET-minutes/week) at follow-up – total PA at baseline (MET-minutes/week). The difference in total PA was divided into quartiles.

Clinical and Laboratory Examinations

Each subject answered a questionnaire about past medical history and diet in addition to the questionnaire about PA. The diet questionnaire included questions regarding coffee consumption (≥ 3/day) and soft drink consumption (≥ 2/week), which are known to be associated with NAFLD [34, 35]. An anthropometric assessment, including height, body weight, waist circumference, and blood pressure, was performed as previously described [12]. The body mass index (BMI) was calculated as follows: BMI = weight (kg)/height squared (m^2). The change in waist circumference or BMI during the follow-up period was calculated by subtracting waist circumference or BMI at baseline from those at follow-up. The presence of hypertension was defined as a systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg more than twice or taking anti-hypertensive medication. The presence of diabetes was defined as either a fasting serum glucose level ≥ 126 mg/dl or taking anti-diabetic medication. Smokers were defined as those who had smoked at least one cigarette per day during the previous year.

Laboratory examinations included assessments of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), total cholesterol, triglycerides, high-density lipoprotein cholesterol, fasting glucose, fasting insulin, hepatitis B surface antigens, and antibodies to hepatitis C virus. All the biochemical evaluations were carried out in the same laboratory using standard laboratory methods. Insulin resistance was evaluated by the homeostasis model assessment-estimated insulin resistance (HOMA-IR) method as follows: HOMA-IR = fasting plasma glucose (mmol/L) x fasting plasma insulin (μIU/ml)/22.5 [36].

Measurement of Adipose Tissue Area

We measured the visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) areas of abdominal fat using computed tomography (CT) following previously described techniques [37]. Briefly, a 16-detector row CT scanner (Somatom Sensation 16; Siemens Medical Solutions, Forchheim, Germany) was used to evaluate the abdominal adipose tissue area of the subjects. The adipose tissue area was measured with commercially available CT software (Rapidia 2.8; INFINITT, Seoul, Korea) that electronically determined the adipose tissue area by setting the attenuation values for the region of interest within a range of -250 to -50 Hounsfield units.

Statistical Analysis

We used chi-square tests for the categorical variables and Student's t-test or the Mann-Whitney U-test for continuous variables to compare the baseline characteristics between the individuals who did and did not develop NAFLD. A Cox proportional hazards model was used to analyze the adjusted hazard ratio (HR) and 95% confidence interval (CI) for incident NAFLD per quartile of baseline PA and per quartile of difference in total PA between the baseline and follow-up evaluations. A two-tailed p-value < 0.05 was considered statistically significant. All the statistical analyses were conducted using IBM SPSS Statistics software ver. 23.0 (IBM Inc., Armonk, NY, USA).

Results

Of the 1,373 subjects (mean age 51.4 ± 9.3 years, males 51.6%) without NAFLD at baseline, 288 subjects had developed NAFLD by the follow-up (Figure 1). Table 1 shows the baseline characteristics of the subjects who did and did not develop NAFLD. The subjects with incident NAFLD at the follow-up included more males; current smokers; individuals with hypertension, metabolic syndrome, a higher BMI, a higher waist circumference, greater soft drink consumption; individuals with higher serum levels of AST, ALT, GGT, triglycerides, fasting glucose, and HOMA index values; and individuals with lower serum levels of HDL cholesterol and increased total, visceral, and subcutaneous adipose tissue areas. Total PA at baseline was lower in the subjects who developed NAFLD (p = 0.045). There was no significant difference in the difference in total PA between the subjects with or without incident NAFLD (p = 0.173).

In the multivariate cox-regression model adjusted for age, gender, BMI, smoking status, hypertension, diabetes, and diet (including soft drink and coffee consumption), total PA at baseline was inversely associated with incident NAFLD (p for trend = 0.005) (Table 2). When difference in waist circumference, VAT and SAT were additionally adjusted, the inverse association between total PA and incident NAFLD was maintained [HR 0.66, 95% CI 0.47-0.94, 4th quartile (highest total PA) vs. 1st quartile (lowest total PA), p for trend = 0.013]. The model adjusted for insulin resistance also showed a significant inverse association between total PA and incident NAFLD (HR 0.66, 95% CI 0.46-0.94 in the 4th quartile vs. the 1st quartile, p for trend = 0.025).

The association between leisure-time PA at baseline and incident NAFLD showed similar trends (Table 3). The leisure-time PA, reflecting moderate to vigorous activity, had an inverse relationship with incident NAFLD (HR 0.55, 95% CI 0.37-0.82 for the 4th quartile vs. the inactive group, *p* for trend = 0.003). This inverse association remained significant even after adjusting for difference in waist circumference, VAT and SAT (HR 0.59, 95% CI 0.39–0.87 for the 4th quartile vs. the inactive group, *p* for trend = 0.008) and insulin resistance (HR 0.57, 95% CI 0.37–0.87 for the 4th quartile vs. the inactive group, *p* for trend = 0.011).

Then, we analyzed the association between incident NAFLD and the change in total PA during the follow-up (Table 4). Approximately one-fourth of the subjects did increase their amount of PA, and more than half of the subjects decreased their amount of PA at the follow-up compared with baseline. When the change in total PA was divided into quartiles, the subjects who did not sustain PA activity at follow-up (quartile 4) were at a higher risk for developing incident NAFLD (HR 1.45, 95% CI 1.04-2.02 for the 4th quartile vs. 1st quartile, *p* = 0.028) based on the multivariate analysis. After difference in waist circumference, VAT and SAT were included in the model, decreased PA at follow-up was associated with newly developed NAFLD (HR 1.48, 95% CI 1.06-2.06 for the 4th quartile vs. 1st quartile, *p* = 0.021). This trend was similarly maintained when insulin resistance was included in the model (HR 1.59, 95% CI 1.11-2.27 for the 4th quartile vs. 1st quartile, *p* = 0.011).

As shown in supplementary figure 1, when we considered the severity of the incident NAFLD according to the total PA and leisure time PA amount at baseline, subjects with highest PA at baseline developed less moderate to severe NAFLD than those with sedentary lifestyle (lower PA amount), and subjects with lowest PA at baseline developed more moderate to severe NAFLD (p=0.032). The association between leisure-time PA at

baseline and severity of incident NAFLD showed similar trends (p=0.062). However, the number of subjects with moderate to severe NAFLD (n=58, 4.2%) was too small to analyze further multivariate analysis.

Discussion

The finding of this study is that various types of PA at baseline were inversely associated with incident NAFLD. Furthermore, sustained or increased PA reduces the risk of incident NAFLD, and reduced PA during follow-up predicts incident NAFLD independent of VAT and insulin resistance. To the best of our knowledge, there has been no study evaluating the effect of sustained PA as a primary prevention for incident NAFLD in adults. These results suggest the importance of PA in the primary prevention of NAFLD development. Preventing incident NAFLD is a fundamental method for reducing the future burden of NAFLD, and we suggest a role for sustained or increased PA as a primary prevention measure for NAFLD development in the general population.

The action mechanism of PA as a primary prevention measure of NAFLD is not fully understood. Possible mechanisms are based on the interaction of skeletal muscle, adipose tissue, and the liver. First, insulin sensitivity is a plausible explanation. PA is known to improve peripheral insulin sensitivity via increases in the levels of glucose transport protein and muscle glycogen synthase activity and decreases in the serum triglyceride concentration [12, 38, 39]. Indeed, the association between PA and incident NAFLD was slightly attenuated after adjustment for insulin resistance in this study, although it was still significant. This result suggests a role for insulin resistance in the association between PA and incident

NAFLD. Second, visceral adiposity is another possible mechanism linking PA and incident NAFLD [40]. PA is associated with decreased visceral adiposity, which may decrease hepatic fat infiltration by decreasing free fatty acid influx from adipose tissue to the liver [41-43]. The slight attenuation of the association after adjustment for VAT in this study implies the participation of VAT in the mechanism of PA in incident NAFLD. Third, PA improved fatty acid and glucose uptake and increased fatty acid and glucose oxidation in skeletal muscle, which in turn decreased the delivery of free fatty acids to the liver. Myokines also have important roles in the maintenance of anti-inflammatory homeostasis [44]. Further studies are warranted to elucidate the exact mechanism of PA associated with incident NAFLD.

This study has several strengths. First, this study was a large-scale longitudinal study with a sufficiently long follow-up period to evaluate the temporal association between PA and NAFLD after adjustment for metabolic risk factors. This study design made it possible to evaluate the temporal relationship of PA and incident NAFLD and to infer causality. Second, the amount of PA was quantified as standard METs. Thus, we could demonstrate the dose-response relationship of baseline PA or PA changes and incident NAFLD. Third, the change in PA was assessed in this study. We evaluated PA by a standard MET quantification at baseline and follow-up; thus, we were able to evaluate the change in PA, which is important in clinical and real-life settings. Fourth, potential confounding variables were comprehensively included in the multivariate analysis. Visceral obesity, which is suggested as an underlying mechanism of NAFLD, was measured using a direct quantitative method. Insulin resistance was also evaluated using HOMA-IR.

This study also has several limitations. First, NAFLD was diagnosed by ultrasonography, not by histology, which is the gold standard for diagnosing NAFLD.

Although, ultrasonography has inter- and intra-observer variability, and cannot detect fatty infiltration below 30%, it has strength in noninvasiveness, low cost, repeatability, and satisfactory sensitivity and specificity [26, 45]. To reduce inter- and intra-observer variation, all ultrasonographic exams were examined by board-certified radiologists using standard methods. Second, there are some concerns regarding the validation of this PA questionnaire. However, PA was measured by a modified Korean version of the PA questionnaire from the National Health and Nutrition Examination Survey, which employs a previously well-validated MET quantification of PA [27-31]. In addition, we could not discriminate independent effect of aerobic versus resistance PA on incident NAFLD due to our PA questionnaire's limitation. Previous studies consistently showed that both aerobic and resistance PA are similarly effective in reducing hepatic fat content [18, 46]. Third, the presence of NAFLD and amount of PA were evaluated two times at 2007-2008 and 2011-2013, and changes between the two periods were not considered in this study. Although, fat deposition in the liver and the amount of PA could have differed between the two periods, the general trends in fat deposition and PA changes may be reflected in the differences in the liver fat and PA questionnaire results between the two periods. Further study with multiple examinations of liver fat and PA are warranted. Fourth, we could not collect data on total calorie intake, which could contribute to the relation between PA and incident NAFLD, because only approximately 5% of subjects in our cohort were included for comprehensive 24-hour diet recall interview. Nevertheless, we did evaluate the relation PA and incident NAFLD adjusted for soft drink reflecting fructose intake and coffee intake, which are known to be associated with NAFLD. Finally, we cannot determine whether the

genetic difference, such as PNPLA3 and TM6SF2, may differently modulate the impact of PA on NAFLD because we did not have genetic data in this cohort.

In conclusion, this study demonstrates that baseline total and leisure-time PA had protective effects on incident NAFLD across a 4-year follow-up period. Furthermore, the greater the PA decrease at follow-up compared with baseline, the greater the development of incident NAFLD. This result demonstrates a primary prevention effect of PA on incident NAFLD.

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Tables

Table 1. Relationship Between Baseline Characteristics and the Development of Nonalcoholic Fatty Liver Disease. (n=1,373)

	No Development of NAFLD (n=1,085)	Development of NAFLD (n=288)	*P*-value
Baseline			
Age (years)	51.2 ± 9.4	51.9 ± 8.8	0.271
Male (%)	511 (47.1)	198 (68.8)	<0.001
Current smoker (%)	275 (25.3)	106 (36.8)	<0.001
Diabetes mellitus (%)	43 (4.0)	18 (6.3)	0.107
Hypertension (%)	157 (14.5)	66 (22.9)	0.001
Systolic blood pressure (mmHg)	114.1 ± 14.1	116.7 ± 14.8	0.006
Diastolic blood pressure (mmHg)	73.4 ± 11.1	75.2 ± 10.9	0.013
Metabolic syndrome (%)	137 (12.6)	69 (24.0)	<0.001
Body mass index (kg/m^2)	22.4 ± 2.4	23.9 ± 2.42	<0.001
Waist circumference (cm)	81.7 ± 7.0	85.8 ± 6.7	<0.001

AST (IU/L)	21.8 ± 7.4	22.9 ± 7.8	0.034
ALT (IU/L)	20.5 ± 13.9	25.1 ± 15.2	<0.001
GGT (IU/L)	24.6 ± 20.8	34.3 ± 30.6	<0.001
Cholesterol (mg/dL)	190.6 ± 31.2	192.4 ± 33.4	0.376
Triglycerides (mg/dL)	91.1 ± 41.9	113.9 ± 61.4	<0.001
HDL cholesterol (mg/dL)	57.7 ± 13.8	52.1 ± 12.4	<0.001
Fasting glucose (mg/dL)	91.5 ± 14.5	96.4 ± 16.6	<0.001
HOMA index	1.76 ± 0.84	2.13 ± 1.03	<0.001
TAT (cm^2)	227.7 ± 78.6	266.9 ± 76.5	<0.001
VAT (cm^2)	88.7 ± 43.7	119.8 ± 47.2	<0.001
SAT (cm^2)	139.0 ± 55.0	147.2 ± 51.2	0.023
Soft drink consumption (%)	116 (10.7)	44 (15.3)	0.038
Coffee consumption (%)	324 (29.9)	101 (35.1)	0.099
Total PA (MET-min/week)	1002 (456, 1695)	852 (327, 1575)	0.045
Leisure-time PA (MET-min/week)	612 (0, 1212)	513 (0, 1080)	0.110
Difference between follow-up and baseline			
Diff of BMI (kg/m^2)	-0.2 (-0.8, -2.8)	0.1 (-0.5, 2.7)	<0.001
Diff of Waist circumference (cm)	-0.2 (-2.8, 2.0)	0.8 (-1.5, 2.7)	<0.001
Diff of total PA (MET-min/week)	-228 (-1029, 411)	-120 (-783, 361)	0.173

NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; TAT, total adipose tissue area; VAT, visceral adipose tissue area; SAT, subcutaneous adipose tissue area; PA, physical activity.

Data are shown as the mean ± SD or median (IQR).

Table 2. Univariate and Multivariate Hazard Ratios of Risk Factors for Incident NAFLD According to the Spectrum of Total Physical Activity at Baseline.

	Univariate Model		Multivariate Model 1		Multivariate Model 2		Multivariate Model 3	
Variable	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
MET-min/week Quartile								
Quartile 1[†]	1	0.003[*]	1	0.005[*]	1	0.013[*]	1	0.025[*]
Quartile 2	0.82 (0.61-1.11)	0.197	0.88 (0.65-1.20)	0.414	0.85 (0.62-1.17)	0.316	0.79 (0.56-1.10)	0.161
Quartile 3	0.62 (0.44-0.88)	0.007	0.69 (0.48-0.99)	0.042	0.71 (0.50-1.02)	0.066	0.75 (0.51-1.10)	0.135
Quartile 4	0.65 (0.47-0.90)	0.009	0.64 (0.46-0.91)	0.012	0.66 (0.47-0.94)	0.020	0.66 (0.46-0.94)	0.022
Difference in WC (per cm)					1.06 (1.03-1.09)	<0.001	1.06 (1.03-1.10)	<0.001

NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; MET, metabolic equivalent; WC, waist circumference

The multivariate model 1 was adjusted for age, gender, body mass index, smoking, hypertension, diabetes, soft drink consumption and coffee consumption.

The multivariate model 2 was adjusted for change in waist circumference during follow-up, visceral adipose tissue area and subcutaneous tissue area in addition to model 1.

The multivariate model 3 was adjusted for change in HOMA-IR index in addition to model 2.

[*] *P* value for the test of trend of odds

[†] Physical activity

Quartile 1, 0-414; quartile 2, 435-995; quartile 3, 996-1740; quartile 4, ≥1741 MET-minutes/week in men

Quartile 1, 0-248; quartile 2, 252-828; quartile 3, 834-1530; quartile 4, ≥1533 MET-minutes/week in women

Table 3. Univariate and Multivariate Hazard Ratios of Risk Factors for Incident NAFLD According to the Spectrum of Leisure-Time Physical Activity at Baseline.

	Univariate Model		Multivariate Model 1		Multivariate Model 2		Multivariate Model 3	
Variable	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
MET-min/week Quartile								
Inactive	1	0.014*	1	0.003*	1	0.008*	1	0.011*
Quartile 1†	0.98 (0.70-1.37)	0.905	0.90 (0.64-1.27)	0.551	0.92 (0.65-1.30)	0.641	0.85 (0.58-1.24)	0.399
Quartile 2	0.85 (0.61-1.18)	0.332	0.85 (0.60-1.18)	0.326	0.86 (0.61-1.20)	0.363	0.86 (0.60-1.23)	0.400
Quartile 3	0.80 (0.56-1.15)	0.225	0.74 (0.52-1.08)	0.115	0.77 (0.54-1.12)	0.171	0.76 (0.51-1.13)	0.170
Quartile 4	0.64 (0.44-0.94)	0.021	0.55 (0.37-0.82)	0.003	0.59 (0.39-0.87)	0.008	0.57 (0.37-0.87)	0.010
Difference in WC (per cm)					1.06 (1.03-1.09)	<0.001	1.06 (1.03-1.10)	<0.001

NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; MET, metabolic equivalent; WC, waist circumference

The multivariate model 1 was adjusted for age, gender, body mass index, smoking, hypertension, diabetes, soft drink consumption and coffee consumption.

The multivariate model 2 was adjusted for change in waist circumference during follow-up, visceral adipose tissue area and subcutaneous tissue area in addition to model 1.

The multivariate model 3 was adjusted for HOMA-IR index in addition to model 2.

**P* value for the test of trend of odds

†Physical activity

Quartile 1, 10-547; quartile 2, 552-996; quartile 3, 1004-1500; quartile 4, ≥1504 MET-minutes/week in men

Quartile 1, 68-480; quartile 2, 486-900; quartile 3, 912-1365; quartile 4, ≥1368 MET-minutes/week in women

Table 4. Multivariate Analyses of the Risk for Incident NAFLD in Subjects without NAFLD at Baseline According to the Difference in Total Physical Activity.

	Univariate Model		Multivariate Model 1		Multivariate Model 2		Multivariate Model 3	
Variable	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
MET-min/week Quartile								
Quartile 1[†]	1		1		1		1	
Quartile 2	0.91 (0.64-1.28)	0.578	0.93 (0.66-1.32)	0.695	0.92 (0.64-1.30)	0.616	0.95 (0.65-1.38)	0.773
Quartile 3	1.30 (0.94-1.79)	0.120	1.35 (0.97-1.87)	0.074	1.34 (0.96-1.87)	0.087	1.21 (0.84-1.73)	0.307
Quartile 4	1.51 (1.09-2.09)	0.014	1.45 (1.04-2.02)	0.028	1.48 (1.06-2.06)	0.021	1.59 (1.11-2.27)	0.011
Difference in WC (per cm)					1.06 (1.03-1.10)	<0.001	1.07 (1.03-1.10)	<0.001

NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; MET, metabolic equivalent; WC, waist circumference

The multivariate model 1 was adjusted for age, gender, body mass index, smoking, hypertension, diabetes, soft drink consumption and coffee consumption.

The multivariate model 2 was adjusted for change in waist circumference during follow-up, visceral adipose tissue area and subcutaneous tissue area in addition to model 1.

The multivariate model 3 was adjusted for HOMA-IR index in addition to model 2.

[†]Physical activity

Quartile 1, ≥564.4; quartile 2, 564.4- -165.0; quartile 3, -165.0- -991.5; quartile 4, ≤-991.5 MET-minutes/week in men

Quartile 1, ≥238.2; quartile 2, 238.2- -247.8; quartile 3, -247.8- -990.0; quartile 4, ≤-990.0 MET-minutes/week in women

Figure legend

Figure 1. Flow diagram of subjects in this study

Supplementary figure

Supplementary figure 1. A) Percentage of incident NAFLD severity according to the total physical activity amount B) Percentage of incident NAFLD severity according to the leisure-time physical activity amount

Fig. 1.