

Supporting information

Low heart rate variability from ten-second electrocardiograms is associated with development of non-alcoholic fatty liver disease

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Supplementary Table 1. Mediation analysis of the association between heart rate variability and the development of hepatic steatosis (HS) or HS with intermediate-to-high probability of advanced fibrosis fibrosis-4 score and NAFLD fibrosis score

Quintiles of heart rate variability	Multivariable-adjusted HR ^a (95% CI)			
	Model 1 (including BMI)	Model 2 (including HOMA-IR)	Model 3 (including hsCRP)	Model 4 (including BMI, HOMA-IR, and hsCRP)
Incident HS				
RMSSD quintiles				
Q1	1.25 (1.20-1.30)	1.23 (1.18-1.28)	1.33 (1.28-1.39)	1.13 (1.08-1.17)
Q2	1.16 (1.11-1.20)	1.15 (1.11-1.20)	1.21 (1.17-1.26)	1.08 (1.04-1.13)
Q3	1.11 (1.07-1.16)	1.10 (1.05-1.14)	1.14 (1.10-1.19)	1.07 (1.02-1.11)
Q4	1.05 (1.00-1.09)	1.04 (1.00-1.08)	1.06 (1.02-1.11)	1.02 (0.98-1.06)
Q5	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
<i>P for trend</i>	< 0.001	< 0.001	< 0.001	< 0.001
HS plus FIB-4 $\geq$ 1.3				
RMSSD quintiles				
Q1	1.20 (0.98-1.46)	1.22 (1.00-1.50)	1.28 (1.05-1.57)	1.14 (0.93-1.39)
Q2	1.17 (0.95-1.43)	1.20 (0.98-1.48)	1.24 (1.01-1.53)	1.13 (0.92-1.39)
Q3	1.16 (0.94-1.43)	1.19 (0.96-1.46)	1.21 (0.98-1.49)	1.14 (0.92-1.41)
Q4	0.98 (0.78-1.23)	0.98 (0.78-1.23)	0.99 (0.79-1.24)	0.97 (0.78-1.22)
Q5	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
<i>P for trend</i>	0.022	0.010	0.002	0.093
HS plus NFS $\geq$ -1.455				
RMSSD quintiles				
Q1		1.27 (1.08-1.51)	1.42 (1.20-1.68)	1.23 (1.04-1.45)
Q2		1.13 (0.95-1.34)	1.22 (1.03-1.45)	1.11 (0.93-1.32)
Q3		1.17 (0.98-1.40)	1.23 (1.03-1.46)	1.16 (0.97-1.38)
Q4		1.05 (0.87-1.26)	1.07 (0.89-1.29)	1.04 (0.87-1.25)
Q5		1.00 (reference)	1.00 (reference)	1.00 (reference)
<i>P for trend</i>		0.002	< 0.001	0.010

^aEstimated from parametric proportional hazard models. Multivariable model 1 was adjusted for age, sex, study centre, year of screening exam, smoking, alcohol consumption, physical activity, depression, sleep duration, education level, total energy intake, SBP, and BMI.; model 2: model 1 plus adjustment for HOMA-IR instead of BMI; model 3: model 1 plus adjustment for hs-CRP instead of BMI; and model 4: model 1 plus adjustment for HOMA-IR and hs-CRP.

BMI, Body mass index; CI, Confidence interval; FIB-4, Fibrosis 4 score; HOMA-IR, Homeostasis model assessment of insulin resistance; HR, Hazard ratio; HS, Hepatic steatosis; hsCRP, High-sensitivity C-reactive protein; PY, Person-year; RMSSD, Root mean square of successive differences in RR intervals; SDNN, Standard deviation of normal-to-normal intervals; SBP, Systolic blood pressure

Supplementary Table 2. Hazard ratios (95% CI) for the development of hepatic steatosis (HS) or HS with intermediate-to-high probability of advanced fibrosis measured by FIB-4 score, according to heart rate variability, among participants without obesity

Quintiles of heart rate variability	Non-obese participants (n = 140,274)	
	Multivariable-adjusted HR ^a (95% CI) for development of HS	Multivariable-adjusted HR ^a (95% CI) for development of HS with intermediate-to-high FIB-4
SDNN quintiles		
Q1	1.30 (1.24-1.36)	1.25 (1.00-1.56)
Q2	1.16 (1.11-1.22)	1.26 (1.01-1.58)
Q3	1.09 (1.04-1.14)	1.01 (0.80-1.29)
Q4	1.02 (0.97-1.07)	1.07 (0.84-1.36)
Q5	1.00 (reference)	1.00 (reference)
<i>P for trend</i>	< 0.001	0.013
RMSSD quintiles		
Q1	1.41 (1.35-1.48)	1.39 (1.10-1.75)
Q2	1.24 (1.19-1.30)	1.19 (0.94-1.51)
Q3	1.14 (1.09-1.20)	1.20 (0.94-1.52)
Q4	1.08 (1.03-1.14)	0.94 (0.73-1.22)
Q5	1.00 (reference)	1.00 (reference)
<i>P for trend</i>	< 0.001	< 0.001

^aEstimated from parametric proportional hazard models. The multivariable model was adjusted for age, sex, study centre, year of screening examination, smoking, alcohol consumption, physical activity, depression, sleep duration, education level, total energy intake, and SBP.

HR, Hazard ratio; CI, Confidence interval; HS, Hepatic steatosis; FIB-4, Fibrosis 4 score; SDNN, Standard deviation of normal-to-normal intervals; RMSSD, Root mean square of successive differences in RR intervals

Supplementary Table 3. The incidence of non-alcoholic fatty liver disease (NAFLD) by heart rate variability when NAFLD was defined as a diagnosis of exclusion during follow-up

Heart rate variability quintiles	PY	Incident cases	Incidence density (/10 ³ PY)	Age- and sex-adjusted HR (95% CI)	Multivariable-adjusted HR ^a (95% CI)	HR (95% CI) ^b in a model with time-dependent variables
SDNN						
Q1	115,340	6,416	55.6	1.38 (1.33-1.44)	1.32 (1.26-1.37)	1.31 (1.26-1.37)
Q2	123,039	5,594	45.5	1.22 (1.17-1.27)	1.20 (1.15-1.25)	1.20 (1.15-1.25)
Q3	125,377	5,081	40.5	1.12 (1.08-1.17)	1.12 (1.08-1.17)	1.10 (1.06-1.15)
Q4	125,365	4,613	36.8	1.04 (1.00-1.09)	1.04 (1.00-1.08)	1.04 (1.00-1.09)
Q5	125,368	4,327	34.5	1.00 (reference)	1.00 (reference)	1.00 (reference)
<i>P for trend</i>				< 0.001	< 0.001	< 0.001
RMSSD						
Q1	114,083	6,795	59.6	1.50 (1.44-1.57)	1.42 (1.37-1.48)	1.44 (1.38-1.51)
Q2	122,922	5,746	46.7	1.29 (1.24-1.35)	1.27 (1.22-1.32)	1.26 (1.20-1.31)
Q3	125,903	5,077	40.3	1.17 (1.12-1.22)	1.16 (1.11-1.21)	1.18 (1.13-1.24)
Q4	125,806	4,511	35.9	1.08 (1.03-1.13)	1.08 (1.03-1.13)	1.08 (1.03-1.13)
Q5	125,776	3,902	31.0	1.00 (reference)	1.00 (reference)	1.00 (reference)
<i>P for trend</i>				< 0.001	< 0.001	< 0.001

^aEstimated from parametric proportional hazard models. The multivariable model was adjusted for age, sex, centre, year of screening examination, smoking, alcohol consumption, physical activity, depressive symptoms, sleep duration, education level, total energy intake, and systolic blood pressure.

^bEstimated from parametric proportional hazard models with quintiles of each heart rate variability (SDNN and RMSSD), smoking, alcohol consumption, physical activity, depressive symptoms, sleep duration, total energy intake, and systolic blood pressure as time-dependent categorical variables; and baseline age, sex, centre, year of screening exam, and education level as time-fixed variables.

CI, confidence interval; HR, hazard ratio; PY, person-year; RMSSD, root mean square of successive differences in RR intervals; SDNN, standard deviation of normal-to-normal intervals

Supplementary Table 4. Hazard ratios (95% CI) for the development of non-alcoholic fatty liver disease (NAFLD) with an intermediate-to-high probability of advanced fibrosis based on fibrosis-4 score and NAFLD fibrosis score by heart rate variability when NAFLD was defined as a diagnosis of exclusion during follow-up

Heart rate variability quintiles	For development of HS with an intermediate-to-high probability of advanced fibrosis based on fibrosis-4 score			For development of HS with an intermediate-to-high probability of advanced fibrosis based on NAFLD fibrosis score		
	Incidence density (/10 ³ PY)	Multivariable-adjusted HR ^a (95% CI)	HR (95% CI) ^b in a model with time-dependent variables	Incidence density (/10 ³ PY)	Multivariable-adjusted HR ^a (95% CI)	HR (95% CI) ^b in a model with time-dependent variables
SDNN						
Q1	2.5	1.12 (0.92-1.37)	1.47 (1.17-1.85)	4.1	1.37 (1.16-1.62)	1.57 (1.31-1.89)
Q2	2.1	1.25 (1.02-1.53)	1.44 (1.13-1.82)	2.8	1.24 (1.05-1.47)	1.39 (1.15-1.68)
Q3	1.6	1.12 (0.91-1.38)	1.41 (1.10-1.80)	2.2	1.13 (0.95-1.35)	1.27 (1.04-1.54)
Q4	1.3	1.03 (0.83-1.28)	1.39 (1.07-1.79)	1.9	1.04 (0.87-1.25)	1.17 (0.95-1.44)
Q5	1.1	1.00 (reference)	1.00 (reference)	1.6	1.00 (reference)	1.00 (reference)
<i>P for trend</i>		0.087	0.008		< 0.001	< 0.001
RMSSD						
Q1	2.8	1.3 (1.05-1.6)	1.69 (1.33-2.16)	4.4	1.46 (1.23-1.74)	1.68 (1.38-2.03)
Q2	1.9	1.23 (0.99-1.52)	1.48 (1.15-1.90)	2.8	1.24 (1.04-1.48)	1.35 (1.11-1.65)
Q3	1.7	1.26 (1.01-1.56)	1.41 (1.09-1.84)	2.4	1.28 (1.07-1.53)	1.26 (1.02-1.55)
Q4	1.2	1.02 (0.81-1.29)	1.37 (1.04-1.80)	1.8	1.09 (0.90-1.32)	1.23 (0.99-1.53)
Q5	1.0	1.00 (reference)	1.00 (reference)	1.4	1.00 (reference)	1.00 (reference)
<i>P for trend</i>		0.004	< 0.001		< 0.001	< 0.001

^aEstimated from parametric proportional hazard models. The multivariable model was adjusted for age, sex, study centre, year of screening examination, smoking, alcohol consumption, physical activity, depressive symptoms, sleep duration, education level, total energy intake, and systolic blood pressure.

^bEstimated from parametric proportional hazard models with quintiles of each heart rate variability (SDNN, RMSSD), smoking, alcohol consumption, physical activity, depressive symptoms, sleep duration, total energy intake, and systolic blood pressure as time-dependent categorical variables; and baseline age, sex, centre, year of screening exam, and education level as time-fixed variables.

CI, confidence interval; HR, hazard ratio; HS, hepatic steatosis; PY, person-year; RMSSD, root mean square of successive differences in RR intervals; SDNN, standard deviation of normal-to-normal intervals

Supplementary Table 5. Incidence of high Hepatic Steatosis Index (>36) heart rate variability

Heart rate variability quintiles	PY	Incident cases	Incidence density (/10 ³ PY)	Age sex adjusted HR (95% CI)	Multivariable-adjusted HR ^a (95% CI)		HR (95% CI) ^b in a model with time-dependent variables
					Model 1	Model 2	
SDNN							
Q1	109,751	3,042	27.7	1.33 (1.26-1.40)	1.30 (1.23-1.37)	1.27 (1.19-1.34)	1.36 (1.30-1.43)
Q2	118,321	2,898	24.5	1.19 (1.13-1.26)	1.19 (1.13-1.26)	1.17 (1.11-1.24)	1.22 (1.17-1.28)
Q3	121,046	2,705	22.3	1.09 (1.03-1.15)	1.10 (1.04-1.16)	1.09 (1.03-1.15)	1.14 (1.08-1.19)
Q4	121,320	2,637	21.7	1.05 (0.99-1.11)	1.05 (1.00-1.11)	1.05 (0.99-1.11)	1.07 (1.01-1.12)
Q5	121,699	2,554	21.0	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
<i>P for trend</i>				< 0.001	< 0.001	< 0.001	< 0.001
RMSSD							
Q1	108,262	3,210	29.7	1.41 (1.33-1.49)	1.37 (1.29-1.44)	1.37 (1.29-1.46)	1.40 (1.33-1.47)
Q2	117,549	2,944	25.0	1.24 (1.17-1.31)	1.24 (1.17-1.31)	1.24 (1.17-1.32)	1.31 (1.24-1.37)
Q3	122,031	2,695	22.1	1.10 (1.04-1.16)	1.11 (1.05-1.17)	1.11 (1.05-1.17)	1.15 (1.09-1.21)
Q4	121,996	2,612	21.4	1.07 (1.02-1.14)	1.09 (1.03-1.15)	1.09 (1.03-1.15)	1.06 (1.01-1.12)
Q5	122,300	2,375	19.4	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
<i>P for trend</i>				< 0.001	< 0.001	< 0.001	< 0.001

^aEstimated from parametric proportional hazard models. The multivariable model was adjusted for age, sex, centre, year of screening examination, smoking, alcohol consumption, physical activity, depressive symptoms, sleep duration, education level, total energy intake, and systolic blood pressure.

^bEstimated from parametric proportional hazard models with quintiles of each heart rate variability (SDNN and RMSSD), smoking, alcohol consumption, physical activity, depressive symptoms, sleep duration, total energy intake, and systolic blood pressure as time-dependent categorical variables; and baseline age, sex, centre, year of screening exam, and education level as time-fixed variables.

CI, confidence interval; HR, hazard ratio; PY, person-year; RMSSD, root mean square of successive differences in RR intervals; SDNN, standard deviation of normal-to-normal intervals

Supplementary Table 6. Hazard ratio (95% CI) of hepatic steatosis by other covariates

	Crude HR (95% CI)	Multivariate-adjusted HR (95% CI)
Age per 5-yr increment	1.15 (1.14-1.16)	1.12 (1.11-1.13)
SBP per 5 mmHg increment	1.19 (1.18-1.19)	1.09 (1.09-1.10)
Sex		
Female	1.00 (reference)	1.00 (reference)
Male	3.26 (3.18-3.34)	2.60 (2.51-2.69)
Alcohol intake		
0 g/day	1.00 (reference)	1.00 (reference)
<10 g/day	1.13 (1.08-1.17)	0.93 (0.90-0.97)
10-19.9 g/day	1.80 (1.73-1.88)	0.98 (0.93-1.02)
≥20 g/day	2.43 (2.31-2.56)	0.93 (0.88-0.98)
Smoking		—
Never	1.00 (reference)	1.00 (reference)
Ever	1.83 (1.78-1.89)	0.98 (0.95-1.01)
Current	2.58 (2.50-2.66)	1.23 (1.19-2.28)
Physical activity		
Inactive	1.00 (reference)	1.00 (reference)
Minimal	1.18 (1.14-1.21)	1.00 (0.98-1.03)
HEPA	1.13 (1.09-1.17)	0.98 (0.94-1.01)
Total energy intake		
Q1	1.00 (reference)	1.00 (reference)
Q2	1.06 (1.00-1.12)	0.97 (0.92-1.03)
Q3	1.18 (1.11-1.25)	1.00 (0.94-1.06)
Q4	1.21 (1.14-1.28)	0.99 (0.93-1.04)
Q5	1.34 (1.27-1.42)	1.03 (0.98-1.09)
Educational level		
<12 years	1.00 (reference)	1.00 (reference)
≥ 12 years	1.07 (1.03-1.11)	0.90 (0.87-0.94)
Sleep duration		
≤6 hours	1.00 (reference)	1.00 (reference)
>6-7 hours	0.96 (0.93-1.00)	0.91 (0.87-0.94)
>7-8 hours	0.79 (0.76-0.82)	0.84 (0.80-0.87)
>8-9 hours	0.61 (0.58-0.64)	0.82 (0.78-0.86)
≥ 9 hours	0.49 (0.44-0.53)	0.80 (0.73-0.87)
CESD-score		
<8	1.00 (reference)	1.00 (reference)
8-15	0.96 (0.93-0.99)	1.03 (1.00-1.06)
16-24	0.82 (0.78-0.86)	1.04 (0.99-1.09)
≥25	0.74 (0.69-0.81)	1.06 (0.98-1.14)

^aEstimated from parametric proportional hazard models. The multivariate models included heart rate variability (SDNN and RMSSD), centre, year of screening examination, and all other variables listed for the model.

CI, confidence interval

Supplementary Table 7. Baseline characteristics of study participants by SDNN quintiles

Characteristics	SDNN quintiles					<i>p</i> for trend
	Q1	Q2	Q3	Q4	Q5	
Number	23,943	30,751	31,054	31,227	31,311	
Age (years) ^a	39.2 (7.4)	37.3 (6.4)	36.1 (6.0)	35.1 (5.7)	33.9 (5.5)	< 0.001
Men (%)	41.6	37.4	36.2	35.9	36.3	< 0.001
Current smoker (%)	15.2	14.2	13.2	13.5	13.4	< 0.001
Alcohol intake (%) ^c	27.2	24.8	24.2	24.2	24.2	< 0.001
HEPA (%)	13.3	13.2	14.0	15.3	16.5	< 0.001
High education (%) ^d	87.9	86.8	88.9	88.6	86.0	< 0.001
Depression (%) ^e	11.6	11.4	12.0	12.4	12.1	< 0.001
Sleep duration (hours) ^a	6.59 (1.13)	6.63 (1.14)	6.63 (1.14)	6.64 (1.14)	6.64 (1.16)	< 0.001
Systolic BP (mmHg) ^a	107.4 (12.2)	105.1 (11.4)	104.2 (11.1)	103.7 (11.0)	103.1 (11.0)	< 0.001
Body mass index (kg/m ²)	22.3 (2.7)	21.9 (2.6)	21.7 (2.6)	21.6 (2.6)	21.5 (2.5)	< 0.001
HOMA-IR ^c	1.09 (0.74-1.54)	1.02 (0.69-1.45)	1.01 (0.68-1.44)	1.00 (0.68-1.42)	0.96 (0.66-1.37)	< 0.001
hsCRP (mg/L) ^c	0.4 (0.2-0.8)	0.3 (0.2-0.6)	0.3 (0.2-0.6)	0.3 (0.2-0.6)	0.3 (0.2-0.6)	< 0.001
Total energy intake ^{b,f}	1494.4 (1139.6-1877.2)	1487.2 (1132.6-1868.6)	1477.9 (1124.9-1859.5)	1479.7 (1114.6-1876.7)	1470.4 (1107.0-1870.0)	< 0.001

Data are expressed as ^amean (standard deviation), ^bmedian (interquartile range), or percentage.

^c≥20 g/day; ^d≥college graduate; ^e≥16 of CES-D score; ^famong 108,475 participants with plausible estimated energy intake levels (within three standard deviations from the log-transformed mean energy intake)

Supplementary Table 8. Baseline characteristics of study participants by RMSSD quintiles

Characteristics	RMSSD quintiles					P for trend
	Q1	Q2	Q3	Q4	Q5	
Number	29,712	30,800	31,086	31,272	31,416	
Age (years) ^a	39.2 (7.4)	37.3 (6.5)	36.2 (6.1)	35.1 (5.7)	33.8 (5.4)	< 0.001
Men (%)	45.4	39.1	36.4	35.0	31.8	< 0.001
Current smoker (%)	15.8	14.7	13.4	13.1	12.5	< 0.001
Alcohol intake (%) ^c	28.4	25.5	24.2	23.7	23.0	< 0.001
HEPA (%)	12.8	13.2	13.9	15.1	17.2	< 0.001
High education (%) ^d	84.3	85.6	85.6	86.8	85.6	< 0.001
Depression (%) ^e	11.0	11.3	11.9	12.6	13.9	< 0.001
Sleep duration (hours) ^a	6.58 (1.12)	6.62 (1.13)	6.64 (1.14)	6.64 (1.15)	6.65 (1.17)	< 0.001
Systolic BP (mmHg) ^a	108.5 (12.3)	105.4 (11.3)	104.2 (11.0)	103.3 (10.8)	102.2 (10.6)	< 0.001
Body mass index (kg/m ²)	22.4 (2.7)	22.0 (2.6)	21.7 (2.6)	21.5 (2.5)	21.4 (2.5)	< 0.001
HOMA-IR ^c	1.12 (0.75-1.59)	1.04 (0.71-1.47)	1.01 (0.68-1.43)	0.98 (0.67-1.40)	0.94 (0.64-1.35)	< 0.001
hsCRP (mg/L) ^c	0.4 (0.2-0.8)	0.3 (0.2-0.7)	0.3 (0.2-0.6)	0.3 (0.2-0.6)	0.3 (0.2-0.5)	< 0.001
Total energy intake ^{b,f}	1502.1 (1150.4-1880.2)	1487.3 (1139.1-1871.1)	1480.8 (1120.5-1868)	1477.9 (1116.9-1877.2)	1459.5 (1093.3-1858.9)	< 0.001

Data are expressed as ^amean (standard deviation), ^bmedian (interquartile range), or percentage.

^c≥10 g/day; ^d≥ college graduate; ^e≥16 of CES-D score; ^famong 108,475 participants with plausible estimated energy intake levels (within three standard deviations from the log-transformed mean energy intake)