

**Long or irregular menstrual cycles and risk of prevalent and incident non-alcoholic fatty liver disease**

**Supplemental Material**

**Supplemental Table 1. Age-adjusted mean values (95% CI) and proportions (95% CI) of baseline characteristics by menstrual cycle category for the cohort study (n=51,118)**

Characteristics	Menstrual cycle (days)					<i>p</i> for trend
	<21	21–25	26–30	31–39	≥40 or irregular	
Number	587	3,127	26,327	7,427	13,650	
Age (years)	31.6 (31.3-31.9)	33.7 (33.6-33.8)	33.2 (33.1-33.2)	32.2 (32.1-32.2)	32.4 (32.3-32.5)	<0.001
Seoul center (%)	48.0 (44.0-52.1)	54.0 (52.2-55.7)	53.8 (53.2-54.4)	54.8 (53.7-56.0)	51.9 (51.1-52.8)	0.021
Current smoker (%)	3.9 (2.3-5.5)	1.7 (1.2-2.1)	1.6 (1.4-1.7)	1.3 (1.0-1.5)	1.8 (1.6-2.1)	0.845
Alcohol intake (%) <sup>a</sup>	14.1 (11.4-16.8)	12.3 (11.1-13.5)	11.8 (11.4-12.2)	11.5 (10.8-12.2)	12.6 (12.1-13.2)	<0.001
HEPA (%)	14.4 (11.6-17.3)	11.8 (10.7-12.9)	11.0 (10.6-11.3)	10.2 (9.5-10.9)	12.0 (11.5-12.6)	0.226
High education level (%) <sup>b</sup>	60.7 (56.8-64.5)	79.4 (78.0-80.9)	85.2 (84.8-85.7)	88.3 (87.6-89.0)	81.9 (81.3-82.5)	0.111
Hypertension (%)	1.7 (0.6-2.7)	1.0 (0.7-1.4)	0.9 (0.8-1.0)	0.9 (0.7-1.1)	1.2 (1.0-1.4)	0.132
Diabetes (%)	0.2 (-0.2-0.5)	0.4 (0.2-0.6)	0.2 (0.1-0.3)	0.1 (0.1-0.2)	0.4 (0.3-0.5)	0.061
History of CVD (%)	0.4 (-0.1-0.8)	0.2 (0.0-0.3)	0.2 (0.2-0.3)	0.2 (0.1-0.3)	0.3 (0.2-0.4)	0.352
Medication for dyslipidemia (%)	0.2 (-0.2-0.6)	0.2 (0.1-0.4)	0.1 (0.1-0.1)	0.1 (0.0-0.2)	0.2 (0.1-0.2)	0.260
Early menarche (%)	5.1 (3.4-6.8)	8.4 (7.4-9.4)	8.8 (8.5-9.2)	7.6 (7.0-8.2)	6.5 (6.1-6.9)	<0.001
Parity (%)	59.6 (56.0-63.1)	59.1 (57.6-60.6)	60.0 (59.5-60.5)	58.8 (57.9-59.7)	59.6 (58.9-60.3)	0.400
Obesity (%) <sup>c</sup>	7.4 (5.2-9.5)	5.6 (4.8-6.4)	5.4 (5.2-5.7)	5.1 (4.6-5.6)	6.3 (5.9-6.7)	0.014
Body mass index (kg/m <sup>2</sup> )	21.2 (21.0-21.4)	20.8 (20.7-20.8)	20.8 (20.7-20.8)	20.7 (20.7-20.8)	20.8 (20.7-20.8)	0.260
Glucose (mg/dl)	89.7 (89.1-90.3)	89.5 (89.2-89.7)	89.3 (89.2-89.3)	89.2 (89.0-89.4)	89.6 (89.5-89.8)	0.001
Total cholesterol (mg/dl)	180.3 (178.0-182.7)	178.3 (177.2-179.3)	179.3 (179.0-179.7)	181.0 (180.3-181.6)	182.4 (182.0-182.9)	<0.001
LDL-C (mg/dl)	104.1 (102.1-106.2)	101.4 (100.5-102.3)	102.8 (102.4-103.1)	104.8 (104.2-105.3)	104.8 (104.4-105.3)	<0.001
HDL-C (mg/dl)	67.7 (66.6-68.9)	68.5 (68.0-69.0)	68.0 (67.8-68.2)	67.7 (67.4-68.1)	68.0 (67.7-68.2)	0.304
Triglycerides (mg/dl)	70.5 (68.0-73.1)	68.0 (66.9-69.1)	69.8 (69.4-70.2)	71.1 (70.4-71.8)	72.7 (72.2-73.2)	<0.001
AST (U/l)	17.0 (16.3-17.7)	17.3 (17.0-17.5)	17.4 (17.3-17.5)	17.6 (17.4-17.8)	17.8 (17.7-18.0)	<0.001
ALT (U/l)	13.1 (12.4-13.7)	12.9 (12.7-13.2)	13.2 (13.1-13.3)	13.5 (13.3-13.7)	13.9 (13.7-14.0)	<0.001
GGT (U/l)	14.2 (13.4-15.0)	13.6 (13.2-13.9)	13.8 (13.6-13.9)	14.1 (13.8-14.3)	14.7 (14.5-14.9)	<0.001
hs-CRP (mg/l)	0.73 (0.50-0.96)	0.69 (0.59-0.79)	0.71 (0.68-0.74)	0.76 (0.70-0.83)	0.80 (0.75-0.84)	<0.001
HOMA-IR	1.27 (1.21-1.33)	1.20 (1.18-1.23)	1.21 (1.20-1.22)	1.19 (1.18-1.21)	1.18 (1.17-1.19)	<0.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CI, confidence interval; CVD, cardiovascular disease; GGT, gamma-glutamyl transferase; HEPA, health-enhancing physical activity; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol.

<sup>a</sup> ≥10 g of ethanol per day.

<sup>b</sup> ≥College graduate.

<sup>c</sup> Body mass index  $\geq 25$  kg/m<sup>2</sup>.

**Supplemental Table 2. Hazard ratio (95% confidence intervals) of incident non-alcoholic fatty liver disease (NAFLD) by menstrual cycle category in clinically relevant subgroups (n=72,092)**

Subgroup	Menstrual cycle (days)					<i>p</i> for linear trend	<i>p</i> for quadratic trend	<i>p</i> for interaction
	<21	21–25	26–30	31–39	≥40 or irregular			
<b>Age</b>								0.225
<30 years (n=12,752)	1.84 (1.21-2.79)	1.27 (0.94-1.71)	1.00 (reference)	1.26 (1.04-1.53)	1.27 (1.09-1.47)	0.123	0.002	
≥30 years (n=38,366)	1.17 (0.84-1.62)	0.93 (0.81-1.08)	1.00 (reference)	1.15 (1.04-1.27)	1.17 (1.09-1.27)	<0.001	<0.001	
<b>Current smoking</b>								0.139
No (n=47,239)	1.38 (1.06-1.80)	0.97 (0.84-1.11)	1.00 (reference)	1.20 (1.09-1.32)	1.22 (1.13-1.31)	<0.001	<0.001	
Yes (n=781)	1.48 (0.45-4.85)	1.84 (0.81-4.19)	1.00 (reference)	2.50 (1.32-4.72)	1.96 (1.20-3.18)	0.043	0.022	
<b>Alcohol intake</b>								0.770
<10 g/day (n=44,956)	1.28 (0.97-1.70)	0.97 (0.85-1.12)	1.00 (reference)	1.19 (1.08-1.31)	1.21 (1.12-1.30)	<0.001	<0.001	
≥10 g/day (n=6,162)	1.70 (0.93-3.11)	0.93 (0.64-1.35)	1.00 (reference)	1.26 (0.96-1.64)	1.34 (1.11-1.61)	0.006	0.001	
<b>HEPA</b>								0.260
No (n=45,287)	1.37 (1.04-1.80)	0.97 (0.84-1.12)	1.00 (reference)	1.22 (1.11-1.34)	1.21 (1.12-1.30)	<0.001	<0.001	
Yes (n=5,723)	1.20 (0.62-2.34)	0.98 (0.69-1.38)	1.00 (reference)	0.93 (0.68-1.26)	1.33 (1.10-1.60)	0.007	0.001	
<b>Early menarche</b>								0.833
No (n=46,856)	1.36 (1.04-1.78)	0.96 (0.84-1.11)	1.00 (reference)	1.18 (1.07-1.3)	1.20 (1.12-1.29)	<0.001	<0.001	
Yes (n=4,042)	1.31 (0.54-3.18)	0.95 (0.61-1.48)	1.00 (reference)	1.17 (0.87-1.56)	1.04 (0.82-1.31)	0.705	0.730	
<b>Parity</b>								0.721
No (n=20,055)	1.56 (1.07-2.28)	1.01 (0.79-1.29)	1.00 (reference)	1.24 (1.07-1.43)	1.23 (1.09-1.38)	0.005	0.001	
Yes (n=29,632)	1.15 (0.80-1.65)	0.92 (0.79-1.09)	1.00 (reference)	1.15 (1.03-1.29)	1.23 (1.13-1.34)	<0.001	<0.001	
<b>Body mass index</b>								0.797
<25 kg/m <sup>2</sup> (n=48,224)	1.25 (0.91-1.70)	0.92 (0.79-1.07)	1.00 (reference)	1.18 (1.06-1.30)	1.29 (1.19-1.40)	<0.001	<0.001	
≥25 kg/m <sup>2</sup> (n=2,894)	1.35 (0.86-2.12)	0.97 (0.75-1.25)	1.00 (reference)	1.22 (1.01-1.47)	1.20 (1.05-1.37)	0.016	0.008	
<b>HOMA-IR</b>								0.001
<2.5 (n=48,760)	1.33 (1.01-1.75)	0.95 (0.82-1.09)	1.00 (reference)	1.18 (1.08-1.30)	1.27 (1.18-1.37)	<0.001	<0.001	
≥2.5 (n=2,358)	2.40 (1.19-4.86)	1.14 (0.79-1.63)	1.00 (reference)	1.22 (0.95-1.57)	0.89 (0.74-1.08)	0.077	0.261	
<b>hs-CRP</b>								0.632
<1.0 mg/L (n=42,765)	1.23 (0.90-1.69)	0.96 (0.83-1.12)	1.00 (reference)	1.20 (1.08-1.33)	1.23 (1.14-1.34)	<0.001	<0.001	
≥1.0 mg/L (n=6,440)	1.59 (0.96-2.61)	0.96 (0.72-1.28)	1.00 (reference)	1.19 (0.99-1.42)	1.12 (0.98-1.28)	0.136	0.047	

Estimated from Cox proportional hazards models. The multivariable model was adjusted for age, center, year of screening examination, alcohol

consumption, smoking, physical activity, education level, parity, age at menarche, body mass index, and HOMA-IR quintile.

Abbreviations: HEPA, health-enhancing physical activity; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

**Supplemental Table 3. Adjusted prevalence ratios (PRs) of non-alcoholic fatty liver disease (NAFLD) by menstrual cycle category among 18,968 women with available pelvic sonography data, after excluding 300 women with PCOS**

Menstrual cycle (days)	Multivariable-adjusted PR <sup>a</sup> (95% CI) (N=18,968)
<21	1.13 (0.67-1.59)
21–25	0.86 (0.67-1.05)
26–30	1.00 (reference)
31–39	1.28 (1.11-1.46)
≥40 or irregular	1.42 (1.27-1.58)
<i>p</i> for linear trend	<0.001
<i>p</i> for quadratic trend	<0.001

<sup>a</sup> Estimated from the logistic regression models. The multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking, physical activity, education level, parity, age at menarche, body mass index, and HOMA-IR quintiles.

Abbreviations: CI, confidence interval; HOMA-IR, homeostatic model assessment of insulin resistance; PCOS, polycystic ovary syndrome.

**Supplemental Table 4. Adjusted hazard (HRs) ratios of non-alcoholic fatty liver disease (NAFLD) by menstrual cycle among 14,378 women with available pelvic sonography data, after excluding 181 women with PCOS**

Menstrual cycle (days)	Multivariable-adjusted HR <sup>b</sup> (95% CI) (N=14,378)
<21	0.89 (0.48-1.67)
21–25	0.92 (0.71-1.19)
26–30	1.00 (reference)
31–39	1.23 (1.03-1.47)
≥40 or irregular	1.32 (1.15-1.52)
<i>p</i> for linear trend	<0.001
<i>p</i> for quadratic trend	<0.001

<sup>a</sup> Estimated from Cox proportional hazards models. The multivariable model was adjusted for age, center, year of screening examination, alcohol consumption, smoking, physical activity, education level, parity, age at menarche, body mass index, and HOMA-IR quintiles.

Abbreviations: CI, confidence interval; HOMA-IR, homeostatic model assessment of insulin resistance; PCOS, polycystic ovary syndrome.

**Supplemental Table 5. Association between menstrual cycle category and severity of non-alcoholic fatty liver disease (NAFLD) based on US at baseline (N= 72,092)**

Menstrual cycle Length (days)	Number	Prevalent cases	Prevalence rate (%)	Age-adjusted PR <sup>a</sup> (95% CI)	Multivariable-adjusted PR <sup>a</sup> (95% CI)	
					Model 1	Model 2
<b>Mild NAFLD</b>						
<21	914	56	6.1	1.36 (1.03-1.79)	0.96 (0.7-1.32)	0.95 (0.69-1.31)
21-25	4,367	198	4.5	0.85 (0.73-0.98)	0.86 (0.73-1.02)	0.86 (0.73-1.02)
26-30	36,378	1,848	5.1	1.00 (reference)	1.00 (reference)	1.00 (reference)
31-39	10,455	650	6.2	1.35 (1.23-1.48)	1.49 (1.34-1.65)	1.47 (1.32-1.64)
≥40 or irregular	19,978	1,468	7.4	1.63 (1.52-1.75)	1.53 (1.41-1.67)	1.51 (1.39-1.65)
<i>p</i> for linear trend				<0.001	<0.001	<0.001
<i>p</i> for quadratic trend				<0.001	<0.001	<0.001
<b>Moderate / severe NAFLD</b>						
<21	914	9	1.0	1.53 (0.78-2.99)	0.80 (0.37-1.73)	0.78 (0.36-1.69)
21-25	4,367	21	0.5	0.71 (0.45-1.11)	0.73 (0.45-1.2)	0.74 (0.45-1.22)
26-30	36,378	243	0.7	1.00 (reference)	1.00 (reference)	1.00 (reference)
31-39	10,455	91	0.9	1.34 (1.05-1.71)	1.78 (1.36-2.33)	1.74 (1.32-2.28)
≥40 or irregular	19,978	450	2.3	3.57 (3.05-4.18)	3.15 (2.61-3.8)	3.03 (2.51-3.66)
<i>p</i> for linear trend				<0.001	<0.001	<0.001
<i>p</i> for quadratic trend				<0.001	<0.001	<0.001

<sup>a</sup> Estimated from multinomial logistic regression models as outcomes categorized as no NAFLD, mild NAFLD, and moderate / severe NAFLD. Multivariable Model 1 was adjusted for age, center, year of examination, alcohol consumption, smoking, physical activity, education level, parity, age at menarche, and BMI; Model 2: Model 1 plus adjustment for HOMA-IR quintile. Abbreviations: BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model assessment of insulin resistance.

**Supplemental Table 6. Development of moderate/ severe non-alcoholic fatty liver disease (NAFLD) based on US by menstrual cycle category at baseline (N= 51,118)**

Menstrual period (days)	Person-years (PY)	Incident cases	Incidence density (/10 <sup>3</sup> PY)	Age-adjusted HR (95% CI)	Multivariable-adjusted HR <sup>a</sup> (95% CI)		HR (95% CI) <sup>b</sup> in a model with time-dependent variables
					Model 1	Model 2	
<21	2,389	10	4.2	2.32 (1.23-4.38)	1.89 (1.00-3.58)	1.84 (0.97-3.49)	0.86 (0.38-1.95)
21–25	14,407	22	1.5	0.92 (0.59-1.43)	0.87 (0.56-1.35)	0.87 (0.56-1.35)	0.56 (0.35-0.91)
26–30	119,973	203	1.7	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
31–39	32,182	53	1.6	0.94 (0.69-1.27)	1.01 (0.74-1.37)	1.01 (0.75-1.37)	1.00 (0.73-1.39)
≥40 or irregular	68,528	187	2.7	1.50 (1.23-1.83)	1.32 (1.07-1.62)	1.32 (1.07-1.62)	1.36 (1.09-1.71)
<i>p</i> for linear trend				0.002	0.031	0.031	<0.001
<i>p</i> for quadratic trend				<0.001	0.003	0.003	0.006

<sup>a</sup> Estimated from Cox proportional hazards models. Multivariable Model 1 was adjusted for age, center, year of examination, alcohol consumption, smoking, physical activity, education level, parity, age at menarche, and BMI; Model 2: Model 1 plus adjustment for HOMA-IR quintile.

<sup>b</sup> Estimated from Cox proportional hazard models with menstrual cycle category, smoking, alcohol consumption, physical activity, parity, HOMA-IR, and BMI as time-dependent variables and baseline age, center, year of examination, education level, and age at menarche as time-fixed variables.

Abbreviations: BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model assessment of insulin resistance; HR, hazard ratio.

**Supplemental Table 7. Adjusted prevalence ratios (PRs) of non-alcoholic fatty liver disease (NAFLD) by menstrual cycle category at baseline (N= 72,092)**

Menstrual cycle Length (days)	Number	Prevalent cases	Prevalence rate (%)	Age-adjusted PR <sup>a</sup> (95% CI)	Multivariable-adjusted PR <sup>a</sup> (95% CI)	
					Model 1	Model 2
<21	914	65	7.1	1.21 (1.07-1.36)	0.95 (0.75-1.14)	0.94 (0.75-1.13)
21-25	4,367	220	5.0	0.77 (0.72-0.81)	0.89 (0.80-0.99)	0.90 (0.80-0.99)
26-30	36,378	2,118	5.8	1.00 (reference)	1.00 (reference)	1.00 (reference)
31-35	9,517	676	7.1	1.34 (1.28-1.40)	1.28 (1.20-1.37)	1.26 (1.18-1.35)
36-40	1,561	132	8.5	1.54 (1.39-1.69)	1.43 (1.24-1.63)	1.40 (1.21-1.59)
>40 or irregular	19,355	1,882	9.7	1.90 (1.84-1.96)	1.38 (1.31-1.45)	1.35 (1.28-1.41)
<i>p</i> for linear trend				<0.001	<0.001	<0.001
<i>p</i> for quadratic trend				<0.001	<0.001	<0.001

<sup>a</sup> Estimated from the logistic regression models. Multivariable Model 1 was adjusted for age, center, year of examination, alcohol consumption, smoking, physical activity, education level, parity, age at menarche, and BMI; Model 2: Model 1 plus adjustment for HOMA-IR quintile. Abbreviations: BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model assessment of insulin resistance.

**Supplemental Table 8. Development of non-alcoholic fatty liver disease (NAFLD) by menstrual cycle category at baseline (N= 51,118)**

Menstrual period (days)	Person-years (PY)	Incident cases	Incidence density (/10 <sup>3</sup> PY)	Age-adjusted HR (95% CI)	Multivariable-adjusted HR <sup>a</sup> (95% CI)		HR (95% CI) <sup>b</sup> in a model with time-dependent variables
					Model 1	Model 2	
<21	2,273	61	26.8	1.54 (1.19-1.99)	1.38 (1.06-1.78)	1.34 (1.04-1.73)	1.20 (0.94-1.54)
21–25	13,794	248	18.0	0.96 (0.84-1.09)	0.97 (0.85-1.10)	0.97 (0.85-1.10)	0.88 (0.78-1.00)
26–30	114,869	2,118	18.4	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
31-35	28,136	569	20.2	1.13 (1.03-1.24)	1.18 (1.07-1.29)	1.19 (1.08-1.31)	1.27 (1.15-1.40)
36-40	4,514	89	19.7	1.10 (0.89-1.36)	1.22 (0.98-1.50)	1.22 (0.99-1.51)	1.36 (1.09-1.70)
>40 or irregular	62,602	1,439	23.0	1.27 (1.18-1.36)	1.22 (1.14-1.31)	1.22 (1.14-1.31)	1.48 (1.38-1.60)
<i>p</i> for linear trend				<0.001	<0.001	<0.001	<0.001
<i>p</i> for quadratic trend				<0.001	<0.001	<0.001	<0.001

<sup>a</sup> Estimated from Cox proportional hazards models. Multivariable Model 1 was adjusted for age, center, year of examination, alcohol consumption, smoking, physical activity, education level, parity, age at menarche, and BMI; Model 2: Model 1 plus adjustment for HOMA-IR quintile.

<sup>b</sup> Estimated from Cox proportional hazard models with menstrual cycle category, smoking, alcohol consumption, physical activity, parity, HOMA-IR, and BMI as time-dependent variables and baseline age, center, year of examination, education level, and age at menarche as time-fixed variables.

Abbreviations: BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model assessment of insulin resistance; HR, hazard ratio.