

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

3

4 **Short title:** Long or irregular menstrual cycles and NAFLD

5

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61

62 **ABSTRACT**

63 **Context**

64 The association of menstrual cycle length and irregularity with the risk of non-alcoholic fatty
65 liver disease (NAFLD) is unknown. We examined this association in large cross-sectional and
66 cohort studies.

67 **Methods**

68 The cross-sectional study included 72,092 women aged <40 years who underwent routine
69 health examinations; the longitudinal analysis included the subset of 51,118 women without
70 NAFLD at baseline. Long or irregular cycles were defined as menstrual cycles of ≥ 40 days or
71 too irregular to estimate. Abdominal ultrasonography was performed to identify NAFLD.
72 Multivariable Cox proportional hazard regression analyses were performed to estimate hazard
73 ratios (HRs) and 95% confidence intervals (CIs) for incident NAFLD according to menstrual
74 cycle regularity and length, with 26–30-day cycles as the reference.

75 **Results**

76 At baseline, 27.7% had long or irregular menstrual cycles and 7.1% had prevalent NAFLD.
77 Long or irregular menstrual cycles were positively associated with prevalent NAFLD. During
78 a median follow-up of 4.4 years, incident NAFLD occurred in 8.9% of women. After
79 adjustment for age, body mass index, insulin resistance and other confounders, the
80 multivariable-adjusted HR for NAFLD comparing long or irregular menstrual cycles to the
81 reference group was 1.22 (95% CI, 1.14–1.31); this association strengthened in the time-
82 dependent analysis with HR of 1.49 (95% CI, 1.38–1.60).

83 **Conclusions**

84 Long or irregular menstrual cycles were associated with increased risk of both prevalent and
85 incident NAFLD in young, premenopausal women. Women with long or irregular menstrual

86 cycles may benefit from lifestyle modification advice to reduce the risk of NAFLD and
87 associated cardiometabolic diseases.

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92 **INTRODUCTION**

93 Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease
94 worldwide (1), can progress to liver cirrhosis and hepatocellular carcinoma and is associated
95 with higher risks of premature mortality (1). NAFLD is also strongly associated with insulin
96 resistance (2), type 2 diabetes mellitus (2), and increased cardiovascular risk (1). Lifestyle
97 modification continues to be the standard of care for NAFLD (3); hence, patients at risk may
98 benefit from the assessment of easily identifiable risk factors for early intervention before
99 progression to adverse outcomes. Reproductive factors and sex hormones have been
100 suggested as risk factors for NAFLD because NAFLD is more prevalent among men than
101 women and among postmenopausal women than premenopausal women (4).

102 Long or irregular menstrual cycles, which may be associated with metabolic or
103 endocrine disorders (5-7), are common among women during the reproductive period, with a
104 reported prevalence of approximately 20% (8). Long or irregular menstrual cycles are
105 associated with cardiometabolic dysfunction, including insulin resistance (9), risk of type 2
106 diabetes mellitus (5), cardiovascular disease (6,10). However, the association between long or
107 irregular menstrual cycles and NAFLD, a metabolic liver disease, has not been described
108 previously. Evidence for an association between polycystic ovary syndrome (PCOS) and
109 increased risk of NAFLD was inconsistent in a large meta-analysis (11). Long or irregular
110 menstrual cycles are common in women with PCOS, which may occur as the ovarian
111 manifestation of metabolic syndrome (12). However, PCOS is a heterogeneous condition that
112 can be difficult to diagnose (13), and not all women with long or irregular menstrual cycles
113 have PCOS. It is also unclear whether the strength of the association with NAFLD varies
114 with the features of PCOS. Women with PCOS with hyperandrogenism had a higher risk of
115 NAFLD than those with PCOS with normal androgen levels or those without PCOS (14,15);

116 however, no previous study has investigated whether menstrual cycle irregularity or
117 oligomenorrhea are risk factors for NAFLD in women with PCOS. Additionally, no study has
118 yet investigated the association between menstrual irregularity and NAFLD in the general
119 population.

120 Hence, we aimed to examine the association between long or irregular menstrual
121 cycles and NAFLD in a cohort of premenopausal women undergoing routine health
122 examinations using cross-sectional and longitudinal study designs.

123

124 **MATERIALS AND METHODS**

125 **Study population**

126 The cohort of this study was derived from the Kangbuk Samsung Health Study and
127 consists of premenopausal Korean women aged <40 years who underwent comprehensive
128 annual or biennial health examinations at one of the healthcare centers in Seoul and Suwon,
129 South Korea (16). The study subjects participated in comprehensive health examinations
130 from 2011 to 2017, with at least one follow-up examination conducted by December 31, 2019
131 (N=135,090). We used data collected from routine health screening examinations, consisting
132 of questionnaires, blood tests, imaging, and procedures (16). The exclusion criteria were as
133 follows: history of liver disease or use of medications for liver disease (n=2,539); confirmed
134 hepatitis B or C (n=3,177); liver cirrhosis (n=4); alcohol ≥ 20 g/day(17) (n=10,434); use of
135 steatogenic medication within the previous year including amiodarone, tamoxifen,
136 methotrexate, or corticosteroids (n=619); history of cancer (n=2,540); thyroid function
137 abnormalities or use of medication for hyperthyroidism or hypothyroidism (n=4,262);
138 premature menopause (n=233); previous oophorectomy or hysterectomy (n=2,592); use of
139 hormone replacement therapy or contraceptives (n=4,153); pregnant or lactating (n=9,448);

140 and missing data on menstrual cycle, abdominal ultrasonography, alcohol intake, body mass
141 index (BMI), and assessment of insulin resistance (n=44,547). Some participants met more
142 than one exclusion criterion, resulting in 72,092 eligible women (**Figure 1**). For the
143 longitudinal analysis of this cohort, we included subjects who were NAFLD-free at baseline
144 with at least one follow-up visit; subjects with NAFLD at baseline (n=5,225) and who did not
145 participate in follow-up examinations (n=17,165) were thus excluded. In total, 51,118 women
146 were finally included in the study of incident NAFLD. This study was approved by the
147 Institutional Review Board of Kangbuk Samsung Hospital (IRB No. KBSMC 2021-09-003),
148 which waived the requirement for informed consent because we used a de-identified dataset
149 retrieved from routine health screening examinations.

150

151 **Data collection**

152 Data on demographics, medical history, and behavioral factors were collected using
153 standardized, self-administered questionnaires. Smoking status was categorized into never,
154 former, or current smokers. Alcohol intake was assessed by estimating the amount of alcohol
155 intake per day based on the frequency and amount consumed per drinking day. Physical
156 activity was based on the Korean version of the International Physical Activity Questionnaire
157 Short Form (18). Health-enhancing physical activity (HEPA) was defined as either vigorous
158 activity ≥ 3 days per week accumulating $\geq 1,500$ metabolic equivalent (MET)-min/week, or
159 7 days of walking or moderate to vigorous intensity activities accumulating $\geq 3,000$ MET
160 min/week (19). Education level was categorized as having a high school degree or lower or a
161 college degree or higher. Parity was assessed based on the number of previous pregnancies,
162 including live births and stillbirths. Early menarche was defined as menarche at < 12 years
163 (20). The women were asked whether their menstrual cycles were regular or too irregular to

164 estimate and were informed that cycles differing by 2–3 days could be considered regular;
165 those with regular menstrual cycles were asked to report the interval in days. The menstrual
166 cycles were categorized as follows: <21-day, 21–25-day, 26–30-day, 31–39-day, and \geq 40-day
167 or too irregular to estimate, similar to those in previous studies including the Nurses' Health
168 Study II (5,21). Menstrual cycle lengths vary widely across populations of women, and while
169 normal cycles can range from 21 to 35 days (22,23), the median menstrual cycle length in our
170 cohort was 28 days; hence, the 26–30-day cycle (28 ± 2 days) was set as the reference group,
171 as in other studies (24,25). However, considering that normal menstrual cycles are defined as
172 21 to 35 days (23), and that the Tremin Trust studies defined >40-day cycles as abnormally
173 long menstrual cycles (26), we performed additional analysis by re-classifying the last two
174 categories as 31–35-day, 36–40-day, and >40-day cycles or too irregular to estimate.

175 Participants' height, weight, and blood pressure (BP) were measured by trained
176 nurses. Obesity was defined as BMI ≥ 25 kg/m², the cutoff value specified for diagnosing
177 obesity in Asians (27). Hypertension was defined as systolic BP ≥ 140 mmHg, diastolic BP
178 ≥ 90 mmHg, or reported use of any antihypertensive medication.

179 Blood samples were drawn from the antecubital vein after ≥ 10 hours of fasting for
180 measurements of fasting blood glucose, total cholesterol, low-density lipoprotein cholesterol,
181 high-density lipoprotein cholesterol, triglycerides, alanine aminotransferase, aspartate
182 transaminase, gamma-glutamyl transferase, and high-sensitivity C-reactive protein. Fasting
183 insulin (Roche catalog No. 12017547, RRID:AB_2756877;
184 http://antibodyregistry.org/AB_2756877) (28) was measured by electrochemiluminescence
185 immunoassay using the Modular Analytics E170 during 2011–2014 and afterwards with the
186 Cobas E602 Analyzer (Roche Diagnostics, Tokyo, Japan). Insulin resistance was determined
187 based on the following homeostatic model assessment of insulin resistance (HOMA-IR)

188 equation: fasting blood insulin ($\mu\text{U}/\text{mL}$) \times fasting blood glucose (mmol/l) / 22.5; and the
189 cutoff value of 2.5 was used (29). Diabetes mellitus was defined as fasting blood glucose
190 ≥ 126 mg/dL, hemoglobin A1c $\geq 6.5\%$, or reported use of any antidiabetic medication.

191 The diagnosis of NAFLD was based on hepatic steatosis identified on abdominal
192 ultrasonography performed by experienced radiologists blinded to the study aims. The
193 diagnosis of hepatic steatosis was based on standard criteria, including diffusely increased
194 fine echogenicity in the liver parenchyma compared to the kidney or spleen parenchyma,
195 deep beam attenuation, and bright vessel walls (30). Radiologists graded hepatic steatosis as
196 mild, moderate, or severe (31). Mild hepatic steatosis was identified by a slight increase in
197 liver echogenicity. Moderate hepatic steatosis was identified by a slightly impaired image of
198 the intrahepatic vasculature and diaphragm, accompanied by increased liver echogenicity.
199 Severe hepatic steatosis was identified by a marked increase in liver echogenicity, impaired
200 penetration of the posterior segment of the right lobe, and poor or no image of the
201 intrahepatic vasculature and diaphragm (32,33). NAFLD severity was categorized as mild or
202 moderate/severe in further analysis. The inter- and intra-observer reliability values for
203 diagnosing hepatic steatosis were substantial (kappa statistic 0.74) and excellent (kappa
204 statistic 0.94), respectively (34). The other requirements for diagnosing NAFLD were met
205 through the exclusion criteria applied at the beginning of this study, including the exclusion
206 of participants with significant alcohol intake, competing etiologies for hepatic steatosis, and
207 other causes of chronic liver diseases (3).

208 In a subsample of women who underwent pelvic ultrasonography examinations,
209 experienced gynecologists who were blinded to the study aims routinely questioned the
210 examinees regarding the diagnosis of gynecologic disorders, including PCOS, and examined
211 for the presence of ovarian cysts, including specific information on the size, echogenicity,

212 echotexture, internal pattern, and content. Previous gynecological disorders or abnormal
213 findings on pelvic ultrasonography were documented in the ultrasonography reports.

214

215 **Statistical analysis**

216 Baseline characteristics were described according to menstrual cycle categories using
217 descriptive statistics with adjustment for age, because age differs across the menstrual cycle
218 categories.

219

220 *Menstrual cycle and prevalent NAFLD: A cross-sectional study*

221 We analyzed the association between menstrual cycle category and NAFLD by
222 performing logistic regression analyses to calculate the prevalence ratios (PRs) and 95%
223 confidence intervals (CIs) for NAFLD, with adjustment for age. We used two models for
224 adjustment of covariates: Model 1 was adjusted for age, center (Seoul or Suwon), year of
225 examination, alcohol consumption, smoking, physical activity, education level, parity, age at
226 menarche, and BMI; Model 2 was adjusted for the variables in Model 1 plus HOMA-IR
227 quintiles because insulin resistance is associated with both NAFLD (2) and PCOS (35). We
228 adjusted for potential confounders that might affect the relationship between menstrual cycles
229 and NAFLD. The confounding variables were defined using the following criteria: 1) causal
230 association with the outcome (NAFLD); 2) non-causal or causal association with exposure
231 (the menstrual cycle); and 3) not being a mediator in the causal pathway between exposure
232 (the menstrual cycle) and the outcome (NAFLD). For the secondary analyses, we estimated
233 the PRs and 95% CIs for mild and moderate/severe NAFLD for the menstrual cycle
234 categories with no NAFLD as the reference group, using multinomial logistic regression
235 models.

236

237 *Menstrual cycle and incident NAFLD: A cohort study*

238 The primary outcome was the development of NAFLD during follow-up among
239 premenopausal women without NAFLD at baseline. The participants were followed up from
240 baseline until the development of NAFLD or the end of 2019, whichever came first. Hazard
241 ratios (HRs) and 95% CIs for the development of NAFLD were calculated using Cox
242 proportional hazards regression analyses, with adjustment for variables as in logistic
243 regression analyses. We also performed time-dependent analyses according to menstrual
244 cycle category, smoking, alcohol consumption, physical activity, parity, BMI, and HOMA-IR
245 quintiles as time-varying covariates. We tested for linear trends by applying menstrual
246 category groups as continuous variables in the regression models. We also tested for a
247 quadratic trend to allow for a J shaped relationship between menstrual cycle length and
248 NAFLD by squaring the linear trend variable, which was centered on the reference value.

249 Predefined subgroup analyses were performed according to age (<30 vs. ≥30 years,
250 since menstrual cycle length differs and decreases with age (36)), current smoking status,
251 alcohol intake (<10 vs. ≥10 g/day, 10 g of ethanol per day for women as the cutoff of light
252 drinking (37)), HEPA (no vs. yes), early menarche, parity, obesity defined using Asian
253 specific criteria (BMI <25 kg/m² vs. ≥25 kg/m² (38,39)), HOMA-IR (<2.5 vs. ≥2.5 (29)), and
254 high-sensitivity C-reactive protein (<1.0 vs. ≥1.0 mg/L, the proposed cutoff as an
255 inflammatory marker for low risk of cardiovascular disease by the Center for Disease Control
256 and American Heart Association (40)). We tested for interactions among the subgroups by
257 performing likelihood ratio tests and compared the models with and without multiplicative
258 interaction terms. We also performed analysis for prevalent and incident NAFLD among
259 women who underwent pelvic ultrasonography examinations and excluded those with a

260 report of PCOS diagnosis or polycystic ovaries on ultrasonography findings.

261 Statistical analyses were performed using Stata version 16.0 (StataCorp LP, College
262 Station, TX, USA). Two-tailed *p* values <0.05 were considered to indicate statistical
263 significance.

264

265 **Patient and public involvement**

266 Patients and the public were not involved in the design, conduct, reporting, or
267 dissemination of this research.

268

269 **RESULTS**

270 **Characteristics of study participants**

271 At baseline, 7.1% had prevalent NAFLD, while 27.7% had long (≥ 40 -day) or
272 irregular menstrual cycles. Compared with 26–30-day menstrual cycles as the reference, long
273 or irregular menstrual cycles were associated with younger age, hypertension, diabetes,
274 obesity, and higher total cholesterol, triglyceride, hsCRP, and HOMA-IR levels (**Table 1**).

275

276 **Association between menstrual cycle and prevalent NAFLD**

277 **Table 2** shows the adjusted PRs of NAFLD based on menstrual cycle categories.
278 Compared with 26–30-day menstrual cycles as the reference, <21-day, 31–39-day, and ≥ 40 -
279 day or irregular menstrual cycles were associated with a higher prevalence of NAFLD, with
280 age-adjusted PRs (95% CIs) of 1.21 (1.07–1.36), 1.35 (1.29–1.41), and 1.89 (1.83–1.95),
281 respectively. After additional adjustments for center, examination year, alcohol consumption,
282 smoking status, HEPA, education level, parity, age at menarche, BMI, and HOMA-IR, 31–
283 39-day and ≥ 40 -day or irregular cycles were associated with NAFLD, with PRs (95% CIs) of

284 1.27 (1.19–1.36) and 1.35 (1.28–1.42), respectively. After categorizing NAFLD as mild or
285 moderate/severe, 31–39-day and \geq 40-day or irregular menstrual cycles were associated with a
286 higher prevalence of mild and moderate/severe NAFLD in a dose-response manner compared
287 to that of the reference group (**Supplemental Table 5**) (41). After reconfiguring the menstrual
288 cycle categories as <21-, 21–25-, 26–30-, 31–35-, 36–40-, >40-day or irregular cycles, 31–
289 35-day, 36–40-day, and >40-day or irregular cycles were also associated with NAFLD
290 (**Supplemental Table 7**) (41).

291

292 **Association between menstrual cycle and incident NAFLD**

293 In the cohort analysis of women without NAFLD at baseline (**Supplemental Table**
294 **1**) (41), 4,524 incident cases of NAFLD occurred during a mean follow-up of 4.4 years.
295 When analyzing the longitudinal associations between menstrual cycle categories and
296 incident NAFLD, <21-day, 31–39-day, and \geq 40-day or irregular menstrual cycles were
297 associated with the development of NAFLD, with age-adjusted HRs (95% CIs) of 1.54 (1.19–
298 1.99), 1.13 (1.03–1.24), and 1.26 (1.18–1.35), respectively (**Table 3**). The associations
299 remained significant after adjusting for additional covariates, including HOMA-IR values. In
300 the time-dependent analysis, 31–39-day and \geq 40-day or irregular cycles were associated with
301 a higher risk of incident NAFLD, with HRs (95% CIs) of 1.27 (1.15–1.39) and 1.49 (1.38–
302 1.60), respectively, compared to the reference group. In a sensitivity analysis using
303 moderate/severe NAFLD as an endpoint, \geq 40-day or irregular menstrual cycles were
304 associated with a higher risk of incident moderate/severe NAFLD in the fully-adjusted model
305 and time-dependent analysis (**Supplemental Table 6**) (41). After reconfiguring the menstrual
306 cycle categories, 21-day, 31–35-day, and >40-day or irregular cycles were associated with
307 NAFLD risk in the fully adjusted model; in the time-dependent analysis, 31–35-day, 36–40-

308 day, and >40-day or irregular cycles were associated with increased NAFLD risk
309 **(Supplemental Table 8)** (41).

310 In subgroup analyses, 31–39-day and \geq 40-day or irregular menstrual cycles were
311 associated with an increased risk of NAFLD when HOMA-IR was <2.5, with adjusted HRs
312 (95% CIs) of 1.18 (1.08–1.30) and 1.27 (1.18–1.37), respectively, but not when HOMA-IR
313 was \geq 2.5 (p for interaction=0.001) **(Supplemental Table 2)** (41). No other significant
314 interactions were observed for the other predefined subgroups.

315

316 **Association between menstrual cycle and NAFLD among women with pelvic** 317 **ultrasonography data**

318 Among the women with pelvic ultrasonography data and gynecologic assessments
319 available, after excluding 300 women with suspected PCOS, 18,968 women were included in
320 the analysis at baseline. In the cross-sectional analysis, 31–39-day and \geq 40-day or irregular
321 menstrual cycles were associated with NAFLD, with adjusted PRs (95% CIs) of 1.28 (1.11–
322 1.46) and 1.42 (1.27–1.58), respectively **(Supplemental Table 3)** (41).

323 In the longitudinal analysis of 14,378 women without either NAFLD or suspected
324 PCOS at baseline, the adjusted HRs (95% CIs) for 31–39-day and \geq 40-day or irregular
325 menstrual cycles were 1.23 (1.03–1.47) and 1.32 (1.15–1.52), respectively **(Supplemental**
326 **Table 4)** (41).

327

328 **DISCUSSION**

329 In our large cohort of premenopausal women, long or irregular menstrual cycles were
330 associated with an increased risk of NAFLD compared to 26–30-day cycles, in both cross-
331 sectional and longitudinal analyses. Long or irregular menstrual cycles were associated with a

332 higher prevalence of mild and moderate/severe NAFLD in a dose-response manner, and the
333 association between long or irregular menstrual cycles and NAFLD risk was more
334 pronounced in time-dependent analyses and were not fully explained by obesity, insulin
335 resistance, or other relevant measured confounders in the subgroup analyses. Importantly, our
336 results indicate that menstrual irregularity, which is easier to diagnose and usually presents
337 earlier than PCOS (42), highlights the possibility of identifying premenopausal women at risk
338 of developing NAFLD.

339 While limited evidence exists regarding how menstrual irregularities may affect the
340 pathogenesis of NAFLD, PCOS has been linked with NAFLD in most of the previous studies
341 included in the meta-analysis by Shengir et al (11). However, although women with long or
342 irregular menstrual cycles are reported to be more likely to have hirsutism and ovulatory
343 infertility than those with regular 26–31-day cycles (5), not all women with long or irregular
344 menstrual cycles have PCOS. The prevalence of PCOS is reported to range from 4% to 21%,
345 depending on the diagnostic criteria applied (43). In our study, 28% of participants had long
346 or irregular menstrual cycles. Meanwhile, previous reports have suggested that the prevalence
347 of PCOS in women with oligomenorrhea is estimated to be approximately 45%–87% (44,45),
348 indicating that PCOS is present in a subgroup of women with abnormal menstrual cycles. On
349 the other hand, women with PCOS and long menstrual cycles, or oligomenorrhea, have
350 poorer metabolic profiles than those without oligomenorrhea (7,46,47). There is also a report
351 that oligomenorrhea predicts the risk of diabetes mellitus in the absence of hyperandrogenism
352 (5), potentially suggesting that menstrual cycle length may be the major determinant of
353 metabolic abnormalities, beyond simply a proxy marker of PCOS. We lacked data to identify
354 hyperandrogenism or hirsutism in our cohort; however, after excluding women with PCOS in
355 our subgroup analysis of women with pelvic ultrasonography data and assessment by a

356 gynecologist, the association between long or irregular menstrual cycles and NAFLD
357 persisted in both the cross-sectional and longitudinal analyses, suggesting that PCOS may not
358 fully explain the relationship.

359 The association between long or irregular menstrual cycles and NAFLD remained
360 after further adjustment for HOMA-IR in both cross-sectional and longitudinal analyses, as
361 well as in time-dependent analysis. Long or irregular menstrual cycles were associated with
362 an increased risk of NAFLD in the subgroup with less insulin resistance (HOMA-IR <2.5)
363 but not in the subgroup with more insulin resistance (HOMA-IR \geq 2.5), although there were
364 only few women in the HOMA-IR \geq 2.5 group. This suggests that insulin resistance, which
365 has been posited to contribute to the association between PCOS and NAFLD,(11) does not
366 fully explain the association between long or irregular menstrual cycles and NAFLD
367 demonstrated in our study. Menstrual irregularity may also be a consequence of unhealthy
368 lifestyle factors such as disordered eating and stress (48), which may increase the risk of
369 NAFLD; however, there was no evidence for effect modification by factors such as smoking,
370 alcohol consumption, HEPA, and obesity.

371 The prevalence of NAFLD in our study (7.0%) is similar to that reported previously
372 for premenopausal women and represents a lower prevalence of NAFLD than that reported in
373 general or male populations, which ranges from 20% to 42% (4). We attempted to exclude
374 women experiencing the perimenopausal transition by excluding women aged >40 years,
375 because hormonal changes that predispose women to NAFLD (49) could confound our
376 analysis of menstrual cycles among premenopausal women. The higher risk of incident
377 NAFLD compared to women with 26-30 day menstrual cycles observed among women with
378 <21-day menstrual cycles in the longitudinal analysis may be attributable to perimenopausal
379 changes, such as lower estradiol and high follicle-stimulating hormone levels(50) and shorter

380 regular menstrual cycles (22). However, this association was not statistically significant in the
381 time-dependent analysis. Interestingly, <21-day menstrual cycles were also associated with
382 higher age-adjusted PRs for NAFLD, higher proportions of diabetes and obesity, and higher
383 mean HOMA-IR values compared to the reference group, as well as higher proportions of
384 current smokers and participants with alcohol intake of 10–19 g/day. Polymenorrhea (<21-
385 day menstrual cycles) may appear to be associated with poor metabolic profiles, similar to
386 those with long or irregular menstrual cycles. However, <21-day menstrual cycles were not
387 associated with NAFLD after adjusting for other covariates. Similarly, previous studies have
388 shown that while oligomenorrhea and amenorrhea were associated with insulin resistance in
389 women with PCOS, the association between polymenorrhea and insulin resistance was
390 comparable to that of women with normal menstrual cycles (47,51).

391 While the mechanisms underlying the association between long or irregular
392 menstrual cycles and NAFLD are unclear, exposure to estrogen may contribute to this
393 association. Low 17β -estradiol levels, as well as the use of antiestrogens such as tamoxifen
394 and aromatase inhibitors, have been associated with NAFLD (52). In contrast, estrogen
395 replacement therapy has been reported to decrease the risk of NAFLD, which is reportedly
396 twice as common in postmenopausal women than in premenopausal women (52,53).
397 Estrogen is suggested to suppress inflammation; improve mitochondrial function; modulate
398 nuclear receptors; and mitigate oxidative stress, insulin resistance, and fibrogenesis, to
399 decelerate the progression of chronic liver diseases including NAFLD (54). The estrogen
400 receptor alpha ($ER\alpha$) expressed in the liver is suggested to contribute to hepatic sexual
401 dimorphism (55) and lower the incidence of hepatic diseases in premenopausal women
402 (56,57). In female mice, $ER\alpha$ was found to counteract the accumulation of lipids in the liver
403 following excessive dietary fat intake, by inhibiting lipid synthesis and promoting

404 mitochondrial fatty acid β -oxidation (57). Although early menopause was not associated with
405 NAFLD in a previous study (58), it may have lacked power due to the limited number of
406 participants with early menopause. Further studies including larger populations are warranted
407 to clarify the role of estrogen insufficiency and sex hormone abnormalities in the
408 development of NAFLD.

409 Besides estrogen exposure, androgen excess and hypogonadotropic hypogonadism
410 may contribute to the association between long or irregular menstrual cycles and NAFLD.
411 Increased luteinizing hormone (59) and androgen (9) levels have been reported in women
412 with irregular menstrual cycles. Normal androgen levels help balance fat and lean mass;
413 conversely, hyperandrogenism may predispose to fat accumulation (53), especially in the
414 abdomen (4). Moreover, hypogonadotropic hypogonadism is common in women who
415 experience significant weight loss, exercise excessively, or are under severe stress and may
416 manifest as long or irregular menstrual cycles (60). Although there was no significant
417 interaction between menstrual length and obesity, the association between long or irregular
418 menstrual cycles and NAFLD was more pronounced in non-obese women, while the
419 association was not significant in obese women. Iron overload may also contribute to the
420 association between long or irregular menstrual cycles and NAFLD; as increased hepatic iron
421 was associated with NAFLD and progression of non-alcoholic steatohepatitis in some studies,
422 regular menstruation may contribute to decreased NAFLD risk (61,62).

423 The strength of our study was the use of both cross-sectional and longitudinal study
424 designs. Our study also has some limitations. First, the menstrual cycle was assessed using
425 self-administered questionnaires. We attempted to avoid misclassification bias by including
426 women aged <40 years and excluding older women who were more likely to be menopausal
427 or who reported the use of estrogen replacement therapy or oral contraceptives. Second, we

428 did not have information on the participants' sex hormone or prolactin levels. Further studies
429 with information on androgen and estrogen levels may help elucidate their influence on the
430 association between menstrual cycles and NAFLD. However, the prevalence of
431 hyperprolactinemia is reported as less than 1% of the general population (63,64); therefore,
432 the overall findings may be less likely to be affected. Third, we could not identify the women
433 meeting the criteria for a diagnosis of PCOS among all women with long or irregular
434 menstrual cycles, because we did not have information on biochemical hyperandrogenism.
435 However, our main findings remained consistent after excluding women with suspected
436 PCOS using data from pelvic ultrasonography examinations and gynecologic assessments,
437 which were available for one-fourth of the women in our cohort. Moreover, our aim was to
438 assess long or irregular menstrual cycles as a risk factor for NAFLD in premenopausal
439 women, regardless of a possible diagnosis of PCOS. Fourth, the diagnosis of NAFLD was
440 based on ultrasonography, instead of histological diagnosis; the latter is the gold standard but
441 is not appropriate for routine health screening examinations. Instead, ultrasonography is
442 employed in epidemiological studies and provides reliable identification of NAFLD (65).
443 Finally, because we included relatively healthy, young premenopausal Korean women, our
444 results may not be generalizable to other populations with comorbidities, older age groups, or
445 women of different ethnicities.

446

447 **CONCLUSION**

448 Our results indicate that long or irregular menstrual cycles may provide an easily
449 identifiable marker for an increased risk of NAFLD in young, premenopausal women.

450 Screening for NAFLD and counseling to promote healthy lifestyle behaviors may benefit

451 women with a history of long or irregular menstrual cycles.

452

453

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456

457 **Data availability:** The datasets generated and analyzed during the current study are not
458 publicly available but are available from the corresponding author on reasonable request.

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660

661 **FIGURE LEGEND**

662 **Figure 1** Selection of study participants

663

664 **Table 1. Age-adjusted mean values (95% CI) and proportions (95% CI) of baseline characteristics by menstrual cycle category**665 **(n=72,092)**

Characteristics	Menstrual cycle (days)					<i>p</i> for trend
	<21	21–25	26–30	31–39	≥40 or irregular	
Number	914	4,367	36,378	10,455	19,978	
Age (years)	31.3 (31.0-31.6)	33.5 (33.4-33.7)	33.0 (33.0-33.1)	32.0 (31.9-32.1)	31.9 (31.8-32.0)	<0.001
Seoul center (%)	51.6 (48.4-54.8)	58.4 (56.9-59.8)	57.9 (57.4-58.4)	58.5 (57.5-59.4)	55.5 (54.8-56.2)	<0.001
Current smoker (%)	4.5 (3.2-5.9)	2.2 (1.7-2.6)	2.0 (1.9-2.2)	1.8 (1.6-2.1)	2.6 (2.4-2.8)	0.074
Alcohol intake (%) ^a	14.8 (12.5-17)	12.9 (11.9-13.9)	12.5 (12.2-12.9)	12.5 (11.9-13.1)	13.8 (13.3-14.3)	0.001
HEPA (%)	14.5 (12.2-16.8)	12.1 (11.1-13.0)	10.9 (10.5-11.2)	10.4 (9.8-10.9)	12.0 (11.6-12.5)	0.133
High education level (%) ^b	60.5 (57.5-63.6)	77.9 (76.7-79.2)	83.6 (83.3-84.0)	86.6 (86.0-87.2)	79.1 (78.6-79.7)	0.085
Hypertension (%)	1.2 (0.4-1.9)	1.3 (1.0-1.7)	1.2 (1.1-1.3)	1.2 (1.0-1.4)	1.7 (1.6-1.9)	<0.001
Diabetes (%)	0.6 (0.1-1.1)	0.4 (0.3-0.6)	0.4 (0.4-0.5)	0.3 (0.2-0.5)	1.0 (0.9-1.2)	<0.001
History of CVD (%)	0.7 (0.1-1.2)	0.2 (0.1-0.3)	0.3 (0.2-0.3)	0.2 (0.1-0.3)	0.3 (0.2-0.4)	0.734
Lipid lowering drug (%)	0.1 (-0.1-0.4)	0.2 (0.1-0.3)	0.1 (0.1-0.1)	0.1 (0.1-0.2)	0.3 (0.2-0.4)	<0.001

Early menarche (%)	5.2 (3.8-6.6)	8.7 (7.8-9.5)	9.3 (9.0-9.6)	8.3 (7.8-8.8)	7.1 (6.7-7.4)	<0.001
Parous (%)	56.4 (53.5-59.3)	55.3 (54.0-56.5)	56.6 (56.2-57.1)	55.9 (55.1-56.7)	56.5 (55.9-57.0)	0.873
Obesity (%) ^c	13.1 (10.9-15.3)	8.0 (7.2-8.8)	8.5 (8.2-8.8)	8.8 (8.3-9.3)	12.2 (11.8-12.7)	<0.001
Body mass index (kg/m ²)	21.7 (21.5-21.9)	21.0 (20.9-21.1)	21.1 (21.0-21.1)	21.1 (21.0-21.1)	21.4 (21.4-21.5)	<0.001
Glucose (mg/dl)	90.0 (89.4-90.6)	89.6 (89.3-89.8)	89.6 (89.5-89.6)	89.6 (89.5-89.8)	90.5 (90.3-90.6)	<0.001
Total cholesterol (mg/dl)	180.4 (178.5-182.3)	178.9 (178.0-179.8)	179.9 (179.6-180.2)	181.9 (181.4-182.5)	184.3 (183.8-184.7)	<0.001
LDL-C (mg/dl)	105.1 (103.4-106.8)	102.5 (101.7-103.2)	103.8 (103.6-104.1)	106.2 (105.7-106.7)	107.6 (107.2-107.9)	<0.001
HDL-C (mg/dl)	66.9 (65.9-67.8)	68.2 (67.7-68.6)	67.5 (67.3-67.6)	67.1 (66.8-67.3)	66.6 (66.4-66.8)	<0.001
Triglycerides (mg/dl)	74.1 (71.6-76.6)	70.2 (69.1-71.4)	71.9 (71.5-72.3)	74.7 (74.0-75.5)	78.7 (78.2-79.3)	<0.001
AST (U/l)	17.2 (16.6-17.9)	17.7 (17.4-18.0)	17.6 (17.5-17.7)	17.9 (17.7-18.1)	18.5 (18.4-18.6)	<0.001
ALT (U/l)	13.8 (13-14.6)	13.9 (13.5-14.2)	13.7 (13.6-13.9)	14.1 (13.9-14.4)	15.5 (15.4-15.7)	<0.001
GGT (U/l)	14.7 (14.0-15.5)	13.9 (13.5-14.3)	14.3 (14.2-14.4)	14.7 (14.4-14.9)	16.1 (15.9-16.3)	<0.001
hs-CRP (mg/l)	0.78 (0.60-0.96)	0.75 (0.67-0.83)	0.77 (0.74-0.80)	0.79 (0.74-0.85)	0.94 (0.90-0.98)	<0.001
HOMA-IR	1.41 (1.33-1.49)	1.28 (1.24-1.31)	1.30 (1.28-1.31)	1.30 (1.27-1.32)	1.40 (1.38-1.42)	0.042

666 Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CI, confidence interval; CVD, cardiovascular disease; GGT,

667 gamma-glutamyl transferase; HEPA, health-enhancing physical activity; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-

668 sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol.

669 ^a ≥ 10 g of ethanol per day.

670 ^b \geq College graduate.

671 ^c Body mass index ≥ 25 kg/m².

672

673

674 **Table 2. Adjusted prevalence ratios (PRs) of non-alcoholic fatty liver disease (NAFLD) by menstrual cycle category at baseline (N=**
 675 **72,092)**

Menstrual cycle Length (days)	Number	Prevalent cases	Prevalence rate (%)	Age-adjusted PR ^a (95% CI)	Multivariable-adjusted PR ^a (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)
31-39	10,455	749	7.2	1.35 (1.29-1.41)	1.30 (1.21-1.38)	1.27 (1.19-1.36)
≥40 or irregular	19,978	1,941	9.7	1.89 (1.83-1.95)	1.38 (1.31-1.45)	1.35 (1.28-1.42)
<i>p</i> for linear trend				<0.001	<0.001	<0.001
<i>p</i> for quadratic trend				<0.001	<0.001	<0.001

676 ^a Estimated from the logistic regression models. Multivariable Model 1 was adjusted for age, center, year of examination, alcohol
 677 consumption, smoking, physical activity, education level, parity, age at menarche, and BMI; Model 2: Model 1 plus adjustment for HOMA-IR
 678 quintile.

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

680 **Table 3. 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 ³ PY)	Age-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	
<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 (0.85-1.1)	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)
31–39	30,749	620	20.2	1.13 (1.03-1.24)	1.18 (1.08-1.30)	1.20 (1.09-1.31)	1.27 (1.15-1.39)
≥40 or irregular	64,503	1,477	22.9	1.26 (1.18-1.35)	1.22 (1.14-1.30)	1.22 (1.14-1.31)	1.49 (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

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

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

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

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