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Clinical Research Article

Long or Irregular Menstrual Cycles and Risk of Prevalent and Incident Nonalcoholic Fatty Liver Disease

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Abstract

Context: The association of menstrual cycle length and irregularity with the risk of non-alcoholic fatty liver disease (NAFLD) is unknown.

Objective: We examined this association in large cross-sectional and cohort studies.

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

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

Conclusion: Long or irregular menstrual cycles were associated with increased risk of both prevalent and incident NAFLD in young, premenopausal women. Women with long or irregular menstrual cycles may benefit from lifestyle modification advice to reduce the risk of NAFLD and associated cardiometabolic diseases.

Key Words: nonalcoholic fatty liver disease, menstruation, menstrual irregularity, cohort study

Abbreviations: BMI, body mass index; BP, blood pressure; HEPA, health-enhancing physical activity; HOMA-IR, homeostatic model assessment of insulin resistance; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; MET, metabolic equivalent; NAFLD, nonalcoholic fatty liver disease; PCOS, polycystic ovary syndrome; PR, prevalence ratio.

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

Long or irregular menstrual cycles, which may be associated with metabolic or endocrine disorders (5-7), are common among women during the reproductive period, with a reported prevalence of approximately 20% (8). Long or irregular menstrual cycles are associated with cardiometabolic dysfunction, including insulin resistance (9), risk of type

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2 diabetes mellitus (5), and cardiovascular disease (6, 10). However, the association between long or irregular menstrual cycles and NAFLD, a metabolic liver disease, has not been described previously. Evidence for an association between polycystic ovary syndrome (PCOS) and increased risk of NAFLD was inconsistent in a large meta-analysis (11). Long or irregular menstrual cycles are common in women with PCOS, which may occur as the ovarian manifestation of metabolic syndrome (12). However, PCOS is a heterogeneous condition that can be difficult to diagnose (13), and not all women with long or irregular menstrual cycles have PCOS. It is also unclear whether the strength of the association with NAFLD varies with the features of PCOS. Women with PCOS with hyperandrogenism had a higher risk of NAFLD than those with PCOS with normal androgen levels or those without PCOS (14, 15); however, no previous study has investigated whether menstrual cycle irregularity or oligomenorrhea are risk factors for NAFLD in women with PCOS. In addition, no study has yet investigated the association between menstrual irregularity and NAFLD in the general population.

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

Materials and Methods

Study Population

The cohort of this study was derived from the Kangbuk Samsung Health Study and consists of premenopausal Korean women younger than 40 years who underwent comprehensive annual or biennial health examinations at one of the health care centers in Seoul and Suwon, South Korea (16). The study individuals participated in comprehensive health examinations from 2011 to 2017, with at least one follow-up examination conducted by December 31, 2019 (N = 135 090). We used data collected from routine health screening examinations, consisting of questionnaires, blood tests, imaging, and procedures (16). The exclusion criteria were as follows: history of liver disease or use of medications for liver disease (n = 2539); confirmed hepatitis B or C (n = 3177); liver cirrhosis (n = 4); alcohol consumption of 20 g/day or more (17) (n = 10 434); use of steatogenic medication within the previous year including amiodarone, tamoxifen, methotrexate, or corticosteroids (n = 619); history of cancer (n = 2540); thyroid function abnormalities or use of medication for hyperthyroidism or hypothyroidism (n = 4262); premature menopause (n = 233); previous oophorectomy or hysterectomy (n = 2592); use of hormone replacement therapy or contraceptives (n = 4153); pregnant or lactating (n = 9448); and missing data on menstrual cycle, abdominal ultrasonography, alcohol intake, body mass index (BMI), and assessment of insulin resistance (n = 44 547). Some participants met more than one exclusion criterion, resulting in 72 092 eligible women (Fig. 1). For the longitudinal analysis of this cohort, we included individuals who were NAFLD free at baseline with at least one follow-up visit; women with NAFLD at baseline (n = 5093) and who did not participate in follow-up examinations (n = 17 165) were thus excluded. In total, 51 118 women were ultimately included in the study of incident NAFLD. This study was approved by the institutional review board of Kangbuk Samsung Hospital (No. KBSMC 2021-09-003), which waived the requirement for informed consent because we used a deidentified data set retrieved from routine health screening examinations.

Data Collection

Data on demographics, medical history, and behavioral factors were collected using standardized, self-administered questionnaires. Smoking status was categorized into never, former, or current smokers. Alcohol intake was assessed by estimating the amount of alcohol intake per day based on the frequency and amount consumed per drinking day. Physical activity was based on the Korean version of the International Physical Activity Questionnaire Short Form (18). Health-enhancing physical activity (HEPA) was defined as either vigorous activity 3 days per week or more accumulating 1500 or more metabolic equivalent (MET)-min/week, or 7 days of walking or moderate to vigorous intensity activities accumulating 3000 or more MET min/week (19). Education level was categorized as having a high school degree or lower or a college degree or higher. Parity was assessed based on the number of previous pregnancies, including live births and stillbirths. Early menarche was defined as menarche at younger than 12 years (20). The women were asked whether their menstrual cycles were regular or too irregular to estimate and were informed that cycles differing by 2 to 3 days could be considered regular; those with regular menstrual cycles were asked to report the interval in days. The menstrual cycles were categorized as follows: less than 21 days, 21- to 25-day, 26- to 30-day, 31- to 39-day, and 40 days or more or too irregular to estimate, similar to those in previous studies including the Nurses' Health Study II (5, 21). Menstrual cycle lengths vary widely across populations of women, and while normal cycles can range from 21 to 35 days (22, 23), the median menstrual cycle length in our cohort was 28 days; hence, the 26- to 30-day cycle (28 ± 2 days) was set as the reference group, as in other studies (24, 25). However, considering that normal menstrual cycles are defined as 21 to 35 days (23), and that the Tremin Trust studies defined greater than 40-day cycles as abnormally long menstrual cycles (26), we performed additional analysis by reclassifying the last 2 categories as 31- to 35-day, 36- to 40-day, and 40-day or more cycles or too irregular to estimate.

Participants' height, weight, and blood pressure (BP) were measured by trained nurses. Obesity was defined as BMI greater than or equal to 25, the cutoff value specified for diagnosing obesity in Asians (27). Hypertension was defined as systolic BP greater than or equal to 140 mm Hg, diastolic BP greater than or equal to 90 mm Hg, or reported use of any antihypertensive medication.

Blood samples were drawn from the antecubital vein after 10 or more hours of fasting for measurements of fasting blood glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, alanine aminotransferase, aspartate transaminase, γ-glutamyl transferase, and high-sensitivity C-reactive protein (hsCRP). Fasting insulin (Roche catalog No. 12017547, RRID:AB_2756877; https://scicrunch.org/resources/Antibodies/search?q=AB_2756877) (28) was measured by electrochemiluminescence immunoassay using the Modular Analytics E170 during 2011–2014 and afterward with the Cobas E602 Analyzer (Roche Diagnostics). Insulin resistance was determined based on the following homeostatic model assessment of insulin resistance (HOMA-IR) equation: fasting

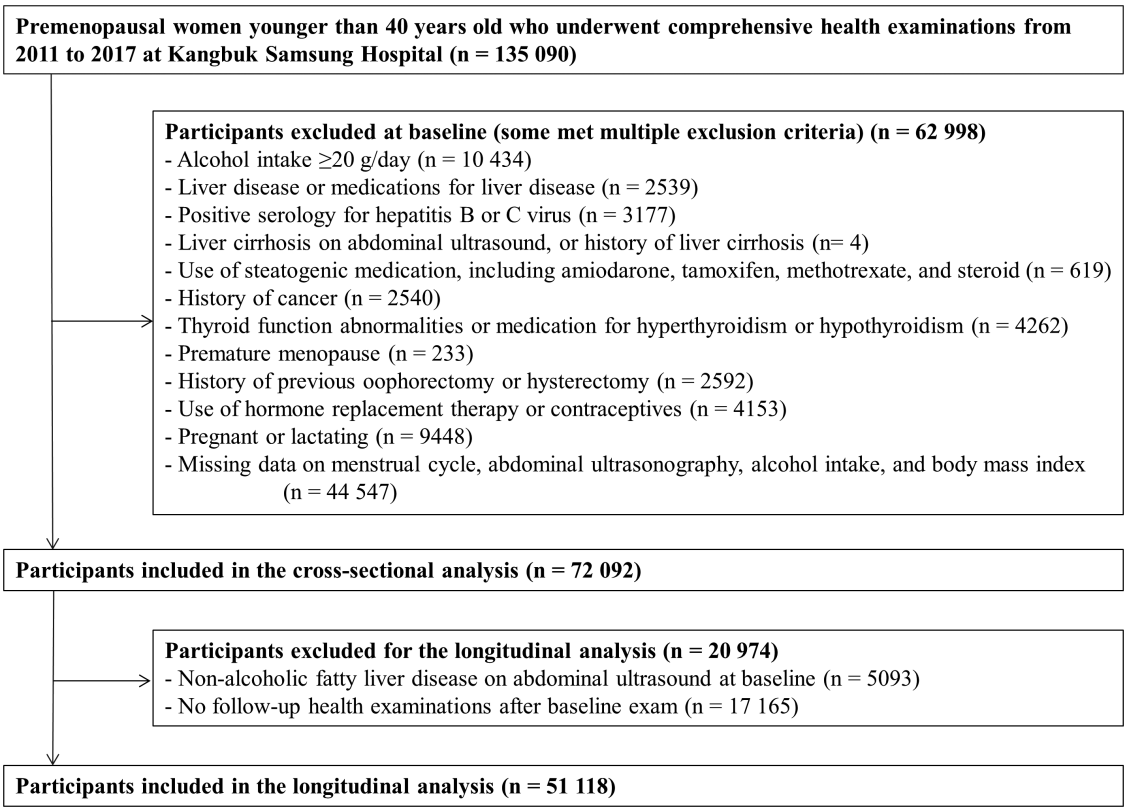

Figure 1. Selection of study participants.

blood insulin (μU/mL) × fasting blood glucose (mmol/L)/22.5; and the cutoff value of 2.5 was used (29). Diabetes mellitus was defined as fasting blood glucose greater than or equal to 126 mg/dL, glycated hemoglobin A_{1c} greater than or equal to 6.5%, or reported use of any antidiabetic medication.

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

In a subsample of women who underwent pelvic ultrasonography examinations, experienced gynecologists who were blinded to the study aims routinely questioned the examinees regarding the diagnosis of gynecologic disorders, including PCOS, and examined for the presence of ovarian cysts, including specific information on the size, echogenicity, echotexture, internal pattern, and content. Previous gynecological disorders or abnormal findings on pelvic ultrasonography were documented in the ultrasonography reports.

Statistical Analysis

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

Menstrual Cycle and Prevalent Nonalcoholic Fatty Liver Disease: A Cross-sectional Study

We analyzed the association between menstrual cycle category and NAFLD by performing logistic regression analyses to calculate the prevalence ratios (PRs) and 95% CIs for NAFLD, with adjustment for age. We used 2 models for adjustment of covariates: Model 1 was adjusted for age, center (Seoul or Suwon), year of examination, alcohol consumption, smoking, physical activity, education level, parity, age at menarche, and BMI; model 2 was adjusted for the variables in model 1 plus HOMA-IR quintiles because insulin resistance is associated both with NAFLD (2) and PCOS (35). We adjusted for potential confounders that might affect the relationship between menstrual cycles and NAFLD. The confounding variables were defined using the following criteria: 1) causal association with the outcome (NAFLD); 2) noncausal or

causal association with exposure (the menstrual cycle); and 3) not being a mediator in the causal pathway between exposure (the menstrual cycle) and the outcome (NAFLD). For the secondary analyses, we estimated the PRs and 95% CIs for mild and moderate/severe NAFLD for the menstrual cycle categories with no NAFLD as the reference group, using multinomial logistic regression models.

Menstrual Cycle and Incident Nonalcoholic Fatty Liver Disease: A Cohort Study

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

Predefined subgroup analyses were performed according to age (< 30 vs ≥ 30 years, since menstrual cycle length differs and decreases with age) (36), current smoking status, alcohol intake (< 10 vs ≥ 10 g/day, 10 g of ethanol per day for women as the cutoff of light drinking) (37), HEPA (no vs yes), early menarche, parity, obesity defined using Asian-specific criteria (BMI < 25 vs ≥25) (27, 38), HOMA-IR (< 2.5 vs ≥ 2.5) (29), and hsCRP (< 1.0 vs ≥ 1.0 mg/L, the proposed cutoff as an inflammatory marker for low risk of cardiovascular disease by the Center for Disease Control and American Heart Association) (39). We tested for interactions among the subgroups by performing likelihood ratio tests and compared the models with and without multiplicative interaction terms. We also performed analysis for prevalent and incident NAFLD among women who underwent pelvic ultrasonography examinations and excluded those with a report of PCOS diagnosis or polycystic ovaries on ultrasonography findings.

Statistical analyses were performed using Stata version 16.0 (StataCorp LP). Two-tailed *P* values of less than .05 were considered to indicate statistical significance.

Patient and Public Involvement

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

Results

Characteristics of Study Participants

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

Association between Menstrual Cycle and Prevalent Nonalcoholic Fatty Liver Disease

Table 2 shows the adjusted PRs of NAFLD based on menstrual cycle categories. Compared with 26- to 30-day menstrual cycles as the reference, less than 21-day, 31- to 39-day, and 40-day or more or irregular menstrual cycles were associated with a higher prevalence of NAFLD, with 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), respectively. After additional adjustments for center, examination year, alcohol consumption, smoking status, HEPA, education level, parity, age at menarche, BMI, and HOMA-IR, 31- to 39-day and 40-day or more or irregular cycles were associated with NAFLD, with PRs (95% CIs) of 1.27 (1.19-1.36) and 1.35 (1.28-1.42), respectively. After categorizing NAFLD as mild or moderate/severe, 31- to 39-day and 40-day or more or irregular menstrual cycles were associated with a higher prevalence of mild and moderate/severe NAFLD in a dose-response manner compared to that of the reference group (Supplementary Table 1) (40). After reconfiguring the menstrual cycle categories as less than 21-, 21- to 25-, 26- to 30-, 31- to 35-, 36- to 40-, and more than 40-day or irregular cycles, 31- to 35-day, 36- to 40-day, and more than 40-day or irregular cycles were also associated with NAFLD (Supplementary Table 2) (40).

Association Between Menstrual Cycle and Incident Nonalcoholic Fatty Liver Disease

In the cohort analysis of women without NAFLD at baseline (Supplementary Table 3) (40), 4524 incident cases of NAFLD occurred during a mean follow-up of 4.4 years. When analyzing the longitudinal associations between menstrual cycle categories and incident NAFLD, less than 21-day, 31- to 39-day, and 40-day or more or irregular menstrual cycles were associated with the development of NAFLD, with age-adjusted HRs (95% CIs) of 1.54 (1.19-1.99), 1.13 (1.03-1.24), and 1.26 (1.18-1.35), respectively (Table 3). The associations remained statistically significant after adjusting for additional covariates, including HOMA-IR values. In the time-dependent analysis, 31- to 39-day and 40-day or more or irregular cycles were associated with a higher risk of incident NAFLD, with HRs (95% CIs) of 1.27 (1.15-1.39) and 1.49 (1.38-1.60), respectively, compared to the reference group. In a sensitivity analysis using moderate/severe NAFLD as an end point, 40-day or more or irregular menstrual cycles were associated with a higher risk of incident moderate/severe NAFLD in the fully adjusted model and time-dependent analysis (Supplementary Table 4) (40). After reconfiguring the menstrual cycle categories, 21-day, 31- to 35-day, and more than 40-day or irregular cycles were associated with NAFLD risk in the fully adjusted model; in the time-dependent analysis, 31- to 35-day, 36- to 40-day, and more than 40-day or irregular cycles were associated with increased NAFLD risk (Supplementary Table 5) (40).

In subgroup analyses, 31- to 39-day and 40-day or more or irregular menstrual cycles were associated with an increased risk of NAFLD when HOMA-IR was less than 2.5, with adjusted HRs (95% CIs) of 1.18 (1.08-1.30) and 1.27 (1.18-1.37), respectively, but not when HOMA-IR was greater than or equal to 2.5 (*P* for interaction = .001) (Supplementary Table 6) (40). No other statistically significant interactions were observed for the other predefined subgroups.

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

Characteristics	Menstrual cycle, d					*P* for trend
	< 21	21-25	26-30	31-39	≥ 40 or irregular	
No.	914	4367	36 378	10 455	19 978	
Age, y	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)	< .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)	< .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)	.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)	.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)	.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)	.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)	< .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)	< .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)	.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)	< .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)	< .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)	.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)	< .001
Body mass index	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)	< .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)	< .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)	< .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)	< .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)	< .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)	< .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)	< .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)	< .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)	< .001
hsCRP, 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)	< .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)	.042

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CVD, cardiovascular disease; GGT, γ-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; HEPA, health-enhancing physical activity; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol.
[a]A total of 10 g or more of ethanol per day.
[b]College graduate or higher.
[c]Body mass index greater than or equal to 25.

Table 2. Adjusted prevalence ratios of nonalcoholic fatty liver disease by menstrual cycle category at baseline (N = 72 092)

Menstrual cycle length, d	No.	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	4367	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	2118	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	1941	9.7	1.89 (1.83-1.95)	1.38 (1.31-1.45)	1.35 (1.28-1.42)
P for linear trend				< .001	< .001	< .001
P for quadratic trend				< .001	< .001	< .001

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; PR, prevalence ratio.
[a]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.

Association Between Menstrual Cycle and Nonalcoholic Fatty Liver Disease Among Women With Pelvic Ultrasonography Data

Among the women with pelvic ultrasonography data and gynecologic assessments available, after excluding 300 women with suspected PCOS, 18 968 women were included in the analysis at baseline. In the cross-sectional analysis, 31- to 39-day and 40-day or more or irregular menstrual cycles were associated with NAFLD, with adjusted PRs (95% CIs) of 1.28 (1.11-1.46) and 1.42 (1.27-1.58), respectively (Supplementary Table 7) (40).

In the longitudinal analysis of 14 378 women without either NAFLD or suspected PCOS at baseline, the adjusted

Table 3. Development of nonalcoholic fatty liver disease by menstrual cycle category at baseline (N = 51 118)

Menstrual period, d	PY	Incident cases	Incidence density (/103 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	2273	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	2118	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	1477	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)
P for linear trend				< .001	< .001	< .001	< .001
P for quadratic trend				< .001	< .001	< .001	< .001

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; HR, hazard ratio; PY, person-year.
[a]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.
[b]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.

HRs (95% CIs) for 31- to 39-day and 40-day or more or irregular menstrual cycles were 1.23 (1.03-1.47) and 1.32 (1.15-1.52), respectively (Supplementary Table 8) (40).

Discussion

In our large cohort of premenopausal women, long or irregular menstrual cycles were associated with an increased risk of NAFLD compared to 26- to 30-day cycles, both in cross-sectional and longitudinal analyses. Long or irregular menstrual cycles were associated with a higher prevalence of mild and moderate/severe NAFLD in a dose-response manner, and the association between long or irregular menstrual cycles and NAFLD risk was more pronounced in time-dependent analyses and were not fully explained by obesity, insulin resistance, or other relevant measured confounders in the subgroup analyses. Importantly, our results indicate that menstrual irregularity, which is easier to diagnose and usually presents earlier than PCOS (41), highlights the possibility of identifying premenopausal women at risk of developing NAFLD.

While limited evidence exists regarding how menstrual irregularities may affect the pathogenesis of NAFLD, PCOS has been linked with NAFLD in most previous studies included in the meta-analysis by Shengir et al (11). However, although women with long or irregular menstrual cycles are reported to be more likely to have hirsutism and ovulatory infertility than those with regular 26- to 31-day cycles (5), not all women with long or irregular menstrual cycles have PCOS. The prevalence of PCOS is reported to range from 4% to 21%, depending on the diagnostic criteria applied (42). In our study, 28% of participants had long or irregular menstrual cycles. Meanwhile, previous reports have suggested that the prevalence of PCOS in women with oligomenorrhea is estimated to be approximately 45% to 87% (43, 44), indicating that PCOS is present in a subgroup of women with abnormal menstrual cycles. On the other hand, women with PCOS and long menstrual cycles, or oligomenorrhea, have poorer metabolic profiles than those without oligomenorrhea (7, 45, 46). There is also a report that oligomenorrhea predicts the risk of diabetes mellitus in the absence of hyperandrogenism (5), potentially suggesting that menstrual cycle length may be the major determinant of metabolic abnormalities, beyond simply a proxy marker of PCOS. We lacked data to identify hyperandrogenism or hirsutism in our cohort; however, after excluding women with PCOS in our subgroup analysis of women with pelvic ultrasonography data and assessment by a gynecologist, the association between long or irregular menstrual cycles and NAFLD persisted both in the cross-sectional and longitudinal analyses, suggesting that PCOS may not fully explain the relationship.

The association between long or irregular menstrual cycles and NAFLD remained after further adjustment for HOMA-IR both in cross-sectional and longitudinal analyses, as well as in time-dependent analysis. Long or irregular menstrual cycles were associated with an increased risk of NAFLD in the subgroup with less insulin resistance (HOMA-IR < 2.5) but not in the subgroup with more insulin resistance (HOMA-IR ≥ 2.5), although there were only few women in the HOMA-IR group with 2.5 or greater. This suggests that insulin resistance, which has been posited to contribute to the association between PCOS and NAFLD (11), does not fully explain the association between long or irregular menstrual cycles and NAFLD demonstrated in our study. Menstrual irregularity may also be a consequence of unhealthy lifestyle factors such as disordered eating and stress (47), which may increase the risk of NAFLD; however, there was no evidence for effect modification by factors such as smoking, alcohol consumption, HEPA, and obesity.

The prevalence of NAFLD in our study (7.0%) is similar to that reported previously for premenopausal women and represents a lower prevalence of NAFLD than that reported in general or male populations, which ranges from 20% to 42% (4). We attempted to exclude women experiencing the perimenopausal transition by excluding women older than 40 years, because hormonal changes that predispose women to NAFLD (48) could confound our analysis of menstrual cycles among premenopausal women. The higher risk of incident NAFLD compared to women with 26- to 30-day menstrual cycles observed among women with less than 21-day menstrual cycles in the longitudinal analysis may be attributable to perimenopausal changes, such as lower estradiol and high follicle-stimulating hormone levels (49) and shorter regular menstrual cycles (22). However, this association was not statistically significant in the time-dependent analysis. Interestingly, less than 21-day menstrual cycles were

also associated with higher age-adjusted PRs for NAFLD, higher proportions of diabetes and obesity, and higher mean HOMA-IR values compared to the reference group, as well as higher proportions of current smokers and participants with alcohol intake of 10 to 19 g/day. Polymenorrhea (< 21-day menstrual cycles) may appear to be associated with poor metabolic profiles, similar to those with long or irregular menstrual cycles. However, less than 21-day menstrual cycles were not associated with NAFLD after adjusting for other covariates. Similarly, previous studies have shown that while oligomenorrhea and amenorrhea were associated with insulin resistance in women with PCOS, the association between polymenorrhea and insulin resistance was comparable to that of women with normal menstrual cycles (46, 50).

While the mechanisms underlying the association between long or irregular menstrual cycles and NAFLD are unclear, exposure to estrogen may contribute to this association. Low 17β-estradiol levels, as well as the use of antiestrogens such as tamoxifen and aromatase inhibitors, have been associated with NAFLD (51). In contrast, estrogen replacement therapy has been reported to decrease the risk of NAFLD, which is reportedly twice as common in postmenopausal women than in premenopausal women (51, 52). Estrogen is suggested to suppress inflammation; improve mitochondrial function; modulate nuclear receptors; and mitigate oxidative stress, insulin resistance, and fibrogenesis, to decelerate the progression of chronic liver diseases including NAFLD (53). The estrogen receptor α expressed in the liver is suggested to contribute to hepatic sexual dimorphism (54) and lower the incidence of hepatic diseases in premenopausal women (55, 56). In female mice, estrogen receptor α was found to counteract the accumulation of lipids in the liver following excessive dietary fat intake by inhibiting lipid synthesis and promoting mitochondrial fatty acid β-oxidation (56). Although early menopause was not associated with NAFLD in a previous study (57), it may have lacked power because of the limited number of participants with early menopause. Further studies including larger populations are warranted to clarify the role of estrogen insufficiency and sex hormone abnormalities in the development of NAFLD.

Besides estrogen exposure, androgen excess and hypogonadotropic hypogonadism may contribute to the association between long or irregular menstrual cycles and NAFLD. Increased luteinizing hormone (58) and androgen (9) levels have been reported in women with irregular menstrual cycles. Normal androgen levels help balance fat and lean mass; conversely, hyperandrogenism may predispose to fat accumulation (52), especially in the abdomen (4). Moreover, hypogonadotropic hypogonadism is common in women who experience considerable weight loss, exercise excessively, or are under severe stress and may manifest as long or irregular menstrual cycles (59). Although there was no statistically significant interaction between menstrual length and obesity, the association between long or irregular menstrual cycles and NAFLD was more pronounced in nonobese women, while the association was not statistically significant in obese women. Iron overload may also contribute to the association between long or irregular menstrual cycles and NAFLD; as increased hepatic iron was associated with NAFLD and progression of nonalcoholic steatohepatitis in some studies, regular menstruation may contribute to decreased NAFLD risk (60, 61).

The strength of our study was the use of both cross-sectional and longitudinal study designs. Our study also has some limitations. First, the menstrual cycle was assessed using self-administered questionnaires. We attempted to avoid misclassification bias by including women younger than 40 years and excluding older women who were more likely to be menopausal or who reported the use of estrogen replacement therapy or oral contraceptives. Second, we did not have information on the participants' sex hormone or prolactin levels. Further studies with information on androgen and estrogen levels may help elucidate their influence on the association between menstrual cycles and NAFLD. However, the prevalence of hyperprolactinemia is reported as less than 1% of the general population (62, 63); therefore, the overall findings may be less likely to be affected. Third, we could not identify the women meeting the criteria for a diagnosis of PCOS among all women with long or irregular menstrual cycles because we did not have information on biochemical hyperandrogenism. However, our main findings remained consistent after excluding women with suspected PCOS using data from pelvic ultrasonography examinations and gynecologic assessments, which were available for one-fourth of the women in our cohort. Moreover, our aim was to assess long or irregular menstrual cycles as a risk factor for NAFLD in premenopausal women, regardless of a possible diagnosis of PCOS. Fourth, the diagnosis of NAFLD was based on ultrasonography, instead of histological diagnosis; the latter is the gold standard but is not appropriate for routine health screening examinations. Instead, ultrasonography is employed in epidemiological studies and provides reliable identification of NAFLD (64). Finally, because we included relatively healthy, young premenopausal Korean women, our results may not be generalizable to other populations with comorbidities, older age groups, or women of different ethnicities.

Conclusion

Our results indicate that long or irregular menstrual cycles may provide an easily identifiable marker for an increased risk of NAFLD in young, premenopausal women. Screening for NAFLD and counseling to promote healthy lifestyle behaviors may benefit women with a history of long or irregular menstrual cycles.

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Author Contributions

Y. Chang and S. Ryu planned, designed, and directed the study, including quality assurance and control. S. Ryu analyzed the data and designed the analytic strategy of the study. J. H. Kang supervised the field activities. All authors

conducted a literature review and prepared the "Materials and Methods" and "Discussion" sections of the text. I. Y. Cho and Y. Chang drafted the manuscript. I. Y. Cho, Y. Chang, J. H. Kang, Y. Kim, E. Sung, H. Shin, and S. Ryu interpreted the results. All authors, including C. Byrne and S. Wild, contributed to the critical revision of the manuscript. All authors read and approved the final manuscript.

Disclosures

The authors have nothing to disclose.

Data Availability

The data sets generated and analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Younossi Z, Tacke F, Arrese M, *et al*. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology*. 2019;69(6):2672-2682.
2. Watt MJ, Miotto PM, De Nardo W, Montgomery MK. The liver as an endocrine organ-linking NAFLD and insulin resistance. *Endocr Rev*. 2019;40(5):1367-1393.
3. Chalasani N, Younossi Z, Lavine JE, *et al*. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2018;67(1):328-357.
4. Lonardo A, Nascimbeni F, Ballestri S, *et al*. Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology*. 2019;70(4):1457-1469.
5. Solomon CG, Hu FB, Dunaif A, *et al*. Long or highly irregular menstrual cycles as a marker for risk of type 2 diabetes mellitus. *JAMA*. 2001;286(19):2421-2426.
6. Gast GC, Grobbee DE, Smit HA, Bueno-de-Mesquita HB, Samsioe GN, van der Schouw YT. Menstrual cycle characteristics and risk of coronary heart disease and type 2 diabetes. *Fertil Steril*. 2010;94(6):2379-2381.
7. Strowitzki T, Capp E, von Eye Corleta H. The degree of cycle irregularity correlates with the grade of endocrine and metabolic disorders in PCOS patients. *Eur J Obstet Gynecol Reprod Biol*. 2010;149(2):178-181.
8. Real FG, Svanes C, Omenaas ER, *et al*. Menstrual irregularity and asthma and lung function. *J Allergy Clin Immunol*. 2007;120(3):557-564.
9. Weiss DJ, Charles MA, Dunaif A, *et al*. Hyperinsulinemia is associated with menstrual irregularity and altered serum androgens in Pima Indian women. *Metabolism*. 1994;43(7):803-807.
10. Solomon CG, Hu FB, Dunaif A, *et al*. Menstrual cycle irregularity and risk for future cardiovascular disease. *J Clin Endocrinol Metab*. 2002;87(5):2013-2017.
11. Shengir M, Chen T, Guadagno E, *et al*. Non-alcoholic fatty liver disease in premenopausal women with polycystic ovary syndrome: a systematic review and meta-analysis. *JGH Open*. 2021;5(4):434-445.
12. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. *Lancet*. 2007;370(9588):685-697.
13. Rao P, Bhide P. Controversies in the diagnosis of polycystic ovary syndrome. *Ther Adv Reprod Health*. 2020;14:2633494120913032.
14. Wu J, Yao XY, Shi RX, Liu SF, Wang XY. A potential link between polycystic ovary syndrome and non-alcoholic fatty liver disease: an update meta-analysis. *Reprod Health*. 2018;15(1):77.
15. Jones H, Sprung VS, Pugh CJ, *et al*. Polycystic ovary syndrome with hyperandrogenism is characterized by an increased risk of hepatic steatosis compared to nonhyperandrogenic PCOS phenotypes and healthy controls, independent of obesity and insulin resistance. *J Clin Endocrinol Metab*. 2012;97(10):3709-3716.
16. Chang Y, Ryu S, Choi Y, *et al*. Metabolically healthy obesity and development of chronic kidney disease: a cohort study. *Ann Intern Med*. 2016;164(5):305-312.
17. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical practice guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol*. 2016;64(6):1388-1402.
18. Chun MY. Validity and reliability of Korean version of International Physical Activity Questionnaire short form in the elderly. *Korean J Fam Med*. 2012;33(3):144-151.
19. Craig CL, Marshall AL, Sjöström M, *et al*. International Physical Activity Questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381-1395.
20. Yu EJ, Choe SA, Yun JW, Son M. Association of early menarche with adolescent health in the setting of rapidly decreasing age at menarche. *J Pediatr Adolesc Gynecol*. 2020;33(3):264-270.
21. Wang YX, Arvizu M, Rich-Edwards JW, *et al*. Menstrual cycle regularity and length across the reproductive lifespan and risk of premature mortality: prospective cohort study. *BMJ*. 2020;371:m3464.
22. Grieger JA, Norman RJ. Menstrual cycle length and patterns in a global cohort of women using a mobile phone app: retrospective cohort study. *J Med Internet Res*. 2020;22(6):e17109.
23. Berek JS. *Berek & Novak's Gynecology*. 15th ed. Lippincott Williams & Wilkins; 2012.
24. Lado-Abeal J, Rodriguez-Arnao J, Newell-Price JD, *et al*. Menstrual abnormalities in women with Cushing's disease are correlated with hypercortisolemia rather than raised circulating androgen levels. *J Clin Endocrinol Metab*. 1998;83(9):3083-3088.
25. Gao YT, Shu XO, Dai Q, *et al*. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. *Int J Cancer*. 2000;87(2):295-300.
26. Harlow SD, Lin X, Ho MJ. Analysis of menstrual diary data across the reproductive life span applicability of the bipartite model approach and the importance of within-woman variance. *J Clin Epidemiol*. 2000;53(7):722-733.
27. World Health Organization, Regional Office for the Western Pacific. *The Asia-Pacific Perspective: Redefining Obesity and Its Treatment*. Health Communications Australia Pty Limit; 2000.
28. RRID:AB_2756877. http://antibodyregistry.org/AB_2756877.
29. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-419.
30. Mathiesen UL, Franzén LE, Aselius H, *et al*. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. *Dig Liver Dis*. 2002;34(7):516-522.
31. Lee T, Yun KE, Chang Y, *et al*. Risk of colorectal neoplasia according to fatty liver severity and presence of gall bladder polyps. *Dig Dis Sci*. 2016;61(1):317-324.
32. Hamaguchi M, Kojima T, Itoh Y, *et al*. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol*. 2007;102(12):2708-2715.
33. Rumack CM, Wilson SR, Charboneau JW. *Diagnostic Ultrasound*. 2nd ed. Mosby; 1998.
34. Chang Y, Ryu S, Sung KC, *et al*. Alcoholic and non-alcoholic fatty liver disease and associations with coronary artery calcification: evidence from the Kangbuk Samsung Health Study. *Gut*. 2019;68(9):1667-1675.
35. Azziz R. Polycystic ovary syndrome. *Obstet Gynecol*. 2018;132(2):321-336.
36. Bull JR, Rowland SP, Scherwitzl EB, Scherwitzl R, Danielsson KG, Harper J. Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. *NPJ Digit Med*. 2019;2(1):83.

37. Fernández-Solà J. Cardiovascular risks and benefits of moderate and heavy alcohol consumption. *Nat Rev Cardiol*. 2015;12(10):576-587.
38. Wen CP, Cheng TYD, Tsai SP, *et al*. Are Asians at greater mortality risks for being overweight than Caucasians? Redefining obesity for Asians. *Public Health Nutr*. 2009;12(4):497-506.
39. Pearson TA, Mensah GA, Alexander RW, *et al*; Centers for Disease Control and Prevention; American Heart Association. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107(3):499-511.
40. Cho IY, Chang Y, Kang JH, *et al*. Supplementary data for "Long or irregular menstrual cycles and risk of prevalent and incident non-alcoholic fatty liver disease." Figshare. Deposited February 17, 2022. https://doi.org/10.6084/m9.figshare.16782886.v3
41. Deligeoroglou E, Karountzos V. Dysfunctional uterine bleeding as an early sign of polycystic ovary syndrome during adolescence: an update. *Minerva Ginecol*. 2017;69(1):68-74.
42. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil Steril*. 2016;106(1):6-15.
43. van Hooff MH, Voorhorst FJ, Kaptein MB, Hirasing RA, Koppenaal C, Schoemaker J. Polycystic ovaries in adolescents and the relationship with menstrual cycle patterns, luteinizing hormone, androgens, and insulin. *Fertil Steril*. 2000;74(1):49-58.
44. Franks S. Polycystic ovary syndrome: a changing perspective. *Clin Endocrinol (Oxf)*. 1989;31(1):87-120.
45. Alebić MŠ, Stojanović N, Baldani DP, Duvnjak LS. Metabolic implications of menstrual cycle length in non-hyperandrogenic women with polycystic ovarian morphology. *Endocrine*. 2016;54(3):798-807.
46. Panidis D, Tziomalos K, Chatzis P, *et al*. Association between menstrual cycle irregularities and endocrine and metabolic characteristics of the polycystic ovary syndrome. *Eur J Endocrinol*. 2013;168(2):145-152.
47. Huhmann K. Menses requires energy: a review of how disordered eating, excessive exercise, and high stress lead to menstrual irregularities. *Clin Ther*. 2020;42(3):401-407.
48. Ryu S, Suh BS, Chang Y, *et al*. Menopausal stages and non-alcoholic fatty liver disease in middle-aged women. *Eur J Obstet Gynecol Reprod Biol*. 2015;190:65-70.
49. Sherman BM, Korenman SG. Hormonal characteristics of the human menstrual cycle throughout reproductive life. *J Clin Invest*. 1975;55(4):699-706.
50. Brower M, Brennan K, Pall M, Azziz R. The severity of menstrual dysfunction as a predictor of insulin resistance in PCOS. *J Clin Endocrinol Metab*. 2013;98(12):E1967-E1971.
51. Morán-Costoya A, Proenza AM, Gianotti M, Lladó I, Valle A. Sex differences in nonalcoholic fatty liver disease: estrogen influence on the liver-adipose tissue crosstalk. *Antioxid Redox Signal*. 2021;35(9):753-774.
52. Lonardo A, Carani C, Carulli N, Loria P. 'Endocrine NAFLD' a hormonocentric perspective of nonalcoholic fatty liver disease pathogenesis. *J Hepatol*. 2006;44(6):1196-1207.
53. Robeva R, Mladenović D, Vesković M, *et al*. The interplay between metabolic dysregulations and non-alcoholic fatty liver disease in women after menopause. *Maturitas*. 2021;151:22-30.
54. Zheng D, Wang X, Antonson P, Gustafsson JÅ, Li Z. Genomics of sex hormone receptor signaling in hepatic sexual dimorphism. *Mol Cell Endocrinol*. 2018;471:33-41.
55. Maggi A, Della Torre S. Sex, metabolism and health. *Mol Metab*. 2018;15:3-7.
56. Meda C, Barone M, Mitro N, *et al*. Hepatic ERα accounts for sex differences in the ability to cope with an excess of dietary lipids. *Mol Metab*. 2020;32:97-108.
57. Park SH, Park YE, Lee J, *et al*. Lack of association between early menopause and non-alcoholic fatty liver disease in postmenopausal women. *Climacteric*. 2020;23(2):173-177.
58. Hull MG. Epidemiology of infertility and polycystic ovarian disease: endocrinological and demographic studies. *Gynecol Endocrinol*. 1987;1(3):235-245.
59. Silveira LFG, Latronico AC. Approach to the patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2013;98(5):1781-1788.
60. Lefebvre P, Staels B. Hepatic sexual dimorphism—implications for non-alcoholic fatty liver disease. *Nat Rev Endocrinol*. 2021;17(11):662-670.
61. Dongiovanni P, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. *J Hepatol*. 2011;55(4):920-932.
62. Biller BM, Luciano A, Crosignani PG, *et al*. Guidelines for the diagnosis and treatment of hyperprolactinemia. *J Reprod Med*. 1999;44(12 Suppl):1075-1084.
63. Soto-Pedre E, Newey PJ, Bevan JS, Greig N, Leese GP. The epidemiology of hyperprolactinaemia over 20 years in the Tayside region of Scotland: the Prolactin Epidemiology, Audit and Research Study (PROLEARS). *Clin Endocrinol (Oxf)*. 2017;86(1):60-67.
64. Hernaez R, Lazo M, Bonekamp S, *et al*. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology*. 2011;54(3):1082-1090.