Mendelian randomization analysis: exploring the causal relationship between menstrual cycle length and bone mineral density

Qi Qu[1], Zhongshan Li[2], Jiang Rui[2], Wensheng Zhu[3]*

[1]College of Medicine, Hubei Minzu University, Enshi, China
[2]Hubei University of Traditional Chinese Medicine, Wuhan, China
[3]Huanggang Hospital of Traditional Chinese Medicine, Huangang, China

*Corresponding author:
Wensheng Zhu
Department of Orthopedics
Huanggang Hospital of
Traditional Chinese
Medicine
19 Dongmen Road
Huanggang 438000, China
E-mail: 1245749181@qq.com

Osteoporosis (OP) arises from an imbalance between bone resorption and formation, leading to diminished bone mass and weakened bone structure [1]. It is the most prevalent bone ailment globally, with over 37.5 million individuals affected in the European Union (EU) and the United States of America (USA) alone by 2010 [2]. Particularly prevalent among postmenopausal women, nearly one in three women over the age of 50 are afflicted by this condition. Therefore, it is essential to recognize potential factors that could impact the onset of OP, and further research into the pathogenesis, prevention, and treatment principles of OP holds significant medical and social value.

Menstrual cyclicity serves as a visible indicator of both health and fertility. The length of the menstrual cycle refers to the duration between the initial day of menstrual bleeding in one cycle and the commencement of menses in the subsequent cycle [3]. Typically, the median duration of a menstrual cycle is 28 days, with most cycles between 25 and 30 days [4]. Individuals experiencing menstrual cycles lasting fewer than 21 days are categorized as polymenorrheic, while those with cycles lasting longer than 35 days are termed oligomenorrheic. Studies indicate that the menstrual cycle entails consistent, cyclic variations in estrogen and progesterone levels, and these hormones are pivotal in bone metabolism, remodeling, and BMD [5, 6].

Mendelian randomization (MR) offers a risk-free method for examining causal effects. It functions as an analytical method that uses genetic traits as instrumental variables (IVs) to explore causal relationships between exposure and result [7]. Our aim was to explore the association between menstrual cycle length and BMD, and to substantiate this relationship through a two-sample MR analysis.

Methods. *Study design.* This study strictly adhered to the three assumptions of MR analysis: (1) The selected IVs were related to the exposure. (2) IVs were not associated with any confounding factors. (3) IVs can influence outcomes solely through the exposure. All datasets utilized in this study are openly accessible. Leveraging these premises, we proceeded to estimate the causal association between menstrual cycle length and total body BMD (TB-BMD), lumbar spine BMD (LS-BMD), femoral neck BMD (FN-BMD), and TB-BMD 0–15 years, TB-BMD 15–30 years, TB-BMD 30–45 years, TB-BMD 45–60 years, and TB-BMD over 60 years.

GWAS Summary Data. The UK Biobank provided the GWAS summary statistics for the menstrual cycle length (ukb-b-3278) (http://www.

AMS

nealelab.is/uk-biobank). The data provide bone density measurements for the total body, lumbar spine and femoral neck with sample sizes of 56,284 (TB-BMD), 28,498 (LS-BMD) and 32,735 (FN-BMD). Furthermore, TB-BMD data across five age groups (0–15 years: n = 11,807 cases; 15–30 years: n = 4,180 cases; 30–45 years: n = 10,062 cases; 45–60 years: n = 18,805 cases; over 60 years: n = 22,504 cases) were also downloaded from MRC-IEU [8] (http://www.bristol.ac.uk/integrative-epidemiology/).

Instrument selection. We established consistent filtering criteria for the tool variables, with statistical significance indicated by $p < 5 \times 10^{-8}$. Linkage disequilibrium analysis of the corresponding SNPs was conducted for each instrumental variable. We enforced a threshold of $r^2 < 0.001$ and kb = 10000. Additionally, we eliminated SNPs that were directly related to OP as well as those that were connected to confounding variables related to OP. Furthermore, we carried out extra screening of the retrieved SNPs to weed out those with palindromic SNPs displaying unclear strands and non-concordant alleles. These rigorously selected SNPs were designated as IVs for subsequent MR analysis. Lastly, the chosen instrumental SNPs ought to show robust correlations with the exposure variable, in line with the MR analysis's underlying assumptions. SNPs with inadequate statistical power ($F < 10$) were excluded. We calculated the F statistic using the formula: $(F = R^2(n - k - 1)/k(1 - R^2))$.

R^2 represents the proportion of exposure variance explained by IVs, where k denotes the number of IVs used in the model, and n represents the sample size [9].

Statistical analysis. The random effects IVW approach was used for the primary study. Additional methodologies such as MR-Egger, weighted median, simple mode, and weighted mode were utilized in order to examine the causal association. For sensitivity analysis, we then utilized the Cochran's Q test as a heterogeneity test, and the intercept from MR-Egger regression was employed to assess the pleiotropy of MR results. Additionally, leave-one-out tests were performed to determine whether single nucleotide polymorphisms altered MR results. All analyses were performed using the TwoSampleMR R package (version 0.5.5) for Mendelian randomization. A significance level of $p < 0.05$ was considered statistically significant.

Results. *Selection of instrumental variables.* A summary of the GWAS data was used to choose qualifying SNPs. Afterward, a cumulative set of 8 SNPs was eventually incorporated into the further analysis, exploring the correlation between menstrual cycle length and TB-BMD, LS-BMD, FN-BMD, as well as TB-BMD across various age intervals (0–15, 15–30, 30–45, 45–60, and over 60).

The F statistics > 10, suggesting that weak instrumental bias may not have been substantial in our study.

MR analysis for causal link of menstrual cycle length with BMD. Utilizing Mendelian randomization, we investigated the associations between menstrual cycle length and BMD. The results of these methods are presented in Table I. In the analysis, the IVW method demonstrated that the genetically predicted menstrual cycle length was negatively associated with the level of BMD (TB-BMD: β = –0.207, 95% CI: [–0.323, –0.091], $p <$ 0.001; LS-BMD: β = –0.140, 95% CI = [–0.267, –0.023], p = 0.031; FN-BMD: β = –0.154, 95% CI = [–0.280, –0.027], p = 0.002) in initial practice.

In the causal analysis of menstrual cycle length and TB-BMD across five age groups, a significant causal negative relationship was found between menstrual cycle length and TB-BMD in the age groups 45–60 and over 60 years. However, no significant causal relationship was found for TB-BMD in the age groups 0–15 years, 15–30 years, and 30–45 years. The IVW method demonstrated the following results: TB-BMD 0–15 years: β = –0.011, 95% CI: [–0.324, 0.125], p = 0.385; TB-BMD 15–30 years: β = –0.158, 95% CI: [–0.639, 0.323], p = 0.520; TB-BMD 30–45 years: β = –0.311, 95% CI: [–0.660, 0.038], p = 0.080; TB-BMD 45–60 years: β = –0.374, 95% CI = [–0.533, –0.215], $p <$ 0.001; TB-BMD over 60 years: β = –0.134, 95% CI = [–0.273, 0.005], p = 0.053.

Sensitivity analyses for causal link of menstrual cycle length with BMD. Refer to Table II for specifics. With the exception of TB-BMD 15–30 and TB-BMD 30–45, the Cochran's Q statistic from both MR-Egger and IVW methods revealed no noteworthy heterogeneity among the instrumental variables.

For TB-BMD 15–30 years: IVW, p = 0.034; MR-Egger, p = 0.047; MR-Egger intercept = 0.031, p = 0.331; and TB-BMD 30–45 years: IVW, p = 0.008; MR-Egger, p = 0.004. Despite observing heterogeneity in some of the results tested with Cochran's Q test, since we used random effects IVW as the main outcome, the level of heterogeneity is acceptable and does not undermine the MR estimates in the present study. Furthermore, the results remain non-significant in leave-one-out sensitivity analyses (Figure 1). Furthermore, no evidence of horizontal pleiotropy was observed in these findings (p > 0.05), suggesting that the results were not influenced by potential confounding pathways, thus affirming their validity and robustness.

Discussion. Our research established a causal link between the length of the menstrual cycle and BMD, encompassing TB-BMD, LS-BMD, and FN-BMD across five different age groups. During the analysis, the IVW method revealed a negative association between menstrual cycle length and

Qi Qu, Zhongshan Li, Jiang Rui, Wensheng Zhu

Table I. MR estimates from different methods for assessing the causal effect of menstrual cycle length on BMD by site

Exposure	Outcome	SNP	Method	Beta	95% CI	*P*-value
Length of menstrual cycle	TB-BMD	8	MR-Egger	−0.362	(−0.613, −0.113)	0.03
			Weighted median	−0.230	(−0.337, −0.124)	< 0.001
			Inverse variance weighted	−0.207	(−0.323, −0.091)	< 0.001
			Simple mode	−0.159	(−0.341, −0.024)	0.133
			Weighted mode	−0.237	(−0.355, −0.121)	0.006
	LS-BMD	8	MR-Egger	−0.254	(−0.551, 0.042)	0.143
			Weighted median	−0.226	(−0.389, −0.063)	0.007
			Inverse variance weighted	−0.140	(−0.267, −0.013)	0.031
			Simple mode	0.060	(−0.263, −0.383)	0.726
			Weighted mode	−0.231	(−0.418, −0.045)	0.045
	FN-BMD	8	MR-Egger	−0.237	(−0.541, −0.066)	0.176
			Weighted median	−0.157	(−0.298, −0.016)	0.029
			Inverse variance weighted	−0.154	(−0.280, −0.027)	0.017
			Simple mode	−0.058	(−0.332, 0.216)	0.691
			Weighted mode	−0.161	(−0.331, 0.009)	0.106
	TB-BMD (0–15)	8	MR-Egger	−0.111	(−0.752, 0.352)	0.504
			Weighted median	−0.129	(−0.378, 0.121)	0.310
			Inverse variance weighted	−0.011	(−0.324, 0.125)	0.385
			Simple mode	−0.228	(−0.598, 0.142)	0.266
			Weighted mode	−0.135	(−0.411, 0.139)	0.366
	TB-BMD (15–30)	8	MR-Egger	−0.684	(−1.769, 0.400)	0.263
			Weighted median	−0.401	(−0.821, 0.019)	0.0613
			Inverse variance weighted	−0.158	(−0.639, 0.323)	0.52
			Simple mode	−0.221	(−0.896, 0.454)	0.542
			Weighted mode	−0.367	(−0.808, 0.075)	0.148
	TB-BMD (30–45)	8	MR-Egger	−0.411	(−1.266, 0.443)	0.382
			Weighted median	−0.304	(−0.580, −0.286)	0.03
			Inverse variance weighted	−0.311	(−0.660, 0.038)	0.08
			Simple mode	−0.158	(−0.717, 0.400)	0.596
			Weighted mode	−0.306	(−0.637, 0.246)	0.113
	TB-BMD (45–60)	8	MR-Egger	−0.477	(−0.855, −0.098)	0.049
			Weighted median	−0.385	(−0.590, −0.179)	< 0.001
			Inverse variance weighted	−0.374	(−0.533, −0.215)	< 0.001
			Simple mode	−0.384	(−0.724, −0.045)	0.062
			Weighted mode	−0.311	(−0.618, −0.182)	0.009
	TB-BMD (over 60)	8	MR-Egger	−0.201	(−0.518, 0.116)	0.260
			Weighted median	−0.143	(−0.326, 0.040)	0.127
			Inverse variance weighted	−0.134	(−0.273, 0.005)	0.05
			Simple mode	−0.081	(−0.347, 0.187)	0.576
			Weighted mode	−0.155	(−0.364, 0.054)	0.19

BMD – bone mineral density, TB-BMD – total body BMD, LS-BMD – L-lumbar spine BMD, FN-BMD – femoral neck BMD, SNP – single nucleotide polymorphism, CI – confidence interval.

BMD level in initial practice. In the causal analysis of menstrual cycle length and age-stratified BMD, a notable negative causal relationship was identified between menstrual cycle length and TB-BMD in age groups 45–60 and over 60 years, but no sig- nificant causal relationship was found for TB-BMD in the age groups 0–15, 15–30, and 30–45.

Numerous studies have investigated the relationship between the length of the menstrual cycle and BMD or OP [10–12]. Several studies gen-

**Table II.** Sensitivity analysis of the MR results for exposures and outcomes

Exposure	Outcome	Pleiotropy test			Heterogeneity test			
		MR-Egger			MR-Egger		IVW	
		Intercept	SE	*P*-value	Q	Q_pval	Q	Q_pval
Menstrual cycle length	TB-BMD	0.009	0.029	0.223	10.759	0.096	14.073	0.059
	LS-BMD	0.007	0.008	0.432	6.506	0.369	7.273	0.401
	FN-BMD	0.005	0.008	0.57	9.131	0.166	9.679	0.207
	TB-BMD (0–15)	0.006	0.015	0.706	9.835	0.132	10.091	0.183
	TB-BMD (15–30)	0.031	0.029	0.331	12.749	0.047	15.131	0.034
	TB-BMD (30–45)	0.006	0.023	0.807	18.881	0.004	19.087	0.008
	TB-BMD (45–60)	0.006	0.01	0.576	7.169	0.37	7.587	0.305
	TB-BMD (over 60)	0.004	0.009	0.66	1.714	0.944	1.928	0.964

BMD – bone mineral density, TB-BMD – total body BMD, LS-BMD – lumbar spine BMD, FN-BMD – femoral neck BMD, SE – standard error, IVW – inverse variance weighted.

erally align with our study results, where participants reported their usual patterns of menstrual cycle regularity and length. Exclusions were made for periods of pregnancy, lactation, or contraceptive use in these studies. A study has shown that women in perimenopause experience a higher rate of bone loss in the spine and femoral neck compared to those in early menopause and premenopause. Irregular menstrual cycles, characterized by lengthening cycles, contribute to a decline in total body, spine, and femoral neck BMD approximately 2 years before the onset of the last menstrual period [13]. A questionnaire survey conducted in China on women aged 40–80 also discovered a close relationship between menstrual cycle length and the prevalence of OP. The survey demonstrated a negative correlation between the menstrual cycle length and the prevalence of OP [12]. Among individuals aged 35–39, cycle length variability was minimal, but it notably increased in those aged 45–49 and 50 years and older [14]. Specifically, the prevalence of OP is the highest when the menstrual cycle is ≥ 29 days. Recker pointed out that abnormal menstrual cycles can lead to a 0.5% decrease in bone density in the femoral neck and a 0.6% decrease in overall mineral content [6].

Perimenopause, often referred to as the menopausal transition, delineates the several-year phase preceding an individual's final menstrual period and is typified by escalating variability in menstrual cycle length. Studies by Parker SE and others [15] have shown that bone loss accelerates threefold in the immediate postmenopausal period, with losses observed at all sites during the ages of 50–59. However, our study did not reveal any causal relationships between TB-BMD and age groups 0–15, 15–30, and 30–45 years. Similarly, in a large cross-sectional study involving 963 healthy women aged 19–35 years, no significant association was found between menstrual status and BMD [16]. Li *et al.* found [14] that the average menstrual cycle length decreases with age across all age groups until reaching age 50, after which it begins to increase for those aged 50 and older.
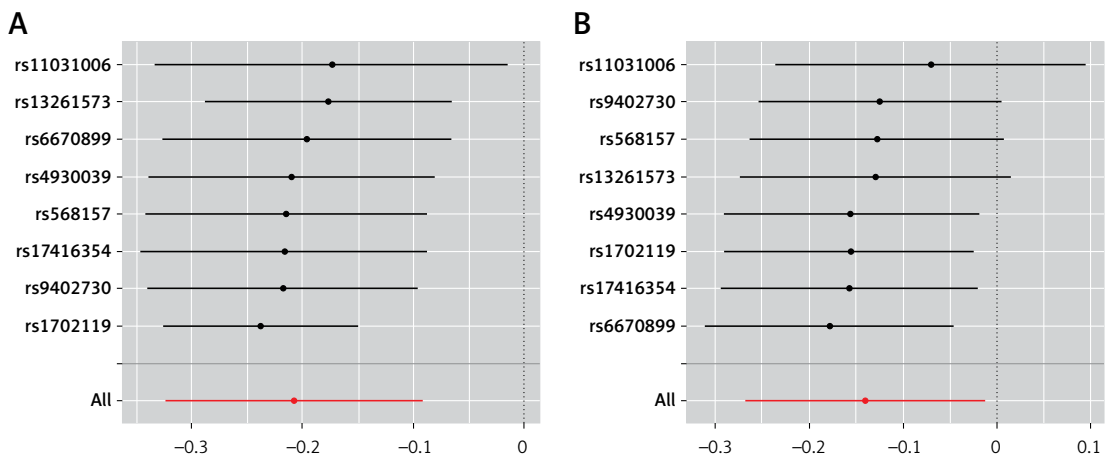
Figure 1. Leave-one-out sensitivity analysis plot for SNP effects on the relationship between length of menstrual cycle and BMD. **A** – Length of menstrual cycle on TB-BMD. **B** – Length of menstrual cycle on LS-BMD
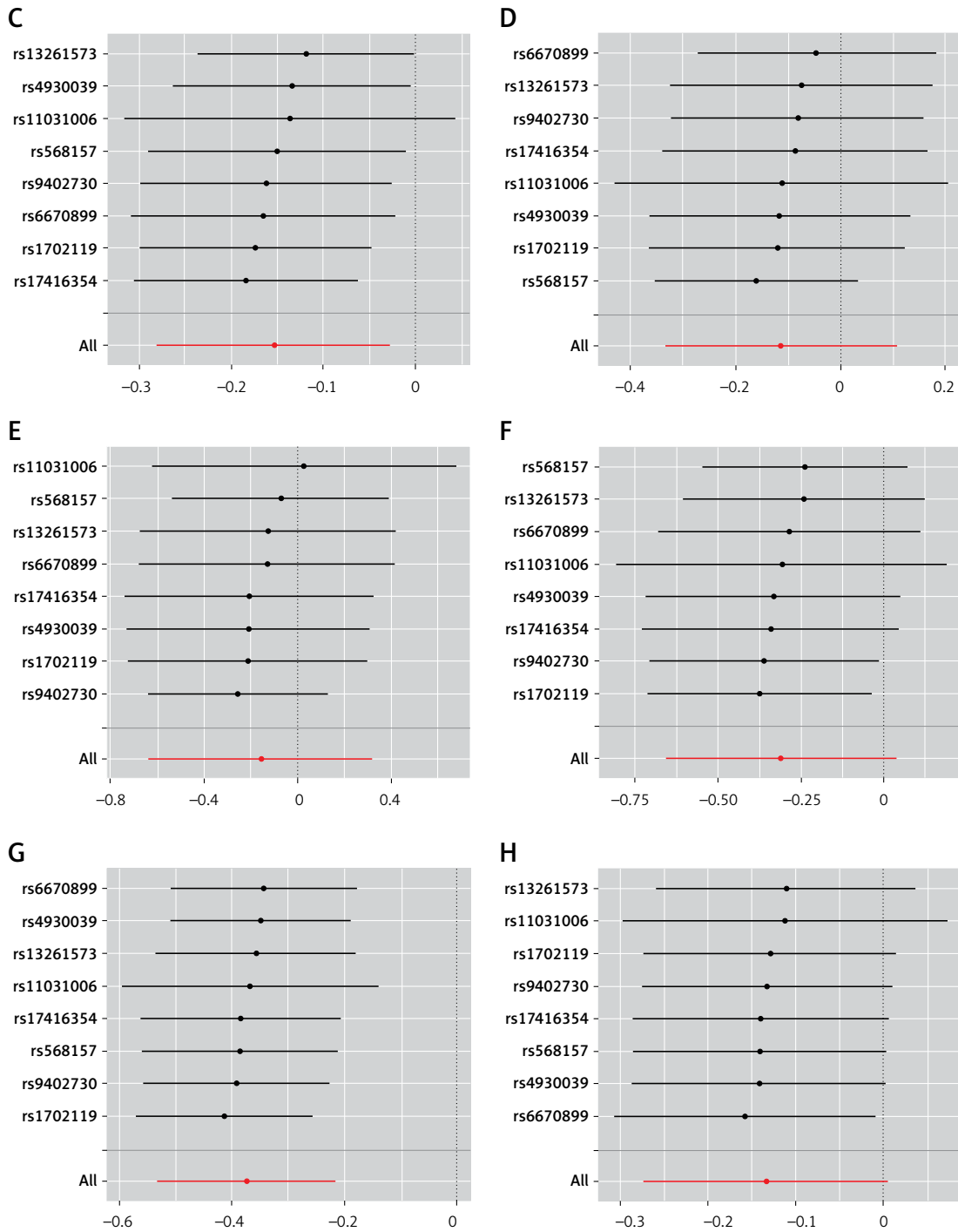
Figure 1. Cont. **C** – Length of menstrual cycle on FN-BMD. **D** – Length of menstrual cycle on TB-BMD (0–15). **E** – Length of menstrual cycle on TB-BMD (15–30). **F** – Length of menstrual cycle on TB-BMD (35–40). **G** – Length of menstrual cycle on TB-BMD (45–60). **H** – Length of menstrual cycle on TB-BMD (over 60)

Irregular and long menstrual cycles, which affect approximately 20% of women, are often attributed to disruptions in the hypothalamic-pituitary-ovarian axis among women of reproductive age. Estrogen has a crucial role in the connection between menstruation and BMD or OP [17]. Research has demonstrated a correlation between decreased bone mass and both extended follic- ular phases and decreased estrogen production [18]. Reduced plasma estradiol levels have been linked to lower bone density and an increased risk of osteopenia or OP in women with oligo- menorrhea, which is characterized by infrequent menstruation, usually defined as cycles occurring 35–40 days apart [19]. Additionally, according to research findings [18], there is a correlation be-

tween increased bone production and decreased bone resorption during the preovulatory and luteal periods, when estrogen levels rise. BMD declines significantly during late perimenopause and continues at a comparable rate through the early postmenopausal period [20]. In addition to the impact of decreased estrogen levels, current evidence suggests that bone loss during menopause may also be associated with increased serum follicle-stimulating hormone (FSH). Elevated FSH levels can stimulate osteoclastogenesis, promoting bone resorption through osteoclast activity [21]. Our research revealed that the chance of BMD loss increased with the length of the menstrual cycle. Longer menstrual cycles are frequently associated with prolonged follicular phases, delayed estrogen peaks, and lower mean estrogen levels across the whole cycle [10]. This may also relate to FSH levels, potentially explaining the negative correlation between menstrual cycle length and BMD. An increasing body of evidence demonstrated that female bone metabolism is also significantly influenced by later menarche, a shorter time from menarche to menopause, and a higher number of births [22]. During pregnancy, the developing fetus relies solely on maternal bone minerals for calcium, challenging maternal BMD. To counteract this fetal-induced bone loss, the maternal body adapts by doubling intestinal calcium absorption. Research in this area has primarily concentrated on parity [23]. A cross-sectional study in Korean women revealed that having more children increases the risk of fractures related to osteoporosis [24]. Moreover, comparing data between studies is complicated by inconsistent definitions of normal, short, or long menstrual cycle (e.g., long menstrual cycle being ≥ 32 days [25] vs. > 45 days [26]) and variability in menstrual cycle length. Hence, additional investigation is warranted to elucidate the physiological mechanisms underlying these alterations.

The MR study has a number of advantages. Firstly, in this study, MR analysis was implemented to elucidate the connection, offering fresh insights into both the influence of menstrual cycle length on BMD and the early prevention of OP. To the best of our knowledge, this is the first MR study to evaluate the link between menstrual cycle length and BMD. Secondly, this study signifies a groundbreaking endeavor in exploring the correlation between menstrual cycle length and BMD by employing MR analyses, thereby expanding the horizon beyond conventional observational studies. However, despite these strengths, our study also had some limitations. Firstly, it is important to note that certain participants in the BMD GWAS did not solely represent individuals of European ancestry. This raises concerns regarding the reli-

ability of our findings, as allele frequencies can vary significantly across different populations. Secondly, we acknowledge the limitations regarding demographic details such as childbirth history, occupation, and exposure to stress factors, which are not fully detailed in the current dataset.

In conclusion, this MR study provides valuable insights into the link between prolonged menstrual cycle length and decreased BMD. Our findings hold significance for clinical practice, suggesting that identifying abnormal menstrual patterns could facilitate the early detection of declining bone density. Such detection may enable timely interventions to prevent OP and promote overall bone health. As such, our study has important implications for both clinical management and public health strategies.

Acknowledgments

Funding

Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Ensrud KE, Crandall CJ. Osteoporosis. Ann Intern Med 2017; 167: ITC17-32.
2. van der Burgh AC, de Keyser CE, Zillikens MC, et al. The effects of osteoporotic and non-osteoporotic medications on fracture risk and bone mineral density. Drugs 2021; 81: 1831-58.
3. Wang YX, Stuart JJ, Rich-Edwards JW, et al. Menstrual cycle regularity and length across the reproductive lifespan and risk of cardiovascular disease. JAMA Netw Open 2022; 5: e2238513.
4. Campbell LR, Scalise AL, DiBenedictis BT, et al. Menstrual cycle length and modern living: a review. Curr Opin Endocrinol Diabetes Obes 2021; 28: 566-73.
5. Sowers M, Randolph JJ, Crutchfield M, et al. Urinary ovarian and gonadotropin hormone levels in premenopausal women with low bone mass. J Bone Miner Res 1998; 13: 1191-202.
6. Recker R, Lappe J, Davies K, et al. Characterization of perimenopausal bone loss: a prospective study. J Bone Miner Res 2000; 15: 1965-73.
7. Jiang R, Qu Q, Wang Z, et al. Association between air pollution and bone mineral density: a Mendelian randomization study. Arch Med Sci 2024; 20: 1334-8.

8. Yu J, Zhuang C, Guo W, et al. Causal relationship between breakfast skipping and bone mineral density: a two-sample Mendelian randomized study. Front Endocrinol 2023; 14: 1200892.

9. Bayer FP, Gander M, Kuster B, et al. CurveCurator: a recalibrated F-statistic to assess, classify, and explore significance of dose-response curves. Nat Commun 202; 14: 7902.

10. Ouyang F, Wang X, Arguelles L, et al. Menstrual cycle lengths and bone mineral density: a cross-sectional, population-based study in rural Chinese women ages 30-49 years. Osteoporos Int 2007; 18: 221-33.

11. Song S, Choi H, Pang Y, et al. Factors associated with regularity and length of menstrual cycle: Korea Nurses' Health Study. BMC Womens Health 2022; 22: 361.

12. He Y, Huang J, Jiang G, et al. Menarche age exceed 17 years and menopausal age smaller than 48 years may affect prevalence of osteoporosis for Chinese women. Arch Osteoporos 2021; 16: 123.

13. Karlamangla AS, Burnett-Bowie SM, Crandall CJ. Bone health during the menopause transition and beyond. Obstet Gynecol Clin North Am 2018; 45: 695-708.

14. Li H, Gibson EA, Jukic A, et al. Menstrual cycle length variation by demographic characteristics from the Apple Women's Health Study. NPJ Digit Med 2023; 6: 100.

15. Parker SE, Troisi R, Wise LA, et al. Menarche, menopause, years of menstruation, and the incidence of osteoporosis: the influence of prenatal exposure to diethylstilbestrol. J Clin Endocrinol Metab 2014; 99: 594-601.

16. Hawker GA, Forsmo S, Cadarette SM, et al. Correlates of forearm bone mineral density in young Norwegian women: the Nord-Trøndelag Health Study. Am J Epidemiol 2002; 156: 418-27.

17. Black DM, Rosen CJ. Clinical practice. Postmenopausal osteoporosis. N Engl J Med 2016; 374: 254-62.

18. Starrach T, Santl A, Seifert-Klauss VR. Perimenopausal Bone loss is associated with ovulatory activity-results of the PeKnO Study (Perimenopausal Bone Density and Ovulation). Diagnostics 2022; 12: 305.

19. Landgren BM, Collins A, Csemiczky G, et al. Menopause transition: annual changes in serum hormonal patterns over the menstrual cycle in women during a nine-year period prior to menopause. J Clin Endocrinol Metab 2004; 89: 2763-9.

20. Cheung E, Tsang S, Bow C, et al. Bone loss during menopausal transition among southern Chinese women. Maturitas 2011; 69: 50-6.

21. AlFaris N, AlKehayez N, AlMushawah F, et al. Anthropometry, bone mineral density and risk of breast cancer in premenopausal and postmenopausal Saudi women. Arch Med Sci 2023; 19: 608-17.

22. Li Y, Lai J, Wu W, et al. Genetic prediction of osteoporosis by Anti-Müllerian hormone levels and reproductive factors in women: a Mendelian randomization study. Calcif Tissue Int 2024; 115: 41-52.

23. Kyvernitakis I, Reuter T C, Hellmeyer L, et al. Subsequent fracture risk of women with pregnancy and lactation-associated osteoporosis after a median of 6 years of follow-up. Osteoporos Int 2018; 29: 135-42.

24. Seo E, Lee Y, Kim HC. Association between parity and low bone density among postmenopausal Korean women. J Prev Med Public Health 2021; 54: 284-92.

25. Roman LA, Pereira A, Garmendia MM. Association between obesity with pattern and length of menstrual cycle: the role of metabolic and hormonal markers. Eur J Obstet Gynecol Reprod Biol 2021; 260: 225-31.

26. Varnell RR, Arnold TJ, Quandt SA, et al. Menstrual cycle patterns and irregularities in hired latinx child farmworkers. J Occup Environ Med 2021; 63: 38-43.