

Cardiovascular Risk Factors at Different Stages of Menopause: A Study among Bengali-Speaking Hindu Ethnic Group, India

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ABSTRACT: A reduction in the estrogen and progesterone levels during midlife leads to adverse changes in body fat distribution, insulin and lipid metabolism, and endothelial dysfunction; all of these increase the risk of cardiovascular disease (CVD). However, scholars are not unanimous on whether menopause enhances the CVD risk, independent of the normal process of aging and other confounding factors. Despite the cardio-protective effect of endogenous estrogen during their premenopausal years, the increased life expectancy of women exposes them to a greater lifetime risk of CVD compared to men. The aim of the present study was to understand the cardiovascular risk factors associated with different stages of menopause. This study was cross-sectional in nature and was carried out in the Howrah district of West Bengal, India. Two hundred and one participants were recruited for the study (Premenopausal 71, Perimenopausal 61, and Postmenopausal 69). Data on body fat distribution, blood glucose levels, and total cholesterol, blood pressure, and socio-demographic, menstrual and reproductive history, and lifestyle characteristics were obtained following standard protocols. Multivariate analysis of covariance was performed to understand how menopausal status impacted CVD risk factors after controlling the effects of the confounders. Menopausal status significantly predicted the CVD risk factors and body fat measures after removing the effects of the confounding variables, reinforcing the role of estrogen in the development of CVD. An overwhelming majority of the participants in our study have central obesity; so, this group is more prone to developing CVD in the near future. Future cross-cultural studies are required to understand how the link between menopause and CVD varies across different cultural groups and throughout the menopausal transition.

KEYWORDS: menopause, midlife health, CVD, blood glucose level, body fat distribution



Original article

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Introduction

Menopause signifies the end of the reproductive phase and the initiation of the post-reproductive phase of a woman's life. An estimated 1.5 million women go through the menopausal transition (MT) each year (Santoro et al. 2005). The phases of MT and postmenopause have a significant impact on women's midlife health. During this menopausal transition, most women passed through some long-term and short-term adverse physiological changes owing to the decline in the sex hormone levels (Carr 2003). The short-term changes may involve the experience of menopausal symptoms, and the long-term changes include the occurrence of cardiovascular risk factors (Matthews et al. 2009). Worldwide, cardiovascular diseases (CVD) are emerging as the primary cause of death, especially among those women living in middle and low-income countries (Matthews et al. 2009). Studies show that women have a reduced risk for CVD than men before the fifth decade of life. After reaching menopause, this gender gap declines, making women more susceptible to CVD risk factors (Satyavani et al. 2015). For instance, menopause results in some adverse biological changes like, redistribution of body fat from periphery to the center, impaired glucose and insulin metabolism, dyslipidemia, fibrinolysis, coagulation, vascular endothelial dysfunction, and low bone mineral density (Carr 2003). The receptors of estrogen are present in the myocardium, tissues of vascular smooth muscle, endothelium, and in coronary arteries and function as a cardio-protective hormone with both long and short-term benefits on the CVD system (Matthews et al. 2009). Furthermore, estrogen helps to maintain a favorable lipid level, changes the vascular tone, pro-

tections the vascular endothelium from the adverse impacts of low-density lipoprotein cholesterol (LDL) by inhibiting the oxidation of LDL, and stabilizes the endothelial cells (Inaraja et al. 2020). All these are the cardio-protective mechanisms of estrogen which is reduced during and after menopause. Thus, the postmenopausal years are linked to cardiovascular health concerns like diabetes, hypertension, dyslipidemia, heart disease, and osteoporosis (Carr 2003; Inaraja et al. 2020). According to the Framingham Heart Study, women with natural menopause (aged 50–59 years) are four times more likely to develop CVD than the age-matched premenopausal women but the result was unadjusted for age and smoking (Lisabeth et al. 2009). Previous cohort studies, including SWAN (El Khoudar et al. 2017), the Melbourne Women's Midlife Health Project (Burger et al. 2007), the Healthy Women Study (Davis et al. 2012), the Penn Ovarian Aging Study (Freeman et al. 2016), and the Seattle Women's Health Study (Thomas et al. 2018) indicated a significant relationship between menopause and CVD risk factors independent of aging; but the trend is not uniform across other studies (Casiglia et al. 2008; Lisabeth et al. 2009; Trikidanathan 2013). Scholars are not unanimous on whether menopause independently enhances the risk of CVD, separate from the normal process of aging (Pardhe et al. 2017). Thus, the question remains unresolved whether natural menopause serves as an independent predictor of CVD across all populations.

Studies in various middle-income countries of the world, including India show that women reach menopause at an advanced age than the developed countries (Syamala and Sivakami 2005). Despite the cardio-protective effect of endogenous estrogen during their pre-

menopausal years, the increased life expectancy of women exposes them to a greater lifetime risk of CVD compared to men (Carr 2003). Thus, the contribution of estrogen deficiency in the occurrence of CVD in women is emerging as a conceivable therapeutic challenge of the present century. Most of the Indian studies related to menopausal women were aimed at understanding the attitude and perception of women regarding menopause, menopausal symptoms, and age at menopause (Dasgupta and Ray 2015; Mozumdar and Aggarwal 2015). Despite few studies (Nag and Ghosh 2013) there is a dearth of data on the relationship between CVD risk factors and menopausal status among Indian populations.

We aimed to compare CVD risk factors and body fat patterning of women of differential menopausal stages and to understand the link between menopausal status and CVD risk factors, after controlling the effects for confounding variables.

Materials and methods

Study area

The study was carried out among the Hindu Bengali-speaking populations of the state of West Bengal, India. Data for this cross-sectional study were collected from the city of Howrah, which is an urban agglomerate of Howrah district of West Bengal, India. The participants were recruited from four municipal wards (namely, ward numbers 42, 44, 47, and 48) of the Howrah Municipal Corporation of Howrah *Sadar* Subdivision. These municipal wards have been selected for operational convenience.

Study participants

Initially, a total number of 300 women were approached for this study based on certain inclusion criteria. The criteria were

as follows: have attained natural menopause (in the case of postmenopausal participants), married with at least one surviving child, and have no documented history of any kind of metabolic diseases. A total number of 99 women were eliminated from the study, either because they did not fit into the inclusion criteria ($n=50$) or they denied participating ($n=49$). Women who reached menopause surgically ($n=15$), or who were on medications ($n=35$) for any metabolic disorders were excluded. Most of the participants who denied participating in the study was reluctant to participate when they learned that the study involves the collection of blood samples. Unmarried and nulliparous women were removed to confirm that all the women had experienced some specific reproductive processes (contraception use, pregnancy, parity, and lactation). The hormonal modifications associated with these reproductive events are found to have an impact on menopause as well as CVD (Patchen et al. 2017). Finally, a total of 201 participants were incorporated in this study (71 premenopausal, 61 perimenopausal, and 69 postmenopausal). The criteria of Stages of Reproductive Aging Workshop (STRAW) were followed to categorize the participants into differential menopausal stages (Soules et al. 2001). Participants who had experienced regular menstrual bleeding during the past three months and were not experiencing any irregularities were considered premenopausal. Participants whose menstruation had stopped for more than three months but less than twelve months and were experiencing irregular period during the last twelve months were considered perimenopausal. Participants who reported that their bleeding has ceased spontaneously for the last twelve months were considered postmenopausal. The purpose

of the research was conveyed to the participants, and they provided written informed consent. The study was approved by the Institutional Human Ethical committee, University of Calcutta (protocol 06/WT/19-20/1763).

Data collection

A structured schedule that was previously applied in the same population was used to obtain data on socio-demographic, menstrual, reproductive, and lifestyle characteristics (Dasgupta and Ray 2015). Data related to socio-demographic characteristics involve participants age at the time of data collection (years), occupational types and educational attainment of participants and their spouses, and monthly per capita household expenditure (in Indian rupees). Reproductive and menstrual history of the participants include age at menarche (years) and age at marriage (years), history of pregnancy and breastfeeding (for the last child), and contraceptive use. The participants were asked to report the actual date when they experienced menarche, or the closest month of the event. Some of the participants could not recall their age at menarche. The first author was required to provide some hint on specific personal events (like her own birthday or academic standards) that occurred around the time of her menarche. This approach helped the participants to report the date of their menarche accurately. Age at marriage was cross-checked by participant's husbands and/or mothers. For postmenopausal participants, they were asked to report how many years prior to the date of the interview their menstrual bleeding had ceased. The first author provided some hints in relation to some personal occasions, like the birthday of her grandchildren or any other specific event that happened around the time of her last menstruation. This

helped the participants to recall the time of their final menstrual bleeding (Kar and Roy 2023). Finally, the participant's age at the time of the interview was subtracted from the number of years following menopause to determine the age at menopause. Pregnancy records include age at first and last pregnancy and total number of pregnancies (live births, stillbirths, and miscarriages). The above-mentioned methodology has been used in our previously published study investigating the same population but addressing different research questions (Kar and Roy 2023).

A pretested food frequency schedule was used to obtain data on the food consumption patterns of the participants (Harmouche-Karaki et al. 2020). The schedule consists of a total of eighteen items that are generally consumed by the Bengali Hindu populations and are available in the study area. The participants were asked to indicate how frequently they consumed these food items in the past week before the date of the interview. Every food item was divided into eight response groups, with zero denoting never and seven denoting every day of the week. Finally, three categories of food consumption pattern were created- regularly (consumption of 5–7 days), occasionally (consumption of 2–4 days) and rarely or never (never or consumption of less than two days).

A pretested physical activity schedule was used to obtain data on the physical activity levels of the participants (Rääsk et al. 2017). The schedule consists of the physical activities of the participants about their daily activities, like cooking, mopping, dusting, ironing, washing clothing, washing dishes, watching television, listening to music, bicycling, exercising, walking, and creating handicrafts. The participants were asked to indicate how many days they got involved in these activities in a typical

week and also how much time (in minutes) on a typical day they spent on these activities. Later, we used the International Physical Activity Questionnaire (IPAQ) to determine the Metabolic Equivalent (MET) score (Rääsk et al. 2017). Metabolic Equivalent minutes per week served as a measure of the degree of physical activity. Metabolic Equivalent minutes exhibited how much energy was required to perform a particular physical activity. The following scores were given for particular physical activities: moderate activities were given 4 METS, walking was given 3.3 METS, vigorous activities were given 8 METS, and sedentary activities (watching television, listening to music, etc.) were given 1 MET. We estimated MET minutes per week by multiplying the provided MET score by the number of minutes the activity was performed in a typical day and by the number of days the activity was carried out in a particular week. For instance, if a participant performed dusting for 20 minutes (in a typical day) for 4 days in a typical week, then the MET score would be 4 (that is the assigned MET score for household chores) $\times 20 \times 4 = 320$ MET. Finally, to obtain a total MET score of a typical week, we added the MET scores of each physical activity category (moderate, sedentary, and vigorous activities).

Total cholesterol (TC) (mg/dl) and random blood glucose levels (mg/dl) were assessed for each participant. The use of random blood glucose testing to estimate diabetes has certain drawbacks. The guidelines of International Diabetes Federation have suggested testing for participants with random glucose values ≥ 200 mg/dl (Engelgau et al. 2010). Accu-check active blood glucose monitoring kit (Model no. GB10803608) and multi-care-in meter (Model no. IN2140129) were used for estimating blood glucose and TC levels,

respectively. We collected blood samples from the tip of the left hand's second finger. An automatic blood pressure monitor (Omron's blood pressure monitor, Model no. HEM-7121) was used to measure the blood pressure level (mmHg) of the participants. After a 10-minute interval, two independent blood pressure readings were obtained, and the average was calculated (Kar and Roy 2023). Each participant's mean arterial pressure (MAP) was determined using the formula $MAP = SBP + 2(DBP)/3$.

Omron's Body Composition Monitor (Model No. HBF-362) was used to measure body weight (to the nearest 0.1 kg), skeletal and subcutaneous fat for the whole body and trunk, visceral fat, and percent body fat (PBF) for each participant who were dressed in minimal clothing and without shoes. The Rossmax Body Fat Monitor was employed to assess the muscle mass content of the body (Model no. WF260). To confirm that the instruments were reliable, each measurement was obtained twice.

A portable GPM anthropometer was used to assess height (to the nearest 0.1 cm) for each participant standing on a horizontal plane without shoes (Lohman et al. 1988). Waist circumference (WC) was determined at the minimum circumference of the torso between the rib cage and the iliac crest with a fiberglass insertion tape over minimal clothing. Hip circumference (HC) was assessed horizontally at the widest extension of the hips around the buttocks. Some of the anthropometric indices such as fat mass (FM), fat-free mass (FFM), and waist-hip ratio (WHR) were assessed following standard formulae:

$$WHR = WC \text{ (cm)} / HC \text{ (cm)}$$

$$FM = PBF / 100 \times \text{Weight (kg)}$$

$$FFM = \text{weight} - FM$$

The study was undertaken during the time period of February 2021 to June 2022.

Statistical analysis

Descriptive statistics were performed to examine the distribution of socio-demographic, reproductive, and menstrual characteristics, food consumption patterns, physical activities, TC level, blood glucose and blood pressure levels, and body fat pattern (skeletal and subcutaneous fat of the whole body and trunk, PBF, WC, HC, visceral fat, WHR, FM, FFM, and muscle mass) of the participants. The Kolmogorov-Smirnov test was applied to check the normality of each variable. We applied the analysis of variance (ANOVA) test to compare the variables of CVD risk factors (total cholesterol, blood glucose, and blood pressure levels) and body fat content across the menopausal groups. A post-hoc test (Scheffe's test) was employed to find out the differences between each menopausal group. The Kruskal-Wallis test was used as an alternative to ANOVA for variables that did not adhere to the normal distribution. Multiple linear regression analysis (stepwise) was employed to identify the predictors of CVD risk factors and body fat patterning. The variables that showed significant differences among the three menopausal groups were incorporated as dependent variables, while socio-demographic, menstrual, and reproductive characteristics, food habits, and physical activities were incorporated as independent variables. The independent variables collinearity was also determined. Multivariate Analysis of Covariance (MANCOVA) was applied to understand how menopausal status impacted CVD risk factors after eliminating the impacts of the confounding variables (socio-demographic, reproductive, and lifestyle variables). Menopausal status was in-

corporated as the grouping variable in MANCOVA. CVD risk factors and body fat measures that differed significantly across the groups in ANOVA (FFM, FM, HC, WHR, PBF, whole body, trunk and leg skeletal fat, and muscle mass, blood pressure, and blood glucose levels) were included as dependent variables. Homogeneity of variances and covariances was checked by performing Box's M test. Mahalanobis distance was calculated to find out the multivariate outliers in the groups of independent variables in the context of each dependent variable. The variables that appeared as significant predictors of body fat measures and CVD risk factors in multiple linear regression were included as confounding variables. Chi-square test was applied to examine the distribution of CVD risk factors across the menopausal groups. The Asian-specific (WHO 2008) cut-off for women has been employed to evaluate the prevalence of CVD risk factors. A WC of >88 cm was considered to be in the risk category, whereas a WHR of ≥ 0.85 was assessed to be in the risk category. Those with blood glucose level of <200 mg/dl were classified in the non-diabetic category, and those with a level of ≥ 200 mg/dl were classified as diabetic; A TC level of ≥ 240 mg/dl was determined as a high cholesterol level, whereas a TC level of <240 mg/dl was assessed as a normal level; SBP value of ≥ 140 mmHg and DBP value of ≥ 90 mmHg and MAP value of ≥ 100 were marked as the hypertensive category (WHO 2008). A minimum 'p' value of 0.05 was determined to be a statistically significant level for all inferential statistics. The whole data was assessed with the help of statistical package for social science version 20.0 (IBM Corporation 2011).

Results

Table 1 shows that the participant's age reported at the time of the interview differed significantly across the menopausal groups. The majority the postmenopausal participants were homemakers (68.2%); while a majority of the partic-

ipants from the premenopausal group were working (46.4%), followed by the perimenopausal participants (44.2%). Table 1 further shows that the majority of the participants and their spouses have completed secondary level education irrespective of their menopausal status (Table 1).

Table 1. Socio-demographic characteristics of the participants (n=201)

Socio-demographic variables	Pre (n=71)	Peri (n=61)	Post (n= 69)	F value/Kruskal-Wallis test/ Chi square test/ Fisher's exact test	p value
Participant's age (years) at the time of interview(mean±SD)	32.28±4.57	42.34±4.34	53.12±7.48	235.01	0.01
Participant's working status				3.52	0.17
Working	33(46.4)	27 (44.2)	22 (31.8)		
Homemaker	38 (53.6)	34(55.8)	47 (68.2)		
Occupational categories of the spouses					
Service	56 (78.9)	49 (80.3)	49 (71.0)	3.57	0.46
Business	13 (18.3)	8 (13.1)	14 (20.3)		
Others*	2 (2.8)	4 (6.6)	6 (8.7)		
Educational categories of the participants					
Non-literate	–	4 (6.6)	7 (10.1)		
Primary	7 (9.9)	9 (14.8)	7 (10.1)	–	–
Secondary	34 (47.9)	31 (50.8)	35 (50.7)		
Higher-secondary	12 (16.9)	7 (11.5)	10 (14.5)		
Graduate and above	18 (25.4)	10 (16.4)	10 (14.5)		
Educational categories of the spouses					
Non-literate	–	4 (6.6)	–		
Primary	8 (11.3)	14 (23.0)	12 (17.4)	–	–
Secondary	31 (43.7)	24 (39.3)	41 (59.4)		
Higher-secondary	12 (16.9)	7 (11.5)	4 (5.8)		
Graduate and above	20 (28.2)	12 (19.7)	12 (17.4)		
Monthly household expenditure (per capita) (INR)	96.39**	97.51	108.83	1.93	0.38

*Others: e.g., non-working, retired, labor; **mean rank; Figures in the parenthesis represent percentage values

Table 2 shows that the age at first and last pregnancies, age at menarche and marriage, and the duration of breastfeeding did not differ significantly across the menopausal groups, while the number of total pregnancies and duration of oral-contraceptive use differed significantly across the menopausal groups. Parity appeared to be significantly higher

among the postmenopausal participants (53.6%), followed by the perimenopausal participants (44.3%). The table further shows that the majority of the participants did not experience any fetal loss irrespective of their menopausal status. The majority of the participants from the postmenopausal group (69.6%) did not use any kind of contraceptive (Table 2).

Table 2. Reproductive and menstrual characteristics of the participants (n=201)

Reproductive and menstrual variables	Pre (n=71)	Peri (n=61)	Post (n= 69)	F value/Kruskal-Wallis test/ Chi square test/ Fisher's exact test	p value
Age at menarche (years) (mean±SD)	12.00±1.39	13.00±1.44	12.00±1.85	1.87	0.15
Age at marriage (years) (mean±SD)	18.00±3.44	19.00±4.17	18.00±4.77	0.12	0.89
Age at first pregnancy (years) (mean±SD)	20.68±3.55	20.00±4.43	20.00±4.99	0.28	0.75
Age at last pregnancy (years) (mean±SD)	27.17±4.31	26.67±4.76	26.45±5.04	0.28	0.76
Number of pregnancies					
One	31 (43.6)	16 (26.2)	11(15.9)	19.09	0.01
Two	25 (35.2)	18 (29.5)	21(30.4)		
More than two	15 (21.2)	27(44.3)	37 (53.6)		
Ever experienced fetal loss					
Yes	12 (16.9)	9 (14.8)	10 (14.5)	0.18	0.91
No	59 (83.1)	52 (85.2)	59 (85.5)		
Duration of breastfeeding(month)	99.15*	103.50	100.70	0.18	0.91
Ever use of contraceptive					
Yes	43 (60.6)	30 (49.2)	21 (30.4)	12.96	0.001
No	28 (39.4)	31 (50.8)	48 (69.6)		
Duration of OCP use (month)	111.40*	103.77	87.85	7.30	0.02

*Mean rank

Table 3 shows that barring regular consumption of soya products, meat, and aerated drinks, the majority of the participants

reported having consumed cereals, pulses, green leafy vegetables, roots and tubers, fish, snacks, and tea on a regular basis ir-

respective of their menopausal status. Further, it appears that the participants differed significantly in roots and tubers, sweets, and egg consumption patterns across the menopausal groups. The participants differed significantly in moderate (household chores) and sedentary activities. Sedentary activities were found to be higher among the premenopausal participants followed by the postmenopausal participants. The participants differed significantly in the

frequency of outside meal consumption. The majority of the participants from the premenopausal group (64.8%) reported consuming outside meals followed by the postmenopausal participants (49.3%). It also appears that 15.9% of the participants from the postmenopausal group reported having chewed tobacco on a daily basis followed by the premenopausal participants (9.9%). None of the participants reported consuming alcohol (Table 3).

Table 3. Food consumption pattern and physical activity level of the participants (n=201) (recall period based on the last seven days)

Items of food	Pre (n=71)	Peri (n=61)	Post (n=69)	Chi-square test/ Fisher's exact test	p value
Consumption of cereals					
Everyday/Regularly	71 (100)	60 (98.4)	69 (100)	—	—
Occasionally	—	1 (1.6)	—		
Never/Rarely	—	—	—		
Consumption of pulses					
Everyday/Regularly	43 (60.6)	41 (67.2)	39 (56.5)	3.75	0.43
Occasionally	7 (9.9)	2 (3.3)	8 (11.6)		
Never/Rarely	21 (29.6)	18 (29.5)	22 (31.9)		
Consumption of green vegetables					
Everyday/Regularly	68 (95.8)	56 (91.8)	63 (91.3)	—	—
Occasionally	—	3 (4.9)	2 (2.9)		
Never/Rarely	3 (4.2)	2 (3.3)	4 (5.8)		
Consumption of roots and tubers					
Everyday	65 (91.5)	57 (93.4)	54 (78.3)	7.66	0.02
Never/Rarely	6 (8.5)	4 (6.6)	15 (21.7)		
Consumption of soya products					
Everyday/Regularly	8 (11.3)	6 (9.8)	4 (5.8)	3.55	0.47
Occasionally	4 (5.6)	8 (13.1)	7 (10.1)		
Never/Rarely	59 (83.1)	47 (77.0)	58 (84.1)		
Consumption of meat					
Everyday/Regularly	8 (11.3)	3 (4.9)	—	—	—
Occasionally	6 (8.5)	5 (8.2)	5 (7.2)		
Never/Rarely	57 (80.3)	53 (86.9)	64 (92.8)		

Items of food	Pre (n=71)	Peri (n=61)	Post (n=69)	Chi-square test/ Fisher's exact test	p value
Consumption of fish					
Everyday/Regularly	45 (63.4)	44 (72.1)	50 (72.5)	7.07	0.13
Occasionally	16 (22.5)	5 (8.2)	7 (10.1)		
Never/Rarely	10 (14.1)	12 (19.7)	12 (17.4)		
Consumption of egg					
Everyday/Regularly	30 (42.3)	25 (41.0)	17 (24.6)	2.13	0.01
Occasionally	8 (11.3)	12 (19.7)	6 (8.7)		
Never/Rarely	33 (46.5)	24 (39.3)	46 (66.7)		
Consumption of fruits					
Everyday/Regularly	25 (35.2)	20 (32.8)	22 (31.9)	3.56	0.47
Occasionally	4 (5.6)	2 (3.3)	8 (11.6)		
Never/Rarely	42 (59.2)	39 (63.9)	39 (56.5)		
Consumption of milk					
Everyday/Regularly	26 (36.6)	19 (31.1)	20 (29.0)	–	–
Occasionally	–	1 (1.6)	1 (1.4)		
Never/Rarely	45 (63.4)	41 (67.2)	48 (69.6)		
Consumption of snacks					
Everyday/Regularly	52 (73.2)	53 (86.9)	60 (87.0)	–	–
Occasionally	–	–	1 (1.4)		
Never/Rarely	19 (26.8)	8 (13.1)	8 (11.6)		
Consumption of sweets					
Everyday/Regularly	21 (29.6)	24 (39.3)	38 (55.1)	11.75	0.01
Occasionally	2 (2.8)	2 (3.3)	4 (5.8)		
Never/Rarely	48 (67.6)	35 (57.4)	27 (39.1)		
Consumption of noodles					
Everyday/Regularly	3 (4.2)	1 (1.6)	4 (5.8)	5.39	0.23
Occasionally	3 (4.2)	7 (11.5)	2 (2.9)		
Never/Rarely	65 (91.5)	53 (86.9)	63 (91.3)		
Consumption of ghee/butter					
Everyday/Regularly	20 (28.2)	12 (19.7)	12 (17.4)	–	–
Occasionally	–	2 (3.3)	1 (1.4)		
Never/Rarely	51 (71.8)	47 (77.0)	56 (81.2)		
Consumption of tea					
Everyday/Regularly	47 (66.2)	52 (85.2)	61 (88.4)	–	–
Occasionally	1 (1.4)	–	–		
Never/Rarely	23 (32.4)	9 (14.8)	8 (11.6)		

Items of food	Pre (n=71)	Peri (n=61)	Post (n=69)	Chi-square test/ Fisher's exact test	p value
Consumption of aerated drinks					
Everyday/Regularly	11 (15.5)	7 (11.5)	5 (7.2)	–	–
Occasionally	1 (1.4)	1 (1.6)	–		
Never/Rarely	59 (83.1)	53 (86.9)	64 (92.8)		
Vigorous physical activity	98.35*	99.20	105.33	1.92	0.38
Moderate physical activity	117.00	95.12	89.73	8.64	0.01
Sedentary activity	113.37	90.34	97.70	5.78	0.05
Habit of chewing tobacco					
Yes	7 (9.9)	3 (4.9)	11 (15.9)	4.11	0.12
No	64 (90.1)	58 (95.1)	58 (84.1)	–	–

*Mean rank; Figures in parenthesis represent percentage values

Table 4 shows that PBF, HC, WHR, skeletal fat of the whole body, leg, and trunk, blood glucose level, SBP, DBP, MAP, muscle mass, and FFM differed significantly across the menopausal groups. It further appeared that PBF, WHR, blood glucose, and blood pressure levels were significantly higher among the postmenopausal participants than the pre and perimenopausal participants. It also appears that for body fat measures like HC, skeletal fat related to the whole body,

leg, and trunk, FFM, and muscle mass, the premenopausal participants showed the highest values, followed by the peri and postmenopausal participants. The post-hoc tests showed significant differences between pre- and post-menopausal participants for the variables of body fat measures (barring a few) and CVD risk factors. Peri and post-menopausal participants differed significantly in the case of the whole body, leg, and trunk skeletal fat, muscle mass, and FFM (Table 4).

Table 4. Distribution of CVD risk factors and fat patterning of the participants (n=201)

Variables	Pre (n=71)	Peri (n=61)	Post (n=69)	F value/ Krus- kal-Wal- lis test	p value	Post-hoc test
	mean±SD					
BMI	26.21±4.41	26.06±3.83	25.22±4.24	1.13	0.33	Pre and Peri (0.98) Pre and Post (0.38) Peri and Post (0.52)
PBF	34.37±4.39	35.35±4.01	37.13±4.25	7.59	0.01	Pre and Peri (0.42) Pre and Post (0.001) Peri and Post (0.05)

Variables	Pre (n=71)	Peri (n=61)	Post (n=69)	F value/ Krus- kal-Wal- lis test	p value	Post-hoc test
	mean±SD					
WC (cm)	88.48±12.13	88.89±9.86	89.10±11.68	0.05	0.95	Pre and Peri (0.98) Pre and Post (0.95) Peri and Post (0.99)
HC (cm)	96.54±7.45	95.23±6.75	93.29±7.83	3.42	0.04	Pre and Peri (0.59) Pre and Post (0.04) Peri and Post (0.33)
WHR	0.91±0.08	0.93±0.08	0.95±0.09	4.29	0.01	Pre and Peri (0.39) Pre and Post (0.01) Peri and Post (0.34)
Visceral fat	7.93±4.13	8.78±4.11	8.86±4.94	0.93	0.39	Pre and Peri (0.55) Pre and Post (0.47) Peri and Post (0.99)
Whole body subcutaneous fat percentage	30.24±5.60	30.49±4.96	31.01±4.71	0.41	0.66	Pre and Peri (0.96) Pre and Post (0.67) Peri and Post (0.84)
Whole body skeletal fat percentage	23.75±2.02	22.92±1.84	21.701±1.79	20.71	0.01	Pre and Peri (0.04) Pre and Post (0.01) Peri and Post (0.01)
Trunk subcu- taneous fat percentage	26.40±5.31	26.92±4.60	27.90±4.57	1.72	0.18	Pre and Peri (0.83) Pre and Post (0.19) Peri and Post (0.52)
Trunk skeletal fat percentage	18.19±2.13	17.29±1.88	16.04±1.92	20.64	0.01	Pre and Peri (0.03) Pre and Post (0.01) Peri and Post (0.01)
Arm subcu- taneous fat percentage	46.73±6.78	47.27±6.15	48.41±5.29	1.35	0.26	Pre and Peri (0.88) Pre and Post (0.27) Peri and Post (0.57)
Arm skeletal fat percentage	24.99±4.64	24.51±3.98	23.61±3.87	1.96	0.14	Pre and Peri (0.80) Pre and Post (0.15) Peri and Post (0.47)
Leg subcu- taneous fat percentage	43.19±7.32	42.59±6.51	42.11±5.43	0.49	0.61	Pre and Peri (0.87) Pre and Post (0.62) Peri and Post (0.91)
Leg skeletal fat percentage	36.69±2.52	35.79±2.41	34.29±2.47	16.88	0.01	Pre and Peri (0.11) Pre and Post (0.01) Peri and Post (0.03)

Variables	Pre (n=71)	Peri (n=61)	Post (n=69)	F value/ Krus- kal-Wal- lis test	p value	Post-hoc test
	mean±SD					
Blood glucose level(mg/dl)	99.00*	105.00	104.00	9.63	0.03	Pre and Peri (0.03) Pre and Post (0.01) Peri and Post (0.94)
Blood total cholesterol level (mg/dl)	190.68±31.34	201.41±40.66	201.90±40.50	1.97	0.14	Pre and Peri (0.27) Pre and Post (0.21) Peri and Post (0.99)
SBP (mmHg)	116.00*	127.00	134.00	31.18	0.01	Pre and Peri (0.01) Pre and Post (0.01) Peri and Post (0.23)
DBP (mmHg)	81.73±8.59	86.72±11.58	87.14±10.73	5.90	0.01	Pre and Peri (0.01) Pre and Post (0.02) Peri and Post (0.97)
MAP (mmHg)	96.67±9.64	101.29±14.43	103.59±13.03	12.19	0.01	Pre and Peri (0.003) Pre and Post (0.01) Peri and Post (0.57)
Muscle mass percentage	32.27±2.67	30.26±2.15	28.85±3.28	27.16	0.01	Pre and Peri (0.01) Pre and Post (0.01) Peri and Post (0.02)
FM (kg)	21.43±6.29	21.43±5.08	21.61±5.79	0.02	0.98	Pre and Peri (1.00) Pre and Post (0.98) Peri and Post (0.99)
FFM (kg)	39.72±5.56	38.53±4.71	35.88±5.46	9.59	0.01	Pre and Peri (0.43) Pre and Post (0.01) Peri and Post (0.02)

*Mean rank

Table 5 shows that PBF, WHR, blood glucose, and blood pressure levels (SBP, DBP and MAP) showed a positive relationship with participants' age, while skeletal fat of whole body and trunk, and muscle mass showed an inverse relationship. Percent body fat showed a positive association with the number of pregnancies, while skeletal fat of the whole body and trunk, and muscle mass showed an inverse association with the number of pregnancies. It also

appears that blood glucose levels were likely to lower with an increase in green-leafy vegetable consumption. Percent body fat, WHR, and blood glucose level shows an inverse association with age at menarche, while skeletal fat of the whole body and trunk showed a positive association with participant's age at menarche. The values of R square represent that the models can explain 6–50 percent of the variability in the dependent variables (Table 5).

Table 5. Predictors of CVD risk factors and fat patterning (n=201)

Dependent variable	Independent variables	Unstandard- ized coefficients	t value	p value	CI at 95%		R square
					lower	upper	
PBF	Participant's age	0.12	3.63	0.0001	0.05	0.19	0.27
	Educational years of the participants	0.33	4.32	0.0001	0.18	0.48	
	Age at menarche	-0.57	-2.95	0.004	-0.96	-0.19	
	Number of pregnancies	1.66	3.67	0.0001	0.76	2.56	
	Total number of wastage	-1.45	-3.02	0.003	-2.41	-0.51	
HC (cm)	Completed years of education	0.82	8.61	0.001	0.48	0.87	0.26
	Weekly duration of walking	-0.009	-4.68	0.001	-0.01	-0.005	
WHR	Exclusively homemaker	0.03	2.41	0.01	0.007	0.06	0.20
	Age at menarche	-0.01	-2.74	0.007	-0.01	-0.003	
	Age at marriage	-0.006	-2.85	0.005	-0.01	-0.002	
	Participant's age	0.002	2.32	0.02	0.0001	0.003	
	Educational years of the participant's husbands	0.004	2.12	0.03	0.0001	0.007	
Whole body skeletal fat percentage	Participant's age	-0.09	-5.78	0.0001	-0.12	-0.06	0.36
	Number of pregnancies	-0.88	-4.30	0.0001	-1.29	-0.47	
	Age at menarche	0.23	2.65	0.009	0.06	0.41	
	Total number of wastage	0.73	3.35	0.001	0.30	1.16	
	Educational years of the participants	-0.08	-2.40	0.01	-0.15	-0.01	
Trunk skeletal fat percentage	Participant's age	-0.10	-6.88	0.0001	-0.13	-0.07	0.38
	Age at menarche	0.29	3.37	0.001	0.12	0.47	
	Exclusively homemaker	-0.93	-3.12	0.002	-1.53	-0.34	
	Number of pregnancies	-0.33	-2.78	0.006	-0.56	-0.09	
Leg skeletal fat percentage	Participant's age	-0.11	-5.28	0.0001	-0.15	-0.07	0.16
Blood glucose level (mg/dl)	Green-leafy vegetables consumption	-7.97	-2.63	0.009	-13.94	-1.99	0.13
	Age at menarche	-7.46	-2.72	0.007	-12.88	-2.04	
	Participant's age	0.89	1.98	0.05	0.002	1.79	
SBP (mmHg)	Participant's age	0.92	5.68	0.0001	0.60	1.24	0.18
DBP (mmHg)	Per capita monthly household expenditure	0.001	2.15	0.03	0.0001	0.001	0.06
	Participant's age	0.18	2.09	0.04	0.01	0.35	
MAP (mmHg)	Participant's age	0.45	4.24	0.0001	0.24	0.66	0.11

(Only significant values are presented)

Table 6 shows that menopausal status significantly predicted CVD risk factors and measures of body fat patterning after omitting the effects of the confounding variables (socio-demographic, reproductive, and lifestyle factors) (Table 6).

Table 7 exhibited that the prevalence of central obesity appeared to be higher among the majority of the participants irrespective of their menopausal status.

It also appears that 60.9% of the postmenopausal participants were hypertensive followed by the perimenopausal participants (54.1%). For DBP, 39.3% of the perimenopausal participants appeared as hypertensive, followed by the postmenopausal participants (34.8%). The prevalence of high total cholesterol levels and diabetes appeared to be lower among all the participants irrespective of their menopausal status (Table 7).

Table 6. Results of Multivariate analysis of covariance of the participants (MANCOVA)

Fixed factors	Dependent variables	Type III sum of squares	F value	p value
Menopausal status	PBF	288.08	7.98	0.0001
	HC	384.22	3.49	0.03
	WHR	0.04	3.46	0.03
	Whole body skeletal fat	161.03	22.73	0.0001
	Trunk skeletal fat	171.79	21.85	0.0001
	Leg skeletal fat	224.75	18.52	0.0001
	Blood glucose level	29550.21	5.62	0.004
	SBP	10765.29	15.36	0.0001
	DBP	1322.56	6.24	0.002
	MAP	3962.95	12.88	0.0001
	Muscle mass percentage	414.69	27.23	0.0001

Table 7. Prevalence of CVD risk factors among the participants (n=201)

CVD risk factors	Pre (n=71)	Peri (n=61)	Post (n= 69)	Chi square test/ Fisher's exact test	p value
Blood glucose level(mg/dl)					
Diabetic	–	5 (8.2)	7 (10.1)	8.61	0.009
Non-diabetic	71 (100)	56 (91.8)	62 (89.9)		
Waist circumference (cm)					
Normal	34 (47.9)	27 (44.3)	37 (39.1)	1.09	0.57
Risk	37 (52.1)	34 (55.7)	42 (60.9)		
WHR					
Normal	12 (16.9)	8 (13.1)	9 (13.0)	0.54	0.76
Risk	59 (83.1)	53 (86.9)	60 (87.0)		

CVD risk factors	Pre (n=71)	Peri (n=61)	Post (n= 69)	Chi square test/ Fisher's exact test	p value
SBP (mmHg)					
Normal	64 (90.1)	43 (70.5)	43 (62.3)	15.09	0.001
Hypertensive	7 (9.9)	18 (29.5)	26 (37.7)		
DBP (mmHg)					
Normal	59 (83.1)	37 (60.7)	45 (65.2)	9.11	0.01
Hypertensive	12 (16.9)	24 (39.3)	24 (34.8)		
MAP (mmHg)					
Normal	54 (76.1)	28 (45.9)	27 (39.1)	21.67	0.0001
Hypertensive	17 (23.9)	33 (54.1)	42 (60.9)		
Total cholesterol level (mg/dl)					
Normal	63 (88.7)	55 (90.2)	58 (84.1)	1.24	0.53
High	8 (11.3)	6 (9.8)	11 (15.9)		

Discussion

We aimed to compare the body fat patterning and CVD risk factors among women of differential menopausal statuses (pre, peri, and post) belonging to the Bengali Hindu ethnic group. Our study indicates that postmenopausal participants have higher PBF and central obesity and reduced lean mass (skeletal fat of whole body and torso) and muscle mass compared to their pre and perimenopausal counterparts as found in studies carried out in India and elsewhere (Ferrara et al. 2002; Ghosh and Bhagat 2010). A decrease in sex hormones after menopause results in fat accumulation, particularly in the abdomen region because sex hormones are required for adipocyte metabolism (Ferrara et al. 2002). The higher prevalence of central obesity among the postmenopausal participants in our study perhaps justifies the sex hormonal depletion as a reason behind the higher prevalence of central obesity. Some longitudinal studies on postmen-

opausal participants from Europe (Poehlman et al. 1995) and the USA (Sowers et al. 2009) reinforce the role of estrogen in central obesity during midlife. Countering these studies, several studies reported that weight gain in middle-aged women was associated with an increase in age, rather than menopause. For example, the Healthy Women's Study exhibited that women accumulate approximately 0.7 kg every year during their fifth and sixth decades of life, independent of menopause (Wing et al. 1991). Despite these inconsistent results, the majority of the cross-sectional (Ghosh and Bhagat 2010) and longitudinal studies (Sowers et al. 2009) have reported an increase in abdominal obesity during menopause, independent of aging which is in agreement with the present study.

Our study reveals that peri- and post-menopausal participants have higher blood glucose levels compared to their premenopausal counterparts. This finding shows consistency with some earlier studies carried out in various parts of the

world, including India (Revis and Keene 2006) though inconsistent results have also been reported (Pandey et al. 2010). Scholars found that a decrease in the sex hormone levels during MT and beyond reduces the sex hormone binding globulin (SHBG) level in the blood, increasing the risk of type II diabetes mellitus (Carr 2003); but the association between menopause and blood glucose level independent of aging remains inconsistent (Kim 2012). Although measuring SHBG concentration is beyond the purview of our research, the findings of our study indicate the role of SHBG in determining blood glucose level.

It appears from our study that postmenopausal participants have significantly higher SBP, DBP, and MAP levels compared to the pre and perimenopausal participants, corroborating with some earlier research findings (Lima et al. 2012). The link between menopausal status and blood pressure levels persisted even after controlling the confounders (socio-demographic, reproductive, and lifestyle variables), which is in agreement with some of the previous studies (Gupta et al. 2014; Son et al. 2015). For instance, a study involving Korean postmenopausal women reported a significant association between menopause and hypertension after controlling for the confounders like age, BMI, WC, vasomotor symptoms, triglycerides, and uric acid which is in partial agreement with the present study (Son et al. 2015). On the contrary, studies reported that the high blood pressure level among midlife women could be explained by factors like age and BMI (Matthews et al. 2009). For example, the SWAN longitudinal study reported that after adjusting for age and other confounders, MT had no effect on blood pressure levels (Matthews et al. 2021). The mechanism through

which menopause and hypertension are related is unclear. This could be attributed to the direct protective mechanism of estrogen on the renin-angiotensin-aldosterone system, which reduces after menopause (Carr 2003). An increase in vasoconstrictors (angiotensin II and endothelin) and a significant drop in nitric oxide took place after menopause; as a result, renal vasoconstriction and endothelial dysfunction occurred, resulting in the development of hypertension (Yanes et al. 2010).

The participants of our study, irrespective of the menopausal status show high level of total cholesterol as found in some other studies (Inaraja et al. 2020; Matthews et al. 2021); but intergroup difference in cholesterol level is not statistically significant, which is partly in agreement with a study conducted in Rajasthan (Kanwar et al. 2014). The SWAN longitudinal study (20 year of follow-up) conducted among 1554 women reported that total cholesterol level increased dramatically throughout the MT until the first year of menopause, then significantly decreased during the postmenopausal years (Matthews et al. 2021); thus, no significant difference persisted between the peri and postmenopausal women which is consistent with the present study. Despite the substantial studies on the effects of estrogen on lipid metabolism, it is not clear whether there is any association between the change in sex steroid concentrations and alterations in the lipid profile. Some large population-based studies reported no association between menopause and HDL levels (Pasquali et al. 1997), but alterations in the proatherogenic lipid profile driven by menopause may have an impact on LDL, triglyceride, and total cholesterol levels (Inaraja et al. 2020). Scholars are of the

opinion that estrogen absorbs free radicals and naturally occurring LDL in the blood, which are capable of damaging the arteries and other tissues. In the absence of estrogen circulation in the blood, these particles build up in the arteries and restrict the blood flow (Carr 2003).

Apart from the hormonal factors, socio-demographic, reproductive, and lifestyle factors significantly contribute to the risk factors related to CVD. In our study, educational attainment of the participants reported a positive link with body fat patterning, corroborating (De Silva et al. 2015) and contradicting (Sabanayagam et al. 2007) with studies from India and elsewhere. For instance, evidence from Asian and Western countries showed a positive link between educational status, income and body fat patterning (De Silva et al. 2015). Additionally, educational attainment of the spouses showed a positive relationship with central body fat of the participants; this is in disagreement with some previous studies (Torssander et al. 2009; Murakami et al. 2017). A study involving Japanese women reported that women whose spouses had only attained high school or less were more likely to be obese than women whose spouses had completed a higher level of education (Murakami et al. 2017); the present study contradicts this finding. Perhaps the completion of higher levels of education by the participants and their husbands is linked to higher income and higher social position, which may result in excess nutrition and the adoption of a sedentary lifestyle. Some Indian studies conducted in urban areas of West Bengal showed 56.1% incidences of central obesity among women, irrespective of their menopausal status, owing to the sedentary lifestyle and the south Asian

genetic predisposition to central obesity (Acharyya et al. 2014); this is consistent with the present study.

Postmenopausal women's risk of midlife obesity and CVD risk factors might also be impacted by pregnancy and childbirth (Patchen et al. 2017). In our study, ages at menarche and marriage showed inverse associations with central obesity, while parity shows an inverse association with lean body mass, corroborating with previous studies (Lakshman et al. 2009). A recent study conducted among 6,103 Iranian midlife women reported that women with an early age at menarche had a higher risk of central obesity compared to those with late age at menarche (after 14 years of age). This finding is aligning with the present study since all the participants had attained menarche before 14 years of age (Kheradmand et al. 2023). On the contrary, some other population-based studies indicated that age at menarche was linked to generalized obesity, but not to central obesity (Trikudanathan et al. 2013). However, the physiological mechanism of this link is not well understood. There may be a shared etiology rather than a direct link between early menarche and midlife obesity. For example, Elks et al. (2011) found that a genetic locus, LIN28B, is linked with both age at menarche and central obesity. Menarche is also associated with a number of neuroendocrinological changes, such as increased levels of adrenal androgen and hyperactivity of the hypothalamic-pituitary-gonadal axis (Trikudanathan et al. 2013). This higher level of androgen promotes the development of central obesity later in life. Additionally, it appears that early age at marriage is associated with early age at first pregnancy, which may lead to disruption in educational and career achievements.

Younger age at childbirth and increased parity cause increased stress and bring changes in lifestyle factors causing hypothalamic-pituitary-adrenal hyperactivity. Both factors independently enhance the development of central obesity in women later in life (Patchen et al. 2017). Mean ages at marriage and first pregnancy were found to be earlier among the participants of the present study. This could partially explain the reason behind the higher incidences of central obesity among the participants, irrespective of their menopausal status. Future prospective studies are needed to establish this association.

Studies revealed that chronological aging is a significant predisposing factor in the development of CVD (Zierer et al. 2016). In the present study, participant's age showed a significant positive relationship with blood pressure levels and body fat patterning. This could be due to the age-related adverse changes at the cellular level, including chronic inflammation, oxidative stress, myocardial deterioration, and changes in calcium plumping capacity (Zierer et al. 2016). All these adverse changes enhance the buildup of diacylglycerol fatty acids and saturated ceramide, leading to an increase in visceral adipose tissue and total body fat content. Aging is also associated with an increase in reactive oxygen and nitrogen species (RONS), which leads to damage in lipids, DNA, and protein, causing dysfunction of vascular tissues and promoting the development of chronic diseases like CVD. However, it is difficult to distinguish between the impacts of aging from menopause because, by definition, post and perimenopausal women are older than the premenopausal women. Future studies are needed to establish the independent association of both these events with CVD risk factors.

The present study exhibited that blood glucose level was likely to increase with a decrease in vegetable consumption, corroborating with previous studies (Kartiko et al. 2020). This could be attributed to the presence of phytochemicals (Vitamin C, beta carotene, etc.) and anti-oxidative nutrients in fruits and vegetables which may improve cardiovascular health. The present study reported that participant's working status is a significant predictor of central obesity (WHR) showing consistency with previous studies (George and Chandan 2016). For example, an Indian study (Mumbai) showed that women who are working have a lower body fat content than non-working women (George and Chandan 2016). A longitudinal cohort study (SWAN study) reported that physical activity is inversely linked to the changes in PBF and WC, independent of menopausal status and aging (Sternfeld et al. 2004). The participants of the present study are mostly non-working; this might be an explanation for the increased level of body fat content among the participants. The present study further reported that walking on a regular basis helps to reduce body fat, conforming to previous studies (Sternfeld et al. 2004; George and Chandan 2016).

Finally, our study concluded that menopausal status is a significant predictor of body fat distribution, blood pressure, and blood glucose levels after controlling the effects of the confounding variables (socio-demographic, reproductive, and lifestyle variables), corroborating with previous studies (Carr 2003; Matthews et al. 2021). Additionally, an overwhelming majority of the postmenopausal Bengali Hindu participants of the present study have central obesity; thus,

this group is more prone to developing CVD in the near future. The likelihood of Indian postmenopausal women suffering from CVD risk factors will increase if menopausal status appears as a strong independent predictor of cardiovascular risk factors, as found in the present study. Policymakers can use baseline information from this study to formulate appropriate health policies that will help postmenopausal women to lead a healthy life beyond menopause. This research underscores the importance of addressing cardiovascular health as a critical component of women's health initiatives. By prioritizing education and access to preventive care, health planners can help postmenopausal women to make informed lifestyle choices that mitigate their risk of CVD and enhance their quality of life. More studies targeting midlife women are crucial to expand our understanding of this seldom-explored field to improve the well-being of postmenopausal women in developing countries.

There are certain limitations in this study. Owing to the COVID-19 pandemic and subsequent nationwide lockdown, the fieldwork was impacted in part. As a result of which the estimated sample size for the present study was not covered. The estimation of the total lipid profile and fasting blood glucose level, along with a larger sample size could have enhanced the findings of this study. The findings might have improved by a more thorough observation of the lifestyle choices (maybe on a subsample) of the participants. Postmenopausal health problems of women could be attributed to genetics, disparities in perception and attitude, and finally, their differential access to health care services. Therefore, incorporating data on these aspects

might enhance the understanding of the link between menopause and risk factors of CVD in the present study.

Conclusions

Prediction of the CVD risk factors among middle-aged women continues to lag behind that of men because women exhibit CVD events a few years later than men. Additionally, the occurrence of CVD risk factors among midlife women might be a complex interaction of menopause, social-cultural factors, differential attitudes and perceptions of women regarding midlife, and access to health care services. Further cross-cultural investigations are needed to better understand how the association between menopause and CVD varies across different cultural groups and throughout the menopausal transition by taking into account both biological and social-cultural aspects of postmenopausal health. An awareness of this complex scenario will help policymakers to develop appropriate strategies for postmenopausal women. These strategies could include targeted health education, accessible healthcare services, and community support programs that can address the unique needs of diverse populations. By fostering a deeper understanding of these cultural differences, the overall health and well-being of postmenopausal women can be enhanced. Additionally, providing awareness and specialized interventions can help mitigate the health risks faced by postmenopausal women. This approach not only encourages improved health outcomes but also empowers women to take charge of their health during this critical phase of life and ensure a successful transition from reproductive to the post-reproductive phase of life.

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Conflict of interests

The authors declared that they have no conflict of interest.

Ethics statement

The study was approved by the Institutional Human Ethical committee, University of Calcutta (protocol 06/WT/19-20/1763). The purpose of the research was explained to, and written informed consent was taken from the participants.

Author contribution

The first author (DK) contributed 50% by collecting data and analyzing the data and partially drafting the manuscript. The second author (SR) contributed 50% by designing the study and reviewing the manuscript.

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