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The impact of endogenous estrogen exposure duration on fracture incidence; a longitudinal cohort study

***Short title*: Endogenous estrogen & fracture incidence**

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Abstract

Context:

Although it is well documented that estrogen hormone is positively associated with bone mineral density and lower risk of fracture, there are limited studies on the association between duration of endogenous estrogen exposure (EEE) and fracture, especially by longitudinal design.

Objective:

This study aimed to investigate the relationship between EEE with fracture incidence by longitudinal design in a community-based study.

Methods:

A total of 5,269 eligible post-menarcheal women, including 2,411 premenopausal and 2,858 menopausal women were recruited from among Tehran-Lipid and Glucose-Study. Cox proportional hazards regression model with adjustment of potential confounders was performed to assess the relationship between duration of EEE and incident of any hospitalized fractures.

Results:

A total of 26.7 % (1409 out of 5269) women were menopause at the baseline and 2858 of the remaining participants reached menopause at the end of follow-up. Results of the unadjusted model demonstrated that the EEE z-score was negatively associated with fracture incidence (unadjusted hazard ratio (HR): 0.81, 95% CI: 0.68-0.96) in post-menarcheal women, indicating that per one SD increase of EEE z-score, the hazard of fracture reduced by 19%. Results remained statistically unchanged after adjustment for potential confounders (adjusted HR: 0.70, 95% CI: 0.58-0.86).

Conclusion:

The findings of this cohort study suggest that a longer duration of EEE has a protective effect on fracture incidence; a point that needs to be considered in fracture risk assessment.

Fractures are one of the major public health concerns and are associated with significant mortality and morbidity, which may adversely affect the patient's socioeconomic, physical, and psychological well-being and quality of life (1, 2). Furthermore, Considering an increasing global life expectancy, the annual incidence of fracture is rapidly increasing worldwide, especially in developing countries and *women* (1-3). However, the findings of studies showed if the age variable is adjusted, this incidence has remained increasing worldwide (especially recent), so, studies predicted this incidence will increase further in the future(1, 2). It is important to note that the lifespan risk of each fracture at age 50 years was more than almost twofold *in women* compared to men (4), because, factors such as bone mineral density, size, and strength between men and *women* are different(5). Many factors can impact the progress of osteoporosis such as smoking, using alcohol, low physical activity, excess calcium loss or calcium malabsorption, vitamin D deficiency, prior bone fractures, corticosteroids consumption, hormonal agents, genetic factors, and *female sex*(6, 7).

Of all the hormones known to affect bone, estrogen is an important key regulator of bone metabolism, bone density accrual, and maintenance (8). It directly affects bone cells to inhibit the osteoclasts activation, bone remodeling, and bone resorption (9). Previous studies have shown not only the lower duration of endogenous estrogen exposure (EEE) is associated with an increased risk of some non-communicable diseases (NCDs) such as cardiovascular disease (CVD) and chronic kidney disease (CKD)(10, 11), but also an increased risk of both of them associated with an increased risk of fractures(12, 13).

Emerging evidence suggests that estrogen levels and their duration of exposure may be associated with bone mineral density (BMD) and subsequent osteoporotic fractures. In this respect, some studies have reported that compared with earlier age at menarche, later age at menarche is associated with increased risk of low BMD and subsequent osteoporotic fractures (14-16). Additionally, it is reported that a shorter reproductive life span among women could be a risk factor for future fracture (16-18). In the present study, we aimed to investigate the associations between the duration of estrogen endogenous exposure and incident fractures, using data of the large and long-term longitudinal population-based Tehran Lipid and Glucose-Study (TLGS).

Materials and Methods

Subjects

The participants in our study were selected from among the TLGS participants. TLGS is an ongoing prospective study, started in 1998, to determine the prevalence and incidence of non-communicable disease risk factors. A total of 15,005 people, aged ≥3 years, were selected via a multistage cluster sampling method and were followed every 3 years follow-up, to document data on demographic, anthropometric, reproductive, and metabolic characteristics, general physical examinations as well as laboratory measurements. In addition, the specific assessments for all obstetrics and reproductive including age at menarche and age at menopause have been documented for all female participants. The ethics committee of the Research Institute for Endocrine Sciences approved the study and written informed consent was obtained from all subjects before the initiation of the study.

The details of TLGS are provided elsewhere (19). The present long-term prospective population-based study included all post-menarcheal women including reproductive and menopausal (both natural and surgical) participants (n = 11,100) who completed related examinations in the first phase of the TLGS (1998–2001) and attended at least one follow-up study Those with lack of sufficient data about endogenous estrogen exposure period (n = 1267) and menarcheal age (n=4282), those receiving hormone replacement therapy (HRT) (n=24), and those without any follow-up assessment (n=258) were excluded from the study. Finally, a total of 5269 met the eligibility criteria and was followed up to the date of incident fracture, censoring (eg, death), or end of the study period, whichever came first. The selection process of participants has shown in Figure 1.

The ethics committee of the Research Institute for Endocrine Sciences approved the study (IR.SBMU.ENDOCRINE.REC.1399.153). Written informed consent was obtained from all subjects before the initiation of the study.

Measurements

During the face-to-face interviews and using a standard questionnaire, all information on demographic and lifestyle variables, various risk factors for non-communicable diseases, familial and individual past medical and reproductive histories were gathered by trained staff.

For all participants, weight and height were measured with minimal clothing, without shoes in a standing position, using standardized procedures and calibrated equipment. Body mass index (BMI) was calculated using the formula kg/m^2 where kg is a person's weight in kilograms and m^2 is their height in meters squared. Waist circumference (WC) was measured midway between the lower rib margin and the iliac crest at the level of the umbilicus, at the end of a

gentle expiration. Hip circumference (HC) was measured using an unstretched measuring tape to the nearest 0.1 cm. Blood pressure (BP) was measured twice in the right arm after a 15-minute rest in a sitting position and was calculated based on the mean of two measurements. Physical activity was assessed using the Modifiable Activity Questionnaire (MAQ), participants with fewer than 600 MET (metabolic equivalent task) minutes per week were classified as low physical activity group (20). Blood samples were collected following a 12–14 h overnight fasting between 7:00 and 9:00 AM. All analysis of blood samples was performed on blood taking day. Other details for measurements of a laboratory containing fasting plasma glucose (FPG) and creatinine (Cr) were presented elsewhere (21).

Definition of Terms

According to the Kidney Disease Outcome Quality Initiative guidelines (K/DOQI), chronic kidney disease was defined as eGlomerular Filtration Rate (GFR) <60 mL/min/1.73 m2 for >3 months occurring at any time during the follow-up period. GFR was estimated using the abbreviated prediction equation, provided by the Modification of Diet in Renal Disease (MDRD) study as follows: GFR = 186 × (serum creatinine (Scr) 2 − 1.154) × (age2 − .203) × 0.742. In this equation, estimated GFR (eGFR) is expressed as mL/min per 1.73 m2 and Scr is expressed as mg/dL (22). Central obesity was defined as WC ≥ 95 centimeters (23). Diabetes mellitus was defined as fasting plasma glucose (FPG) ≥ 7 mmol/L and/or 2-hour post-challenge plasma glucose (2-hPCPG) ≥ 11.1 mmol/L or the use of anti-diabetic medications (24). Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or using anti-hypertensive medications (25).

Fracture outcome

Individually participants are followed for any medical event resulting in hospitalization through the prior year via a telephone call, based on the previously published elsewhere(21). A trained nurse from all participants asked for any medical condition and next, a trained physician gathered supplementary data about that event via the achievement of data from medical files and a home visit. An internist, endocrinologist, cardiologist, epidemiologist, and other experts, if required formed an outcome committee that evaluates the collected data to allocate a special outcome per event. It is important to note that the status of baseline risk factors becomes blinded for the outcome committee. The outcome committee registered and adjudicated fractures in each extremity (upper or lower or other sites) needing at least one day of hospitalization. According to the claim of the patient and the hospital discharge abstract the diagnosis of fracture was specified. We subdivided all fractures into four main classifications: upper extremity (containing the upper

humerus, wrist, hand, scapula, clavicle, elbow, and forearm), lower extremity (including the pelvis, hip, femur, patella, tibia, fibula, ankle, or foot), vertebral, and other fractures (including ribs, scalp, fascial or sternum)(22, 23).

Exposure

In the present study, duration of EEE was defined as the time interval between age at menarche and age at menopause or age at incident fracture or end of follow-up, whichever occurred earlier.

The cumulative duration of pregnancies (40 weeks per birth or 20 weeks per abortion), duration of oral contraceptive pill consumption, duration of breastfeeding (number of months per child), and duration of progesterone dominant (luteal) phases of menstrual cycles (2 weeks per menstrual cycle) were deducted from the primary EEE variable to evaluate only E2 dominant (follicular) phases of menstrual cycles.

Statistical analysis

All continuous variables were examined in terms of normal distribution, using one-sample Kolmogorov–Smirnov test. Data are expressed as mean (standard deviation) for variables with a normal distribution or median, interquartile range, 25th to 75th percentile (IQR 25%-75%) for variables with a skewed distribution. Categorical variables are also expressed as a percentage.

The Cox regression model was used to assess the hazard ratios and 95% confidence intervals (CIs) for the association between EEE and the risk of fracture. The event date was defined as the date of the incident fracture and age at fracture was computed. Women, who did not develop fracture by the end of the follow-ups, were considered as lost to follow-up or censored. For individuals with incident any fracture, survival time was defined as the interval between the age at menarche and fracture age (in years). Additionally, for the censored participants, the survival time was defined as the difference between the age at menarche and age, at last, follow up.

Both unadjusted and adjusted Cox regression models were applied. Potential confounding factors, including age, BMI, smoking status, education status, and steroid and aspirin drug treatment, physical activity, marital status, CKD, CVD, diabetes, and hypertension were entered in the multivariate Cox model as time-varying covariates.

Since the reason for menopause may affect the results, menopausal women were classified into two groups of natural and surgical menopause. Further, we performed a subgroup analysis in both groups of non-menopausal in addition to natural menopausal women (n = 4634) and non-menopausal in addition to surgical menopause women (n = 3046). Moreover, EEE Z-score was compared among four prespecified categories of the fracture using the Kruskal-Wallis

test. All analyses were conducted using STATA version 13 SE (Stata crop, TX, USA) and a two-tailed p-value<0.05 was considered significant.

Results

The characteristics of the study participants at baseline and date of incident fracture/end of follow-up are shown in Table 1. The mean (SD) age of participants at baseline and date of incident fracture/end of follow-up were 38.3 (14.1) and 52.4 (13.8) years, respectively. The overall mean (SD) age at menarche was 13.6 (1.4) years. A total of 26.7 % (1409 out of 5269) women were menopause at the baseline and 2736 (71%) of the remaining participants reached menopause at the end of follow-up.

The median (IQR 25%-75%) of EEE duration at baseline and date of incident fracture/end of follow-up were 489.9 (268.9-735.6) and 696.8 (553.5-811.8) weeks, respectively.

Table 2 shows the hazard ratios for the effect of endogenous estrogen exposure Z-score on fracture incidence among study participants. Results of the unadjusted model demonstrated that the EEE z-score was negatively associated with fracture incidents (unadjusted hazard ratio (HR): 0.81, 95% CI: 0.68-0.96) in post-menarcheal women, indicating that per one SD increase of EEE z-score, the hazard of fracture reduced by 19%. Results remained statistically unchanged after adjustment for potential confounders (adjusted HR: 0.70, 95% CI: 0.58-0.86).

Results of subgroup analyses showed that the EEE z-score were negatively associated with fracture incidence in both subgroups of non-menopausal women in addition to natural menopause (unadjusted HR: 0.78, 95% CI: 0.64-0.96), and in non-menopausal women in addition to surgical menopause (unadjusted HR: 0.65, 95% CI: 0.48-0.88), indicating that the risk of fracture reduced by 22% and 35% for each SD increase in EEE z-score in those both groups respectively. Adjustment for potential confounders did not statistically change the results (Table 2).

Excluding women with surgical menopause resulted in an HR of 0.78 (95% CI: (0.64,0.96), this means each one SD increase in EEE z-score, decreased the hazard of fracture by 22%. Excluding women with natural resulted in an HR of 0.65(95%CI: 0.48,0.88); this means that per 1 SD increase in EEE z-score, the hazard of fracture is decreased by 35% After adjusting, findings indicated that the HR (95% CI) for fracture associated with EEE z-score was 0.70 (0.58, 0.86), 0.66 (0.52, 0.85) and 0.57 (0.40,0.81) and per one SD increase of EEE duration the hazard of fracture reduced by 30%, 34%, 43% in all participants, Non-Menopausal women+ women with natural menopause and Non-Menopausal women+ women with surgical menopause, respectively.

Box plots of EEE duration among fracture causes were shown in figure 2. Based on the Kruskal-Wallis test, there was no significant difference between the duration of EEE and various fracture categories in different study groups.

Discussion

Our findings supported our assumption that a reduction in EEE duration is associated with an increased risk of fracture incidence among women. However, the type of surgical or natural menopause in postmenopausal women did not have any effect on fracture incidence. Additionally, the results were similar across various types of fracture.

Estrogen is one of the most important main hormonal regulators of bone metabolism. It directly prevents bone remodeling and bone resorption and also modulates osteoblast/osteocyte and T-cell regulation of osteoclasts (8, 9, 24, 25). In addition, estrogen increases calcium reabsorption in kidneys (26, 27). However, lack of estrogen could lead to increased bone resorption, decreased deposition of new bone particularly in weight-bearing bones (9), and also imbalanced renal calcium and phosphate excretion, all involved in the pathogenesis of osteoporotic fracture (28). Furthermore, estrogen deficiency can lead to an increase in oxidative stress and the generation of advanced glycation end products, and a subclinical inflammatory bone microenvironment state (29, 30).

In women, exposure to endogenous estrogen occurs mostly during the reproductive lifespan, mainly from age at menarche until the age at menopause. Due to strong evidence regarding the effect of estrogen on bone metabolism, it is hypothesized that the duration of estrogen exposure potentially affects fracture incidence. In this regard, the present study revealed significant negative associations between increased EEE and risk of fractures. In agreement with our findings, Shimizu et al. in a large-scale population-based cohort study reported that late menarche at ≥16 years could increase the risk of vertebral fractures in Japanese women (15). In another recently published population-based cohort study of 1272115 postmenopausal Korean women, Yoo et al. reported that later age at menarche ≥17 years and earlier age at menopause < 40 years, and shorter reproductive span < 40 years were each independently associated with increased risk of any fracture (16).

Additionally, we evaluated the effect of type of menopause on the risk of fracture among women. In this respect, it is well documented that the physiologic changes associated with natural and surgical menopause are different (31). Although women who undergo both natural and surgical menopause experience the loss of cyclic ovarian production of estrogen, but that estrogen deprivation occurs quickly in surgical menopause whereas it happens gradually in natural menopause (32), which may potentially influence the risk of fracture in these women. The present study provides

further new evidence that those types of menopause may not have any effect on fracture incidence. In agreement with these findings, Fakkert et al. in a meta-analysis of ten studies showed that BMD after menopause was significantly lower compared to premenopausal age-matched women, irrespective of the type of menopause. They argued that the effect of surgical or natural menopause on BMD and fracture prevalence are comparable (33).

At menopause, estrogen level reduction is associated with low bone density (34-36). This bone density loss can lead to osteoporosis which may result in an osteoporotic fracture that occurs in almost 50% of elderly postmenopausal women(37-39). As estrogen among all hormones which affect bone is important for bone density and strength (40) we expect a decrease in the duration of EEE leads to an increase in fracture incidence. So, an association is between low estradiol levels and bone density reduction (41) which can lead to an increased rate of the hip, but not the spine, fracture (7), and since the fat mass is considered as the source of endogenous estrogen an impact of low estrogen levels on hip fracture in elderly women it is attributed to variations in body weight (42). Furthermore, in postmenopausal women, the impact of low EEE concentrations has been shown on increased hip, vertebral(43), and low-trauma clinical fractures incidence (44), but no report for an increase in nonvertebral fracture incidence (45). Finigan et al. reported low endogenous concentration of estrogen can predict incident vertebral fractures separately from other influencing factors (46). In addition, the incidence of vertebral (47) and hip (48) fractures in women increases with age (over 50 years) are almost twice men. In consequence, endogenous estrogen levels have an important physiological effect on bone mass. However, we did not find the relationship between types of fracture and EEE duration, maybe due to the lack of an adequate number of events in each fracture category. In this respect, since, we included all severe forms of fracture which needed to hospital admission, it might be possible that milder and asymptomatic forms of fracture have been missed in this study which potentially could affect these findings. The small number of vertebral fracture in the current study may be due to no need for hospitalization in majority of such cases (49, 50).

Limitations & strengths

To our knowledge, this is one of the largest population-based prospective study with long-term follow up that assessed the duration of endogenous estrogen exposure and risk of fracture incidence. We evaluated the risk of fracture as a clinical outcome directly rather than surrogate markers of fracture such as BMD. In addition, using hospital records for the outcome of interest, let us present a reliable estimation. In addition, we adjusted the most important potential risk factors that potentially affect the results.

The current study has some limitations as well. Firstly, we used a self-report questionnaire for evaluation of the age at menarche and menopause, breastfeeding duration, and hormonal contraceptive consumption. That information may be affected by recall bias. Considering a fixed value for appraising the follicular phase can be considered as a second limitation. Thirdly, we included only hospitalized fractures during study follow-up, therefore, we may have missed milder and asymptomatic fractures. Additionally, due to a lack of data, we could not differentiate between osteoporotic and traumatic fractures. Moreover, we did not evaluate the effect of nutritional factors such as vitamin D on the findings. However, since more than half of the Iranian population suffers from vitamin D deficiency (51), it seems the lack of data on vitamin D in our data set could not affect our findings. Our findings can not be extrapolated in rural areas, for the reason that we performed our study in the urban city of Tehran. Eaghtly, the present study is a retrospective study, and the results should be perceived as associations, not as causality.

As a consequence, the health providers and clinicians have to know fracture risk factors to prevent this major issue which threatens health seriously, and a shorter duration of EEE can be an important risk factor in women.

Conclusion

the results of this large, population-based cohort study showed EEE duration was negatively associated with fracture incidence. In this respect, a longer duration of endogenous estrogen exposure has a protective effect on fracture incidence. The results of this study will help healthcare providers and policymakers to do some intervention to reduce the risk of fracture risk for women at high risk, including those with or without osteoporosis.

Abbreviations

EEE endogenous estrogen exposure

HR hazard ratio

NCDs non-communicable diseases

CVD cardiovascular disease

CKD chronic kidney disease

BMD bone mineral density

TLGS Tehran Lipid and Glucose Study

HRT hormone replacement therapy

CI confidence interval

MAQ Modifiable Activity Questionnaire

MET metabolic equivalent task

BP Blood pressure

SBP systolic blood pressure

DBP diastolic blood pressure

BMI Body mass index

WC Waist circumference

HC Hip circumstance

WHR Waist to hip ratio

KDOQI Kidney Disease Outcome Quality Initiative guidelines

CI confidence interval

MDRD Modification of Diet in Renal Disease

Scr serum creatinine

eGFR estimated glomerular filtration rate

FPG fasting plasma glucose

2-hPCPG 2-hour post-challenge plasma glucose

SD standard deviation

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Additional Information

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Data Availability: The data sets produced through the current study are not publicly available but are available from the corresponding author on reasonable request.

Figure1. Study flowchart.

Abbreviation: TLGS, Tehran lipid and glucose study; HRT, Hormone replacement therapy.

Figure 2. Box plot of endogenous estrogen exposure (EEE) duration among fracture causes (a) All participants, (b) Excluding surgical menopausal women (c) Excluding natural menopausal women.

Table1. Characteristics of the study participants at baseline and date of incident fracture/end of follow-up

Values are presented as mean (SD)*, median (IQR 25%-75%)**, or percentage*** as appropriate.

$: Just for menopausal participants.

Abbreviations: BMI, Body mass index; WC, Waist circumference; HC, Hip circumstance; WHR, Waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, Cardiovascular disease; CKD, Chronic kidney disease; EEE, endogenous estrogen exposure.

Table 2. Hazard ratios and 95% confidence intervals (CI) from the unadjusted and adjusted analysis of EEE with any fracture incidence by menopause reasons

Abbreviations: HR, Hazard ratio; CI, Confidence interval; EEE, Endogenous estrogen exposure; BMI, Body mass index; WC, Waist circumference; CVD, Cardiovascular disease; CKD, Chronic kidney disease. The survival time was defined as the difference between the age at menarche and age at the last follow up.

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Table1. Characteristics of the study participants at baseline and date of incident fracture/end of follow-up

	N	Baseline	N	Date of incident fracture/end of follow-up
Age,* years	5269	38.3 (14.1)	5269	52.4 (13.8)
Age at menarche, * years	5269	13.6 (1.4)	-	-
Menopausal women***	1409	26.7	2858	54.2
Age at menopause *$	1409	49 (45-52)	2858	50 (46-53)
BMI, * kg/m^2	5269	27.2 (5)	5033	29.6 (5.2)
WC,* cm	5269	86.7 (12.9)	5033	96.2 (12.7)
HC,* cm	5269	103.5 (9.6)	5032	104 (9.8)
WHR*	5269	0.83 (0.08)	5032	0.93 (0.13)
SBP,* mmHg	5269	116.3 (18.7)	5220	117.1 (20.1)
DBP,* mmHg	5269	76.5 (10.7)	5219	75.8 (10.5)
Marital status***				
Living a lone	1083	20.5	984	18.7
Married	4186	79.5	4278	81.3
Ever smokers***	222	4.2	281	5.4
Educational level, ***				
< 6 years	484	9.2	433	8.2
6-12 years	4352	82.6	3817	72.6
> 12 years	433	8.2	1009	19.2
Low physical activity***	3526	66.9	3575	69.9
Comorbidities				
Diabetes***	535	10.1	1282	24.4
Central obesity ***	5269	2215 (42.0)	5033	3488 (69.3)
Hypertension***	993	18.8	2222	42.5
CVD history***	162	3.1	639	12.1
CKD***	675	12.8	2207	41.9
Asprin consumption***	462	8.8	997	18.9
Steroid medication***	85	1.6	134	2.5
Total EEE duration** (weeks)	5269	489.9 (268.9-735.6)	5269	696.8 (553.5-811.8)

Values are presented as mean (SD)*, median (IQR 25%-75%)**, or percentage*** as appropriate.
$: Just for menopausal participants.
Abbreviations: BMI, Body mass index; WC, Waist circumference; HC, Hip circumstance; WHR, Waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, Cardiovascular disease; CKD, Chronic kidney disease; EEE, endogenous estrogen exposure.

Table 2. Hazard ratios and 95% confidence intervals (CI) from the unadjusted and adjusted analysis of EEE with any fracture incidence by menopause reasons.

Participants' groups	Non-Menopausal women+ women with all menopausal reasons Survival time median(Q_{25}- Q_{75}) 38.1 (28_50) (N=5269)		Non-Menopausal women+ natural menopausal women Survival time median(Q_{25}- Q_{75}) 36 (26_48) (N=4634)		Non-Menopausal women+ surgical menopausal women surgical Survival time median(Q_{25}- Q_{75}) 30 (23_37) (N=3046)	
unadjusted	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
EEE Z score, weeks	0.81 (0.68,0.96)	0.02	0.78 (0.64,0.96)	0.02	0.65(0.48,0.88)	0.005
adjusted	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
EEE Z score, weeks	0.70(0.58,0.86)	0.001	0.66(0.52,0.85)	0.001	0.57(0.40,0.81)	0.002
Age, years	0.90(0.89,0.92)	<0.001	0.90(0.89,0.92)	<0.001	0.90(0.87,0.93)	<0.001
BMI, kg/m^2	0.98(0.95,1.03)	0.5	0.98(0.94,1.03)	0.5	1.00(0.99,1.01)	0.4
Central obesity	2.21(1.53,3.20)	<0.001	2.47(1.64,3.72)	<0.001	2.28(1.31,3.96)	0.003
Marital status						
Living alone	Reference	-	Reference	-	Reference	-
Married	0.85(0.62,1.20)	0.4	0.76(0.53,1.10)	0.1	1.19(0.61,2.33)	0.6
Ever smokers	1.32(0.58,2.98)	0.5	1.10(0.46,2.59)	0.8	1.25(0.19,8.00)	0.8
Educational level, years						
<6	Reference	-	Reference	-	Reference	-
6-12	1.86(1.26,2.74)	0.002	1.78(1.15,2.77)	0.01	2.84(1.44,5.60)	0.003
>12	2.11(0.95,4.71)	0.06	2.42(1.04,5.64)	0.04	1.00(0.22,4.42)	0.9
Low physical activity	0.82(0.65,1.01)	0.06	0.87(0.68,1.11)	0.3	0.75(0.54,1.05)	0.1
Comorbidities						
CKD	1.15(0.83,1.60)	0.4	1.07(0.74,1.56)	0.7	1.23(0.72,2.10)	0.4
CVD	0.80(0.51,1.26)	0.3	0.77(0.45,1.34)	0.4	0.95(0.40,2.26)	0.9
Diabetes	0.91(0.63,1.32)	0.6	0.85(0.54,1.34)	0.5	0.95(0.49,1.83)	0.9
Hypertension	1.29(0.93,1.78)	0.1	1.30(0.92,1.85)	0.1	1.36(0.73,2.51)	0.3
Steroid medication	1.18(0.55,2.53)	0.7	1.19(0.52,2.72)	0.7	2.27(0.64,8.13)	0.2
Aspirin consumption	1.10(0.83,1.45)	0.5	1.05(0.76,1.43)	0.8	1.19(0.69,2.04)	0.5

Abbreviations: HR, Hazard ratio; CI, Confidence interval; EEE, Endogenous estrogen exposure; BMI, Body mass index; WC, Waist circumference; CVD, Cardiovascular disease; CKD, Chronic kidney disease. The survival time was defined as the difference between the age at menarche and age at the last follow up.

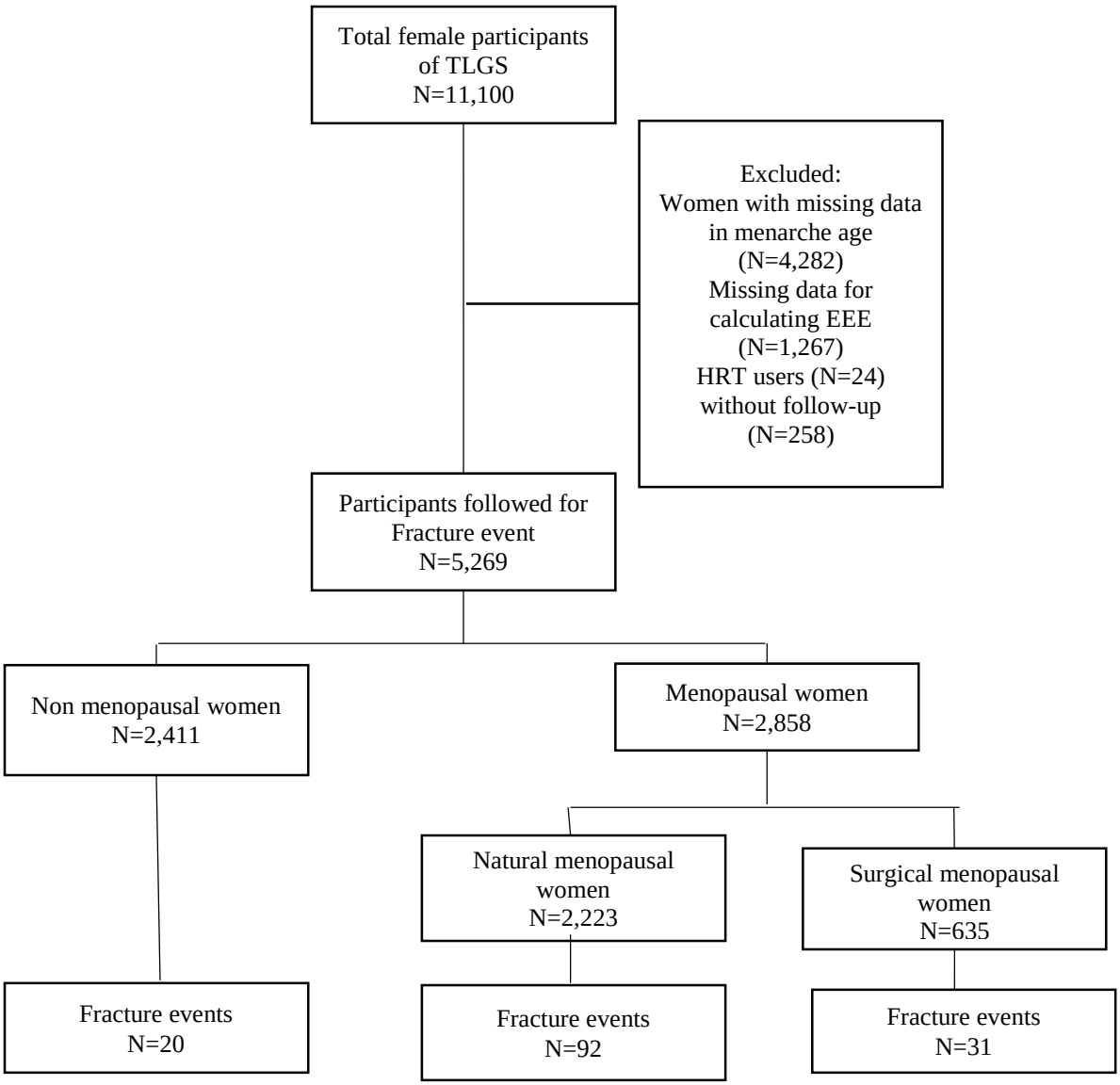

Figure1. Study flowchart.

Abbreviation: TLGS, Tehran lipid and glucose study; HRT, Hormone replacement therapy.

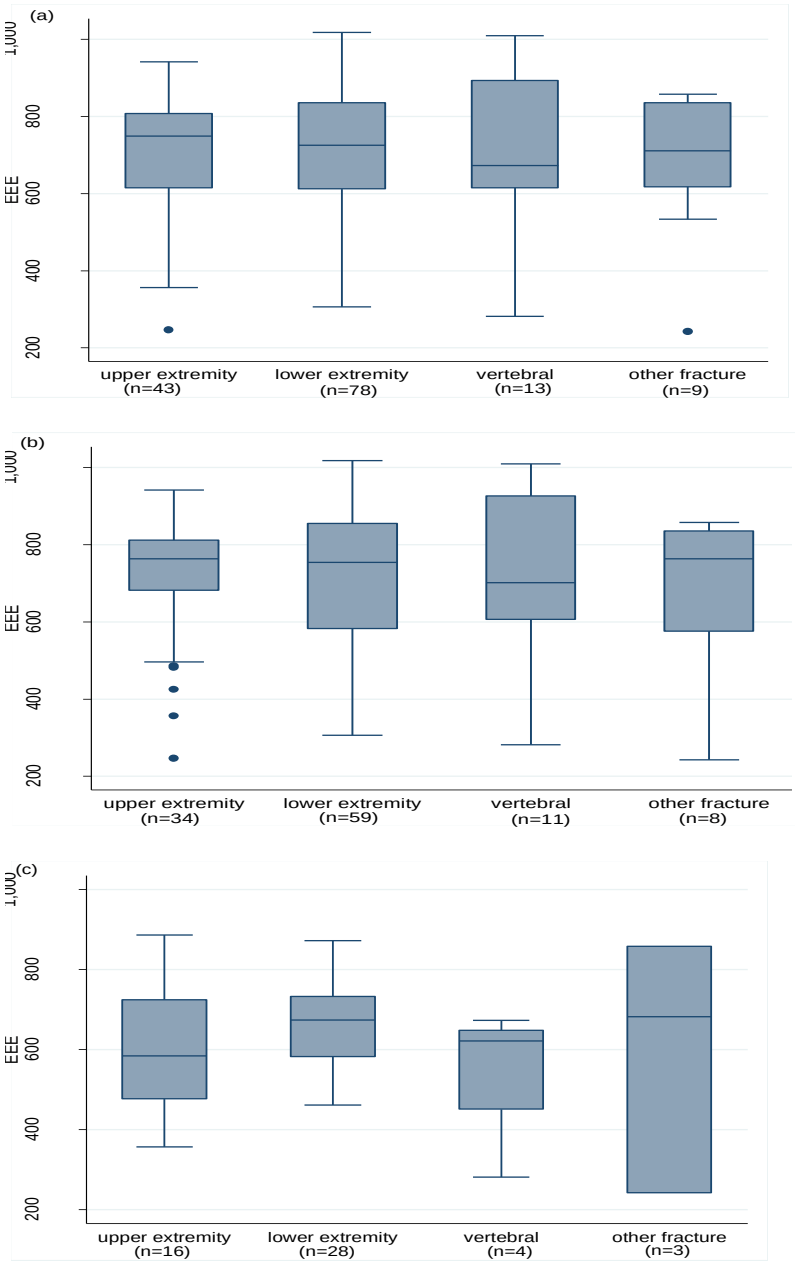

Figure 2. Box plot of endogenous estrogen exposure (EEE) duration among fracture causes (a) All participants, (b) Excluding surgical menopausal women (c) Excluding natural menopausal women