STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

1a. This cross-sectional study aimed to evaluate the association between serum estradiol (E2) level and appendicular lean mass index (ALMI) in middle-aged postmenopausal women using population-based data.
1b. Our results demonstrated an inverted U-shaped curve relationship between serum E2 levels and ALMI in middle-aged postmenopausal women, suggesting that low serum E2 levels play an important in the loss of muscle mass in middle-aged postmenopausal women.
2. Compared with the anabolic effects of androgens on the skeletal muscle mass in men, the effects of estrogens on the skeletal muscle mass in women are less clearly understood. Moreover, previous studies on the association between the loss of estrogen at menopause and skeletal muscle mass or function came to contradictory conclusions.
3. As the most potent estrogen hormone, estradiol (E2) is responsible for the maintenance of sexual characteristics and muscle health. Thus, we aimed to evaluate the association between serum E2 level and appendicular lean mass index (ALMI) in middle-aged postmenopausal women using population-based data.
4. The National Health and Nutrition Examination Survey (NHANES) is a large, ongoing cross-sectional survey conducted annually in a nationally representative sample of the non-institutionalized United States population.
5. Data for this study were pooled from the NHANES between 2013 and 2016. The study population was restricted to postmenopausal women aged 40–59 years. Individuals with a regular period in the past 12 months (n = 840), or with an unrecorded menopausal status (n =287), as well as those with missing serum E2 levels (n=69) or ALMI data (n = 171) were excluded. Finally, 673 women were included in the analysis.
6a. Individuals with a regular period in the past 12 months (n = 840), or with an unrecorded menopausal status (n =287), as well as those with missing serum E2 levels (n=69) or ALMI data (n = 171) were excluded. Finally, 673 women were included in the analysis.
7. The exposure variable was the serum E2 level, which was measured based on the reference method of the National Institute for Standards and Technology, using isotope dilution liquid chromatography tandem mass spectrometry. The outcome variable was ALMI, which was measured by dual-energy X-ray absorptiometry whole-body scans and calculated as the appendicular lean mass (kg) divided by height squared (m2). The covariates included in this study were age, race, educational level, body mass index (BMI), ratio of family income to poverty, moderate activities, total protein, blood urea nitrogen, and serum uric acid and calcium levels.
8. Detailed information on these variables can be found on the NHANES website (https://www.cdc.gov/nchs/nhanes/).
9. We constructed three models: Model 1, no covariates were adjusted; Model 2, age and race were adjusted; and Model 3.
10. This study included 673 postmenopausal women, aged 40–59 years, from the National Health and Nutrition Examination Survey between 2013 and 2016.
11. Weighted multivariable linear regression models were used to evaluate the association between serum E2 level and ALMI. We constructed three models: Model 1, no covariates were adjusted; Model 2, age and race were adjusted; and Model 3, all covariates presented in Table 1 were adjusted.
12a. Weighted multivariable linear regression models were used to evaluate the association between serum E2 level and ALMI. When non-linear associations were found by using weighted generalized additive model and smooth curve fitting, two-piecewise linear regression models were further applied to examine the threshold effects.
12b. In the subgroup analysis stratified by BMI and race.
12c. Individuals with a regular period in the past 12 months (n = 840), or with an unrecorded menopausal status (n =287), as well as those with missing serum E2 levels (n=69) or ALMI data (n = 171) were excluded.
12d. All estimates were applied with weights, in accordance with the guidelines edited by the NCHS.
12e. In the subgroup analysis stratified by BMI and race.

13. Individuals with a regular period in the past 12 months (n = 840), or with an unrecorded menopausal status (n =287), as well as those with missing serum E2 levels (n=69) or ALMI data (n = 171) were excluded. Finally, 673 women were included in the analysis.
14. Demographic characteristics of the participants subclassified based on the serum E2 level quartiles (Q1: ≤3.80 pg/mL; Q2: 3.88–7.42 pg/mL; Q3: 7.45–17.50 pg/mL; and Q4: ≥17.60 pg/mL) are shown in Table 1.
15. Compared with the Q1 group, individuals in other groups were younger, and had lower levels of blood urea nitrogen, and higher levels of income to poverty ratio, BMI, total protein, serum uric acid, and ALMI.
16. The association between serum E2 level and ALMI was positive in each model, with a significant P for trend among the different serum E2 level quartile groups (Table 2). In the subgroup analysis stratified by BMI and race, this positive association was significant in the group with BMI < 25 kg/m2 (Table 3). An inverted U-shaped curve relationship between serum E2 level and ALMI was found, as shown in Figure 1, and the inflection point was identified at a serum E2 level of 85 pg/mL (Table 4).
17. In the subgroup analysis stratified by BMI and race, this positive association was significant in the group with BMI < 25 kg/m2 (Table 3). An inverted U-shaped curve relationship between serum E2 level and ALMI was found, as shown in Figure 1, and the inflection point was identified at a serum E2 level of 85 pg/mL (Table 4).
18. This study evaluated the association between serum E2 level and ALMI in middle-aged postmenopausal women, and found an inverted U-shaped curve relationship between them, with the point of inflection at a serum E2 level of 85 pg/mL.
19. However, the limitations of this study should also be noted. First, a causal relationship between serum E2 level and ALMI in middle-aged postmenopausal women could not be determined due to the cross-sectional design of the NHANES surveys. Second, biases caused by unmeasured confounding factors cannot be excluded. Third, the conclusion cannot be generalized to older women because the population of this study was restricted to middle-aged postmenopausal women.
20. Overall, this study showed an inverted U-shaped curve relationship between serum E2 levels and ALMI in middle-aged postmenopausal women, suggesting that low serum E2 levels play a crucial role in the loss of muscle mass in middle-aged postmenopausal women.
21. Third, the conclusion cannot be generalized to older women because the population of this study was restricted to middle-aged postmenopausal women.
22. This study received no funding.