Dear Editor and Reviewer(s),

We appreciate the opportunity allowing us to revise our manuscript and thanks for reviewer's comments and suggestions. We would like to submit our revised manuscript, entitled "Association between serum estradiol level and appendicular lean mass index in middle-aged postmenopausal women" for consideration for publication. In the revised manuscript, we have carefully addressed all comments and questions raised by reviewer(s) point-by-point. We greatly appreciate your time and efforts to improve our manuscript for publication.

Reply to Reviewers

Reviewer 1

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Reply: In line with the Reviewer's feedback, a scientific native speaker has rechecked the language and streamlined it to make the manuscript more scientific (all changes marked in yellow in the revised manuscript).

Conclusion: Accept (General priority)

Specific Comments to Authors: I think the topic of study is interesting. However, I think that the authors need to add a list of abbreviations at the end of the manuscript.

Reply: Thanks for your suggestion, we have added a list of abbreviations at the end of the manuscript in the revised manuscript.

We marked the changes in yellow in the revised paper.

These comments are all valuable and enable us to greatly improve the quality of our manuscript. We tried our best to improve the manuscript. These changes will not influence the content and framework of the paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the corrections will meet with approval. Please do not hesitate to contact us with any further questions or recommendations.

Once again, thank you very much for your comments and suggestions.

Association between serum estradiol level and appendicular lean mass index in middle-aged postmenopausal women

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Author contributions

JF, YFW and ZXZ contributed to data collection, analysis and writing of the manuscript. ZXZ contributed to study design and editing of the manuscript.

Funding

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethical Approval and consent to participate.

The ethics review board of the National Center for Health Statistics approved all NHANES protocols and written informed consents were obtained from all participants.

Availability of Data and Materials

The datasets analysed during the current study are available at NHANES website (https://www.cdc.gov/nchs/nhanes/index.htm).

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The authors appreciate the time and effort given by participants during the data collection phase of the NHANES project.

Abstract

Background: Previous studies investigating the association between loss of estrogen at menopause and skeletal muscle mass came to contradictory conclusions. 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.

Methods: This study included 673 postmenopausal women, aged 40–59 years, from the National Health and Nutrition Examination Survey between 2013 and 2016. 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.

Results: There was a positive association between serum E2 level and ALMI. Compared to individuals in quartile 1 group, those in other quartiles had higher ALMI levels. An inverted U-shaped curve relationship between serum E2 level and ALMI was found on performing weighted generalized additive model and smooth curve fitting, and the inflection point was identified as a serum E2 level of 85 pg/mL.

Conclusions: 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.

Keywords: Estradiol, skeletal muscle, menopause, health, the National Health and Nutrition Examination Survey.

Background

Most women experience menopausal transition in middle age, when aging-related hormonal changes accelerate[1]. The onset of sarcopenia, a multifactorial condition related to the loss of muscle mass and quality, has been intimately linked to menopause[2, 3].

Compared with the anabolic effects of androgens on the skeletal muscle mass in men[4, 5], the effects of estrogens on the skeletal muscle mass in women are less clearly understood[6]. Moreover, previous studies on the association between the loss of estrogen at menopause and skeletal muscle mass or function came to contradictory conclusions[7]. As the most potent

estrogen hormone, estradiol (E2) is responsible for the maintenance of sexual characteristics and muscle health[8]. 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.

Materials and Methods

Data source and study population

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. 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.

Written informed consent was obtained from all participants and the Institutional Review Board of the National Center for Health Statistics (NCHS) approved the survey protocols (Protocol #2011-17).

Study variables

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 (m^2). 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. Detailed information on these variables can be found on the NHANES website (https://www.cdc.gov/nchs/nhanes/).

Statistical analyses

All estimates were applied with weights, in accordance with the guidelines edited by the

NCHS[9], to account for the NHANES sampling method. All analyses were performed using EmpowerStats software (http://www.empowerstats.com) and R software (version 3.4.3). The statistical significance was set at $P<0.05$. Weighted multivariable linear regression models were used to evaluate the association between serum E2 level and ALMI. Following the Strengthening the Reporting of Observational Studies in Epidemiology statement[10], 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. 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.

Results

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. 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.

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/m^2 (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).

Discussion

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.

Estrogens, especially E2, are known to play an important role in the preservation of muscle

health. Several studies have investigated the effects of hormone replacement therapy (HRT) and found that it has a positive and measurable impact on muscle function[11, 12]. Conversely, other studies found that HRT does not protect against muscle loss[13, 14]. Moreover, it was reported that menopausal HRT was associated with an increased risk of adverse events, such as dementia[15], stroke[16], and breast cancer[17]. Therefore, it is important to balance the potential benefits against risks. Our results revealed an inverted U-shaped curve relationship between serum E2 level and ALMI, suggesting that adequate E2 supplementation may be a useful adjunct therapy for individuals with a low serum E2 level. The exact mechanism underlying the effects of E2 on skeletal muscle remains unclear. A possible explanation for the potentially beneficial effect is that E2 can stimulate the proliferative activity of the muscle satellite cells (stem cells) that are responsible for muscle tissue maintenance[18, 19]. Another possible explanation is that estrogen deficiency results in the loss of muscle mass through apoptotic mechanisms[20, 21]. Despite these possibilities, the molecular mechanism of the impact of E2 on muscle function needs to be further explored. Data from the NHANES surveys were acquired following standard protocols, which ensured that the data were accurate and consistent. 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.

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.

Abbreviations

E2: estradiol

ALMI: appendicular lean mass index

NHANES: The National Health and Nutrition Examination Survey

NCHS: the National Center for Health Statistics

BMI: body mass index

HRT: hormone replacement therapy

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Figure legends

Figure 1 The association between serum estradiol level and appendicular lean mass

index. a Each black point represents a sample. b Solid red line represents the smooth curve fit between variables.

age, race, educational level, body mass index, ratio of family income to poverty, moderate activities, total protein, blood urea nitrogen, serum uric acid, and serum calcium were adjusted.