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Effectiveness of Selenium Supplement Combined with Exercise on Ankle Brachial Index and Bone Mineral Density

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Abstract

Selenium (Se) is an essential trace element involved in fatty acid metabolism and bone formation. Arterial disease resulting from excessive fat accumulation and low bone mineral density (BMD) are prevalent health concerns in the general population. This study aimed to investigate the effectiveness of Se supplementation combined with exercise on the Ankle-Brachial Index (ABI) and BMD. Sixty participants were randomly assigned to either a control group (n = 30) or an intervention group (n = 30). Both groups followed a structured exercise program for 12 weeks. In addition to the exercise regimen, participants in the intervention group received Se supplementation in the form of selenomethionine (200 µg per capsule daily) for the same duration, while the control group received no supplementation. Data on ABI, BMD, and body composition were collected from all participants at baseline and at the end of the study. At the study endpoint, participants in the intervention group showed significantly higher right and left ABI scores compared to the control group ($p < 0.05$). Similarly, BMD measurements at the wrist and ankle were significantly higher in the intervention group than in the control group ($p < 0.05$). Furthermore, significant positive correlations were observed between Se intake and both ABI (left ABI: $r = 0.30$) and BMD improvements (wrist: $r = 0.47$; ankle: $r = 0.31$; $p < 0.05$). In conclusion, the findings suggest that Se supplementation combined with exercise is more effective in improving ABI and BMD than exercise alone.

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Introduction

Selenium (Se) is a vital trace element essential for the physiological functioning of the human body. It plays important roles in various biological systems, including the thyroid gland, reproductive system, and immune system, among others.[1] A common method for assessing Se status is through blood Se concentration, which should not fall below 120 µg/L.[2] Previous studies have reported the prevalence of Se deficiency across populations in various countries. For instance, a study conducted in Spain among children found that 13.93% had blood Se levels below 60 µg/L.[3] Similarly, research in Saudi Arabia revealed that 56% of the adult population had Se levels below 56 µg/L.[4] In Thailand, data on Se intake among the general population are limited. However, one study on HIV-infected children in Thailand reported that 56% of participants were so deficient.[5] In addition, previous research has highlighted the benefits of Se in relation to vascular health. Se, particularly in the form of selenoproteins, has been shown to stimulate intracellular fat metabolism, potentially aiding in the regulation of blood lipid levels and supporting normal blood circulation.[6] For example, a longitudinal study conducted in China among adults monitored blood Se levels over time and found that individuals with normal Se levels exhibited lower triglyceride and cholesterol levels compared to those with Se deficiency, who were more likely to experience lipid abnormalities.[7] These findings suggest that adequate Se intake plays a key role in lipid regulation and contributes to the maintenance of healthy vascular function.

Peripheral Arterial Disease (PAD) is caused by the accumulation of fatty deposits on the inner walls of arteries, leading to arterial hardening and the narrowing or blockage of peripheral arteries in the arms and legs. The severity of PAD varies depending on the extent of arterial obstruction. Common symptoms include leg and calf pain, particularly during walking, which may progress to chronic wounds on the feet or toes due to reduced blood flow resulting from narrowed arteries.[8] The etiology of PAD involves several risk factors, including diabetes, kidney disease, and elevated blood lipid levels, all of which contribute to the deposition and accumulation of lipids on the inner vascular walls.[9] PAD significantly impairs patients' quality of life, with global estimates indicating that approximately 236 million individuals are affected worldwide.[10] In Thailand, one study reported a PAD prevalence rate of 7.9% in non-urban areas of the lower northern region.[11] Screening for PAD can be conducted by assessing peripheral arterial circulation using the Ankle-Brachial Index (ABI), a non-invasive method that evaluates arterial patency in the arms and legs. This test is painless, safe, provides immediate results, and does not require fasting beforehand..[12] According to diagnostic criteria, a normal ABI ranges from 0.9 to 1.4. An ABI value below 0.9 indicates peripheral arterial stenosis and is considered abnormal. This measurement technique has been widely accepted in previous research as a reliable and accurate indicator of PAD risk.[13,14] Furthermore, previous studies have suggested that Se plays a role in stimulating fatty acid metabolism in the human body.[15] Therefore, adequate Se intake may help reduce fat accumulation in the bloodstream. In addition to regulating lipid levels, regular physical exercise is also recognized as an effective strategy for improving peripheral arterial circulation.[16]

Low bone mineral density (BMD) and osteoporosis are significant global health concerns. These conditions are primarily associated with increased bone turnover, often resulting from physiological changes—particularly hormonal fluctuations—alongside insufficient physical activity and inadequate intake of nutrients essential for bone health, especially calcium, during early life.[17,18] The consequences of low BMD substantially affect individuals' quality of life, leading to pain, increased risk of fractures, and diminished capacity for self-care.[19] Current data indicate that, in the United States, approximately 10 million individuals aged 50 and older suffer from osteoporosis.[20] In Japan, the number of osteoporosis cases is estimated at 15 million.[21] In Thailand, the prevalence of bone fragility and osteoporosis is increasing, largely due to the country's transition to an aging society. For example, a previous study reported that 21.3% of early postmenopausal women attending a menopausal clinic at a tertiary care hospital in Thailand were diagnosed with osteoporosis.[22] In addition to inadequate intake of bone-supporting nutrients such as calcium and vitamin D, and lack of exercise, Se has been identified in previous studies as a mineral associated with BMD. Se contributes to antioxidant defense and inhibits the activity of inflammatory mediators, including cytokines released during systemic inflammation.[23] These inflammatory substances have been linked to accelerated bone turnover.[24] Se is believed to mitigate internal inflammation by suppressing the release of these cytokines, thereby potentially slowing bone loss.[25] Thus, prior research suggests that adequate Se intake may be associated with reduced bone resorption and improved bone health.[26] Given the relevance of both peripheral arterial circulation and BMD in public health, and considering the possible beneficial role of Se, this study aims to investigate the effectiveness of Se supplementation combined with exercise in improving ABI and BMD values. Despite emerging evidence, studies specifically exploring the combined effects of Se and exercise on these outcomes remain limited.

Materials and Methods

Study tools

Basic Information Questionnaire for Participants

A closed-ended questionnaire was designed and developed to collect basic demographic information from participants, such as gender, age, body mass index (BMI), education level, and so on. The participants completed this questionnaire by themselves.

Three-Days Food Record Interview Form

A closed-ended questionnaire was developed for participants to record their food intake over three days following their participation in the study and after receiving education on food portion calculations. Participants recorded their food intake for two weekdays and one weekend day during the final week of the study.[27] The recorded data were then submitted for analysis to calculate energy intake and nutrient consumption, providing information on total energy intake, energy distribution, and daily Se intake. This nutritional data, particularly Se levels, was used to support the interpretation of ABI measurements in the final week of the study. The dietary energy and Se intake data from this questionnaire were analyzed using the INMUCAL-Nutrient Version 4.0 food analysis program, developed by the Institute of Nutrition, Mahidol University, Thailand, in conjunction with the United States Department of Agriculture (USDA) database.

Se Dietary Supplement

A dietary supplement containing Se in capsule form was obtained from general pharmacies. The Se in this product is in the organic form of selenomethionine (SeMet), which has the highest absorption and utilization rate in the human body compared to other forms of Se.[28] Each capsule contains 200 µg of Se as SeMet. Participants in the intervention group took one capsule daily after lunch. This dosage of Se has been shown to be effective in various clinical contexts, as demonstrated by multiple previous studies.[29,30]

ABI Testing Device

This device (HBP-8000, OMRON, Kyoto, Japan) is used to measure peripheral arterial function. It undergoes regular calibration and maintenance to ensure accurate and reliable results. The device measures the ABI of peripheral arteries in various areas, including both the left and right arms and legs, and prints the results along with their interpretation. A normal ABI value ranges from 0.9 to 1.4, while a value below 0.9 indicates a risk of arterial disease. Additionally, the device measures the Arterial Stiffness Index (ASI), which reflects the pressure range within the arterial volume. An optimal ASI value is less than 70; values between 70 and 180 indicate a moderate risk of arterial stiffness, while values above 180 indicate a high risk.[31]

Bone Mineral Density Measurement Device

A DEXA-based bone density measurement device (Osteo Checker, Ampall Co. Ltd., Seoul, South Korea) was used to measure bone density at the wrist and ankle. The device reports results as T-scores and bone mineral density (BMD) values. The instrument was calibrated before each use.

Body Composition Analysis Devices

All participants underwent body composition analysis using the BOD POD device (COSMED Inc., Rome, Italy), which was calibrated before each use, and the InBody270 device (InBody Co., Seoul, South Korea) to measure parameters such as body fat percentage, muscle mass, and body fluid percentage, both before and after the study.

The study instruments, including questionnaires, were validated for accuracy and appropriateness by three qualified experts in nutrition and dietetics. After incorporating their suggested revisions, the questionnaires and detailed study procedures—conducted in accordance with the Declaration of Helsinki—were submitted for ethical approval to the Human Research Ethics Committee of Burapha

University, Thailand (approval number IRB1-099/2567).

Participants

After enrollment, a total of 60 healthy participants residing in Saensuk Sub-District Municipality, Mueang District, Chonburi Province, were recruited for this study. The sample size was determined based on a power analysis using G*Power software (version 3.1), which indicated that a minimum of 52 participants was required to detect a medium effect size (d = 0.5) with 80% power at a significance level of 0.05. To account for potential dropouts and incomplete data, the target sample size was increased by approximately 15%, resulting in the recruitment of 60 participants. The inclusion criteria were: Thai ethnicity and nationality, good health with no chronic diseases, legal adults aged 18 to 60 years, and the ability to read and write Thai. The exclusion criteria were: a history of allergies to food or dietary supplements; muscle or bone injuries that impede exercise; serious communicable diseases; oral problems affecting chewing or swallowing; current use of other dietary supplements or herbal products; pregnancy or breastfeeding; intellectual disabilities affecting communication; withdrawal from the study before completion; and incomplete questionnaire responses or data. All participants signed informed consent forms prior to participation.

Study Procedures

At the first appointment, all 60 participants were invited to a designated room at the Nutrition and Dietetics Division, Faculty of Allied Health Sciences, Burapha University, where they were provided with study details and each participant signed the informed consent form. Subsequently, participants were divided into two equal groups using quota sampling: an intervention group (n=30) and a control group (n=30). After group allocation, baseline data were collected using the aforementioned instruments. These measurements included weight and height, peripheral arterial function assessed by an ABI device (measuring ASI and ABI), body composition parameters (% body fat, % fat-free mass, muscle mass, % body fluid, thoracic gas volume) measured using BOD POD and InBody270, and wrist and ankle bone density (T-score and BMD) assessed by the Osteo Checker. Participants then completed a basic information questionnaire and received education on food exchange lists using food models. They were provided with a 3-day food record questionnaire to document their intake over two weekdays and one weekend day during the final week of the study.

Both groups were assigned to exercise according to WHO guidelines,[32] which included 30–45 minutes of aerobic exercise 4–5 days per week and strength training 1–2 days per week for 12 weeks. The control group was asked to exercise only, while the intervention group additionally received Se supplements (200 µg SeMet per capsule) to be taken once daily after any meal for 12 weeks.[33] In the final week (week 12), participants submitted their 3-day food records for calculation of energy intake, energy distribution, and Se intake. Final measurements were taken, including peripheral arterial function (ABI), body composition (BOD POD and InBody), and bone mineral density (Osteo Checker), for subsequent statistical analysis.

Statistical Analyses

Participants' data on sex and education were reported as percentages, and differences were assessed using Fisher's Exact Test. Duration of weekly exercise, daily sun exposure, and other ABI and BMD scores were reported as mean ± standard deviation (SD). Skewness and kurtosis were used to assess data normality, and the Mann-Whitney U test was applied to determine significant differences between groups. Pearson correlation coefficients were used to examine the relationships between Se intake and ABI and BMD scores at the study endpoint. Statistical analyses were performed using SPSS software (version 26.0). A p-value of less than 0.05 was considered statistically significant.

Results

At the end of the final week of the study (week 12), all participants completed the study with no dropouts. The background data collected during the first week indicated no significant differences between the two groups in terms of general characteristics. The average age of participants in the control group was 26.36 years, while that of the intervention group was 25.20 years. The majority were female in both the control group (70%) and the intervention group (86.67%). Twenty-six participants (86.67%) in the control group and 28 participants (93.33%) in the intervention group held a bachelor's degree. The average weekly exercise duration was 60.66

minutes in the control group and 68.50 minutes in the intervention group. Daily sun exposure averaged 22.50 minutes in the control group and 30.16 minutes in the intervention group (Table 1).

Table 1: Background characteristics of participants

Characteristics	Control group (n=30)	Intervention group (n=30)	p value
Age (year) [1], mean (SD)	26.36 (5.15)	25.20 (5.39)	0.15
Sex[2]			
Male, n (%)	9 (30.00)	4 (13.33)	0.2
Female, n (%)	21 (70.00)	26 (86.67)	
Education[2]			
Bachelor degree, n (%)	26 (86.67)	28 (93.33)	0.67
Graduate degree, n (%)	4 (13.33)	2 (6.67)	
Length of weekly exercise (minute) [1], mean (SD)	60.66 (56.19)	68.50 (79.71)	0.58
Length of daily sun exposure (minute) [1], mean (SD)	22.50 (19.06)	30.16 (30.89)	0.33

Table 2: ABI scores, BMD scores, and body composition of participants

Parameters	Baseline		p value	Endpoint		p value
	Control (n=30)	Intervention (n=30)		Control (n=30)	Intervention (n=30)	
Right ABI, mean (SD)	1.08 (0.14)	1.04 (0.12)	0.24	1.19 (0.13)^	1.33 (0.08)^	<0.05*
Left ABI, mean (SD)	1.09 (0.15)	1.02 (0.13)	<0.05*	1.21 (0.13)^	1.30 (0.10)^	<0.05*
Right brachial ASI, mean (SD)	34.60 (8.88)	36.43 (10.83)	0.67	32.13 (6.04)	31.46 (8.03)^	0.18
Left brachial ASI, mean (SD)	36.76 (7.59)	38.13 (8.89)	0. 29	34.23 (8.95)	32.56 (11.79)^	0.12
Right ankle ASI, mean (SD)	89.40 (26.01)	85.90 (21.78)	0.66	80.50 (21.51)^	70.53 (21.51)^	<0.05*
Left ankle ASI, mean (SD)	83.83 (18.11)	82.83 (16.30)	0.98	80.03 (14.96)	68.30 (14.42)^	<0.05*
T-score of wrist bone, mean (SD)	-1.18 (0.80)	-1.24 (0.67)	0.85	-1.42 (0.66)^	-1.20 (0.69)	0.19
BMD value of wrist (g/cm2), mean (SD)	0.47 (0.07)	0.51 (0.09)	0.06	0.46 (0.06)	0.55 (0.09)^	<0.05*
T-score of ankle bone, mean (SD)	-1.36 (0.82)	-1.25 (0.77)	0.59	-1.42 (0.84)	-1.23 (0.84)	0.22
BMD value of ankle	0.47 (0.04)	0.50 (0.06)	0.08	0.50 (0.05)^	0.55 (0.07)^	<0.05*
BMI, mean (SD)	21.86 (2.93)	22.10 (2.63)	0.28	22.33 (2.59)	22.40 (2.42)	0.74
Body fluid (L), mean (SD)	28.95 (3.00)	29.59 (2.67)	0.34	28.77 (3.04)	29.66 (2.46)	0.29

Muscle mass (kg), mean (SD)	19.55 (2.32)	19.83 (2.04)	0.59	19.57 (2.06)	20.16 (1.87)	0.41
Body fat mass (kg), mean (SD)	20.45 (3.35)	20.55 (2.49)	0.66	21.04 (3.34)	20.07 (2.12)	0.37
Body weight by mineral (kg), mean (SD)	2.52 (0.18)	2.55 (0.24)	0.92	2.44 (0.22)	2.88 (0.16)^	<0.05*
Thoracic gas volume (L), mean (SD)	2.93 (0.32)	2.94 (0.31)	0.8	3.12 (0.31)^	3.11 (0.23)^	0.72
Estimated daily total kcal requirement (kcal), mean (SD)	1,810.66 (143.00)	1,832.36 (156.44)	0.34	2,282.36^ (156.44)	2,313.50^ (167.74)	0.63

* Significant difference between groups using Mann-Whitney U test.
^ Significant difference within group when compared with baseline using Mann-Whitney U test.

At the end of the study, the right and left ABI values at week 12 (final week) for participants in the intervention group were 1.33 and 1.30, respectively, which were significantly higher than those in the control group (1.19 and 1.21, respectively; $p < 0.05$). For the ASI, the right and left ASI values of participants in the intervention group were 70.53 and 68.30, respectively, which were significantly lower than the corresponding values in the control group (80.50 and 80.03; $p < 0.05$). Regarding BMD, the wrist and ankle BMD values at the endpoint for participants in the intervention group were 0.55 and 0.55 g/cm², respectively, significantly higher than those in the control group (0.46 and 0.50 g/cm², respectively; $p < 0.05$). Moreover, the body mineral weight of participants in the intervention group was 2.88 kg, significantly greater than that of the control group (2.44 kg; $p < 0.05$). Additionally, when examining changes within each group at the endpoint, several parameters showed significant improvement compared to baseline in both groups, including right and left ABI, right ankle ASI, ankle BMD values, and thoracic gas volume ($p < 0.05$) (Table 2).

Table 3: Dietary habits of participants

Nutrients	Control group (n=30)	Intervention group (n=30)	p value
Total kcal consumed (kcal), mean (SD)	2,234.53 (248.42)	2,269.33 (189.84)	0.48
%kcal from carbohydrate, mean (SD)	55.66 (6.12)	59.83 (7.34)	<0.05*
%kcal from protein, mean (SD)	13.06 (5.16)	12.93 (5.57)	0.69
%kcal from fat, mean (SD)	31.26 (3.90)	27.23 (4.42)	<0.05*
Amount of Se intake (µg), mean (SD)	63.50 (10.97)	268.80 (13.97)	<0.05*

* Significant difference between groups using Mann-Whitney U test.

The 3-day food records completed by the participants were used to assess their dietary habits during the study. Results showed that participants in the intervention group derived 59.83% of their total calories from carbohydrates, which was significantly higher than the control group (55.66%; $p < 0.05$). The intervention group obtained 27.23% of their total calories from fat, significantly lower than the control group (31.26%; $p < 0.05$). Additionally, with Se supplementation, the average daily Se intake of participants in the intervention group was 268.80 µg, significantly higher than that of the control group (63.50 µg; $p < 0.05$) (Table 3).

When examining the correlations between Se intake and arterial health outcomes among all 60 participants with similar exercise habits, results showed a significant positive correlation between Se intake and left ABI ($r = 0.30$; $p < 0.05$), as shown in Figure 1B, and a significant negative correlation between Se intake and left ASI ($r = -0.35$; $p < 0.05$), as shown in Figure 1F.

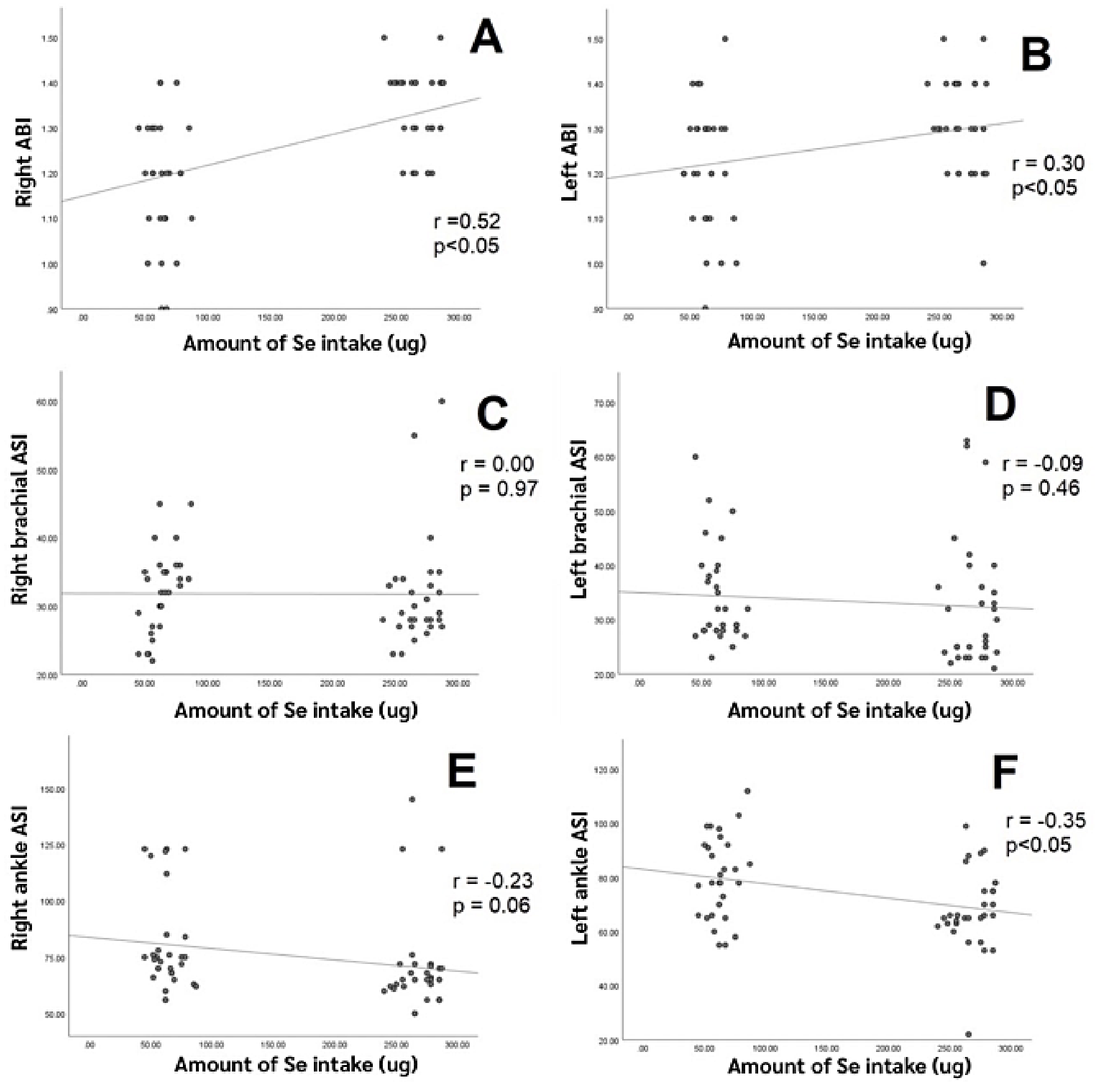

Fig. 1: Correlation of the amount of Se intake and ankle brachial indexes (A) Right ABI and amount of Se intake; (B) Left ABI and amount of Se intake; (C) Right brachial ASI and amount of Se intake; (D) Left brachial ASI and amount of Se intake; (E) Right ankle ASI and amount of Se intake; (F) Left ankle ASI and amount of Se intake.

For BMD, the results indicated significant positive correlations between Se intake and wrist BMD ($r = 0.47$; $p<0.05$) and ankle BMD ($r = 0.31$; $p<0.05$) among all 60 participants with similar exercise habits (Fig. 2B and 2D, respectively).

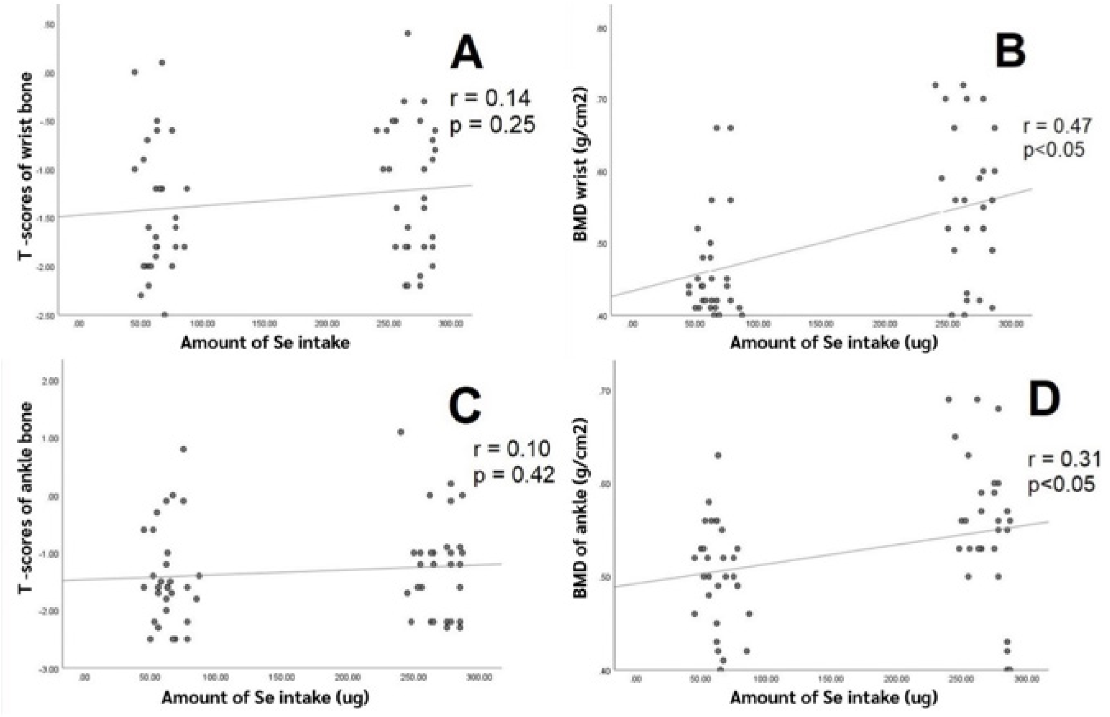

Fig. 2: Correlations of amount of Se intake and bone mineral density among participants at endpoint: (A) T-scores of wrist bone and amount of Se intake; (B) BMD of wrist bone and amount of Se intake; (C) T-scores of ankle bone and amount of Se intake; (D) BMD of ankle bone and amount of Se intake

Discussion

In this study, participants were assigned to follow the same exercise regimen according to WHO guidelines throughout the study to minimize confounding factors arising from differing physical activity levels. This was reflected in the lack of significant difference in total energy expenditure between groups. Se supplementation combined with exercise in the intervention group was observed to be more effective in improving arterial function than exercise alone. Evidence suggests that Se plays a crucial role in metabolic processes within several tissues, including the liver, white adipocytes, and muscles. It is particularly important in the thermogenesis of adipocytes, encompassing both brown and white adipose tissue, making Se a potential dietary intervention for treating obesity and metabolic disorders. Specifically, in the thermogenesis of brown adipose tissue (BAT), the Se-containing enzyme type 2 iodothyronine deiodinase (DIO2) is critical for initiating adaptive thermogenesis.[34,35] Se may influence thermogenesis in BAT through multiple mechanisms. One key pathway involves selenoproteins, particularly DIO2, which has long been recognized as essential for thermogenic adaptation. Additionally, Se intake can enhance the activity of another selenoprotein, glutathione peroxidase 1 (GPX1), leading to reduced glutathione levels and increased reactive oxygen species (ROS) production, ultimately triggering thermogenesis.[36,37] For these reasons, the improved arterial function observed in the intervention group receiving Se supplementation may be attributable to enhanced blood lipid metabolism. However, participants in the control group also showed improved ABI values at the endpoint compared to baseline, supporting previous studies that reported the benefits of exercise on vascular and cardiac function.[38,39] Previous studies have demonstrated the anti-inflammatory benefits of Se and exercise, particularly through the suppression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), which are known to adversely affect arterial wall tissues.[40,41] These mechanisms may underlie the findings of the present study, wherein participants in the intervention group—who received both Se supplementation and engaged in exercise—exhibited superior arterial function compared to the control group, who participated in exercise alone. Regarding bone mineral density (BMD), although the control group showed some improvement in bone health at the study endpoint compared to their baseline values, the intervention group demonstrated a greater enhancement. This improvement in the control group may be attributed to the role of exercise in promoting bone formation through osteoblast stimulation.[42]

However, the more pronounced improvement in the intervention group may be explained by prior research suggesting that Se can suppress inflammatory cytokines such as TNF-α and IL-6, both of which are implicated in bone resorption.[43,44] Therefore, the administration of Se in the intervention group may have contributed to a downregulation of these cytokines, thereby reducing bone resorption and promoting osteoblast activity.[45,46] This dual action of Se and exercise likely accounts for the more significant improvement in BMD observed in the intervention group compared to the control group, who received exercise only.

At the endpoint, participants showed no significant changes in body composition, suggesting that Se may not play a role in muscle building when combined with exercise. Previous studies have demonstrated that combining adequate intake of high-biological-value protein with weight training is most effective in inducing skeletal muscle hypertrophy.[47] While the control group consumed a higher percentage of calories from fat than the intervention group, and the intervention group consumed a higher percentage of calories from carbohydrates than the control group, their regular exercise routine was likely the primary factor in maintaining their body fat mass.[48] Results from the final week showed that both groups increased their estimated energy expenditure compared to baseline due to regular exercise, but their BMI remained unchanged. The balance of calories consumed may be key to maintaining body weight.[49]

Interestingly, participants in the control group who did not receive Se supplementation had a daily Se intake of 63.50 µg, which slightly exceeds the recommended daily allowance of 55 µg/day for adults.[50] This may be attributed to the study location—an urban seaside area where participants have easy access to a variety of foods. Moreover, Se-rich foods such as fish and other seafood are readily available at reasonable prices in this coastal setting.[51-53] Additionally, fish commonly consumed in Thailand from both freshwater and marine sources exhibit high Se bioavailability and lower levels of toxic heavy metal contamination.[54,55] Limitations of this study include the lack of biochemical marker data related to Se status, such as GPX1, selenoprotein P, and superoxide dismutase, as well as missing information on blood lipid profiles (which are involved in arterial function), serum vitamin D, and parathyroid hormone (which are involved in bone health). Future studies are recommended to investigate these biochemical markers and different types of exercise to explore the best practical approaches for improving ABI and BMD values.

Conclusion

The findings of this study showed that Se supplementation at 200 µg per day for 12 weeks combined with exercise tends to be effective in improving arterial function (as measured by ABI) and promoting BMD (as measured by DEXA) compared with exercise alone. The supplemented amount exceeds the recommended daily intake of 55 µg for adults but remains well below the established upper intake limit of 400 µg. These results support the importance of regularly adequate Se intake (at least 12 weeks) at an optimal level to enhance arterial function and BMD. Foods rich in Se, such as seafood, marine fish, and certain vegetables, should be promoted in diverse portions to help individuals achieve sufficient Se intake. Importantly, regular exercise, in combination with adequate Se intake, is a key factor in improving these health parameters.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This study obtained ethical approval by the Human Research Ethics Committee of Burapha University, Thailand (approval number IRB1-099/2567).

Informed Consent Statement

All participants were signed the informed consent before participated in this study.

Clinical Trial Registration

This trial is registered at Thai Clinical Trials Registry with the registration number TCTR20250214001.

Permission to Reproduce Material from Other Sources

Not applicable.

Author Contributions

- **Arluk Udomwongyon:** Conceptualized and collected the data
- **Busakorn Yindee:** Conceptualized and collected the data
- **Chanidapa Yathuam:** Conceptualized and collected the data
- **Chonnikarn Limpaninchart:** Conceptualized and collected the data
- **Juthathip Nakieam:** Conceptualized and collected the data
- **Oradee Saikiseng:** Conceptualized and collected the data
- **Surangkhana Phalaket:** Conceptualized and collected the data
- **Thanaphorn Pumlamjeak:** Conceptualized and collected the data
- **Jörg Feldmann:** Gave advice about the study protocol and proofread the manuscript draft
- **Nunnapus Laitip:** Gave advice about the study protocol and proofread the manuscript draft
- **Alongkote Singhato:** Conceptualization of methodology, statistical analyses, and manuscript writing

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