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*La mayor frecuencia de añadir sal a los alimentos aumenta el riesgo de baja densidad mineral ósea en los individuos mayores de 60 años: estudio de aleatorización mendeliana*

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*Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request. All data used in the current study are publicly available GWAS summary data.*

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**ABSTRACT**

**Background**: Adding salt to foods is associated with an increased osteoporosis risk, but the causality of this relationship remains unknown.

**Methods**: in this study, we conducted a two-sample Mendelian randomization (MR) study to investigate the potential causal effect of adding salt to foods on bone mineral density (BMD). Utilizing data from the UK Biobank to estimate adding salt to foods based on self-reported consumption and genetic association data for BMD from the Genetic Factors for Osteoporosis (GEFOS) consortium, we examined various BMD sites: forearm (distal 1/3 radius), lumbar spine (L1-4), femoral neck, total body BMD (TB-BMD), and age-specific TB-BMD (0-

15, 15-30, 30-45, 45-60, and over 60 years). The primary analysis used the inverse variance weighted method, supplemented by sensitivity analyses employing multiple MR methods, MR-PRESSO, and leave-one-out approach. Pleiotropy and heterogeneity were assessed using MR-Egger intercept, funnel plots, Cochran's Q, and Rucker's Q.

**Results**: we found a suggestive association between higher frequency of adding salt to foods and decreased TB-BMD in Europeans over 60 (OR = 0.84, 95 % CI = 0.721-0.979, *p* = 0.026). This association remained robust across different methods and sensitivity analyses, showing no apparent heterogeneity or pleiotropy. However, no causal effect was detected on BMD in other age groups or skeletal sites.

**Conclusion**: this MR study suggests a higher frequency of adding salt to foods significantly increases low BMD risk in individuals over 60, underscoring the importance of reducing salt consumption in this demographic for osteoporosis prevention.

**Keywords**: Adding salt to foods. Bone mineral density. Mendelian randomization. Osteoporosis.



## RESUMEN

**Introducción**: añadir sal a los alimentos se asocia con un aumento del riesgo de osteoporosis, pero la causalidad de esta relación sigue siendo desconocida.

**Métodos**: realizamos un estudio de aleatorización mendeliana (MR) de dos muestras para investigar el posible efecto causal de añadir sal a los alimentos sobre la densidad mineral ósea (DMO). Utilizando datos del UK Biobank para estimar la adición de sal a los alimentos sobre la base del consumo autoinformado y los datos de asociación genética de la DMO del consorcio Genetic Factors for Osteoporosis (GEFOS), examinamos diversos sitios de DMO: antebrazo (radio distal, 1/3), columna lumbar (L1-4), cuello femoral, DMO total del cuerpo

(TB-DMO) y TB-DMO por edades (0-15, 15-30, 30-45, 45-60 y mayores de 60 años). El análisis principal utilizó el método de ponderación inversa de varianza, complementado por análisis de sensibilidad utilizando varios métodos de MR, MR-PRESSO y el enfoque de dejar uno fuera. Se evaluó la pleiotropía y heterogeneidad utilizando el intercepto de MR-Egger, gráficos de embudo, la Q de Cochran y la Q de Rucker.

**Resultados**: encontramos una asociación sugestiva entre la mayor frecuencia de añadir sal a los alimentos y la disminución de la TB-DMO en europeos mayores de 60 años (OR = 0,84, IC 95 % = 0,721-0,979, $p$ = 0,026). Esta asociación se mantuvo robusta en los diferentes métodos y análisis de sensibilidad, sin mostrar heterogeneidad o pleiotropía aparente. Sin embargo, no se detectó un efecto causal sobre la DMO en otros grupos de edad o sitios esqueléticos.

**Conclusiones**: este estudio MR sugiere que una mayor frecuencia de añadir sal a los alimentos aumenta significativamente el riesgo de baja DMO en individuos mayores de 60 años, lo que subraya la importancia de reducir el consumo de sal en esta población para la prevención de la osteoporosis.

**Palabras clave:** Añadir sal a los alimentos. Densidad mineral ósea. Aleatorización mendeliana. Osteoporosis.

## INTRODUCTION

Osteoporosis is a prevalent bone metabolic disorder defined by a decrease in bone density and degradation of bone tissue microarchitecture, resulting in heightened vulnerability and risk of fractures (1,2). The World Health Organization reports that osteoporosis affects almost 200 million individuals globally, resulting in over 8.9 million fractures per year (3), with one-third of these

occurring in Europe (4). The high costs of hospitalization and treatment for fractures place a significant economic burden on individuals and society (5). Annually, approximately 3.5 million fractures in the 27 EU countries are attributable to osteoporosis, with related healthcare costs reaching 36 billion euros (4). Therefore, identifying osteoporosis risk factors and taking preventive measures are crucial for maintaining bone health in Europe.

Increasing evidence suggests that sodium is important for regulating blood pressure and is also a key factor in bone mineral metabolism (6-9). Nevertheless, the correlation between the addition of salt to foods and the risk of osteoporosis is still a subject of discussion (10). On the one hand, epidemiological studies have found that increased salt intake may reduce bone mineral density (BMD) by inducing calciuria or enhancing bone resorption (11-13). However, some studies suggest that a low-salt diet may cause health issues, such as inadequate intake of energy and nutrients, hyponatremia, and activation of the renin-angiotensin-aldosterone system, potentially leading to abnormally low BMD (14-16).

One possible reason for the inconsistent findings in previous studies could be the inadequate accuracy of salt measurement. Salt intake fluctuates significantly on a daily basis, and most previous studies estimated it using only one-day urine collections or dietary surveys, making it difficult to assess an individual's regular consumption level (11-13). Furthermore, existing techniques for quantifying dietary salt and potassium fail to distinguish between their respective impacts (17), given the interconnectedness of salt consumption and renal metabolism with potassium (18,19). Since that these two crucial positively charged ions have contrasting impacts on human health, their correlation may complicate the connection between salt consumption and health results.

Considering the deficiencies in traditional measurement methods, this study employs a new evaluation approach. Adding salt to foods, typically at the table, is a common dietary behavior closely linked to

an individual's preference for salty foods and habitual salt intake (20,21). In Western diets, table salt addition contributes to 6-20 % of total salt consumption (22,23). Furthermore, typical table salt is made up of 97-99 % sodium chloride (20), reducing the potential for confounding effects from other dietary factors like potassium. Therefore, evaluating the association between the frequency of adding salt to foods and low BMD can provide a unique and accurate approach.

Notably, BMD varies significantly with age and skeletal site. Differences in bone mineralization and remodelling processes at various times and sites may result in heterogeneous impacts of adding salt to foods on BMD (24). Most studies have focused on BMD in specific age groups or skeletal regions, with few systematically evaluating salt intake's overall impact on bone density across different ages and sites.

Therefore, this study intends to use two-sample Mendelian randomization (MR) analysis with genetic variation as instrumental variables to establish causal relationships (25,26). This comprehensive investigation will explore the causal association between adding salt to foods and BMD at various sites, including the forearm (distal 1/3 of the radius), lumbar spine (L1-4), femoral neck, total body BMD (TB-BMD), and age-specific TB-BMD groups (0-15, 15-30, 30-45, 45-60, and over 60 years old). Employing a two-sample MR design can partially overcome the constraints of conventional epidemiological studies (27,28). The random distribution of genetic variation in MR makes it less likely to encounter confounding and reverse causality (28). This study will help clarify the relationship between frequency of adding salt to foods and BMD, provide scientific evidence for public health policies and dietary guidelines, and play a significant role in preventing osteoporosis and maintaining bone health.

## METHODS

## Research design

A two-sample MR analysis was used to assess the causal relationship between adding salt to foods and BMD at various sites, including the forearm (distal 1/3 of radius), lumbar spine (L1-4), femoral neck, TB-BMD, and age-specific TB-BMD groups (0-15, 15-30, 30-45, 45-60, and over 60 years old). Relevant details are given in supplementary table I (https://www.nutricionhospitalaria.org/files/8731/ADMA1-05492-03.pdf). Genetic variants were used as risk factors, and the selection of effective instrumental variables (IVs) met three key assumptions: I) IVs are strongly associated with and adding salt to foods ($p < 5 \times 10^{-8}$); II) IVs are not related to confounders; and III) the association of genetic instruments with the outcome is mediated only through adding salt to foods (29). The study design is depicted in figure 1. Given the public availability of this data, our study did not necessitate ethical approval.

## Data sources

Summary-level data on adding salt to foods and TB-BMD across all age groups were obtained from the IEU OpenGWAS project. The data on adding salt to foods was sourced from the United Kingdom Biobank (MRC-IEU), encompassing up to 462,630 participants of European ancestry. Dietary data were categorized and evaluated using questionnaires. Notably, the UK Biobank is a cohort study of individuals aged 40-69 in the UK. The question regarding adding salt to foods was: “Do you add salt to your foods? (Do not include salt used in cooking).” The available responses were: “never/rarely,” “usually,” “sometimes,” “always,” and “prefer not to answer” (https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=1478) (20).

Genetic association data on BMD were provided by the Genetic Factors for Osteoporosis (GEFOS) consortium (http://www.gefos.org), with the GEFOS-seq project investigating the association of SNPs with BMD in European populations (31). In 2015, the genetic associations of SNPs with BMD, adjusted for age, age squared, sex, and weight in

additive models (18), were made publicly available for alleles with a frequency of ≥ 0.5 % (32). BMD was measured at three skeletal sites (31) (forearm, lumbar spine, and femoral neck) using dual-energy X-ray absorptiometry (DXA) and standardized to account for systematic differences between DXA machines (31).

TB-BMD data were derived from a meta-analysis of 30 genome-wide association studies (GWAS), including various age ranges: 11,807 individuals aged 0-15 years, 4,180 aged 15-30 years, 10,062 aged 30-45 years, 18,805 aged 45-60 years, and 22,504 individuals over 60 years (33). TB-BMD is a reliable indicator for assessing osteoporosis and predicting fractures. BMD T-scores consistent with the WHO standards were used to determine individuals who have osteopenia or osteoporosis (34). A T-score range of -1 to -2.5 defines osteopenia, while a T-score below -2.5 characterizes osteoporosis (34). For pediatric individuals aged 0-15 years, TB-BMD measurement excluded the head (35). TB-BMD was typically measured using DXA (Hologic Inc, Waltham, MA) in g/cm$^2$. To minimize potential bias and confounding factors, the study focused exclusively on participants of European ancestry.

**Selection of genetic instruments**

To minimize the potential impact of linkage disequilibrium (LD) on the analysis, we selected independent SNPs ($p < 5 \times 10^{-8}$), LD $r^2 < 0.001$, and clustering windows < 10,000 kb and excluded dependent SNPs using the PLINK clustering method. Initially, 106 SNPs associated with adding salt to foods ($p < 5 \times 10^{-8}$) were extracted as IVs for the preliminary study. Subsequently, we collected the outcome data linked to the preserved SNPs. In order to maintain consistency of effect alleles between the exposure and outcome datasets, we removed palindromic and ambiguous SNPs that had inconsistent alleles (Supplementary Tables II-X - https://www.nutricionhospitalaria.org/files/8731/ADMA1-05492-03.pdf). The strength of each SNP was assessed using the F statistic,

calculated as $F = R^2 (N - 2) / (1 - R^2)$, where $R^2$ represents the variance in exposure explained by genetic instruments, and N is the total sample size (36,37). We then matched outcome data with exposure SNPs, identifying eligible SNPs for further MR analysis. All of the F statistics for these SNPs surpassed the threshold of 10, indicating the robustness of the instrumental variables (Supplementary Tables II-X - https://www.nutricionhospitalaria.org/files/8731/ADMA1-05492-03.pdf).

**Statistical analysis**

The analyses were performed using the two-sample MR (38) and MR-PRESSO packages (39) in R software (version 4.3.3). The study reported odds ratios (OR) with 95 % confidence intervals (CIs) to assess the relationship between adding salt to foods and BMD. In our MR analysis, to enhance result robustness, we employed the inverse variance weighted (IVW) method as the primary analysis, supplemented with various sensitivity analyses (MR-Egger, weighted median, simple mode, weighted mode, MR-PRESSO and MR-PRESSO outlier-corrected) (40-42). The IVW method provided fixed and random effects estimates, integrating individual Wald estimates for a comprehensive assessment of exposure-outcome effects (43). IVW and MR-Egger methods were used to evaluate heterogeneity among instrumental variables using Cochran's Q and Rucker's Q statistics. When the Q test did not detect significant heterogeneity ($p > 0.05$), a fixed-effects model was implemented; otherwise, a random-effects model was used (44). In MR-Egger regression, the intercept represents the average pleiotropic effect of the instrumental variables, with a significant deviation from zero indicating pleiotropy (45). Asymmetric funnel plots may also indicate pleiotropy (38). MR-Egger regression and MR-PRESSO methods were employed to identify and address pleiotropic effects (46). A significance level of $p < 0.05$ in MR-Egger regression indicates pleiotropy (47), while MR-PRESSO identifies and corrects horizontal pleiotropic outliers. Subsequent

evaluations determined whether significant differences in causal effects remained after removing outliers (48). To further ensure the reliability of the analysis, a "leave-one-out" sensitivity analysis was performed to explore the potential impact of individual SNPs on bias introduction and influence on the overall causal effect (49).

## RESULTS

The MR results of adding salt to foods on nine bone density indicators are shown in figure 2 and supplementary table XI (https://www.nutricionhospitalaria.org/files/8731/ADMA1-05492-03.pdf). For forearm BMD and TB-BMD, the Q test showed $p < 0.05$, indicating heterogeneity; therefore, we used the IVW random effects model. For the remaining bone density indicators, we applied the IVW fixed effects model.

For three skeletal sites, the IVW method indicated that higher frequency of adding salt to foods may be associated with low BMD in femoral neck bone density (OR = 0.861, 95 % CI = 0.764-0.969, $p$ = 0.013). Sensitivity analyses showed inconsistent directions and no statistically significant trends (MR Egger: OR = 1.206, 95 % CI = 0.764-0.969, $p$ = 0.294; weighted median: OR = 0.923, 95 % CI = 0.767-1.11, $p$ = 0.396; simple mode: OR = 0.619, 95 % CI = 0.382-1.003, $p$ = 0.054; weighted mode: OR = 1.067, 95 % CI = 0.78-1.458, $p$ = 0.686). Rucker's Q test and Cochran's Q test did not show evidence of heterogeneity ($p$ = 0.535, 0.444). MR-PRESSO detected two outliers, rs9375448 and rs976179 ($p$ = 0.008 for the global test of pleiotropy), and provided the original estimates (Supplementary Table XII - https://www.nutricionhospitalaria.org/files/8731/ADMA1-05492-03.pdf). After excluding the outliers, a second round of MR-PRESSO analysis showed no heterogeneity in the global test. Leave-one-out analysis indicated that rs9611875 might impact our IVW results, with a dispersed distribution of black dots in the funnel plot, and MR-Egger intercept analysis suggested directional pleiotropy ($p$ = 0.045) (Supplementary Table XIII -

https://www.nutricionhospitalaria.org/files/8731/ADMA1-05492-03.pdf). Therefore, the causal effect estimate of adding salt to foods on femoral neck bone density may be biased.

In the age-stratified analysis of TB-BMD, the IVW method showed that each standard deviation increase in adding salt to foods may be associated with low BMD in individuals over 60 years old (OR = 0.84, 95 % CI = 0.721-0.979, $p$ = 0.026). The effect estimates from various sensitivity analysis methods were consistent in direction, all suggesting that higher frequency of adding salt to foods is associated with reduced TB-BMD in individuals over 60 years old. Both the weighted median method (OR = 0.766, 95 % CI = 0.602-0.974, $p$ = 0.029) and MR-PRESSO analysis (OR = 0.836, 95 % CI = 0.708-0.988, $p$ = 0.04) reached statistical significance. Rucker's Q test and Cochran's Q test did not find evidence of heterogeneity ($p$ = 0.173, 0.19), and the funnel plot and MR-Egger intercept analysis did not show evidence of directional pleiotropy ($p$ = 0.818). Leave-one-out analysis also did not reveal that a single SNP dominated the results. These results suggest that excessive salt intake in daily life may accelerate bone loss and increase the risk of osteoporosis in individuals over 60 years old. The scatter plots, funnel plots, forest plots, and leave-one-out plots of adding salt to foods and TB-BMD in individuals over 60 years old are shown in supplementary figures 1-9 (https://www.nutricionhospitalaria.org/files/8731/ADMA1-05492-03.pdf). It can be seen that for individuals over 60 years old, excessive salt intake in daily life may accelerate bone loss, thereby increasing the risk of osteoporosis.

## DISCUSSION

The association between adding salt to foods and bone health has recently gained widespread attention (50,51). S.-J. Kwon's cross-sectional study (50), using data from the 2008-2011 Korea National Health and Nutrition Examination Survey, found that higher salt intake in postmenopausal women negatively correlated with bone

mineral content (BMC) and BMD. Takase's study (7), which assessed salt intake using spot urine samples and measured calcaneal bone density through quantitative ultrasound, revealed that excessive salt intake was significantly associated with lower bone density among 884 women aged 60.1 ± 10.1 years, regardless of age and lifestyle-related diseases. Lin (52) highlighted the negative association between high salt intake and bone health in older people, suggesting that a low-sodium diet (such as the DASH diet) could positively impact BMD.

Munesada's research (53) found that although high salt intake significantly increased the total salt content in rat bones, it did not alter the sodium content in specific areas such as the scapula, pelvis, vertebrae, and limbs, indicating heterogeneity in sodium intake effects on different bone types or densities. Weili Feng and colleagues (54) reviewed the role of diet in osteoporosis development. They found that while dietary factors are associated with osteoporosis incidence in humans, observational studies did not show a significant impact of salt intake on bone density in adults already diagnosed with osteoporosis.

Our MR study results support the findings of most previous epidemiological studies. Similarly, our Mendelian randomization analysis confirmed that higher frequency of adding salt to foods increases the risk of reduced TB-BMD in individuals over 60 years old, but no significant causal relationship was found across different skeletal sites. Although no significant associations were found in other age groups, similar trends were observed. The F-statistics of the selected SNPs were all above 10, indicating indicating that the selected SNPs are robust instruments of adding salt to foods. Multiple sensitivity analyses further confirmed the robustness of these findings.

Previous studies have found that a high salt diet exacerbates bone loss by promoting the development of osteoclastogenic Th17 cells and inhibiting anti-osteoclastogenic Treg cells in mice, thereby

disturbing the Treg-Th17 balance and affecting the host immune system (55). Additionally, a high salt diet increases urinary calcium excretion, resulting in net calcium loss (56). Since effective calcium absorption depends on vitamin D, a high salt diet may indirectly reduce calcium absorption efficiency by lowering serum levels of active vitamin D (56). Moreover, excessive salt intake may affect vitamin D metabolism, further reducing calcium absorption and ultimately impacting BMD (14). As people age, the body's gastrointestinal absorption capacity gradually declines, and a high salt diet further reduces their ability to absorb calcium and other essential nutrients, negatively affecting BMD.

Additionally, with age, the levels of estrogen and testosterone in the elderly decline, both of which have protective effects on BMD (57,58). The reduction of estrogen leads to increased bone resorption and decreased BMD, especially in women, where the sharp decline in estrogen levels after menopause is the main cause of osteoporosis (50). A high-salt diet exacerbates this natural bone loss associated with ageing. Overall, controlling adding salt to foods is crucial for preventing osteoporosis and maintaining bone health, involving multiple complex physiological processes.

Our study has several strengths. First, we provide additional evidence supporting the causal relationship between excessive adding salt to foods and low BMD. Second, we discuss the impact of frequency of adding salt to foods on nine different BMD indicators, with varying results potentially providing more detailed reference information for future research. Furthermore, by the random distribution of genetic variation within the population, we minimized the chances of reverse causation and residual bias. Importantly, all the data analyzed in our study were from individuals of European ancestry, which helps to mitigate potential bias due to racial heterogeneity.

Inevitably, several limitations were noted. Firstly, participants in the UK Biobank self-reported the frequency of adding salt to foods, which could potentially introduce recall bias and impact the accuracy of the

results. Furthermore, employing the frequency of salt addition to meals as the exposure variable does not enable a quantitative evaluation of the correlation between salt consumption and low BMD risk. Thirdly, BMD is closely related to age and gender. Although age stratification was performed in this study, gender differences in BMD were not considered.

## CONCLUSION

In summary, the current MR study suggests that higher frequency of adding salt to foods significantly increases the risk of low BMD in individuals over 60 years old. This underscores the importance of moderate salt consumption within this demographic as a preventive measure against osteoporosis.

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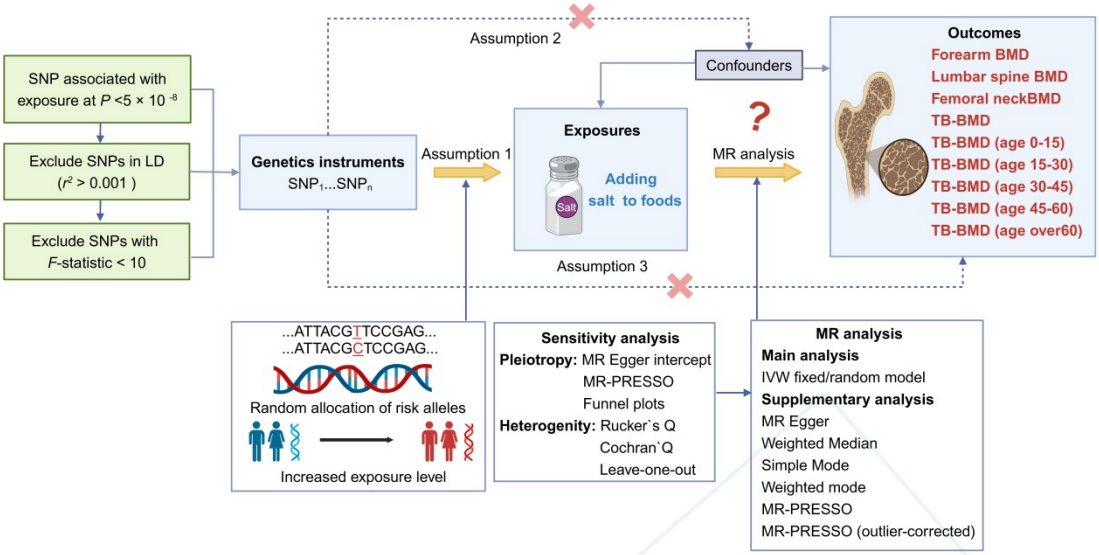


Figure 1. Overview of research design and MR design assumptions: Assumption 1 states that the genetic variation suggested as an instrumental variable must have a strong correlation with the risk factor being studied. Assumption 2 posits that this genetic variation should not have any correlation with any confounding influences. Assumption 3 posits that the selected genetic variation impacts the likelihood of the result solely through the risk factor, without exerting any influence on other routes (MR: Mendelian randomization; LD: linkage disequilibrium; BMD: bone mineral density).

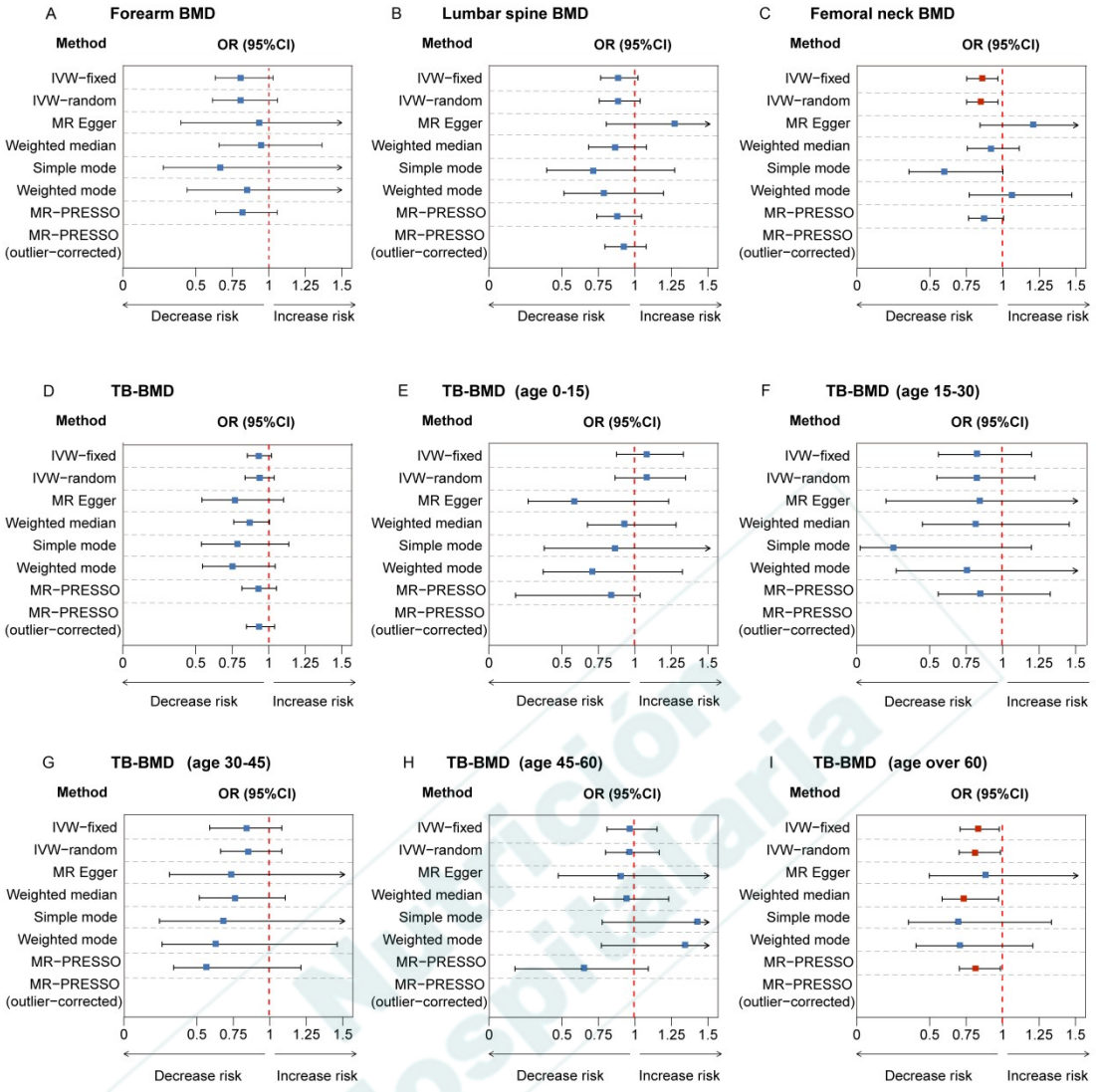


Figure 2. The causal association between adding salt to foods and various BMD measures was estimated using different methods, including inverse-variance-weighted (IVW - fixed effects and random effects), MR-Egger, Weighted Median, Simple Mode, Weighted Mode, MR-PRESSO, and MR-PRESSO (outlier-corrected). The X-axis displays odds ratios, with data displayed as odds ratio (OR) and 95 % confidence intervals (CI). Red squares indicate *p*-values < 0.05; otherwise, blue squares.