International Journal of General Medicine

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Association of a Sulfur-Containing Diet and a CTH Polymorphism with Bone Density in the Uyghur Population of China: A Preliminary Study

Lingna Fang[1], Zhiqin Zhang[2], Dawei He[3], Yanming Hao[4], Yan Gao[4], Rongzhu Lu[5], Chong Li[4,6]

[1]Department of Geriatrics, Kunshan Hospital Affiliated of Jiangsu University, Kunshan, 215300, People's Republic of China; [2]Biobank, Kunshan Hospital Affiliated of Jiangsu University, Kunshan, 215300, People's Republic of China; [3]Central Laboratory, Kunshan Hospital Affiliated of Jiangsu University, Kunshan, 215300, People's Republic of China; [4]Department of Orthopaedics, Kunshan Hospital Affiliated of Jiangsu University, Kunshan, 215300, People's Republic of China; [5]Department of Preventive Medicine and Health Inspection, Jiangsu University, Zhenjiang, 310030, People's Republic of China; [6]Department of orthopaedics, Atushi People's Hospital, Atushi, 845350, People's Republic of China

Correspondence: Chong Li, department of orthopaedics, Kunshan Hospital Affiliated of Jiangsu University Kunshan, Kunshan, 215300, People's Republic of China, Tel +8618906263110, Fax +86512 57501112, Email f30907988@163.com

Background: The aim of this study was to examine the impact of a sulfur-containing diet and a CTH polymorphism (G1208T; rs1021737) on bone density.

Methods: A total of 200 Uyghur residents aged over 50 from Xinjiang, China, were recruited for this study. Fasting blood samples were collected from the participants to measure serum hydrogen sulfide (H_2S) levels and perform CTH polymorphism sequencing. Dietary sulfur intake was assessed using a food frequency questionnaire and categorized into animal protein-derived and non-animal protein-derived sources. Skeletal health of the calcaneus was evaluated using quantitative ultrasound scanning.

Results: The study included a total of 200 participants, comprising 81 males and 119 females. Participants were stratified based on osteopenia status, with a T-score ≥ -1.0 indicating normal bone density and a T-score < -1.0 indicating osteopenia. Individuals in the osteopenia group exhibited significantly lower bone density markers, including broadband ultrasound attenuation (BUA) and speed of sound (SOS), as well as lower total weekly dietary sulfur intake and weekly dietary sulfur intake from animal protein. Additionally, they had significantly higher serum H_2S levels compared to participants with normal bone density. However, no differences in CTH genotype were observed between the normal bone density group and the osteopenia group. Participants were further categorized into tertiles (Q1 to Q3) based on weekly dietary sulfur intake from animal protein. Compared to the Q1 group, the Q3 group showed significantly higher T-scores and BUA values. Binary logistic regression analysis revealed that, compared to the group with high weekly dietary sulfur intake from animal protein, the low and middle intake groups had 3.252 times and 4.330 times higher risks of osteopenia, respectively.

Conclusion: Dietary sulfur intake from animal protein may exert a protective effect on bone density.

Keywords: sulfur, hydrogen sulfide, CTH, gene polymorphism, bone mineral density

Introduction

Osteoporosis is a metabolic bone disease characterized by a reduction in bone mass and the deterioration of bone microarchitecture, resulting in increased bone fragility and a heightened risk of fractures. It is closely associated with aging, and the occurrence of complex fractures significantly contributes to increased mortality rates among the elderly.[1] Hydrogen sulfide (H_2S), recognized as the third endogenous gaseous signaling molecule after carbon monoxide and nitric oxide, exerts a wide range of biological effects. In recent years, H_2S has been identified as a key regulator in bone metabolism and osteoporosis. Specifically, H_2S promotes osteogenic differentiation and stimulates mineralization;[2] inhibits osteoclast maturation and bone resorption;[3] and enhances osteogenic differentiation while suppressing cellular aging in mesenchymal stem cells (MSCs).[4] H_2S donors have been shown to increase bone density in an ovariectomized

Received: 14 February 2025
Accepted: 23 May 2025
Published: 6 June 2025

animal model,[2] with similar results observed in an animal model of osteoporosis induced by hyperhomocysteinemia.[5] These studies highlight the potential role of H_2S in the prevention and treatment of osteoporosis.

H_2S in the human body is a byproduct of essential amino acid metabolism, primarily generated by the enzymatic activity of cystathionine beta-synthase (CBS), cystathionine gamma-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST). The proper functioning of these enzymes is crucial for maintaining adequate endogenous H_2S production. The CTH gene encodes CSE, and the single nucleotide polymorphism (SNP) c.1364G>T in exon 12 of CTH has been widely investigated in clinical research. This SNP has been linked to plasma total homocysteine levels in the Caucasian population, with individuals carrying the homozygous CTH 1364T/T genotype exhibiting significantly higher serum total homocysteine concentrations compared to those with other genotypes.[6] The SNP c.1364G>T is cataloged in the NCBI SNP database under the reference number rs1021737. In the updated SNP database, rs1021737 is designated as CTH G1208T. Recent clinical studies have explored the relationship between CTH gene polymorphisms and cardiovascular diseases. Notably, a case–control study reported that the CTH G1208T polymorphism is associated with an increased risk of fatal myocardial infarction in women.[7] Given the role of this gene in H_2S metabolism, the CTH G1208T SNP may also play a significant role in the development of osteoporosis.

In humans, dietary sulfur is obtained from both organic and inorganic sources. Organic sulfur primarily originates from sulfur-containing amino acids, such as methionine, cysteine, and cystine, as well as other organic sulfur compounds found in foods like garlic and cruciferous vegetables. Through the enzymatic actions of CBS and CSE, L-cysteine contributes to the production of approximately 70% of endogenous H_2S.[8] According to reports, compounds such as diallyl trisulfide (DATS) and isothiocyanates could release H_2S. These molecules are naturally present in edible garlic and cruciferous vegetables.[9,10]

To date, the influence of CTH gene polymorphisms and sulfur-containing diets on bone density, mediated through the regulation of endogenous H_2S levels, remains unclear. This study aims to explore the effects of CTH gene polymorphisms and sulfur-containing diets on serum H_2S levels and bone density.

Materials and Methods

Participants

Between November 2016 and November 2019, a total of 200 Uyghur individuals aged over 50 years undergoing medical examinations were recruited from Atushi People's Hospital in the Kezhou District of Xinjiang. The exclusion criteria included the presence of hypertension, diabetes, hyperlipidemia, coronary heart disease, stroke, acute or chronic kidney disease, rheumatoid arthritis, and long-term use of glucocorticoids. This study was conducted in accordance with the principles of the Helsinki Declaration and was approved by the Ethics Committee of Atushi People's Hospital (Ethics Approval No. 201609001). Written informed consent was obtained from all participants prior to enrollment.

Parameters

The general information collected included the participants' age, sex, height, and weight.

Determination of Serum H_2S Level

Fasting blood samples were collected from participants, and the serum was stored at −80°C. The serum H_2S levels were subsequently measured using the H_2S ELISA Kit (TSZ, USA).

Investigation of Dietary Sulfur Intake

A previously developed food frequency questionnaire (FFQ), tailored to the dietary habits of Uyghur residents, was utilized in this study. The validity and reproducibility of the FFQ were assessed among 50 Uyghur participants. The FFQ encompassed seven food categories: staple foods, vegetables, animal-based foods, beans and their products, fruits, snacks, and beverages, comprising a total of 50 items. Food intake frequency was recorded on either a daily or weekly basis. Weekly dietary sulfur intake was calculated based on total food consumption and the sulfur content of each food item. To evaluate the effects of sulfur from different dietary sources on bone mass, dietary sulfur was categorized into

animal protein-derived sulfur and non-animal protein-derived sulfur. The former primarily originated from lamb, eggs, and milk.

Determination of Dietary Sulfur Content

The most consumed local foods in Xinjiang, as identified by the FFQ, were sent to the Comprehensive Technical Center of Zhenjiang Customs in Jiangsu Province for analysis. Following wet digestion, the sulfur content in the food samples was determined using ion chromatography (Dionex ICS-3000, Dionex, USA).

Bone Mass Measurements

The skeletal health of the calcaneus was assessed using a quantitative ultrasound scan (QUS) with a bone densitometer (Medilink, Pegasus, France). Two key parameters, broadband ultrasound attenuation (BUA) and speed of sound (SOS), were measured by the QUS. BUA reflects bone mineral density and structural characteristics of the bone, while SOS evaluates bone mineral density and elasticity. All measurements were performed using the same instrument and operated by a single investigator to ensure consistency. The QUS score was utilized to classify participants into bone health categories based on the World Health Organization diagnostic criteria: a T-score ≥ -1.0 indicated normal bone density, whereas a T-score < -1.0 indicated osteopenia.

CTH Polymorphism Sequencing

Fasting blood samples were collected in EDTA anticoagulation tubes and stored at −80°C. Prior to analysis, the samples were thawed and gently inverted 8–10 times to ensure thorough mixing. Subsequently, 300 μL of the sample was diluted with Ga buffer. Proteinase K was added, and the sample tube, pipette tip, and collection tube were placed in the designated positions of a preheated MagCore automated nucleic acid extractor. Genomic DNA was then extracted and purified, and its concentration was measured using a Nanodrop 2000 spectrophotometer.

Primers were designed near the G1208T site of the CTH gene (CTH G1208T); forward primer: 5'-GCTTTTGGATGGGGG-3', reverse primer: 5'-TCAGGCGA AACATEGAGA-3'. For PCR amplification, the reaction mixture consisted of 2 μL of DNA sample, 0.5 μL of Taq polymerase, 1 μL of forward primer, 1 μL of reverse primer, 5 μL of buffer, and 50 μL of ultrapure water. The amplification conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes. The amplified products were subsequently sent for Sanger sequencing. PCR analysis of patient DNA extracts confirmed the presence of three expected genotypes in the study population: 1208GG, 1208GT, and 1208TT.

Statistical Analysis

Statistical analyses were performed using SPSS 23.0 software (IBM, USA), with a significance level set at $P < 0.05$. Continuous variables were presented as mean ± standard deviation for normally distributed data, and as median with interquartile ranges for skewed distributions. Categorical variables were expressed as percentages (%). Independent Student's t-tests and ANOVA were used to compare continuous variables with normal distributions, while the Mann–Whitney *U*-test was applied for non-normally distributed continuous variables. Categorical variables were compared using the Chi-square test. Participants were categorized into tertiles (Q1 to Q3) based on total weekly dietary sulfur intake, weekly dietary sulfur intake from animal protein, and weekly dietary sulfur intake from non-animal protein. A binary logistic regression model was employed to estimate the odds of osteopenia across tertiles of weekly dietary sulfur intake, adjusting for age, sex, height, and weight.

Results

In this study, 200 Uyghur participants from southern Xinjiang were enrolled, comprising 81 males and 119 females, with a mean age of 58.90 ± 8.22 years. Based on T-scores, participants were categorized into a normal bone density group and an osteopenia group. Normal bone density was defined as a T-score ≥ -1.0, while osteopenia was defined as a T-score < -1.0.

Our findings revealed that participants with osteopenia had significantly lower BUA, SOS, total weekly dietary sulfur intake, and weekly dietary sulfur intake from animal protein compared to those in the normal bone density group. Conversely, participants in the osteopenia group exhibited significantly higher age and serum H_2S levels than those in the normal bone density group. No significant differences were observed between the two groups in terms of height, weight, body mass index, or CTH genotype (Table 1).

The sulfur content of the primary foods consumed by the sampled Xinjiang population is presented in Table 2. These foods included mutton, eggs, broccoli, and garlic. Among them, mutton, dried pepper, and garlic were identified as having the highest sulfur content.

Participants were further classified into tertiles (Q1 to Q3) based on total weekly dietary sulfur intake, with Q1, Q2, and Q3 representing low-, middle-, and high-sulfur intake groups, respectively. Compared to the low-sulfur intake group, participants in the high-sulfur intake group had significantly higher T-scores and BUA, suggesting that higher sulfur intake was associated with greater bone mineral density. However, there were no significant differences in serum H_2S levels or CTH genotype among the groups (Table 3).

Participants were also stratified into tertiles (Q1 to Q3) based on weekly dietary sulfur intake from animal protein and non-animal protein. The high-sulfur intake from animal protein group exhibited significantly higher bone density markers, including T-score, BUA, and SOS, compared to the low-sulfur intake from animal protein group, and these

Table 1 Characteristics of Participants Stratified by T Score

		T score ≥ −1.0	T score < −1.0	P
N		121	79	
Female (%)		78 (64.5%)	41 (51.9%)	0.081
Age (y)		57.96±8.22	60.84±7.95*	0.031
Height (cm)		165.16±6.87	164.52±6.28	0.510
Weight (kg)		72.64±11.61	70.95±13.09	0.392
BMI (kg/m^2)		26.67±4.07	26.17±3.96	0.452
T value		−0.10(−0.57, 1.14)	−1.61(−2.13, −1.21)*	0.000
BUA (dB/MHz)		73.34(64.67, 87.59)	42.26(32.38, 48.19)*	0.000
SOS (m/s)		1372.62 (1342.81, 1407.93)	1339.68(1116.383, 1374.39)*	0.000
Serum H_2S (pg/mL)		14.78(9.83, 19.91)	31.05(14.08, 52.01)*	0.000
Total weekly dietary sulfur intake (mg)		3202.60(2918.55, 3559.59)	2941.92(2389.05, 3243.36)*	0.000
Weekly dietary sulfur intake from animal protein		2340.13(2047.43, 2662.73)	2188.20(1499.21, 2444.58)	0.002
Weekly dietary sulfur intake from non-animal protein		826.05(730.35, 1036.35)	826.05(730.35, 916.55)	0.294
Gene				
	GG	62	48	0.354
	GT	53	29	
	TT	6	2	

Notes: Data are expressed as the mean ± standard deviation or median and interquartile ranges (IQRs) or %.* Refers that compare with normal, P < 0.05.
Abbreviations: BMI, body mass index; BUA, broadband ultrasound attenuation; SOS, speed of sound; H_2S, hydrogen sulfide.

Table 2 Sulfur Content of Main Food in Xinjiang

Food	Sulfide (mg/kg)	Food	Sulfide(mg/kg)
Mutton	5.21×10^3	Celery	0.332×10^3
Egg	1.07×10^3	Leek	1.30×10^3
Milk	0.0383×10^3	Shallot	1.67×10^3
Carrot	0.163×10^3	Onion	1.02×10^3
Broccoli	1.01×10^3	Dry pepper	5.13×10^3
Chinese cabbage	0.801×10^3	Garlic	3.82×10^3
Pancake	0.957×10^3	Grape	0.0691×10^3

Table 3 Characteristics of Participants Stratified by Total Weekly Dietary Sulfur Intake

Weekly Dietary Sulfur Intake	Q1	Q2	Q3	P
N	67	67	66	
Female (%)	45 (67.2%)	42(62.7%)	32(48.5%)	0.073
Age (y)	59.27±8.40	58.00±8.67	59.49±7.55	0.576
Height (cm)	164.97±6.15	164.63±5.14	165.30±6.79	0.841
Weight (kg)	71.95±12.09	73.32±13.18	70.85±10.87	0.557
BMI (kg/m^2)	27.00±4.04	25.77±3.73	26.50±4.03	0.257
T value	−0.86(−1.72, 0.11)	−0.54(−1.20, 0.51)	−0.10(−0.77, 0.52)*	0.005
BUA (dB/MHz)	53.94(38.40, 73.23)	64.41(47.82, 82.73)	70.72(61,76, 83,96)*	0.001
SOS (m/s)	1349.20(1292.66, 1400.05)	1364.87(1331.89, 1399.92)	1374.99(1347.78,1405.53)*	0.050
Serum H_2S (pg/mL)	19.35(11.55, 43.70)	16.10(9.71, 26.60)	15.70(9.35, 24.33)	0.152
Gene				
	39	35	36	0.709
	27	29	26	
	1	3	4	

Notes: Data are expressed as the mean ± standard deviation or median and interquartile ranges (IQRs) or %.
Abbreviations: BMI, body mass index; BUA, broadband ultrasound attenuation; SOS, speed of sound; H_2S, hydrogen sulfide. Weekly sulfide intake was divided into tertilesQ1: total weekly dietary sulfur intake <= 2918.55 mg, Q2: 2918.55 mg < total weekly dietary sulfur intake < 3354.40 mg, Q3: total weekly dietary sulfur intake ≥ 3354.40 mg.* Refers that compare with Q1, P < 0.05.

differences were statistically significant (Table 4). In contrast, no significant differences in bone density markers, serum H_2S levels, or CTH genotype were observed among groups classified by weekly dietary sulfur intake from non-animal protein.

Additionally, participants were stratified by CTH genotype into three groups: GG, GT, and TT. No significant differences in any measured parameters were found among these genotype groups.

To further investigate the association between weekly dietary sulfur intake from animal protein and the risk of osteopenia, a binary logistic regression model was constructed. The results indicated that the risk of osteopenia was 3.252

Table 4 Characteristics of Participants Stratified by Weekly Dietary Sulfur Intake from Animal Protein

Weekly Dietary Sulfur INTAKE from Protein		Q1	Q2	Q3	P
N		67	72	61	
Female (%)		43 (64.2%)	47(65.3%)	29(47.5%)	0.073
Age (y)		60.55±7.73	56.14±8.43#^	60.04±7.89	0.006
Height (cm)		164.83±6.11	164.64±5.50	165.41±6.45	0.776
Weight (kg)		71.91±12.29	73.02±12.92	71.30±11.15	0.747
BMI (kg/m^2)		26.92±3.91	26.92±4.47	25.89±3.64	0.367
T value		−0.79(−1.61, −0.26)	−0.67(−1.54, 1.18)	−0.25(−0.77, 0.42)*	0.006
BUA (dB/MHz)		55.92(39.57, 71.61)	60.32(445.27, 86.72)	70.69(59.42, 84.71)*	0.003
SOS (m/s)		1349.46(1316.58, 1396.04)	1364.87(1326.86, 1402.06)	1373.73(1350.13,1408.72)*	0.039
Serum H_2S (pg/mL)		16.38(11.46, 41.19)	18.13(11.66, 28.96)	14.70(8.46, 23.98)	0.243
Gene					
	GG	37	41	32	0.673
	GT	29	28	25	
	TT	1	3	4	

Notes: Data are expressed as the mean ± standard deviation or median and interquartile ranges (IQRs) or %.
Abbreviations: BMI, body mass index; BUA, broadband ultrasound attenuation; SOS, speed of sound; H_2S, hydrogen sulfide. Weekly sulfide intake was divided into tertiles, Q1: weekly dietary sulfur intake from animal protein <= 2122.30 mg, Q2: 2122.31 mg < weekly dietary sulfur intake from animal protein < 2509.20 mg, Q3: weekly dietary sulfur intake from animal protein ≥ 2509.20 mg. * Refers that compare with Q1, P < 0.05.

Table 5 Binary Logistic Regression, Adjusted Relationships of Weekly Dietary Sulfur Intake from Animal Protein Tertiles with Osteopenia Among Participants

		OR	(95% CI)	P
Osteopenia	Weekly dietary sulfur intake for protein			
	Q1	3.252	(1.331–7.946)	0.010
	Q2	4.330	(1.698–11.043)	0.002
	Q3	Reference	Reference	Reference

Notes: Adjusted for age, sex, and weight. Weekly sulfide intake from animal protein was divided into tertiles, Q1: weekly dietary sulfur intake from animal protein <= 2122.30 mg, Q2: 2122.31 mg < weekly dietary sulfur intake from animal protein < 2509.20 mg, Q3: weekly dietary sulfur intake from animal protein ≥ 2509.20 mg. The normal bone mass is defined as T-score> −1.0, osteopenia is defined as T-score < −1.0.

times higher in the low-sulfur intake from animal protein group and 4.330 times higher in the middle-sulfur intake from animal protein group compared to the high-sulfur intake from animal protein group (Table 5).

Discussion

In this study, we found that both total weekly sulfur intake and weekly sulfur intake from animal protein were significantly higher in individuals with normal bone density compared to those with osteopenia among Uyghur residents. Conversely, serum H_2S levels were significantly lower in individuals with normal bone density than in those with osteopenia. When total weekly sulfur intake and weekly sulfur intake from animal protein were categorized into tertiles, the T-scores and BUA of the Q3 groups were significantly higher than those of the Q1 groups. Further logistic regression analysis revealed that the risk of osteopenia was 3.252 times higher in the low-sulfur intake from animal protein group and 4.330 times higher in the middle-sulfur intake from animal protein group compared to the high-sulfur intake from animal protein group. These findings suggest that sulfur intake from animal protein is a significant predictor of osteopenia.

We observed elevated serum H_2S levels in participants with osteopenia. This finding aligns with previous studies, which have demonstrated that serum H_2S levels are influenced by various pathological conditions. In low-grade inflammatory diseases, such as hypertension and type 2 diabetes, serum H_2S levels are typically reduced. In contrast, conditions such as multiple myeloma, acute myocardial infarction, and chronic obstructive pulmonary disease are associated with increased serum H_2S levels.[11] Elevated Serum H_2S Levels in Chinese Han Patients with Osteoporosis or Osteopenia,[12] consistent with our results. In patients with acute myocardial infarction and chronic obstructive pulmonary disease, the elevation in serum H_2S levels may serve as a compensatory mechanism to mitigate the extensive damage induced by the rapid activation of oxidative stress and pro-inflammatory pathways.[13,14] Oxidative stress and inflammation are known to precede the development of osteoporosis. On the other hand, H_2S, as a gaseous molecule, exhibits “Janus - faced”. At physiological concentrations, H_2S generally plays a protective role against oxidative stress and inflammation. However, elevated levels of H_2S especially under pathophysiological conditions can lead to increased oxidative stress.[15] The increased serum H_2S levels observed in patients with osteopenia may also reflect a disruption of systemic homeostasis and contribute to further bone loss.The relationship between elevated serum H_2S levels and reduced bone mass remains to be further elucidated.

Dietary sulfur is primarily derived from sulfur-containing amino acids and organic sulfides, with sulfur-containing amino acids predominantly found in animal-based proteins. Our findings indicate that participants with higher sulfide intake from animal protein exhibited greater bone density, whereas those with lower sulfide intake from animal protein faced an increased risk of osteopenia. Both dietary intake of sulfur-containing amino acids and their levels in blood serum are closely associated with skeletal health. A clinical study investigating the relationship between dietary protein intake and the risk of hip fracture in the Han Chinese population revealed that women with higher sulfur-containing amino acid intake had a significantly reduced risk of hip fracture. Furthermore, the protective effect of sulfur-containing amino acids against fractures was more pronounced in individuals with a lower body mass index and a dietary calcium

intake below 400 mg/day.[16] A three-year prospective study examined the association between dietary protein intake and changes in bone mass. The study identified a positive correlation between methionine and cysteine levels and femoral neck bone density, with regression coefficients of 1.59 ± 0.79 and 3.82 ± 1.30, respectively.[17] In Singaporean Chinese menopausal women, a positive correlation was observed between femoral neck bone density and serum methionine levels.[18] Similarly, a clinical study conducted in Japan found that women with low estrogen levels and low bone mass had significantly lower serum cysteine levels compared to those in the normal bone mass control group.[19] Two metabolomics studies have also reported a positive correlation between serum homocysteine concentrations and bone mass in Caucasian women and postmenopausal women.[20,21] A study investigating the effects of a methionine-restricted (MR) diet found that 14 weeks of MR in mice led to a reduction in femoral length and diameter, as well as a decrease in bone density, compared to the normal diet group. These findings suggest that methionine plays a positive role in bone growth.[22] This is further supported by in vitro evidence: supplementation with lysine, serine, methionine, or tryptophan enhanced cell growth and alkaline phosphatase activity in primary osteoblasts derived from newborn Sprague-Dawley rats. Additionally, a reduced level of collagen synthesis was observed, contributing to bone formation.[23] Increased methionine intake has been shown to enhance cartilage formation in ovariectomized rats and to inhibit the development of osteoclasts in monocytes.[24] However, excessive methionine intake can result in hyperhomocysteinemia, which negatively impacts bone microstructure and disrupts collagen regulation in the bone matrix.[25] A recent study demonstrated that RUNX2, a key transcription factor for the osteoblast lineage, decreased under both methionine depletion and excessive supplementation. This suggests the existence of an optimal range of methionine concentration for osteoblast differentiation.[26] Therefore, it is essential to determine the optimal level of protein intake to promote bone health.

Currently, there is limited research on the impact of diet on endogenous H_2S levels. However, in biological models such as yeast, fruit flies, and Caenorhabditis elegans, the effects of dietary restriction (DR) and MR on stress response and lifespan have attracted considerable attention. Both DR and MR have been shown to confer beneficial effects, partly through the regulation of endogenous H_2S levels. DR promotes H_2S biosynthesis by upregulating the reverse transsulfuration pathway,[27] while MR increases hepatic H_2S production.[28] Additionally, a high-salt diet reduces renal CBS expression and endogenous H_2S production in rats,[29] while a high-fat diet decreases the ability of mice to synthesize H_2S.[30] Organic sulfides derived from the Allium and Brassica genera have the potential to act as natural donors of H_2S. Notably, intracellular H_2S can be detected in cultured cells treated with oils extracted from these plants.[31] In addition, DATS modulates H_2S levels in animal blood and cardiac tissues,[32] while organic sulfides also influence the activity of endogenous H_2S metabolic enzymes.[33] Recently, a self-spray coating system based on DATS has been reported to possess foaming capabilities. This system produces nano-sized micelle particles within the colorectal lumen, which are subsequently internalized by colonic epithelial cells, leading to the production of H_2S.[34] Our study aimed to investigate whether dietary sulfur influences the skeleton by modulating endogenous H_2S levels. Dietary sulfur intake from animal and non-animal protein sources was divided into tertiles, but no significant differences in serum H_2S levels were observed among the groups. Previous research indicates that the relationship between diet and endogenous H_2S production is highly complex. For example, certain dietary patterns, such as DR and MR, have been shown to enhance endogenous H_2S levels, whereas other dietary factors, such as high salt and high fat intake, suppress H_2S production. Organic sulfides from Allium and Brassica genera have been reported to increase endogenous H_2S levels, although this effect has so far been demonstrated only in animal studies. Additional clinical research is needed to clarify the effects of specific dietary patterns or components on endogenous H_2S production. Given that dietary sulfur primarily originates from sulfur-containing amino acids, the protective effects of dietary sulfur on the skeleton observed in this study are likely attributable to protein intake rather than endogenous H_2S production.

We further aimed to investigate the impact of a SNP in a gene involved in H_2S metabolism on bone health. H_2S-metabolizing enzymes are expressed in bone tissue and play a role in bone metabolism. CBS, CSE, and 3-MST are all expressed in MSCs, with CBS and CSE contributing to H_2S production and promoting osteogenic differentiation in MSCs.[35] CSE is the primary regulator of H_2S production in MSCs. Knockout or inhibition of the CSE gene results in reduced alkaline phosphatase activity and impaired mineralized nodule formation in bone marrow-derived MSCs.[36] CBS and CSE are expressed in rat osteoblasts and contribute to the production of H_2S, with approximately 70% of H_2S derived from CSE activity. In mouse osteoblasts, CSE is the primary enzyme responsible for H_2S production, whereas

CBS plays a less significant role. Elevated CSE-mediated H_2S production in osteoblasts has been shown to enhance alkaline phosphatase activity, promote calcium nodule formation, and increase matrix protein expression, thereby facilitating bone formation.[37] CSE-derived H_2S promotes bone remodeling by increasing both the number and activity of osteoclasts. While knockout of the CSE gene results in a reduction in osteoclast number and activity, this impairment can be mitigated through supplementation with H_2S donors.[38] These findings suggest that CSE plays a critical regulatory role in maintaining H_2S levels within bone tissue. Genetic defects or abnormal expression of CSE significantly impact bone formation and remodeling processes, potentially leading to skeletal damage. However, our study found no differences in the rs1021737 genotypes between patients with normal bone density and those with osteopenia. Similarly, no significant differences in bone mass or serum H_2S levels were observed among the different rs1021737 genotype groups. While the CTH rs1021737 polymorphism has shown potential as a target for mechanistic research and therapeutic intervention in cardiovascular diseases,[39] previous studies have not identified an association between CTH rs1021737 and other conditions, such as preeclampsia or primary hypertension.[40,41] The expression of CSE plays a critical role in regulating bone metabolism by influencing H_2S production; however, it is not the sole enzyme involved in H_2S synthesis. Consequently, compensatory effects from other H_2S-producing enzymes may occur in the human body in response to CSE loss-of-function mutations.

Our study has several limitations. First, we utilized ion chromatography to measure the sulfur content in the diet; however, this method does not allow for differentiation between sulfur derived from various dietary components. Second, this is a preliminary study with a relatively small sample size. Third, the cross-sectional design of our study precludes the establishment of a causal relationship between dietary sulfur intake and bone mass. Lastly, participants were selected from a health examination cohort, and bone mass was assessed using QUS, which may have lower precision compared to dual-energy X-ray absorptiometry.

Conclusion

In this clinical study investigating the association between CTH gene polymorphisms and dietary sulfur intake with bone density, we identified significant associations between dietary sulfur derived from animal protein and bone density maintenance. However, no such associations were observed for CTH SNPs. These findings suggest that a diet rich in sulfur from animal protein may play a preventive role in osteoporosis.

Data Sharing Statement

The data and materials used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study followed the principles of the Helsinki Declaration and was approved by the Ethics Committee of Atushi People's Hospital (ethics approval no. 201609001). All enrolled patients provided written informed consent.

Artificial Intelligence (AI) Policy

Not using AI tools.

Consent for Publication

All authors in this study agreed to publication.

Acknowledgments

The authors thank all of the doctors and participants who were involved in the study. The authors also thank Philippa Gunn, D.Phil., from Liwen Bianji (Edanz) (www.liwenbianji.cn) for editing the English text of a draft of this manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Medical Education Collaborative Innovation Fund of Jiangsu University (JDY2022013); Clinical Medical Science and Technology Development Fund Project of Jiangsu University (JLY2021060); Clinical Medical Science and Technology Development Fund Project of Jiangsu University(JLY 2021069); Suzhou Science and Technology Development Plan Project (SKYD2022055).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Clynes MA, Harvey NC, Curtis EM, Fuggle NR, Dennison EM, Cooper C. The epidemiology of osteoporosis. *Br Med Bull*. 2020;133(1):105–117. doi:10.1093/bmb/ldaa005
2. Grassi F, Tyagi AM, Calvert JW, et al. Hydrogen sulfide is a novel regulator of bone formation implicated in the bone loss induced by estrogen deficiency. *J Bone Miner Res*. 2016;31(5):949–963. doi:10.1002/jbmr.2757
3. Lee SK, Chung JH, Choi SC, et al. Sodium hydrogen sulfide inhibits nicotine and lipopolysaccharide-induced osteoclastic differentiation and reversed osteoblastic differentiation in human periodontal ligament cells. *J Cell Biochem*. 2013;114(5):1183–1193. doi:10.1002/jcb.24461
4. Yang S, Su YY, Li XY, et al. Hydrogen sulfide attenuates mesenchymal stem cell aging progress via the calcineurin-NFAT signaling pathway. *Stem Cells*. 2023;41(10):916–927. doi:10.1093/stmcls/sxad056
5. Behera J, George AK, Voor MJ, Tyagi SC, Tyagi N. Hydrogen sulfide epigenetically mitigates bone loss through OPG/RANKL regulation during hyperhomocysteinemia in mice. *Bone*. 2018;114:90–108. doi:10.1016/j.bone.2018.06.009
6. Wang J, Huff AM, Spence JD, Hegele RA. Single nucleotide polymorphism in CTH associated with variation in plasma homocysteine concentration. *Clin Genet*. 2004;65:483–486. doi:10.1111/j.1399-0004.2004.00250.x
7. Elisabet S, Jonas A, Stefan S, Bethany VG, Torbjörn KN, Johan H. CTH G1208T and MTHFR A1298C polymorphisms are associated with a higher risk of a first myocardial infarction with fatal outcome among women. *Drug Metab Pers Ther*. 2022;38(1):57–63. doi:10.1515/dmpt-2022-0119
8. McBean GJ. The transsulfuration pathway: a source of cysteine for glutathione in astrocytes. *Amino Acids*. 2012;42:199–205. doi:10.1007/s00726-011-0864-8
9. Jin S, Pu SX, Hou CL, et al. Cardiac H2S generation is reduced in ageing diabetic mice. *Oxid Med Cell Longev*. 2015;2015:758358. doi:10.1155/2015/758358
10. Tocmo R, Liang D, Lin Y, Huang D. Chemical and biochemical mechanisms underlying the cardioprotective roles of dietary organopolysulfides. *Front Nutr*. 2015;2:1. doi:10.3389/fnut.2015.00001
11. Eugenia P, Marco AM, Ersilia L, Alma M, Vincenzo C. Circulating levels of hydrogen sulfide (H2S) in patients with age-related diseases: a systematic review and meta-analysis. *Biomolecules*. 2023;13(7):1023.
12. Hao YM, He DW, Gao Y, et al. Association of hydrogen sulfide with femoral bone mineral density in osteoporosis patients: a preliminary study. *MedSci Monit*. 2021;27:e929389.
13. Gaddam RR, Chambers S, Murdoch D, Shaw G, Bhatia M. Circulating levels of hydrogen sulfide and substance P in patients with sepsis. *J Infect*. 2017;75:293–300. doi:10.1016/j.jinf.2017.07.005
14. Gambari L, Grigolo B, Grassi F. Hydrogen sulfide in bone tissue regeneration and repair: state of the art and new perspectives. *Int J Mol Sci*. 2019;20:5231. doi:10.3390/ijms20205231
15. Munteanu C, Turnea MA, Rotariu M. Hydrogen sulfide: an emerging regulator of oxidative stress and cellular homeostasis - a comprehensive one - year review. *Antioxidants*. 2023;12:1737. doi:10.3390/antiox12091737
16. Liu ZM, Huang Q, Li SY, et al. A 1:1 matched case-control study on dietary protein intakes and Hip fracture risk in Chinese elderly men and women. *Osteoporos Int*. 2021;32(11):2205–2216. doi:10.1007/s00198-021-05960-0
17. Liu ZM, Huang Q, Long HH, et al. Increased dietary intakes of total protein, animal protein and white meat protein were associated with reduced bone loss-a prospective analysis based on Guangzhou health and nutrition cohort, South China. *Nutrients*. 2023;15(6):1432. doi:10.3390/nu15061432
18. Diana C, Marlena K, Frances MW, et al. Association of plasma lipids and polar metabolites with low bone mineral density in Singaporean-Chinese menopausal women: a pilot study. *Int J Environ Res Public Health*. 2018;15(5):1045. doi:10.3390/ijerph15051045
19. Miyamoto T, Hirayama A, Sato Y, et al. Metabolomics-based profiles predictive of low bone mass in menopausal women. *Bone Rep*. 2018;9:11–18. doi:10.1016/j.bonr.2018.06.004
20. Zhao Q, Shen H, Su KJ, et al. Metabolomic profiles associated with bone mineral density in US Caucasian women. *Nutr Metab*. 2018;15:57. doi:10.1186/s12986-018-0296-5
21. Miyamoto T, Hirayama A, Sato Y, et al. A serum metabolomics-based profile in low bone mineral density postmenopausal women. *Bone*. 2017;95:1–4. doi:10.1016/j.bone.2016.10.027

22. Li MX, Zhai LD, Wei WF, Dong JM. Effect of methionine restriction on bone density and nk cell activity. *Biomed Res Int*. 2016;2016:3571810. doi:10.1155/2016/3571810
23. Conconi MT, Tommasini M, Muratori E, Parnigotto PP. Essential amino acids increase the growth and alkaline phosphatase activity in osteoblasts cultured in vitro. *Farmaco*. 2001;56:755–761. doi:10.1016/S0014-827X(01)01126-0
24. Vijayan V, Khandelwal M, Manglani K, Gupta S, Surolia A. Methionine down-regulates TLR4/MyD88/NF-κB signalling in osteoclast precursors to reduce bone loss during osteoporosis. Br. *J Pharmacol*. 2014;171:107–121.
25. Tania A, Naveen GK, Natalie LD, et al. Methionine as a regulator of bone remodeling with fasting. *JCI Insight*. 2024;9:e177997. doi:10.1172/jci.insight.177997
26. Petar M, Dragan H, Ksenija R, et al. Moderate hyperhomocysteinemia induced by short-term dietary methionine overload alters bone micro-architecture and collagen features during growth. *Life Sci*. 2017;191:9–16. doi:10.1016/j.lfs.2017.10.008
27. Fereshte A, Hassan MK, Mohammad HA, Reza F, Javad ZR. The effect of consumption of garlic tablet on proteins oxidation biomarkers in postmenopausal osteoporotic women: a randomized clinical trial. *Electron Physician*. 2017;9(11):5670–5675. doi:10.19082/5670
28. Hine C, Harputlugil E, Zhang Y, et al. Endogenous hydrogen sulfide production is essential for dietary restriction benefits. *Cell*. 2015;160:132–144. doi:10.1016/j.cell.2014.11.048
29. Yang Y, Wang Y, Sun J, et al. Dietary methionine restriction reduces hepatic steatosis and oxidative stress in high-fat-fed mice by promoting H 2 S production. *Food Funct*. 2019;10(1):61–67. doi:10.1039/C8FO01629A
30. Huang P, Chen S, Wang Y, et al. Down-regulated CBS/H2S pathway is involved in high-salt-induced hypertension in Dahl rats. *Nitric Oxide*. 2015;46:192–203. doi:10.1016/j.niox.2015.01.004
31. Peh MT, Anwar AB, Ng DS, Atan MS, Kumar SD, Moore PK. Effect of feeding a high fat diet on hydrogen sulfide (H2S) metabolism in the mouse. *Nitric Oxide*. 2014;41:138–145. doi:10.1016/j.niox.2014.03.002
32. Liang D, Wang C, Tocmo R, Wu H, WenDeng L, Huang D. Hydrogen sulphide (H2S) releasing capacity of essential oils isolated from organosulphur rich fruits and vegetables. *J Functional Foods*. 2015;14:634–640. doi:10.1016/j.jff.2015.02.007
33. Predmore BL, Kondo K, Bhushan S, et al. The polysulfide diallyl trisulfide protects the ischemic myocardium by preservation of endogenous hydrogen sulfide and increasing nitric oxide bioavailability. *Am J Physiol Heart Circ Physiol*. 2012;302:H2410–2418. doi:10.1152/ajpheart.00044.2012
34. Chuah SC, Moore PK, Zhu YZ. S-allylcysteine mediates cardioprotection in an acute myocardial infarction rat model via a hydrogen sulfide-mediated pathway. *Am J Physiol Heart Circ Physiol*. 2007;293:H2693–2701. doi:10.1152/ajpheart.00853.2007
35. Lin WC, Pan WY, Liu CK, et al. In situ self-spray coating system that can uniformly disperse a poorly water-soluble H2S donor on the colorectal surface to treat inflammatory bowel diseases. *Biomaterials*. 2018;182:289–298. doi:10.1016/j.biomaterials.2018.07.044
36. Liu Y, Yang R, Liu X, et al. Hydrogen sulfide maintains mesenchymal stem cell function and bone homeostasis via regulation of Ca(2+) channel sulfhydration. *Cell Stem Cell*. 2014;15:66–78. doi:10.1016/j.stem.2014.03.005
37. Song AH, Hua YM. Cystathionine γ-lyase-H2S facilitates mandibular defect healing via inducing osteogenic differentiation of bone marrow mesenchymal stem cells. *Arch Oral Biol*. 2020;117:104821. doi:10.1016/j.archoralbio.2020.104821
38. Zheng Y, Liao F, Lin XJ, et al. Cystathionine γ-lyase–hydrogen sulfide induces runt-related transcription factor 2 sulfhydration, thereby increasing osteoblast activity to promote bone fracture healing. *Antioxid Redox Signal*. 2017;10(11):742–753. doi:10.1089/ars.2016.6826
39. Mo SZ, Hua YM. Cystathionine gamma lyase-H2S contributes to osteoclastogenesis during bone remodeling induced by mechanical loading. *Biochem Biophys Res Commun*. 2018;501(2):471–477. doi:10.1016/j.bbrc.2018.05.015
40. Zhou W, Yang Q, Yu H, et al. Association between an indel polymorphism within CTH and the risk of sudden cardiac death in a Chinese population. *Leg Med*. 2020;46:101736. doi:10.1016/j.legalmed.2020.101736
41. Przemysław MM, Anna B, Magdalena O, et al. The importance of rs1021737 and rs482843 polymorphisms of cystathionine gamma-lyase in the etiology of preeclampsia in the Caucasian population. *Ginekol Pol*. 2015;86;2:119–125.