Serum zinc levels and multiple health outcomes: Implications for zinc-based biomaterials

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\textbf{ABSTRACT}

\textbf{Background:} Zinc-based biomaterials, including biodegradable metal, nanoparticles, and coatings used in medical implants release zinc ions that may increase the whole-body and serum zinc concentrations. The impact of serum zinc concentrations on major health outcomes can provide insights for device design and clinical transformation of zinc-based biomaterials.

\textbf{Methods:} This nationally representative cross-sectional study enrolled participants from the National Health and Nutrition Examination Survey (NHANES, 2011-2014) including 3607 participants. Using unadjusted and multivariate-adjusted logistic regression analyses, two-piecewise linear regression models with a smoothing function and threshold level analysis, we evaluated the associations between elevated serum zinc levels and major health outcomes.

\textbf{Results:} Elevated serum zinc levels were significantly associated with an increase in total spine and total femur bone mineral density (BMD). Every 10 μg/dL increase was associated with a 1.12-fold increase in diabetes mellitus (DM) and 1.23-fold and 1.29-fold increase in cardiovascular diseases (CVD) and coronary heart disease (CHD), in participants with serum zinc levels ≥ 100 μg/dL. It had no significant linear or nonlinear associations with risk of fractures, congestive heart failure, heart attack, thyroid disease, arthritis, osteoarthritis, rheumatoid arthritis, dyslipidemia and cancer.

\textbf{Conclusion:} Serum zinc levels are significantly associated with increased BMD in the total spine and total femur, and risk of DM, and CVD/CHD among participants with serum zinc levels ≥ 100 μg/dL.

\textbf{1. Background}

Zinc-based biomaterials including biodegradable zinc-based metal, nanoparticles, and coatings have been researched and explored in the field of medical implants in recent years [1–5], especially for tissue engineering, cardiovascular, orthopedic, anti-tumor and anti-infective applications [6–9]. Most of the zinc-based biomaterials were designed to be biodegradable [10–13], and therefore, release zinc ions upon degradation [14,15]. While they have several biological functions, including anti-bacterial, osteogenic, and angiogenic, high doses of zinc-based biomaterials can also have negative effects, such as damage to the liver, spleen, and pancreas in mice, disruption of energy metabolism, and impairment of the mitochondria and cell membrane in rat kidney [16–18]. Application of these biomaterials in clinical practice, therefore, leads to the concern whether elevated local or whole-body zinc concentrations will have a significant impact on human health and risk for certain diseases.

How to accurately assess the whole-body zinc content? A number of
methods are used to assess zinc concentrations in the body. These include measuring its levels in the serum, plasma, hair, nails, and assessing a variety of zinc-binding proteins, such as metallothionein and other zinc metalloenzymes as possible indicators of body zinc status [2,19]. Serum zinc levels generally reflect changes in the whole-body zinc status [2]. Lowe et al. measured changes in total body zinc content using metabolic balance techniques in their depletion study, and found a strong correlation between the changes in total body zinc content and serum zinc concentration ($r^2 = 0.826$, $p < 0.001$) [20]. They concluded that acute perturbations in its short-term intake and in the absence of confounding factors, changes in serum zinc concentrations might accurately reflect changes in the whole-body zinc status.

Will implantation of zinc-based biomaterials increase serum zinc levels? Wang et al. evaluated the long-term toxicity of oral zinc oxide nanoparticles (Nano-ZnO) and zinc sulfate ($\text{ZnSO}_4$) in mice and found that both increased the serum zinc concentrations after 7 weeks (24.82 μmol/L in control group vs. 31.99 ± 1.32 μmol/L in Nano-ZnO and 37.04 ± 2.43 μmol/L in $\text{ZnSO}_4$ groups) of oral consumption [21].

Although there are no published studies to date demonstrating the effects of implantation of biodegradable zinc-based metal on zinc levels in the body. However, some researchers believe that more attention should be paid to the zinc toxicity after degradation of zinc-based metal compared to magnesium-based biomaterials, since the recommended daily intake of magnesium for adults (240–420 mg/day) is up to 52.5 times higher than that of zinc (8–11 mg/day) [12,22]. Pure zinc implants may be a concern because a daily intake of 100–300 mg of zinc can cause health problems and higher doses can be more harmful.

Therefore, investigating the impact of serum zinc concentrations on major health outcomes can provide additional insights for device design and clinical transformation of zinc-based biomaterials. Based on the National Health and Nutrition Examination Survey (NHANES, 2011-2014), our study mainly evaluated the associations between serum zinc levels and major human diseases such as fractures, cardiovascular diseases, stroke, arthritis, diabetes mellitus, thyroid diseases, and cancer and indicators of major outcomes such as bone mineral density, blood glucose, and blood lipids. We also aimed to ascertain the presence of non-linear correlations between serum zinc concentrations and the above-mentioned diseases and outcomes indicators and to explore possible threshold effects.

2. Methods

2.1. Study population

We analyzed the data from NHANES 2011-2014, which is an independent cross-sectional study conducted by the Centers for Disease Control and Prevention (CDC) National Center for Health Statistics (NCHS) designed to assess health and nutritional status in a nationally representative sample of non-institutionalized, civilian general population in the United States. The survey details regarding its plan, operation, and design have been described previously [23–26]. Questionnaire surveys, physical examinations, household interviews including demographic, dietary, health-related questions and examinations, and laboratory tests were performed. From a total of 4848 participants who were tested for serum zinc levels, we finally selected 3607 adults after excluding those who were under 18 years of age. Among the 3607 participants, few of them have multiple conditions. The NHANES 2011-2014 was approved by the NCHS Ethics Review Board, and informed consent was obtained from all participants.

2.2. Measurement of serum zinc concentrations

Serum zinc concentrations were measured by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS), which is a multi-element analytical technique capable of trace level elemental analysis. Detailed instructions on specimen collection and processing can be found on the NHANES website [https://www.cdc.gov/Nchs/Nhanes/2011-2012/CUSEZN_G.htm and https://www.cdc.gov/Nchs/Nhanes/2013-2014/CUSEZN_H.htm].

2.3. Assessments

The demographics data (age, sex, and race), physical examination data (standing height [cm], weight [kg], body mass index [kg/m²], waist circumference [cm], systolic blood pressure [SBP, mm Hg], diastolic blood pressure [DBP, mm Hg], bone mineral density [BMD, g/cm²]), bone mineral content [BMC, g], and bone mineral area [BMA, cm²]), laboratory data (serum lipids [mg/dL], fasting glucose [mmol/L], glycohemoglobin [%], and insulin [pmol/L]), and questionnaire data (smoking status, alcohol drinking, activity status, fractures and major diseases, including cardiovascular diseases [congestive heart failure, coronary heart disease and heart attack], stroke, diabetes mellitus, thyroid disease, arthritis, osteoarthritis, rheumatoid arthritis, gout, and cancer) are all described on the NHANES website at https://wwwn.cdc.gov/Nchs/Nhanes/continuousnhanes/default.aspx?BeginYear=2011 and https://wwwn.cdc.gov/Nchs/Nhanes/continuousnhanes/default.aspx?BeginYear=2013.

For all participants, race (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, other race), smoking status (yes or no), alcohol drinking (yes or no), and moderate physical activity (yes or no) were defined. BMD, BMC, and BMA in the total spine, total femur, and femoral neck were examined by dual-energy x-ray absorptiometry (Hologic QDR-4500A fan-beam densitometers, software version Hologic APEX 3.2). Serum lipids, including total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-C) were analyzed using the Roche Modular P chemistry analyzer (enzymatic method). Whole blood glycohemoglobin was analyzed using the Tosoh G8 glycohemoglobin analyzer. Serum insulin was measured by an immunoenzymometric assay using the TOSOH AIA-900 chemistry analyzer.

Self-reported fractures were assessed by the questionnaire. Prior low trauma fractures were defined as self-reported fractures that occurred at an age ≥ 40 years due to a fall from a standing height or less, a trip/slip, or a fall out of bed (hip, wrist, spine) or at an age ≥ 20 years and were not due to severe trauma such as a car accident, hard fall down steps, or from a ladder (fractures other than hip, wrist, and spine). Cardiovascular diseases (CVD) were defined as congestive heart failure (CHF), coronary heart disease (CHD), and heart attack (HA). Arthritis included osteoarthritis/ degenerative, rheumatoid arthritis (RA), psoriatic, and other forms of arthritis. Diabetes mellitus (DM) was defined as a self-reported diagnosis of DM and assessed by measuring blood glycohemoglobin, fasting plasma glucose levels, 2-h glucose (Oral Glucose Tolerance Test) levels, and serum insulin in participants. Dyslipidemia was defined as having high TG (≥ 200 mg/dL), TC (≥ 240 mg/dL), and LDL-C (≥ 160 mg/dL), or low high-density lipoprotein-cholesterol (HDL-C) (< 40 mg/dL).

2.4. Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD), while categorical variables were presented as numbers and their proportion. We used the Chi-squared tests for categorical variables, one-way ANOVA for normally continuous variables, and the Kruskal-Wallis test for the skewed continuous variables. Regression coefficient and corresponding 95% confidence intervals (CI) were calculated using unadjusted and multivariate-adjusted logistic regression analyses to determine associations between 10 μg/dL increases in serum zinc levels and major diseases. The crude model was adjusted for no variables. We selected these confounders on the basis of their associations with the outcomes of interest or a change in effect estimate of more than 10%. The multivariate model was adjusted for sex, age, race, smoking, alcohol drinking, moderate activity, body mass index (BMI, 411

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Table 1
Participants characteristics. Values are mean ± SD or n (%).

| Serum Zinc (µg/dL) | < 71.50 | 71.50 to 80.3 | 80.3 to 90.3 | ≥ 90.3 | P-value |
|-------------------|---------|---------------|--------------|--------|---------|
| N                 | 892     | 891           | 915          | 909    |         |
| Age (y)           | 47.23 ± 18.68 | 47.40 ± 18.74 | 47.18 ± 18.16 | 45.86 ± 18.07 | 0.258   |
| Standing Height (cm) | 166.17 ± 9.93 | 166.66 ± 9.88 | 167.44 ± 10.04 | 168.75 ± 10.17 | < 0.001 |
| Weight (kg)       | 81.78 ± 24.07 | 81.46 ± 23.35 | 79.73 ± 20.61 | 80.41 ± 20.49 | 0.180   |
| Body Mass Index (kg/m²) | 29.55 ± 8.00 | 20.20 ± 7.54 | 28.36 ± 6.52 | 28.12 ± 6.23 | < 0.001 |
| Waist Circumference (cm) | 99.31 ± 17.82 | 98.59 ± 17.17 | 97.48 ± 16.40 | 97.46 ± 15.84 | 0.062   |
| Systolic blood pressure (mm Hg) | 123.26 ± 19.03 | 122.21 ± 17.98 | 121.33 ± 17.35 | 122.88 ± 17.89 | 0.134   |
| Diastolic blood pressure (mm Hg) | 70.00 ± 12.74 | 69.19 ± 13.51 | 69.24 ± 13.81 | 69.48 ± 13.45 | 0.588   |
| Bone mineral density (g/cm²) |         |               |              |        |
| Total spine       | 0.98 ± 0.15 | 1.00 ± 0.16 | 1.04 ± 0.17 | 1.01 ± 0.15 | 0.021   |
| Femoral neck      | 0.70 ± 0.13 | 0.72 ± 0.13 | 0.72 ± 0.13 | 0.72 ± 0.12 | 0.373   |
| Trochanter        | 0.60 ± 0.18 | 0.62 ± 0.19 | 0.61 ± 0.19 | 0.62 ± 0.17 | 0.323   |
| Bone mineral content (g) |       |             |              |        |
| Total spine       |         |               |              |        |
| Femoral neck      | 4.02 ± 0.84 | 4.14 ± 0.97 | 4.17 ± 1.01 | 4.19 ± 0.87 | 0.169   |
| Trochanter        | 8.37 ± 2.27 | 8.74 ± 2.61 | 8.73 ± 2.53 | 8.89 ± 2.37 | 0.107   |
| Wards triangle    | 0.70 ± 0.23 | 0.74 ± 0.25 | 0.73 ± 0.23 | 0.73 ± 0.23 | 0.355   |
| Bone mineral area (cm²) |         |             |              |        |
| Total spine       |         |               |              |        |
| Femoral neck      | 5.20 ± 0.57 | 5.23 ± 0.60 | 5.27 ± 0.56 | 5.28 ± 0.57 | 0.338   |
| Trochanter        | 22.98 ± 6.79 | 23.09 ± 6.66 | 23.03 ± 6.92 | 23.61 ± 6.49 | 0.704   |
| Wards triangle    | 11.87 ± 2.23 | 12.07 ± 2.43 | 12.06 ± 2.20 | 12.24 ± 2.13 | 0.318   |
| Serum lipids (mg/dL) |       |             |              |        |
| Total Cholesterol |         |               |              |        |
| Triglyceride      | 104.92 ± 74.38 | 102.65 ± 57.01 | 121.39 ± 100.02 | 132.62 ± 109.81 | < 0.001 |
| HDL-Cholesterol   | 52.20 ± 15.36 | 53.17 ± 15.59 | 52.43 ± 14.70 | 53.02 ± 15.77 | 0.480   |
| LDL-Cholesterol   | 185.10 ± 40.44 | 188.47 ± 40.14 | 189.69 ± 41.97 | 193.80 ± 41.50 | < 0.001 |
| Fasting Glucose (mmol/L) |         |               |              |        |
| Insulin (µmol/L)  |         |               |              |        |
| Sex               |         |               |              |        |
| Smoking           |         |               |              |        |
| Alcohol drinking  |         |               |              |        |
| Moderate activity |         |               |              |        |
| Total Fracture    |         |               |              |        |
| Spine Fracture    |         |               |              |        |
| Hip Fracture      |         |               |              |        |
| Cardiovascular diseases |       |             |              |        |
| Stroke            |         |               |              |        |
| Congestive heart failure |       |             |              |        |

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kg/m²), waist circumference (cm), SBP (mm Hg), and DBP (mm Hg), CVD, DM, thyroid disease; arthritis; cancer; dyslipidemia and BMD. A two-sided p value < 0.05 was considered to be statistically significant. To examine the nonlinear association between serum zinc levels and major diseases (logOR), we further applied a two-piecewise linear regression model using a smoothing function. The threshold level was determined using trial and error, including selection of turning points along a pre-defined interval and then choosing the turning point that gave the maximum model likelihood. In addition, we conducted a log-likelihood ratio test comparing the one-line linear regression model with the two-piecewise linear model. Statistical analyses were performed using R packages (http://www.r-project.org) and Empower (R) (www.empowerstats.com, X&Y solutions Inc., Boston, MA.

3. Results

3.1. Descriptive analysis

The participant characteristics are presented in Table 1. This study included 3607 participants with fractures (n = 335), CVD (n = 229), stroke (n = 109), DM (n = 425), thyroid diseases (n = 330), arthritis (n = 864; 397 cases of osteoarthritis and 152 of RA), cancer (n = 297) and dyslipidemia (n = 1064). The average standing height, serum TC, and serum LDL-C increased across the serum zinc quartiles. BMI decreased across the serum zinc quartiles.

| Table 1 (continued) | Serum Zinc (ug/dL) | < 71.50 | 71.50 to 80.3 | 80.3 to 90.3 | ≥ 90.3 | P-value |
|---------------------|------------------|---------|---------------|-------------|--------|---------|
| No                  | 808 (90.58%)     | 823 (92.37%) | 839 (91.69%) | 833 (91.64%) | 0.647 |
| Yes                 | 32 (3.59%)       | 25 (2.81%)  | 23 (2.51%)    | 17 (1.87%)   |        |
| Coronary heart disease | No             | 810 (90.81%) | 814 (91.36%) | 834 (91.15%) | 0.886 |
| Yes                 | 34 (3.81%)       | 32 (3.59%)  | 25 (2.73%)    | 29 (3.19%)   |        |
| Heart attack        | No              | 813 (91.14%) | 819 (91.92%) | 830 (90.71%) | 0.059 |
| Yes                 | 32 (3.59%)       | 29 (3.25%)  | 33 (3.61%)    | 29 (3.19%)   |        |
| Diabetes            | No              | 760 (85.20%) | 766 (85.97%) | 772 (84.37%) | 0.103 |
| Yes                 | 95 (10.65%)      | 102 (11.45%)| 116 (12.68%)  | 112 (12.32%) |        |
| Thyroid disease     | No              | 766 (85.87%) | 753 (84.51%) | 775 (84.70%) | 0.432 |
| Yes                 | 78 (8.74%)       | 93 (10.44%) | 89 (9.73%)    | 70 (7.70%)   |        |
| Arthritis           | No              | 621 (69.62%) | 618 (69.36%) | 646 (70.60%) | 0.559 |
| Yes                 | 219 (24.55%)     | 229 (25.70%)| 215 (23.50%)  | 201 (22.11%) |        |
| Gout                | No              | 814 (91.26%) | 811 (91.02%) | 833 (91.04%) | 0.303 |
| Yes                 | 31 (3.48%)       | 37 (4.15%) | 31 (3.39%)    | 47 (5.17%)   |        |
| Osteoarthritis      | No              | 621 (69.62%) | 618 (69.36%) | 646 (70.60%) | 0.559 |
| Yes                 | 98 (10.99%)      | 114 (12.79%)| 95 (10.38%)   | 90 (9.90%)   |        |
| Rheumatoid arthritis| No             | 621 (69.62%) | 618 (69.36%) | 646 (70.60%) | 0.930 |
| Yes                 | 38 (4.26%)       | 37 (4.15%)  | 36 (3.93%)    | 41 (4.51%)   |        |
| Cancer              | No              | 767 (85.99%) | 768 (86.20%) | 792 (86.56%) | 0.719 |
| Yes                 | 78 (8.74%)       | 80 (8.98%) | 72 (7.87%)    | 67 (7.37%)   |        |
| Dyslipidemia        | No              | 648 (72.65%) | 637 (71.49%) | 649 (70.93%) | 0.049 |
| Yes                 | 244 (27.35%)     | 254 (28.51%)| 266 (29.07%)  | 300 (33.00%) |        |
| High TC level (mg/dL) | < 200          | 795 (90.14%) | 788 (89.14%) | 810 (89.50%) | 0.043 |
| ≥ 240              | 87 (9.86%)      | 96 (10.86%) | 95 (10.50%)   | 124 (13.79%) |        |
| High TG level (mg/dL) | < 200         | 170 (92.39%) | 334 (93.82%) | 467 (88.28%) | < 0.001|
| ≥ 200              | 14 (7.61%)      | 22 (6.18%) | 62 (11.72%)   | 92 (14.22%)  |        |
| Low HDL-C level (mg/dL) | ≥ 40       | 718 (81.41%) | 721 (81.56%) | 751 (82.98%) | 0.815 |
| < 40               | 164 (18.59%)    | 163 (18.44%)| 154 (17.02%)  | 160 (17.80%) |        |
| High LDL-C level (mg/dL) | < 160     | 165 (91.16%) | 328 (92.13%) | 475 (91.00%) | 0.294 |
| ≥ 160              | 16 (8.84%)      | 28 (7.87%) | 47 (9.00%)    | 71 (11.32%)  |        |
3.4. Serum zinc levels, cardiovascular diseases, and stroke

Table 2
Multivariate regression analysis for effect of serum zinc on multiple health outcomes.

| Bone related diseases | Bone mineral density (g/cm²) | β/OR (95% CI) | P value | β/OR (95% CI) | P value | β/OR (95% CI) | P value |
|-----------------------|-----------------------------|---------------|---------|---------------|---------|---------------|---------|
| Total Spine           | 0.006 (−0.002, 0.014)       | 0.119         | 0.009 (0.002, 0.016) | 0.011 | 0.009 (0.001, 0.016) | 0.020 |
| Total femur           | 0.004 (−0.002, 0.010)       | 0.242         | 0.005 (0.000, 0.011) | 0.034 | 0.005 (0.000, 0.010) | 0.049 |
| Femoral neck          | 0.003 (−0.003, 0.009)       | 0.321         | 0.005 (0.000, 0.010) | 0.063 | 0.005 (−0.001, 0.010) | 0.080 |
| Fracture              |                             |               |         |               |         |               |         |
| Total Fracture        | 0.938 (0.863, 1.019)        | 0.131         | 0.915 (0.832, 1.006) | 0.065 | 0.930 (0.841, 1.030) | 0.164 |
| Spine Fracture        | 0.855 (0.655, 1.117)        | 0.251         | 0.872 (0.643, 1.182) | 0.378 | 0.818 (0.424, 1.576) | 0.548 |
| Hip Fracture          | 0.899 (0.690, 1.172)        | 0.433         | 0.956 (0.698, 1.310) | 0.781 | 1.042 (0.742, 1.462) | 0.814 |

Cardiovascular diseases and stroke

| Model I adjust for: Sex; Age (y); Race; Smoking; Alcohol drinking; Moderate activity; Body Mass Index (kg/m²); Waist Circumference (cm); Systolic blood pressure (mm Hg); Diastolic blood pressure (mm Hg). Model II adjust for. |
|-------------------|----------------|---------------|---------|----------------|---------------|---------|---------|
| β/OR (95% CI)     | P value | β/OR (95% CI) | P value | β/OR (95% CI) | P value |
| Arthritis and gout |                             |               |         |               |         |         |
| Arthritis         | 0.970 (0.923, 1.021) | 0.242         | 0.994 (0.933, 1.060) | 0.857 | 0.992 (0.930, 1.058) | 0.807 |
| Osteoarthritis    | 0.984 (0.918, 1.054) | 0.640         | 0.996 (0.913, 1.086) | 0.926 | 0.993 (0.922, 1.070) | 0.854 |
| Rheumatoid arthritis | 1.003 (0.902, 1.114) | 0.963         | 1.039 (0.921, 1.174) | 0.532 | 1.004 (0.921, 1.118) | 0.936 |
| Cancer            | 0.982 (0.909, 1.061) | 0.650         | 1.013 (0.928, 1.106) | 0.776 | 1.001 (0.924, 1.085) | 0.980 |

Table 2, S2 [see Supplementary data] and Fig. 1 present the association between serum zinc levels and fractures. In the multivariate logistic regression analysis after being adjusted for sex, age, race, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, CVD, DM, thyroid disease, arthritis, cancer and BMD (total spine/total femur/femoral neck), serum zinc levels were not associated with risk of total, spine, or hip fractures (all p > 0.05).

3.3. Serum zinc levels and fractures

Table 2, S3 [see Supplementary data] and Fig. 2 present the association between serum zinc levels and risk of CVD and stroke. There were no significant linear or nonlinear associations between serum zinc levels, CHF and HA in multivariate logistic regression analysis after multivariate adjustment for sex, age, race, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, DM, thyroid disease, arthritis, cancer and dyslipidemia. However, every 10 μg/dL increase in the serum zinc level was associated with a 1.23-fold (p = 0.041, 95% CI = 1.04–1.41) increase in risk of CVD and CHF risk, respectively in participants with serum zinc levels ≥ 100 μg/dL. Every 10 μg/dL increase was associated with a 1.40-fold (p = 0.045, 95% CI = 1.01–1.85) increase in stroke, in participants with serum zinc levels ≥ 120 μg/dL.

3.4. Serum zinc levels, cardiovascular diseases, and stroke

Table 2 and S4 [see Supplementary data] and Fig. 3 present the association of serum zinc levels with DM and thyroid disease. There were no significant linear or nonlinear associations between serum zinc levels and diabetes mellitus, and thyroid disease.

3.5. Serum zinc levels, diabetes mellitus, and thyroid disease
levels and thyroid disease in multivariate logistic regression analysis after multivariate adjustment for sex, age, race, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, CVD, DM, arthritis, cancer and dyslipidemia. However, every 10 μg/dL increase in serum zinc level was associated with a 1.12-fold (p = 0.005, 95% CI = 1.04–1.21) increase in the risk of DM after multivariate adjustment for sex, age, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, CVD, DM, arthritis, cancer and dyslipidemia. Though multivariate logistic regression analysis revealed no significant linear or nonlinear associations between serum zinc levels and fasting glucose and insulin. And it showed that every 10 μg/dL increase in serum zinc levels, resulted in a glycohemoglobin increase by 0.034% (p = 0.003, 95% CI = 0.012–0.056).

### 3.6. Serum zinc levels, arthritis, and gout

There were no significant linear or nonlinear associations between serum zinc levels and risk of arthritis, osteoarthritis, and RA in multivariate logistic regression analysis after multivariate adjustment for sex, age, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, CVD, DM, thyroid disease, cancer and dyslipidemia. (Table 2 and S5 [see Supplementary data] and Fig. 4). However, multivariate logistic regression analysis and threshold level analysis suggested that in participants with serum zinc levels < 99 μg/dL, for every 10 μg/dL increase in serum zinc levels, the risk of gout increased by 1.24-fold (p for nonlinearity = 0.043).

### 3.7. Serum zinc levels and cancer

There were no significant linear or nonlinear associations between serum zinc levels and incidence of cancer in multivariate logistic regression analysis after multivariate adjustment for sex, age, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, CVD, DM, thyroid disease, arthritis, cancer, and dyslipidemia. (OR 1.001, 95% CI: 0.924, 1.085, p = 0.980) (Table 2 and S6 [see Supplementary data] and Fig. 5).

### 3.8. Serum zinc levels and dyslipidemia

Table 2 and S7 [see Supplementary data] and Fig. 6 present the associations of serum zinc levels with dyslipidemia and serum lipids. There were no significant linear or nonlinear associations between serum zinc levels and total dyslipidemia in multivariate logistic regression analysis after multivariate adjustment for sex, age, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, CVD, DM, thyroid disease, arthritis, and cancer. However, every 10 μg/dL increase in serum zinc level was associated with a 1.10-fold (p = 0.011, 95% CI = 1.022–1.182), and a 1.12-fold (p = 0.033, 95% CI = 1.010–1.248) increase in high TC level and high TG level, respectively. Every 10 μg/dL increase in serum zinc levels was also associated with 2.219 mg/dL, 4.962 mg/dL, 0.355 mg/dL and...
Fig. 2. Multivariate adjusted smoothing spline plots of serum zinc levels and cardiovascular diseases and stroke. This model was adjusted for sex, age, race, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, DM, thyroid disease, arthritis, cancer, and dyslipidemia. The red line represents the best-fit line, and the blue lines are 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3. Multivariate adjusted smoothing spline plots of serum zinc levels and diabetes mellitus, thyroid disease, and levels of fasting glucose, glycohemoglobin, and insulin. This model was adjusted for sex, age, race, smoking status, alcohol drinking, moderate activity, BMI, waist circumference, SBP, DBP, CVD, DM/thyroid disease, arthritis, cancer, dyslipidemia. The red line represents the best-fit line, and the blue lines are 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
1.602 mg/dL increase in serum TC, serum TG, HDL-Cholesterol and LDL-cholesterol.

4. Discussion

This study analyzed the data from the NHANES (2011-2014) including 3607 participants and found that the high serum zinc levels were significantly associated with an increase in the total spine and total femur BMD; risk of DM; and CVD/CHD in participants with serum zinc levels 100 μg/dL. It had no significant linear or nonlinear associations with risk of fractures, CHF, HA, thyroid disease, arthritis, osteoarthritis, RA, dyslipidemia and cancer (Fig. 7 and Fig. 8).

Zinc-based biomaterials, especially zinc-based nanoparticles, can increase the concentration of zinc ions in the blood, raising concerns about their whole-body zinc ion status and toxicity [21,27–29]. A study has shown that high doses of nano-ZnO can cause acute toxicity, which can damage the lung, liver, spleen, and pancreas in mice, disturb energy metabolism and impair the mitochondria and cell membrane in rat kidney. They were also shown to increase lactate dehydrogenase (LDH) leakage and cause genotoxicity to primary mouse embryo fibroblasts [16–18]. Studying the long-term toxicity of nano-ZnO, Wang et al. reported that 7 weeks after their gastrointestinal administration, reduction in body weight was seen from weeks 8–11, and reported increased serum glutamic-pyruvic transaminase activity, and elevated zinc concentrations in the serum, liver, and kidney [21]. Some studies provided convincing evidence that zinc ions played important roles in the toxicity of zinc-based nanoparticles [30,31]. George et al. have reported that decreasing the nano-ZnO dissolution rate could slow the release of zinc ions, thereby reducing their toxicity [30,31].

Furthermore, it is estimated that around two billion people in the developing world have zinc deficiency due to high phytate-containing cereal protein intake [32]. On the other hand, in the developed countries there are rising concerns over excessive levels of zinc in the body [33]. The use of zinc-based biomaterials may further increase the risk of excessive zinc. Therefore, investigating the relationship between serum zinc ions level and human health outcomes can provide evidence and standards for the future designs, applications, contraindications, and post-implantation strategies for zinc-containing biomaterials.

It has been suggested that zinc deficiency can increase the risk of chronic diseases, though its toxicity and possible negative effects on
The benefits of zinc for bone health have been demonstrated by many studies [37–39], and our findings indicate that serum zinc levels were significantly associated with the total spine and total femur BMD. Bone zinc content has been shown to decrease with aging, skeletal unloading, and following menopause, suggesting its role in bone physiology [37–39]. Zinc has been demonstrated to have a stimulatory effect on osteoblastic proliferation, differentiation, and mineralization, which might be related to zinc-induced activation of a variety of osteogenesis-related genes and proteins thereby promoting bone formation [38,40]. It has also been shown to activate the expression of ALP, osteocalcin, Runx2 genes, and proteins, which play a role in the differentiation of osteoblastic cells [41]. Moreover, zinc enhances protein synthesis in osteoblastic cells by activating aminocacyl-tRNA synthetase, a rate-limiting enzyme in the translational machinery and therefore has a potent stimulatory effect on the osteoblastic bone formation. Zinc activates MAPK kinase and has a stimulatory effect on the proliferation of osteoblastic cells in the bone tissues of newborn rats. It also increases the production of IGF-I and TGF-b1, which activate osteoblastic cells and modulate cell proliferation [42–44]. Zinc has also been shown to inhibit osteoclastic bone resorption by inhibiting the formation of osteoclasts from the bone marrow cells and inducing apoptosis of mature osteoclasts. It has a suppressive effect on the receptor activator of nuclear factor kappa-B ligand (RANKL) and TNFα-induced osteoclastogenesis. Zinc also stimulates OPG gene expression in osteoblastic cells, which can inhibit the binding of RANKL to RANK receptors in pre-osteoclastic cells [41,45].

Our study found that every 10 μg/dL increase in serum zinc levels was associated with a 1.12-fold increase in the incidence of DM. Another prospective study including 2220 Finnish men from the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) has demonstrated that higher serum zinc levels are significantly and independently associated with an increased risk of incident type 2 DM in middle-aged and older men even after adjustment for potential confounders [33]. However, the potential mechanisms underlying this relationship between higher serum zinc levels and DM are not elucidated. Previously published studies have also shown that high levels of zinc can target hormones such as leptin and impact the hormonal homeostasis which in turn could result in insulin resistance. Zinc has been shown to play an important role in the function of β-cells and secretion of insulin [46]. The activity of β-cells is suggested to be increased in order to manage glucose levels in subjects with DM [46]. Excess zinc can cause hyperactivity of β-cells and the resultant increase in insulin production can lead to insulin resistance through exhaustion of insulin receptors. Overstimulation by long-term elevated zinc levels, therefore, can be harmful to the β-cells [33]. Previous studies have shown lower levels of serum zinc and higher levels of urinary zinc in subjects with DM compared to control subjects [47], suggesting that this could be a mechanism to release excess zinc and avoid toxicity [33].

Our study also found that every 10 μg/dL increase was associated with a 1.23-fold and 1.29-fold increase in CVD and CHD, in participants with serum zinc levels ≥ 100 μg/dL. Some studies have explored the association between serum zinc levels and CVD, and the results are controversial [48]. Most of these studies are based on patient-based case-control studies, and the conclusions are difficult to generalize to the general population. A large prospective study conducted by Milton et al. found a positive association between dietary zinc intake and incidence of CVD in women even after adjusting for potential confounders. Compared to those in the lowest quintile of zinc intake, those in the highest quintile (odds ratio [OR] = 1.67, 95% CI = 1.08–2.62) had almost twice the odds of developing CVD (p = 0.007) [49]. However, the potential mechanisms underlying this relationship are not clarified. Our study findings are preliminary and need to be investigated further. Future research is necessary to confirm this association in other populations as well.

4.1. Implications and conclusion

Our study found that serum zinc ion concentrations are associated with BMD, which strongly supports the use of zinc-based biomaterials for the treatment of bone-related diseases, bone fixation, bone repair materials, and bone loss prevention. While our study found no correlation between serum zinc levels and most of the health outcomes we evaluated, we did find associations with DM and CHD, which indicated that patients at high risk of DM or CHD should be extremely cautious to control serum zinc ions when they were using products that increase serum zinc ions.

Future in vivo studies are recommended to focus on changes in serum zinc concentrations (total zinc, free Zn ion and protein-bound Zn) following implantation of zinc-based biomaterials. At the same time, an evaluation of the dose-effect relationship, the relationship
between different zinc biomaterials, different treatment methods (such as oral and surgical implantation), and different implanted sites (cardiovascular, cortical bone marrow cavity, muscle, subcutaneous) is also required. Further studies focusing on the application of zinc biomaterials in pre-diabetic, diabetic, and CHD animal models is suggested. Glycated hemoglobin testing should be considered in pre-diabetic or diabetic animal models. Investigations into the impact of zinc from zinc-based biomaterials on human health are also needed to evaluate and modify the currently recommended guidelines for the clinical transformation of zinc-based biomaterials.

However, in our study, some potential confounding factors were possibly omitted due to inherent bias of representative cross-sectional study. Although most confounding factors were adjusted, other known and unknown risk factors cannot be excluded as a potential explanation for the observed findings.

In conclusion, our study demonstrates that serum zinc levels are significantly associated with (a) increased BMD in the total spine and total femur and (b) risk of DM, and CVD/CHD among participants with serum zinc levels ≥100 μg/dL. However, there were no significant associations between serum zinc levels and risk of fractures, CHF, HA, stroke, thyroid disease, arthritis, dyslipidemia, and cancer. This study reveals an association between serum zinc concentration and BMD, which indicate that biodegradable zinc-based materials have good prospects for orthopedic applications. In future studies, the application of zinc-based biomaterials in pre-diabetic or diabetic models should be further evaluated. In addition, further exploration of the threshold of zinc release from zinc-based biomaterials in cardiovascular disease models can aid in the application of zinc-based biomaterials for development of cardiovascular stents.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request. Online Supplementary data are available.

Ethics approval

The NHANES 2011-2014 was approved by the NCHS Ethics Review Board, and informed consent was obtained from all participants.
### Fracture
- Total Fracture: 0.93 (0.84, 1.03)
- Spine Fracture: 0.82 (0.42, 1.58)
- Hip Fracture: 1.04 (0.74, 1.46)

### Cardiovascular diseases and stroke
- Cardiovascular diseases: 1.06 (0.96, 1.17)
- Stroke: 1.09 (0.95, 1.26)
- Coronary heart disease: 1.06 (0.94, 1.21)
- Congestive heart failure: 0.95 (0.81, 1.10)
- Heart attack: 1.05 (0.92, 1.20)

### Diabetes and Thyroid disease
- Diabetes: 1.12 (1.03, 1.21)
- Thyroid disease: 1.00 (0.91, 1.09)

### Arthritis and gout
- Arthritis: 0.99 (0.93, 1.06)
- Gout: 1.09 (0.99, 1.21)
- Osteoarthritis: 0.99 (0.92, 1.07)
- Rheumatoid arthritis: 1.00 (0.90, 1.12)

### Cancer
- All kind of cancer: 1.00 (0.92, 1.08)

### Dyslipidemia
- Dyslipidemia: 1.05 (1.01, 1.10)
- High TC level: 1.09 (1.03, 1.16)
- High TG level: 1.18 (1.07, 1.30)
- Low HDL-C level: 0.98 (0.93, 1.04)
- High LDL-C level: 1.08 (0.97, 1.19)

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**Fig. 7.** Forest plots of serum zinc levels and major human diseases.

**Fig. 8.** Conceptual diagram of serum zinc levels and major human diseases.
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