The association of calf circumference and all-cause, cardiovascular and cerebrovascular mortality: Results from the National Health and Nutrition Examination Surveys

Type
Research paper

Keywords
Mortality, National Health and Nutrition Examination Survey (NHANES), Anthropometric markers

Abstract
Introduction
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Material and methods
The data was retrieved from the 1999-2006 National Health and Nutritional Examination Surveys (NHANES), composing of 20,214 individuals aged ≥ 18 years with CC being measured. We performed multivariate Cox regression models to examine the associations, then stratified the regression models into subgroups to test for interactions.

Results
Among 20,214 participants, 47.25% were men and the mean age was 45.8 years. In the fully adjusted model, each 1 cm increment in CC was inversely associated with the risk of all-cause mortality (HR = 0.92, 95%CI = 0.90-0.94, P < 0.0001) and cardiovascular mortality (HR = 0.90, 95%CI = 0.84-0.97, P = 0.0056). Meanwhile, the highest quartile of CC had 50% (HR = 0.50, 95%CI = 0.40-0.64, P trend < 0.001) lower risk of all-cause mortality and 57% (HR = 0.43, 95%CI = 0.21-0.88, P trend = 0.045) lower risk of cardiovascular mortality, compared to the lowest quartile of CC. For cerebrovascular mortality, CC did not have significant associations with mortality.

Conclusions
Our results suggested an independently inverse association between CC and all-cause and cardiovascular mortality.
The association of calf circumference and all-cause, cardiovascular and cerebrovascular mortality: Results from the National Health and Nutrition Examination Surveys

Running title: Calf circumference and mortality

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Key words: Anthropometric markers; Mortality; NHANES
Introduction

Cardiovascular and cerebrovascular death remains the leading cause of mortality in worldwide [1]. Plentiful factors have been reported to be associated with all cause and cause-specific mortality, such as high density lipoprotein cholesterol (HDL-C)[2], serum uric acid and apolipoprotein B (apoB)[3], and lower carbohydrate diets[4]. Another risk factor that worth concerning is the anthropometric index, which may serve as an easy-to-use indicator to identify high-risk population. Calf circumference (CC) is an index reflecting size of lower-limb, which represents peripheral fat and lean mass. Previous studies indicated that CC was a potential indicator to assess nutritional status and physical function, as well as an important predictor for mortality[5]. In addition, CC is easier to assess than body mass index (BMI) in frail and ill elderly patients, since the measurement of height and weight is often inaccurate or unavailable[6]. There is cumulating evidence to suggest body composition and fat distribution are important in determining the risk of mortality, whereas global body mass may not be the best predictor [7]. Noticeably, decreased CC mainly reflect muscle loss and nutritional status. Several previous studies have found that increased size of larger limbs was associated with a reduced risk of metabolic disorder or cardiovascular disease[8].

Currently, numerous studies have focused on anthropometric markers of abdominal obesity (waist circumference, waist-to-hip ratio, waist-to-height ratio) and the long-term health[9,10], but it is unclear whether markers of body composition other than abdominal obesity, namely CC, is another important indicator. To address this research gap, we have conducted this study using data from the National Health and Nutritional Examination Surveys (NHANES) population.

Material and methods
Study population

In the present study, we used publicly available data from NHANES which the study methods and procedures for data collection have been described in detail elsewhere[11]. The current study was based on the analysis of NHANES data collected from 1999 to 2006 (N= 41, 474). We selected participants aged ≥ 18 years (N = 22, 624), and excluded participants who had missing data on calf circumference (N=2, 389) or mortality (N = 21), making a final sample of 20, 214 eligible participants. The selection of study cohort is demonstrated in Figure 1. The ethics approval for NHANES had been obtained by the Institutional Review Board of the Centers for Disease Control and Prevention. Written informed consent was obtained from all participants.

Data collection

Demographic data (age, sex, race), results on physical examination and laboratory test (systolic blood pressure (SBP), BMI, diastolic blood pressure (DBP), fasting blood glucose, total cholesterol (TC), triglyceride, HDL-C, low density lipoprotein-cholesterol (LDL-C), estimated glomerular filtration rate (eGFR), medical history (hypertension, diabetes, cardiovascular diseases), and prescription medication information (anti-hypertensive drugs, anti-diabetes drugs) were retrieved from the NHANES data. Cigarette smoking status was categorized as smoker, ex-smoker or non-smoker. eGFR was calculated by Modification of Diet in Renal Disease (MDRD) formula [12]. To measure CC, technicians slid the tape to measure upside and downside of the calf in order to find the widest point for accurate measurement. Hypertension was classified by the presence of SBP ≥ 130 mmHg and/or DBP ≥ 80 mm Hg, and/or currently use of anti-hypertension medication[13]. Diabetes was based on
self-reported history of diabetes or the presence of any one of the following criteria: 1. fasting glucose $\geq 126$ mg/dL; 2. non-fasting glucose $\geq 200$ mg/dL; 3. HbA1c $\geq 6.5\%$; 4. taking medication to lower blood glucose; or 5. consuming insulin[14]. BMI was calculated as weight in kilogram divided by squared height in meters (kg/m$^2$). Obesity was defined as BMI $\geq 30$ kg/m$^2$ while overweight as BMI $\geq 25$ kg/m$^2$, and normal weight was defined as $18.5$ kg/m$^2 \leq$ BMI $< 25$ kg/m$^2$, underweight was defined as BMI $< 18.5$ kg/m$^2$[15].

Outcome

The anonymize data of NHANES 1999–2006 participants were linked to longitudinal Medicare and mortality data. Data on survival status was collected from the date of survey participation until 31 December 2015. Outcomes of our study were all-cause mortality, mortality due to cardiovascular diseases (I00-I09, I11, I13, I20-I51), and cerebrovascular diseases (I60-I69). The cause of death was determined using the 10th revision of the International Classification of Diseases (ICD-10).

Statistical analysis

Continuous variables were expressed as means $\pm$ standard deviation (SD), whereas categorical variables were presented as numbers with corresponding percentages. CC was divided into quartiles (Q1-Q4). Baseline characteristics were summarized based on CC quartiles. The Kruskal-Wallis $H$, one-way ANOVA and $X^2$ test was applied to determine whether the subgroup difference was significant.
Multivariate Cox regression was performed to assess the association between CC and the risk of all-cause, cardiovascular and cerebrovascular mortality. CC was treated as both continuous (per 1 cm increment) and categorical variables (Q1-Q4) and was put into separate models. We computed three regression models which were adjusted for different sets of confounders. Crude model only included CC. Model 1 was further adjusted for age, gender and BMI. Meanwhile, Model 2 was adjusted for age, gender, BMI, SBP, TC, HDL-C, use of antihypertensive drugs and anti-diabetic drugs, eGFR, alcohol drinking, serum albumin, smoking status, race; history of diabetes, hypertension or cardiovascular diseases. The results were reported as hazard ratio (HR) with 95% confidence interval (CI).

To test the robustness of our results, we further stratified the regression models into subgroups and tested for interaction. The cohort was divided into groups respectively based on gender, age, hypertension diabetes, BMI, SBP and smoking status. When analysis a subgroup variable, age, gender, BMI, SBP, TC, HDL-C, use of antihypertensive drugs and anti-diabetic drugs, eGFR, alcohol drinking, smoking status, race, history diabetes, hypertension or cardiovascular diseases were all adjusted except the variable itself. The Kaplan-Meier analysis was conducted to compare the difference in survival rates by CC quartiles, and estimated the intergroup differences by log-rank test. Cubic spline models were used to estimate the HRs for mortality associated with increasing CC. A two-sided p value < 0.05 was considered statistically significant. Data management and analyses were performed using the statistical software package R version 3.32 (http://www.R-project.org, The R Foundation, Vienna, Austria).

Results
Baseline characteristics

Table 1 summarized the characteristics of participants based on CC quartiles. Overall, 20,214 participants were included, with a mean age of 45.8 years, 47.75% were men. During the follow-up period of 11.82 years, 3,655 cases of deaths were recorded, including 466 cardiovascular disease deaths and 152 cerebrovascular disease deaths. Participants with the highest quartile of CC had the lowest proportion of all-cause (11.25%), cardiovascular (1.36%) and cerebrovascular mortality (0.37%). All variables were significantly different by quartiles (all \( P < 0.05 \)).

Relationships between calf circumference and all-cause, cardiovascular and cerebrovascular disease mortality

Results from Cox regression models are shown in Table 2. When CC was expressed as continuous variable, each 1 cm increment of CC was inversely associated with all-cause mortality. The corresponding HR was 0.90 (95% CI = 0.90-0.91, \( P < 0.0001 \)) in crude model, 0.91 (95% CI = 0.90-0.92, \( P < 0.0001 \)) in Model 1 and increased CC still significantly associated with lower risk of all-cause mortality in Model 2 (HR = 0.92, 95% CI = 0.90-0.94, \( P < 0.0001 \)). Regarding to cardiovascular death, it showed the similar pattern, CC was significantly associated with cardiovascular mortality in Model 2 (HR = 0.90, 95% CI = 0.84-0.97, \( P = 0.0056 \)). For cerebrovascular death, significant association was found in crude model and Model 1, but not Model 2.

When CC was expressed as categorical variable, the risk of all-cause mortality was significantly lower for participants with the highest quartile of CC in Model 2 (HR = 0.50, 95% CI = 0.40-0.64, \( P \) trend < 0.001) when using the lowest quartile as referent. Similarly, the highest quartile of CC was
significantly associated with a lower risk of cardiovascular mortality in Model 2 (Q4: HR = 0.43, 95%CI = 0.21-0.88, P trend=0.045). CC did not associate with cerebrovascular mortality when being treated as categorical variable.

In Figure 2, the Kaplan-Meier survival curves plotting quartiles of CC showed that the rates of all-cause and cardiovascular death were statistically different by CC quartiles (both log rank: p < 0.001). Figure 3 demonstrated the cubic spline models estimated the HRs for all-cause and causes specific mortality associated with increasing CC.

Subgroup analysis

Table 3 summarized the association between CC and mortality in subgroups. Among all the subgroups, only BMI significantly modified the associations between CC and mortality risk. The HR of all-cause mortality was 0.80 (95%CI = 0.79-0.82) in the subgroup with BMI < 25kg/m², which was significantly lower than subjects with BMI ≥ 25kg/m² (HR = 0.88; 95%CI = 0.87-0.89) (P for interaction < 0.0001). Similar pattern also found on the association between CC and cardiovascular mortality. When subgroup with BMI < 25kg/m², the HR was 0.74 (95%CI = 0.70-0.78) and when BMI ≥ 25kg/m², the HR was 0.88 (95%CI = 0.85-0.91) (P for interaction=0.0005).

Discussion

Current study investigated the association between CC and all-cause, cardiovascular and cerebrovascular mortality. We found that CC was inversely associated with all-cause and
cardiovascular mortality. For every 1cm increment in CC, there was 8% reduction in the risk of all-cause mortality and 10% reduction for cardiovascular mortality. For subjects with the highest quartile of CC (≥ 40.70 cm), there was 50% reduction in the risk of all-cause mortality, and 57% reduction in the risk of cardiovascular mortality.

CC is an indicator that is simple, non-invasive and easy to obtain in routine clinical practice. However, limited evaluated the ability of CC in predicting mortality. Previous studies have illuminated that CC was closely related to lean muscle mass[16], but the relationship between CC and cause-specific mortality has not been investigated in detail. Prior studies mainly investigated CC among elderly and fragile patients, and found that low CC was suggested to be an indicator of malnutrition in elderly population[17,18]. In contrast, a study reported that CC was not an independent predictor of mortality risk after adjusting for other prognostic factors in older patients with cardiovascular disease[19]. The present study focused on the general population and included relatively younger and healthier individuals. The results of this study proposed the usage of CC might be applied to a larger scale of population.

Regarding to subgroup analysis, the relationships between CC and mortality differed by BMI. CC had stronger inverse association with mortality among participants with BMI < 25 kg/m². This is consistent to the well-established theory that BMI is an independent risk factor for mortality[20], which might weaken the protective effects of increased CC. Besides, a study conducted among 160 patients aged ≥ 65 years revealed CC to be a better indicator than BMI in predicting mortality[21]. CC is potentially a protective factor for mortality particular for those with lower BMI.
The underlying mechanism of CC relating to all-cause and cardiovascular mortality has not been fully investigated, but several potential mechanisms have been proposed. CC is a marker of peripheral subcutaneous fat, besides lean mass[22]. Several studies addressed that adiposity distribution affect mortality might be partially explained by inflammation[23]. A study revealed that a larger leg fat mass was related to lower fasting and postprandial glucose levels[8]. Some other previous studies shown that greater CC might have antiatherogenic effect[24,25]. For example, larger CC reduce the frequency of carotid plaques, which give a plausible reason for a lower risk for cardiovascular mortality[25]. Few studies suggested that peripheral fat mass could protect against unstable glucose and lipid metabolites, which in turn reduce adverse effect of fat on cardiometabolic risk[26]. Some other studies implied that polymorphisms was associated with hypertension and, consequently, with a higher risk of cardiovascular mortality[27]. Similarly, CC might also be affected by genetic composition. These results may explain the protective role of greater CC in all-cause and cardiovascular mortality.

Further prospective studies are expected to elucidate the link between CC and mortality among general population with various demographics, and to reveal the underlying mechanism between CC and mortality. There were several limitations of our study. First, the CC values would change if the participant was standing or laying. Second, we did not obtain the status of chronic heart failure and chronic kidney disease. Third, some data were self-reported and might subject to recall bias. Forth, more sophisticated anthropometric measurements such as dual energy X-ray absorptiometry was not available in this study. Fifth, the optimal cut off values of CC were calculated from the NHANES data, which mainly represent US citizens. Replication studies are required in external population.
Conclusions

CC was inversely associated with the risk of all-cause and cardiovascular mortality. CC had more significant protective effect on those who with BMI < 25kg/m².

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Fig. 1 Procedure of selecting participants.

Fig. 2 The Kaplan-Meier survival curves plotting quartiles of calf circumference.

Legend: $Q$, quartile, $m$, month.

Fig. 3 Cubic spline of hazard ratios for all-cause and cause specific mortality associated with increasing calf circumference.

Legend: Age, gender, BMI, SBP, TC, HDL-C, antihypertensive drugs, anti-diabetic drugs, eGFR, serum albumin, alcohol drinking, smoking status, race, diabetes, hypertension, cardiovascular diseases were all adjusted.

$CI$, confidence interval, $A$, association of calf circumference (cm) and hazard ratios of all-cause mortality, $B$, association of calf circumference (cm) and hazard ratios of cardiovascular mortality, $C$, association of calf circumference (cm) and hazard ratios of cerebrovascular mortality.
|                          | Total  | Q1: < 35.20cm | Q2: 35.20-37.70cm | Q3: 37.70-40.70cm | Q4: ≥ 40.70cm | P-value |
|--------------------------|--------|---------------|-------------------|------------------|---------------|---------|
| **Number**               | 20214  | 4923          | 4982              | 5178             | 5131          |         |
| **Age (years)**          | 45.80 ± 20.21 | 48.96 ± 23.25 | 46.27 ± 20.80    | 45.27 ± 18.84   | 42.83 ± 17.11 | <0.001  |
| **Male (n,%)**           | 9652 (47.75%) | 1849 (37.56%) | 2365 (47.47%)    | 2826 (54.58%)   | 2612 (50.91%) | <0.001  |
| **Drinking (gm)**        | 8.64 ± 27.74 | 7.59 ± 28.73  | 8.82 ± 27.70     | 9.84 ± 27.81    | 8.26 ± 26.69  | <0.001  |
| **Smoking status (n,%)** |        |               |                   |                  |               |         |
| **Non-smoker**           | 9351 (51.83%) | 2151 (50.60%) | 2249 (51.10%)    | 2447 (51.87%)   | 2504 (53.61%) | <0.001  |
| **Ex-smoker**            | 4745 (26.30%) | 1005 (23.64%) | 1179 (26.79%)    | 1306 (27.68%)   | 1255 (26.87%) |         |
| **Current smoker**       | 3945 (21.87%) | 1095 (25.76%) | 973 (22.11%)     | 965 (20.45%)    | 912 (19.52%)  |         |
| **Race (n,%)**           |        |               |                   |                  |               | <0.001  |
| **Black**                | 4282 (21.18%) | 840 (17.06%)  | 965 (19.37%)     | 1070 (20.66%)   | 1407 (27.42%) |         |
| **Mexican American**     | 4743 (23.46%) | 1521 (30.90%) | 1317 (26.44%)    | 1143 (22.07%)   | 762 (14.85%)  |         |
|                          | Other Hispanic | White | Other race | BMI (kg/m²) | SBP (mmHg) | DBP (mmHg) | FBG (mg/dl) | TC (mg/dl) | HDL-C (mg/dl) | TG (mg/dl) | LDL-C (mg/dl) | eGFR (ml/min/1.73m²) | Hypertension (n,%) | Cardiovascular diseases (n,%) | Diabetes (n,%) | Antihypertensive drugs (n,%) |
|--------------------------|----------------|-------|------------|-------------|------------|------------|-------------|------------|----------------|------------|----------------|------------------------|---------------------|-----------------------------|----------------|---------------------------|
|                          | 831 (4.11%)    | 9588 (47.43%) | 242 (4.92%) | 2073 (42.11%) | 2297 (46.11%) | 2568 (49.59%) | 2650 (51.65%) | 28.11 ± 6.33 | 22.73 ± 3.37 | 25.70 ± 3.48 | 28.61 ± 3.81 | 35.03 ± 6.26 | <0.001 | 6620 (32.89%) | 756 (4.21%)    | 2377 (11.83%) | 3934 (19.46%) |
|                          | 242 (4.92%)    |     | 230 (4.62%) | 205 (3.96%) | 173 (3.47%) | 192 (3.71%) | 158 (3.08%)   | 247 (5.02%) | 173 (3.47%) | 154 (3.00%) | 154 (3.00%) |             |          | 1534 (31.38%) | 216 (5.12%)    | 593 (12.10%) | 989 (20.09%)  |
|                          | 230 (4.62%)    |     | 205 (3.96%) | 173 (3.47%) | 192 (3.71%) | 158 (3.08%) | 154 (3.00%)   | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) |             |          | 1500 (30.29%) | 187 (4.26%)    | 524 (10.58%) | 938 (18.83%)  |
|                          | 205 (3.96%)    |     | 173 (3.47%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%)   | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) |             |          | 1643 (31.79%) | 199 (4.23%)    | 576 (11.18%) | 948 (18.31%)  |
|                          | 173 (3.47%)    |     | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%)   | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) |             |          | 1943 (37.96%) | 154 (3.31%)    | 684 (13.42%) | 1059 (20.64%) |
|                          | 154 (3.00%)    |     | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%)   | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) | 154 (3.00%) |             |          |                 |              |               | 0.010       |

BMI (kg/m²): The mean BMI ± standard deviation for each group. SBP (mmHg): Systolic Blood Pressure ± standard deviation. DBP (mmHg): Diastolic Blood Pressure ± standard deviation. FBG (mg/dl): Fasting Blood Glucose ± standard deviation. TC (mg/dl): Total Cholesterol ± standard deviation. HDL-C (mg/dl): High Density Lipoprotein ± standard deviation. TG (mg/dl): Triglycerides ± standard deviation. LDL-C (mg/dl): Low Density Lipoprotein ± standard deviation. eGFR (ml/min/1.73m²): Estimated Glomerular Filtration Rate ± standard deviation. Hypertension (n,%) and Cardiovascular diseases (n,%) refer to the number and percentage of patients with hypertension and cardiovascular diseases, respectively. Antihypertensive drugs (n,%) indicate the number and percentage of patients treated with antihypertensive drugs.
|                                | n    | %    | n    | %    | n    | %    | n    | %    | p     |
|--------------------------------|------|------|------|------|------|------|------|------|-------|
| **Anti-diabetic drugs(n,%)**   | 1175 | 5.81 | 300  | 6.09 | 253  | 5.08 | 287  | 5.54 | 335   | 6.53  | 0.011 |
| **All-cause mortality(n,%)**   | 3655 | 18.08| 1394 | 28.32| 920  | 18.47| 764  | 14.75| 577   | 11.25 | <0.001|
| **Cardiovascular mortality(n,%)** | 466  | 2.31 | 212  | 4.31 | 97   | 1.95 | 87   | 1.68 | 70    | 1.36  | <0.001|
| **Cerebrovascular mortality(n,%)** | 152  | 0.75 | 66   | 1.34 | 42   | 0.84 | 25   | 0.48 | 19    | 0.37  | <0.001|

Values are means ± SD or numbers of patients (%). Q-quartile, BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure, FBG-fasting blood glucose, TC-total cholesterol, HDL-C-high density lipoprotein-cholesterol, LDL-C-low density lipoprotein-cholesterol, TG-triglyceride, eGFR-estimated glomerular filtration rate.
Table II. Cox regression analysis for all-cause, cardiovascular and cerebrovascular disease mortality

| Exposure                              | Non-adjusted | Model 1 | Model 2 |
|---------------------------------------|--------------|---------|---------|
| All-cause mortality                    | HR(95%CI), P-value | HR(95%CI), P-value | HR(95%CI), P-value |
| Calf Circumference (per 1cm increment) | 0.90 (0.90, 0.91), <0.0001 | 0.91 (0.90, 0.92), <0.0001 | 0.92 (0.90, 0.94), <0.0001 |
| Calf circumference groups(cm)         |              |         |         |
| Q1                                    | 1.0          | 1.0     | 1.0     |
| Q2                                    | 0.61 (0.56, 0.67), <0.0001 | 0.66 (0.61, 0.73), <0.0001 | 0.70 (0.60, 0.81), <0.0001 |
| Q3                                    | 0.48 (0.44, 0.53), <0.0001 | 0.57 (0.51, 0.63), <0.0001 | 0.64 (0.54, 0.75), <0.0001 |
| Q4                                    | 0.36 (0.33, 0.40), <0.0001 | 0.49 (0.43, 0.57), <0.0001 | 0.50 (0.40, 0.64), <0.0001 |
| P for trend                            | <0.001       | <0.001  | <0.001  |
| Cardiovascular mortality               |              |         |         |
| Calf Circumference (per 1cm increment) | 0.88 (0.86, 0.90), <0.0001 | 0.87 (0.84, 0.91), <0.0001 | 0.90 (0.84, 0.97), 0.0056 |
| Calf circumference groups(cm)         |              |         |         |
| Q1                                    | 1.0          | 1.0     | 1.0     |
| Q2                                    | 0.43 (0.34, 0.54), <0.0001 | 0.48 (0.37, 0.62), <0.0001 | 0.34 (0.21, 0.56), <0.0001 |
| Q3                                    | 0.36 (0.28, 0.47), <0.0001 | 0.44 (0.33, 0.60), <0.0001 | 0.38 (0.22, 0.64), 0.0003 |
| Q4                                    | 0.29 (0.22, 0.38), <0.0001 | 0.41 (0.28, 0.62), <0.0001 | 0.43 (0.21, 0.88), 0.0205 |
| P for trend                            | <0.001       | <0.001  | 0.045   |
Cerebrovascular mortality

| Calf Circumference (per 1cm increment) | Non-adjusted | Adjusted for age, gender and BMI. | Adjusted for age, gender, BMI, SBP, TC, HDL-C, antihypertensive drugs, anti-diabetic drugs, eGFR, drinking, serum albumin, smoking status, race, diabetes, hypertension, cardiovascular diseases. |
|---------------------------------------|--------------|----------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Q1                                    | 0.86 (0.83, 0.90) | 0.90 (0.84, 0.96) | 0.91 (0.80, 1.03) |
| P for trend                           | <0.0001      | 0.0028                           | 0.1201 |
| Q2                                    | 0.59 (0.40, 0.88) | 0.76 (0.49, 1.17) | 0.64 (0.28, 1.46) |
| P for trend                           | 0.0085       | 0.2118                           | 0.2893 |
| Q3                                    | 0.34 (0.21, 0.53) | 0.55 (0.32, 0.94) | 0.89 (0.37, 2.16) |
| P for trend                           | <0.0001      | 0.0293                           | 0.8052 |
| Q4                                    | 0.25 (0.15, 0.42) | 0.52 (0.25, 1.10) | 0.70 (0.19, 2.56) |
| P for trend                           | <0.0001      | 0.0862                           | 0.5943 |
| P for trend                           | <0.001       | 0.377                           | 0.602 |

Non-adjusted adjusted for none.
Model 1 was adjusted for age, gender and BMI.
Model 2 was adjusted for age, gender, BMI, SBP, TC, HDL-C, antihypertensive drugs, anti-diabetic drugs, eGFR, drinking, serum albumin, smoking status, race, diabetes, hypertension, cardiovascular diseases.

Q-quartile, HR-hazard ratio, CI-confidence interval.
Table III. Subgroup analysis for calf circumference with all-cause and cardiovascular mortality

|                      | All-cause mortality | Cardiovascular mortality |
|----------------------|---------------------|-------------------------|
|                      | Number              | HR(95%CI), P-value      | P interaction |
| Gender               |                     |                         |               |
| Male                 | 9652                | 0.89 (0.88, 0.90) <0.0001 | 0.87 (0.84, 0.90) <0.0001 |
| Female               | 10562               | 0.90 (0.89, 0.91) <0.0001 | 0.86 (0.83, 0.89) <0.0001 |
| Age                  |                     |                         |               |
| <50                  | 11856               | 0.96 (0.94, 0.98) <0.0001 | 0.99 (0.92, 1.08) 0.8686 |
| >=50                 | 8358                | 0.91 (0.90, 0.92) <0.0001 | 0.89 (0.87, 0.91) <0.0001 |
| Diabetes             |                     |                         |               |
| No                   | 17978               | 0.89 (0.88, 0.90) <0.0001 | 0.88 (0.85, 0.90) <0.0001 |
| Yes                  | 2080                | 0.92 (0.91, 0.94) <0.0001 | 0.88 (0.84, 0.92) <0.0001 |
| Hypertension         |                     |                         |               |
| No                   | 14210               | 0.88 (0.87, 0.90) <0.0001 | 0.79 (0.76, 0.82) <0.0001 |
| Yes                  | 5914                | 0.90 (0.89, 0.91) <0.0001 | 0.90 (0.88, 0.93) <0.0001 |
| BMI                  |                     | <0.0001                 | 0.0005        |
| <25                  | 6888                | 0.80 (0.79, 0.82) <0.0001 | 0.74 (0.70, 0.78) <0.0001 |
| >=25                 | 13122               | 0.88 (0.87, 0.89) <0.0001 | 0.88 (0.85, 0.91) <0.0001 |
| SBP                  |                     |                         |               |
| <140                 | 15470               | 0.92 (0.91, 0.93) <0.0001 | 0.89 (0.86, 0.92) <0.0001 |
| >=140                | 612                 | 0.90 (0.86, 0.93) <0.0001 | 0.86 (0.78, 0.94) 0.0012 |
| Smoking              |                     |                         |               |
| Non-smoker           | 9351                | 0.90 (0.88, 0.91) <0.0001 | 0.88 (0.85, 0.91) <0.0001 |
| Ex-smoker            | 4745                | 0.89 (0.87, 0.90) <0.0001 | 0.85 (0.82, 0.89) <0.0001 |
| Current smoker       | 3945                | 0.90 (0.88, 0.92) <0.0001 | 0.87 (0.82, 0.92) <0.0001 |
When analysis a subgroup variable, age, gender, BMI, SBP, TC, HDL-C, antihypertensive
drugs, anti-diabetic drugs, eGFR, serum albumin, drinking, smoking status, race; diabetes,
hypertension, cardiovascular diseases were all adjusted except the variable itself.

HR-hazard ratio, CI-confidence interval, BMI-body mass index, SBP-systolic blood pressure.
Subjects were from the 1999–2006 National Health and Nutrition Examination Survey (n=41474)

Excluded:
Aged <18 years (n=18850)
Missing calf circumference (n=2389)
Missing mortality data (n=21)

Enrolled analysis (n=20214)

Fig. 1 Procedure of selecting participants.
Fig. 2 The Kaplan-Meier survival curves plotting quartiles of calf circumference. Legend: Q, quartile, m, month.
Fig. 3 Cubic spline of hazard ratios for all-cause and cause specific mortality associated with increasing calf circumference.

Legend: Age, gender, BMI, SBP, TC, HDL-C, antihypertensive drugs, anti-diabetic drugs, eGFR, serum albumin, drinking, smoking status, race, diabetes, hypertension, cardiovascular diseases were all adjusted.
CI, confidence interval, A, association of calf circumference (cm) and hazard ratios of all-cause mortality, B, association of calf circumference (cm) and hazard ratios of cardiovascular mortality, C, association of calf circumference (cm) and hazard ratios of cerebrovascular mortality.