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Low to moderate toenail arsenic levels in young adulthood and incidence of diabetes later in life: findings from the CARDIA Trace Element study

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Abstract

Some studies suggest a positive association between arsenic exposure and risk of diabetes. However, the findings are inconsistent and inconclusive, particularly at a low to moderate arsenic exposure level, and longitudinal data are lacking. We examined toenail arsenic at low to moderate level in young adulthood in relation to incidence of diabetes later in life. This study included 4,102 black and white participants aged 20–32 at baseline (1987–88) who completed up to 7 follow-up exams through 2015–16. Toenail arsenic was measured by collision-cell inductively-coupled-plasma mass-spectrometry. Incident diabetes was defined as fasting glucose ≥126 mg/dL, non-fasting glucose ≥200 mg/dL, 2-hour postchallenge glucose ≥200 mg/dL, hemoglobin A1c ≥6.5%, or use of glucose-lowering medications. Cox proportional hazards model and generalized estimating equations (GEEs) were used to determine the associations of quintiles of toenail arsenic with incident diabetes and other metabolic parameters. The median (inter-quartile range) toenail

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Duality of Interest

No potential conflicts of interest relevant to this article were reported.

arsenic levels was 0.097 (0.065–0.150) ppm in this study. During the follow-up period, 599 incident cases of diabetes were identified. After adjustment for potential confounders, the hazards ratio (95% confidence interval) was 0.96 (0.73, 1.27) (*P* for linear trend=0.85) comparing the highest to the lowest quintile of toenail arsenic levels. No significant association was observed between toenail arsenic and levels of fasting glucose, insulin, homeostatic model assessment of insulin resistance, homeostatic model assessment of beta cell function, or C-reactive protein. The null associations persisted across subgroups of age, sex, race, and body mass index. Findings from this longitudinal study do not support the hypothesis that low to moderate toenail arsenic levels in young adulthood is associated with diabetes risk later in life.

Keywords

Arsenic; Insulin resistance; Incidence of diabetes; Toenail; Young adulthood

1. Introduction

Arsenic is a naturally occurring toxic metalloid (metal-like element) in the environment [1]. It exists in both organic and inorganic forms, and its health effects mainly result from its inorganic forms as arsenite (iAsIII) and arsenate (iAsV). Humans are exposed to arsenic mainly through food, water, and air, in which arsenic level depends on its environmental background value, and human activities (such as mining, coal-fired power plants, smelting operations, pesticide use, etc.). The arsenic in soil and water could enter into food supply when food crops are growing or food are processed and prepared. Marine products, followed by grains, legumes, and seeds, are the major food sources that contribute to arsenic exposure in the general population [2, 3]. High levels of arsenic are present in the groundwater of many countries, such as Bangladesh [4] and China [5].

Diabetes is a serious public health issue worldwide [6]. Although lifestyle, diet, and genetic factors are major determinants of diabetes risk, studies have indicated that environmental factors are also important determinants in the diabetes epidemic. Evidence from *in vitro* and *in vivo* studies indicates that arsenic alters signal transduction and transcription factors that are related to insulin pathways and impairs pancreatic beta cell function, particularly insulin synthesis and secretion [7, 8]. Although some studies suggest that the risk of diabetes is higher among those who have a high level of arsenic exposure through drinking water in arsenic-contaminated areas or through occupational contact [9, 10], findings are inconsistent and inconclusive in populations with low to moderate arsenic exposure. Two cross-sectional studies did not find statistically significant differences in plasma/urine arsenic levels between patients with diabetes and the healthy controls [11, 12]. In contrast, two other cross-sectional studies [13, 14] and one nested case-control study [15] reported a positive association between arsenic exposure and the risk of diabetes. Also, one prospective cohort study conducted among 2,203 Utah residents with a 19-year follow-up found that the arsenic exposure index, derived from the length of residence and the median arsenic level of drinking water in the community, was not associated with diabetes incidence or mortality [16]. Of note, longitudinal data linking low to moderate arsenic exposure measured at the individual level to the incidence of diabetes are lacking.

To measure arsenic exposure among individuals, biomarkers are required, because of the dietary and non-dietary exposure and the large variation of arsenic content in food and water. Blood and urine are widely used as markers of arsenic exposure. However, they may not be reliable markers for chronic arsenic exposure since arsenic only remains in blood for 10 hours and in urine for 96 hours after exposure cessation [17]. Hair is also used as a marker of arsenic exposure, but it is prone to be contaminated by the environment because of its high surface/volume ratio, which prevents it from being a reliable biomarker [18]. By contrast, toenail arsenic provides a relatively longer measure of body exposure as compared to arsenic levels in the blood or urine, and it has been identified as a reliable and long-term biomarker of arsenic exposure even for quantifying low exposure [19, 20].

Therefore, our objective was to examine arsenic exposure measured in toenail clippings from young adulthood in relation to incidence of diabetes later in life using data from the Coronary Artery Risk Development in Young Adults (CARDIA) study.

2. METHODS

2.1 Study Design

The CARDIA study is a multicenter, longitudinal cohort study that was originally designed to explore risk factors for the development of cardiovascular disease. In 1985–86 (Y0), 5,114 African-American and white men and women (aged 18–30 years) were recruited from 4 cities in the US: Birmingham, Alabama; Chicago, Illinois; Minneapolis, MN; and Oakland, CA. Participants were reexamined 2 (1987–88), 5 (1990–91), 7 (1992–93), 10 (1995–96), 15 (2000–01), 20 (2005–06), 25 (2010–11), and 30 (2015–16) years after baseline. The detailed study design and recruitment protocol were published previously [21]. The study was approved by the Institutional Review Boards of the participating centers.

CARDIA exam Y2 (1987–88) was the baseline of the present study since toenail samples were collected at that time. Of the 4,623 participants who attended exam Y2, 4,361 provided toenail clippings. We excluded individuals with diabetes at baseline (n=11) and missing values on key covariates [body mass index (BMI) (n=2), glucose (n=62), lipid profile (n=6), alcohol consumption (n=1), and family history of diabetes (n=6)], and another 171 participants without sufficient information for defining incident diabetes over the follow-up period. A total of 4,102 participants remained in the final analysis after these exclusions.

2.2 Assessment of toenail arsenic

Toenail clippings were collected and stored at room temperature and ambient humidity. Detailed procedure of toenail collection has been published elsewhere [22]. All samples were washed with deionized water in a sonicator. Toenail levels of arsenic and cadmium were measured by collision-cell inductively-coupled-plasma mass-spectrometry (CC-ICP-MS), and mercury was quantified by instrumental neutron-activation analysis (INAA) at the University of Missouri-Columbia Research Reactor Center. The samples were assayed randomly by laboratory personnel who were blinded to other measures. The detection limit for arsenic was 0.01 ppm. The coefficient of variation of duplicated subsamples from the same participants was 8.8%. In a validation study among 127 pre- and postmenopausal

women, the correlation coefficient for the reproducibility of toenail arsenic over 6 years apart was 0.54 [23].

2.3 Measurements of glucose, insulin, HbA1c, OGTT, HOMA-IR, HOMA-β, and CRP

Plasma glucose concentrations were measured at exam Y0, Y7, Y10, Y15, Y20, Y25, and Y30 using the hexokinase method and were recalibrated to assure comparability of plasma glucose across examinations. Two-hour plasma glucose levels from OGTT were measured at exam Y10, Y20, and Y25 in both genders and at exam Y30 only in women. Fasting plasma insulin was analyzed with a radioimmunoassay kit (Linco Research Inc., St. Charles, MO) at exam Y7, Y10, Y15, Y20, Y25, and Y30. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated with the equation: glucose (mmol/L) × insulin (mU/L)/22.5. HOMA beta cell function (HOMA-β) was computed with the equation: (20×insulin) / (glucose − 3.5). Hemoglobin Alc (HbA1c) was assessed using a Tosoh G7 high-performance liquid chromatography instrument (Tosoh Bioscience) at exam Y20, Y25, and Y30, and the inter-assay coefficient of variations were 2.0–3.0%. Serum high-sensitivity C-reactive protein (*hs*-CRP) level was measured in samples collected at exam Y7, Y15, Y20, and Y25 using a new enzyme-linked immunosorbent assay method improved with a nephelometry-based high-throughput assay that offers greater sensitivity and reproducibility [24].

2.4 Ascertainment of incident diabetes cases

During any follow-up examination, incident cases of diabetes were identified by demonstrating any one of the following: 1) fasting plasma glucose ≥7.0mmol/L (126 mg/dL); 2) non-fasting plasma glucose ≥11.1mmol/L (200 mg/dL); 3) postprandial 2-h plasma glucose ≥11.1 mmol/L from an oral glucose tolerance test (OGTT); 4) HbA1c ≥ 6.5%; or 5) reported use of glucose-lowering medications, which were verified by drug names [21].

2.5 Assessment of other covariates at baseline

CARDIA measurements at baseline have previously been reported in detail [21]. Briefly, body weight was measured with a calibrated balance-beam scale, and height was measured with a vertical ruler. BMI was calculated as weight in kilograms divided by the square of height in meters. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured 3 times by sphygmomanometer, and the average of the second and third measurements was recorded.

A self-administered questionnaire was used to collect demographic information, including age, sex, race, and education level. Smoking status was determined based on self-report as current, former, or never smoker. Alcohol consumption was assessed through a self-administered questionnaire. Diet was assessed by an interview-based diet history questionnaire, which was evaluated previously [25, 26]. Physical activity represented by the amount of time per week spent in 13 categories of physical activities was assessed by the CARDIA Physical Activity History Questionnaire [27]. Family history of diabetes was defined as either of the parents having diabetes.

Fasting blood samples were collected following standardized procedures, and those biomarkers with fasting time less than 8 hours were replaced with missing values. Plasma lipid profiles, including total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol, were measured at central laboratories [21].

2.6 Statistical analysis

According to quintiles of toenail arsenic levels, participants were divided into five groups. Differences in baseline characteristics were compared across groups by using analysis of variance, the chi-squared test, or the Kruskal-Wallis equality-of-populations rank test, as appropriate.

Cox proportional hazards regression models were used to estimate the association of toenail arsenic levels with the incidence of diabetes. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated for the 2nd to 5th quintiles of toenail arsenic levels with the lowest quintile as the reference. Potential confounding variables were adjusted sequentially in multiple models (age, sex, race, study center, baseline glucose, BMI, education, smoking status, alcohol consumption, physical activity, and family history of diabetes). A few sensitivity analyses were conducted to ensure the robustness of the results.

In addition, generalized estimating equations (GEEs) with an exchangeable correlation structure were used to examine the associations of toenail arsenic concentrations with repeated measurements of fasting glucose, insulin, HOMA-IR, and HOMA-β, respectively [28]. Suppose that Y_{ij} is the jth (j=1, …, t) response (fasting glucose, insulin, HOMA-IR, or HOMA-β) of subject i (i=1, …, n) at time j, $\boldsymbol{X}$ is a p×1 vector of toenail arsenic as well as other covariates, and the marginal expectation of Y_{ij} is μ_{ij} [$E(Y_{ij}) = \mu_{ij}$], then the marginal model that relates μ_{ij} to a linear combination of the covariates can be written as:

$$g\left(\mu_{ij}\right) = \boldsymbol{X}'\boldsymbol{\beta}$$

where $\boldsymbol{\beta}$ is unknown p×1 vector of regression coefficients and g(.) is known as linkage function, which is log linkage in this study. A logarithmic transformation was used to improve the normality of distribution of these outcome variables. In GEE modelling, the quasi-likelihood estimate of $\boldsymbol{\beta}$ is obtained by solving a set of p "quasi-score" differential equations [28]. To explore the potential mechanisms, we assessed the association between arsenic and *hs*-CRP by using GEE, considering that arsenic may increase the risk of diabetes via the inflammatory pathway [29]. Moreover, we investigated whether pre-specified factors, including age, sex, race, and BMI, modified the associations of interest. Interaction terms between the exposure and these potential effect modifiers were created, and their significance was examined by using the likelihood ratio test, comparing the models with and without the interaction term.

All the analyses were conducted by using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina). Two-sided P values were reported, and a $P \leq 0.05$ was considered statistically significant.

3. Results

The median toenail arsenic levels were 0.047, 0.071, 0.097, 0.135, and 0.237 ppm from the lowest to the highest quintile, respectively (Table 1). Compared with those in the lowest quintile of toenail arsenic levels, participants in the highest quintile were slightly younger; more likely to be males, white, and current smokers; physically more active; and less likely to have a family history of diabetes. Those in the highest quintile also had lower levels of education, BMI, waist circumference, and LDL cholesterol and higher levels of SBP.

During the 28 years of follow-up (82,765.50 person years), 599 participants developed diabetes. After adjustment for potential confounders, toenail arsenic levels were not significantly associated with incidence of diabetes. The multivariable adjusted HR (95% CI) of diabetes comparing the highest to the lowest quintile of toenail arsenic was 0.96 (0.73, 1.27) (*P* for linear trend=0.85) (Table 2). In sensitivity analyses, the results were not appreciably altered when adjusted for cumulative averages for BMI, education, physical activity, and alcohol intake instead of baseline variables. Also, the results did not change materially after further adjustment for other dietary and non-dietary variables, such as blood pressure, cholesterol levels, long-chain omega-3 fatty acids/seafood, and other heavy metals, e.g., mercury and cadmium. In addition, we conducted a propensity score (PS) matching analysis for all the covariates at baseline in our final model within a caliper of 0.2*standard deviation of logit(PS), and ran Cox proportional hazards model with consideration of the paired nature of the data [30,31], the results essentially remained.

Similarly, no significant associations were observed between toenail arsenic levels and fasting glucose, insulin, HOMA-IR, HOMA-β, and *hs*-CRP with adjustment for potential cofounders (Table 3).

In stratified analyses by pre-specified factors, the observed null associations were not appreciably modified by age, sex, race, BMI, or smoking status (data not shown).

4. Discussion

In this 28-year longitudinal follow-up study, we did not find statistically significant associations of toenail arsenic levels with incidence of diabetes, insulin resistance, and beta cell function as well as inflammatory marker *hs*-CRP among African-American and white young adults. Results from this study do not support that toenail arsenic in young adulthood is associated with diabetes incidence later in life.

4.1 Comparison with findings from other studies

There is a growing body of research documenting an elevated risk of diabetes among people living in arsenic-contaminated areas [32, 33]. However, much of this research has been carried out in areas of the world where the arsenic level tends to be much higher than the guideline (<10 μg/L for drinking water) issued by the US Environmental Protection Agency (EPA) [34] and the World Health Organization [35]. In the present study, the median toenail arsenic concentration is approximately 0.10 ppm, which is close to the median level (0.12 ppm) among individuals exposed to drinking water with an average arsenic concentration of

2.72 μg/L [36]. In addition, for populations who are not heavily exposed, arsenic levels in nails are generally less than 0.50 ppm, and the average level is approximately 0.10 ppm [37]. Thus, participants in the present study were considered having a low to moderate level of arsenic exposure.

Studies conducted in the general population at low to moderate levels of arsenic exposure have produced inconsistent and inconclusive results on arsenic exposure and diabetes risk. In 2006, a systematic review examined 4 case-control studies in general populations from countries with low to moderate arsenic exposure and did not find a significant association between arsenic exposure and risk of diabetes [32]. In 2011, the US National Toxicology Program (NTP) reviewed the literature and concluded that the available evidence was insufficient to determine an association between low to moderate arsenic exposure and risk of diabetes [38]. In 2014, a meta-analysis reported an overall positive association between arsenic levels in drinking water and risk of diabetes based on 12 cross-sectional studies, 3 case-control studies, and 2 cohorts [39]. However, the association was substantially attenuated and was not significant at low to moderate levels of arsenic exposure.

Of note, the majority of previous research comprises cross-sectional or case-control studies. One small case-cohort study found a modest positive association when the arsenic level in drinking water was ≥20 μg/L [40]. Similarly, another large cohort study reported a modest positive association between arsenic exposure in drinking water and diabetes risk. However, the association was substantially attenuated and was not statistically significant after excluding possible “false positive” diabetes cases [41]. Notably, both studies did not have data on baseline glucose levels, which might have confounded their results. In addition, these two studies estimated the time-weighted average arsenic exposure using arsenic data from either drinking or groundwater by combining the information of residential and employment history as well as school location and diet information (if available). As noted by the authors, the assessment did not take other major sources of arsenic exposure into account, such as food, inhalation of dust or soil, or tobacco products. One study conducted in Michigan, USA, found that more than 1/3 of arsenic exposure was from food sources [3]. Another study conducted in Mexico found that the correlation coefficient between arsenic in water and in urine changed from 0.50 to 0.35 with or without adjustment for the amount of water intake [42]. Thus, arsenic exposure derived from drinking or groundwater at the community level may not accurately reflect individual arsenic exposure. This measurement error might substantially bias the findings in the previous studies.

Two recent prospective studies [43, 44] reported the associations between arsenic metabolites in urine and incident diabetes, indicating that increased MMA% (or decreased iAs%, or DMA%) was associated with lower HOMA-IR, and lower MMA%, was related to elevated incidence of diabetes. Consistent with the results from the present study, one study also found no significant association between urinary arsenic levels and incident diabetes [44]. This discrepancy in the correlations between different arsenic metabolites and incident diabetes merits further investigation.

4.2 Strengths and limitations

To the best of our knowledge, this is the longest prospective follow-up study measuring arsenic biomarker to assess its association with diabetes risk and metabolic parameters. In particular, toenail arsenic was employed as a relatively longer measure of individual-level arsenic exposure mainly from drinking water and diet as compared to arsenic levels in other biospecimens, e.g., blood or urine. Also, CARDIA participants were followed for 28 years from young adulthood to midlife with standardized protocols and quality control procedures for data collection. Additionally, the CARDIA study included a large sample size, balanced by sex and race, from 4 geographic regions in the US, which enabled us to conduct stratified analyses. The low to moderate level of arsenic exposure observed in the CARDIA study is comparable to reported low to moderate arsenic exposure levels in the general US population living in urban area.

One possible concern is that arsenic speciation was not assessed in toenails. However, toenails have been suggested to preferentially sequester inorganic arsenic and its metabolites [45]. For example, study in low to moderate arsenic exposure populations has indicated that toenail arsenic is correlated with concentrations of inorganic arsenic and its metabolites [46], supporting that toenail arsenic well-captures inorganic arsenic exposure. Notably, toenail arsenic level may not be a measure of the absolute inorganic arsenic exposure. However, toenail arsenic levels are generally proportional to the levels of inorganic arsenic exposure so that we are able to rank participants based on toenail/inorganic arsenic levels and calculate the relative risk or examine the association. On the other hand, in a sensitivity analysis, the observed associations were not appreciably changed with adjustment for fish/seafood consumption, a major source of organic arsenic exposure [47]. Another possible concern is that toenails were only collected at baseline, which is common in a large prospective cohort study because of budget constraint. The single measurement may attenuate any possible association due to non-differential variation. Of note, after using propensity score matching at baseline variables, our main findings and conclusion remained. Nevertheless, we acknowledge that baseline only measurement limits our ability to take changes in arsenic exposure into account. An individual’s arsenic exposure status is generally stable as long as his/her residence does not change. However, participants in this study, who were centered at 4 field centers at baseline, had spread across all 50 states by year 30. Therefore, a cofounding “effect” from the residence change over years could not be excluded without considering detailed geographic markers of residence. However, when we further limited our analysis to those who showed greatest residential stability, the conclusion remained. In addition, type of diabetes could not be clearly defined in the present study because some participants were young at diagnosis. However, the majority of the incident cases are likely to be type 2 diabetes, given that the majority of diabetes is this classification and the average age of participants was 27 at baseline in 1987. When we excluded 44 incident diabetes cases that occurred in the first five years of follow-up, the results were not appreciably altered. Moreover, participants with high arsenic exposure at baseline tended to have lower BMI, higher alcohol consumption, and less likely to have family history of diabetes. Although we have adjusted for those potential confounders in the model, the residual cofounding could not be completely ruled out. To further make sure our results are robust, we conducted a

propensity score matching at baseline covariates, the results were essentially the same. Furthermore, using the following equation provided by Moon *et al.* [48]:

$$Water\ arsenic(\mu g/L) = 10^{\wedge}(1.4 + 0.9 * log10(toenail\ arsenic, ppm))$$

we estimated that 20 μg/L arsenic in drink water is approximately equivalent to 0.776 ppm in toenails, which is 98.8th percentile among participants in the present study. Therefore, we do not have sufficient power to estimate the association with relatively high level of arsenic exposure, e.g., equivalent to 20 μg/L arsenic in drink water[40].

4.3 Generalizability of the findings

As mentioned, arsenic exposure in the US general population living at urban area is considered to be at a low to moderate level. Thus, the finding from this study is incapable of excluding any possible association between high levels of arsenic exposure and incidence of diabetes. Also, the findings do not overturn the common perception about the toxic effects of arsenic.

5. Conclusion

In summary, toenail arsenic levels in young adulthood was not significantly associated with incident diabetes, insulin resistance, or beta cell function later in life. The observed null association remained consistent across subgroups of age, sex, race, and BMI. Since a randomized clinical trial is not feasible for studying the health impact of arsenic exposure, additional longitudinal cohort studies with biomarkers for arsenic species and its metabolites among individuals in the general population are warranted.

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Abbreviations

CARDIA	Coronary Artery Risk Development in Young Adults study
HOMA-IR	Homeostatic model assessment of insulin resistance
HOMA-β	HOMA beta cell function
CRP	C-reactive protein
SBP	Systolic blood pressure

DBP	Diastolic blood pressure
GEEs	Generalized estimating equations

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Highlights

- The median toenail arsenic levels were 0.047, 0.071, 0.097, 0.135, and 0.237 ppm from the lowest to the highest quintile among the participants.
- No significant association was observed between toenail arsenic and levels of fasting glucose, insulin, homeostatic model assessment of insulin resistance, homeostatic model assessment of beta cell function, or C-reactive protein.
- Findings from this prospective cohort study do not support the hypothesis that low to moderate toenail arsenic levels in young adulthood is associated with diabetes risk later in life.

Table 1.

Baseline characteristics of the study population by quintiles of toenail arsenic levels, the CARDIA study, 1987 to 2015 (n=4,102)[a]

Characteristics	Total	Quintiles of toenail arsenic levels					*P* value *
		1 (lowest)	2	3	4	5 (highest)	
No. of participants	4,102	821	819	820	822	820	NA
Toenail arsenic, ppm	0.097 (0.065–0.150)	0.047 (0.038–0.054)	0.071 (0.065–0.077)	0.097 (0.089–0.105)	0.135 (0.122–0.150)	0.237 (0.195–0.341)	NA
Toenail cadmium, ppm	0.009 (0.004–0.019)	0.006 (0.004–0.014)	0.007 (0.004–0.015)	0.009 (0.004–0.019)	0.009 (0.005–0.020)	0.011 (0.006–0.028)	<0.0001
Toenail mercury, ppm	0.215 (0.125–0.375)	0.186 (0.108–0.317)	0.220 (0.130–0.374)	0.233 (0.131–0.404)	0.232 (0.131–0.387)	0.211 (0.126–0.371)	<0.0001
Toenail mass, g	0.024±0.013	0.022±0.012	0.023±0.013	0.025±0.014	0.026±0.014	0.025±0.014	<0.0001
Age, year	27.0±3.6	27.0±3.8	27.3±3.5	27.1±3.6	26.8±3.5	26.7±3.6	<0.01
Female, %	54.8	69.3	59.2	53.2	50.4	41.7	<0.0001
African-American, %	47.8	61.0	49.5	45.0	42.3	41.0	<0.0001
Education, year	14.2±2.4	14.3±2.2	14.3±2.3	14.4±2.4	14.0±2.4	14.0±2.4	<0.01
Current smoker, %	20.5	13.7	17.4	18.4	25.2	28.0	<0.0001
Alcohol, g/day	5.1 (0.0–15.6)	0.0 (0.0–6.8)	3.0 (0.0–11.2)	5.4 (0.0–16.2)	8.1 (0.0–22.0)	9.5 (0.0–24.3)	<0.0001
Physical activity score exercise units	319 (168–533)	248 (120–445)	306 (178–513)	332 (163–535)	348 (196–573)	365 (196–599)	<0.0001
BMI, kg/m^2	25.2±5.3	26.1±6.2	25.4±5.8	25.1±5.2	24.8±4.9	24.5±4.4	<0.0001
Waist circumference, cm	79.7±12.1	80.6±13.9	79.6±12.2	79.6±11.8	79.4±11.5	79.5±10.7	0.27
SBP, mmHg	107.8±10.7	107.2±10.8	106.7±10.4	107.6±10.3	108.1±11.3	109.4±10.7	<0.0001
DBP, mmHg	67.5±9.5	67.7±9.5	67.3±9.5	67.1±9.4	67.1±9.4	68.3±9.8	0.03
Total cholesterol, mg/dL	182.7±34.5	184.0±33.3	183.1±33.7	184.4±34.6	181.8±34.1	180.2±36.5	0.08
Triglycerides, mg/dL	77.4±48.5	73.8±41.2	76.0±45.4	77.3±51.7	78.9±49.6	81.0±53.3	0.03
HDL cholesterol, mg/dL	54.7±13.9	54.0±13.1	54.3±13.5	55.2±14.1	55.1±14.3	54.6±14.6	0.34
LDL cholesterol, mg/dL	112.7±32.7	115.3±31.7	113.7±32.0	114.0±32.1	111.0±32.6	109.6±35.0	<0.01
Glucose, mg/dL	82.1±12.1	81.3±10.4	81.6±14.4	81.8±8.7	83.4±16.0	82.4±8.7	<0.01
Family history of diabetes, %	13.5	16.1	11.6	15.4	12.4	12.1	0.02
Dietary intake							
Magnesium, mg/day	387.2±196.5	332.9±160.5	366.3±190.0	396.5±195.0	414.3±211.7	426.6±215.3	<0.0001
LCω_3PUFA, g/day	0.12±0.18	0.10±0.12	0.12±0.24	0.12±0.18	0.12±0.17	0.11±0.15	0.06

Abbreviations: BMI, body mass index; CARDIA, Coronary Artery Risk Development in Young Adults; HDL, high-density lipoprotein; DBP, diastolic blood pressure; IQR, inter-quartile range; LCω_3PUFAs, long-chain omega-3 polyunsaturated fatty acids; LDL, low-density lipoprotein; NA, not applicable; SBP, systolic blood pressure; SD, standard deviation.

[a] Data are means ±SDs, medians (IQRs), or proportions, unless otherwise specified.

[*] *P*-values were for difference across quintiles of toenail arsenic levels using analysis of variance, chi-squared test, or Kruskal-Wallis equality-of-populations rank test.

Table 2.

Multivariable-adjusted HRs (95% CIs) of diabetes by quintiles of toenail arsenic levels, the CARDIA study, 1987 to 2015 (n=4,102)[a]

	Quintiles of toenail arsenic levels					
	1 (lowest)	2	3	4	5 (highest)	*P* for linear trend*
Arsenic, ppm	<0.0593	0.0593–0.0827	0.0828–0.1119	0.1120–0.1691	≥0.1692	
No. of participants	821	819	820	822	778	NA
No. of events	149	122	122	111	95	NA
Incidence rate,/1000 person years	8.96	7.22	7.32	6.70	5.93	
Model 1[b]	1 (Ref.)	0.85 (0.67, 1.08)	0.93 (0.73, 1.18)	0.84 (0.65, 1.08)	0.77 (0.59, 1.01)	0.08
Model 2[c]	1 (Ref.)	0.95 (0.75, 1.22)	1.05 (0.82, 1.35)	1.00 (0.77, 1.29)	0.90 (0.69, 1.19)	0.48
Model 3[d]	1 (Ref.)	0.95 (0.75, 1.22)	1.04 (0.81, 1.33)	1.00 (0.77, 1.30)	0.96 (0.73, 1.27)	0.85

Abbreviations: BMI, body mass index; CARDIA, Coronary Artery Risk Development in Young Adults; CI, Confidence interval; HR, Hazard ratio; LCω3PUFA, long-chain omega-3 polyunsaturated fatty acids; LDL, low-density lipoprotein; NA, not applicable.

[a] All models were constructed using the Cox proportional hazards regression analysis.

[b] Model 1: adjustment for age (continuous), sex, race, study center, and baseline glucose levels (continuous).

[c] Model 2: model 1 with additional adjustment for baseline BMI (continuous).

[d] Model 3: model 2 with additional adjustment for education (<12, 12, 12.1–15.9, 16, or ≥16 years), current smoking status (yes or no), alcohol consumption (0, 0.1–9.9, 10.0–19.9, or ≥20 g/day), physical activity (quintiles), and family history of diabetes (yes or no).

* *P* for linear trend was examined by using medians of toenail arsenic across its quintiles in the models.

Table 3.

Multivariable-adjusted associations [beta coefficients (95% CIs)] between toenail arsenic concentrations and levels of fasting glucose and insulin, HOMA-IR and HOMA beta cell function, as well as hs-CRP, the CARDIA study, 1987 to 2015 (n=3,666)[a]

	Quintiles of toenail arsenic levels					
	1 (lowest)	**2**	**3**	**4**	**5 (highest)**	***P* for linear trend***
Arsenic, ppm	<0.060	0.060–0.083	0.084–0.113	0.114–0.171	≥0.172	NA
No. of participants	733	734	733	733	733	NA
No. of observations	3,237	3,303	3,255	3,213	3,127	NA
Ln (Glucose, mg/dL)[b]	0 (Ref.)	−0.001 (−0.009, 0.007)	0.003 (−0.005, 0.012)	0.001 (−0.008, 0.009)	0.008 (−0.002, 0.017)	0.070
Ln (Insulin, μU/mL)[b]	0 (Ref.)	−0.013 (−0.054, 0.028)	−0.020 (−0.061, 0.021)	0.004 (−0.039, 0.046)	0.001 (−0.043, 0.045)	0.579
Ln (HOMA-IR)[b]	0 (Ref.)	−0.012 (−0.057, 0.033)	−0.009 (−0.054, 0.037)	0.008 (−0.039, 0.056)	0.015 (−0.034, 0.064)	0.280
Ln (HOMA-β)[b]	0 (Ref.)	−0.009 (−0.048, 0.031)	−0.027 (−0.067, 0.014)	0.002 (−0.039, 0.044)	−0.013 (−0.055, 0.029)	0.810
Ln (hs-CRP, μg/mL)[c]	0 (Ref.)	0.014 (−0.082, 0.109)	−0.032 (−0.128, 0.064)	0.007 (−0.093, 0.107)	0.002 (−0.099, 0.103)	0.934

Abbreviations: BMI, body mass index; CARDIA, Coronary Artery Risk Development in Young Adults; CI, Confidence interval; HOMA-β, homeostatic model assessment of beta cell function index, HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high sensitivity C-reactive protein; NA, not applicable.

[a] All of the models were constructed by using generalized estimating equations.

[b] Participants were excluded if they took antidiabetic medication, their fasting levels were <8 h, or they were pregnant women. The adjusted variables included age (continuous), sex, race (African American or Caucasian), study center, baseline glucose (continuous), baseline BMI (continuous), education (<12, 12, 12.1–15.9, 16, or ≥16 years), current smoking status (yes or no), alcohol consumption (0, 0.1–9.9, 10.0–19.9, or ≥20 g/day), physical activity (quintiles), family history of diabetes (yes or no), and measurement years (year 7, 10, 15, 20, 25, or 30).

[c] The analysis was conducted with a sample size of 3,963. Participants were excluded if they had no baseline arsenic; had arthritis, asthma, or any other reported allergic diseases at baseline; did not have hs-CRP measured at any follow-up examination; or were pregnant women. The adjusted variables included age (continuous), sex, race (African American or Caucasian), study center, baseline BMI (continuous), education (<12, 12, 12.1–15.9, 16, or ≥16 years), current smoking status (yes or no), alcohol consumption (0, 0.1–9.9, 10.0–19.9, or ≥20 g/day), physical activity (quintiles), and measurement years (year 7, 15, 20, 25, or 30).

[*] *P* for linear trend was examined by using medians of toenail arsenic across its quintiles in the models.