BMJ Open

Analyzing the relationship between occupational exposure of heavy metals and diabetes type 2 diabetes in large-scale cohort.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039541
Article Type:	Original research
Date Submitted by the Author:	18-Apr-2020
Complete List of Authors:	Ji, Jun Ho ; Samsung Changwon Hospital, Internal medicine Jin, Mi Hyeon ; Samsung Changwon Hospital Kang, Jung-Hun ; Gyeongsang National University College of Medicine, Internal Medicine Lee, Soon Il ; Dankook University College of Medicine, Internal Medicine Lee, Suee ; Dong-A University Medical Center, Internal medicine Kim, Sung-Hyun ; Dong-A University Medical Center, Internal Medicine Oh, Sung Yong; Dong-A University Medical Center
Keywords:	Diabetes & endocrinology < INTERNAL MEDICINE, SOCIAL MEDICINE, EPIDEMIOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Analyzing the relationship between occupational exposure of heavy metals and diabetes type 2 diabetes in large-scale cohort.

Jun Ho Ji[1], Mi Hyeon Jin[2], Jung-Hun Kang[3], Soon Il Lee[4],

Suee Lee[5], Sung-Hyun Kim[5], Sung Yong Oh[5#]

[1]Department of Internal Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea;

[2]Department of Biostatistics, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea;

[3]Department of Internal Medicine, Gyeongsang National University Scholl of Medicine, Jinju, Korea;

[4]Department of Internal Medicine, Dankook University College of Medicine, Cheonan;

[5]Department of Internal Medicine, Dong-A University College of Medicine, Busan

Running title: Relationship between heavy metal exposure and diabetes

Word count: 2974

Corresponding author: Sung Yong Oh

Address: Department of Internal Medicine, Dong-A University College of Medicine, 26 Daesingongwon-Ro, Seo-Gu, Busan 49201, Korea

Mobile: +82-10-8624-9818

E-mail:drosy@dau.ac.kr

Abstract

Objectives: We investigated the association between heavy-metal exposure and serum ferritin, physical measurements, and type 2 diabetes (DM).

Design: Retrospective longitudinal cohort study.

Setting: Changwon, the location of the study, is a representative industrial city in Korea. Data was based on the medical checkups at single secondary hospital between 2002 and 2018.

Participants: There were included 34,814 subjects; of these, 1,035 with lead exposure were grouped as cohort A, 200 with cadmium exposure as cohort B, and the remaining 33,579 as the control cohort. Data including age, HbA1c, fasting glucose, ferritin, height, weight, follow-up duration, and blood level of heavy metals (lead and cadmium) within one year from exposure were collected.

Interventions: Medical data including age, HbA1c, fasting glucose, ferritin, height, weight, follow-up duration, and blood level of heavy metals (lead and cadmium) within one year from exposure were collected.

Results: In cohort A, DM was diagnosed in 33 subjects, and 1,002 subjects were not diagnosed with DM; there was a significant difference in lead concentration (3.94 ± 2.92 versus 2.81 ± 2.03, $p = 0.002$) between subjects diagnosed with DM and those without DM during the follow-up period. Simple exposure to lead and cadmium was not found to be associated with DM in Cox regression models (lead exposure, hazard ratio [HR] 1.02 (0.60-1.76), $p = 0.930$; cadmium exposure, HR 1.23 (0.51-2.93) $p = 0.646$). Annual changes in fasting blood glucose according to the concentration of lead at the beginning of exposure showed a weak positive correlation (R = 0.072, $p = 0.032$).

Conclusion: Our findings demonstrate that simple occupational exposure to lead or cadmium is not associated with prevalence of DM, but lead concentration at the beginning of exposure may be an indicator of DM and glucose elevation.

Keywords: diabetes, heavy-metal exposure, HbA1c, body mass index, ferritin

Strengths and limitations of this study

- This cohort study was conducted on data from mega-sized population.
- It was carried out in one institution in a consistent laboratory test manner with long serial follow up.
- It was retrospective medical data review.
- Because of the possibility of iron deficiency during menstruation, female subjects were excluded, and young subjects who had low incidence of DM were included, mainly because of the occupational characteristics of a workplace with metal exposure.

Introduction

Diabetes mellitus (DM), a common and rising global problem, is one of the leading causes of death, blindness, and chronic renal failure, and a major risk factor for vascular diseases, such as myocardial infarction, stroke, and peripheral vascular disease. The increase in social costs because of DM-related morbidity or mortality has intensified the efforts to reduce the incidence of DM. The rising incidence of DM is considered to be associated with alterations in lifestyle and other contributing factors, including exposure to several environmental pollutants and industrial chemicals. With the recent, rapid industrial development, exposure levels to various environmental toxic materials have risen alongside DM incidence. These environmental substances causing endocrine disruptions have been defined as endocrine-disrupting chemicals (EDC) by the U.S. Environmental Protection Agency (EPA) [1]. Metals are naturally existing inorganic elements, present in very small amounts in the body, and are essential for vital processes. Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. Heavy metals and metalloids (e.g., lead, mercury, cadmium, and metalloid arsenic) may have hormonal activity, suggesting that these compounds are EDCs as well as more generalized toxicants. These heavy metals have negative effects on physiology and may be associated with the incidence of DM in some populations. In this study, we particularly focused on the association between heavy metals and DM. In recent decades, the environmental exposure of heavy metals has been declining, because industrialization has already occurred, and many countries have begun to pay attention to environmental problems rather than to the development of industry. However, there is the possibility of natural exposures in the environment, such as exposure to heavy metals in older households, exposure through drinking water as in the case of Flint, Michigan, in the United States [2], and exposure because of illegal, unauthorized disposal of toxic materials, including heavy metals from industries. In Korea, occupational exposures are more common than are random environmental exposures. For occupational heavy-metal exposures, there were relatively few studies reporting on whether the degree of exposure is direct or indirect, on changes in the body after exposure, or on the influence of the exposure on specific diseases. A few population-based studies have focused on the association between metal exposure and diabetes, but the existing studies were not consistent [3-9]. Most previous studies have examined the association of DM with heavy-metal concentrations in blood

or urine at one specific moment [6, 7]. Intense exposure to heavy metals results in high levels in the blood or urine, whereas light exposure results in extremely low levels. Therefore, long-term light exposure to heavy metals leads to low levels of heavy metals in the blood or urine, and heavy metals deposited in the organs may be harmful. Deposition of heavy metals in the liver and pancreas alters gluconeogenesis in the liver, and insulin secretion maybe affected as well, eventually influencing the incidence of DM. Although this study was designed as a retrospective study of long-term occupational heavy-metal (lead, cadmium) exposure, instead of measuring the concentration of heavy metals in organs, such as the liver, bone and pancreas, we measured the concentration of heavy metals in the blood during the beginning of exposure (within 1one year) and compared the changes in fasting glucose, HbA1c, and incidence of DM with those of the general population who were not exposed to heavy metals during the same period.

Material and Methods

1) Study population

Changwon, the location of the study, is a representative industrial city in Korea, with many occupations involving heavy-metal exposure, including battery-manufacturing plants. This cohort study was based on data from the general population of 403,253 who underwent medical checkups at Samsung Changwon Hospital between 2002 and 2018. The schematic flow chart for the selection of subjects is shown in Fig 1. All participants underwent a physical exam, blood sampling in the morning following an overnight fast, and filled out a questionnaire. Among the 403,253 subjects, 89,826 who had taken a blood test for ferritin were included, and 38,039 women were excluded. In the occupational screening, most of the women were fertile, and the results of ferritin may be inaccurate because of menstruation. In all, 269 subjects were excluded because of unavailability of HbA1c and fasting blood glucose (FBS) data. Furthermore, 2709 subjects who were already diagnosed with DM were excluded (DM was defined as FBS ≥ 126 mg/dl, HbA1c ≥ 6.5%, or history of DM in the questionnaire). Additionally, 28,151 subjects were excluded, because only one screening result was available without follow-up data. Finally, 34,814 subjects were included in the analysis. Of these, 1,035 subjects with lead exposure were grouped as cohort A, 200 subjects with cadmium exposure as cohort 2, and the remaining 33,579 as the control group. This study collected subjects' data, including age, HbA1c, FBS, ferritin, height, body weight, follow-up duration, and concentration of heavy metals (lead and cadmium). The study's protocol was approved by the Samsung Changwon Medical Center institutional review board (SCMC-2019-04-014).

2) Measurement and collection of lead and cadmium in the blood

For the measurement of lead and cadmium concentrations, 3 ml of blood samples from each subject were collected in vacuum bottles using heparin as the anticoagulant in the morning following an overnight fast. Blood samples were diluted 1:15 and 1:10 for the measurement of lead and cadmium concentrations, respectively, with 2.5 ml of 10% Triton X-100, 0.1 ml of concentrated nitric acid, and 1 ml of 10% ammonium di-hydrogen phosphate as a modifier. Graphite-furnace atomic absorption

spectrometry with Zeeman background correction (PinAAcle 9i00z Atomic absorption spectrometer, PerkinElmer, USA) was used for measuring lead and cadmium levels, which in all subjects were measured within the first year of heavy-metal exposure.

3) Statistical analyses

Continuous variables are presented as means ± standard deviation and categorical variables as the number of cases and percentage. An independent t-test was used for evaluating the significance of mean differences between continuous variables for demographical factors, such as age and body mass index (BMI). Of the baseline characteristics, the Cox proportional hazard model was used to identify potential predictors of type 2 DM in subjects who were not diagnosed with DM. The exposure levels of lead and cadmium in consecutive blood tests were set as dependent variables, and FBS and HbA1c were set as independent variables. The mixed model was used to assess the effects of heavy-metal exposure and ferritin on FBS and HbA1c, respectively. The annual change of FBS and HbA1c with the concentration of lead is shown in a scatter plot. Stata 14.0 software (Stata Corporation, College Station, TX, USA) was used in all statistical analysis.

Results

1) Baseline characteristics of the study

The baseline characteristics of each cohort are shown in Table 1. Of the 34,818 subjects, 1034 were diagnosed with DM during the follow-up, and 33,780 were not diagnosed with DM. In cohort A, which included 1035 lead-exposed subjects, 1,034 were confirmed to have DM, and of the 1,034, 33 were exposed to lead. In the control group without heavy-metal exposure, age, HbA1c, FBS and ferritin were associated with DM, as we already know. In the heavy-metal-exposed subjects, only HbA1c, FBS, and BMI were significantly associated with DM. An interesting aspect in cohort A is that the concentration of the initial lead exposure (within one year) was significantly higher in subjects who were later diagnosed with DM (2.81 ± 2.03 in non-diabetes and 3.94 ± 2.92 in diabetes, p = 0.002). In contrast, early blood levels of cadmium exposure did not differ between the group with subjects progressing to diabetes and that with subjects not progressing to diabetes. The follow-up period was shorter, and the mean age was higher in the subjects progressing to diabetes in both cohorts.

2) Risk of developing DM from lead/cadmium exposure and serum ferritin

Cox-regression models showed crude and adjusted hazard ratios of variables predicting the development of DM (Table 2). Age, HbA1c, FBS, BMI, and ferritin were considered to be predictors of developing DM in both crude and adjusted, but simple exposure to lead and cadmium was not associated with DM. Ferritin level had a positive relationship with FBS and HbA1c elevation during the follow-up period in both cohorts A and B (Figure 2-A. 2-D, Figure 3-A, 3-D). The FBS elevation of subjects with simple lead exposure showed a slower pattern than did those without lead exposure (Figure 2-B). However, in HbA1c elevation, simple lead exposure did not have a significant effect (Figure 2-E). The result of the early exposure to cadmium did not differ from that of lead. In cohort B, ferritin also had a significant effect on the rate of elevation of FBS and HbA1c (Figure 3-A, Figure 3-D), and the early exposure to cadmium was positively correlated with the rate of FBS change, but negatively correlated with HbA1c change (Figure 3-B, Figure 3-E).

The unusual finding in both the cohorts was that all subjects were healthy without DM at the time of

enrollment, but subjects with elevated ferritin and heavy-metal exposure had higher baseline values of FBS and HbA1c than did those who did not (Figure 2-C. 2-F Figure 3-C, 3-F).

Regarding the concentration of heavy metals, the annual variation of FBS according to the initial concentration of lead showed a weak but positive correlation. (0.072 of R, p = 0.032, Figure 4.)

Discussion

Many studies have attempted to explain the relationship between heavy-metal exposure and hyperglycemia. There are several plausible hypotheses in the background of such research; first, oxidative stress by heavy metals directly damages the beta cells of the pancreas, leading to elevated serum glucose levels [10-17], and this oxidative stress may also increase blood glucose by decreasing insulin release, impairing insulin receptors, disrupting the glucose uptake, increasing hepatic gluconeogenesis and pancreatic glucagon secretion, and decreasing peripheral glucose use [16, 18-22]. Another hypothesis is about competitive inhibition of the toxic metals, which states that essential trace metals at normal levels play a key role in glucose homeostasis, because those are essential cofactors in glucose metabolism, pancreatic beta cell function, and the insulin signaling cascade [18, 19, 23, 24]. Toxic metals compete with these essential metals for various physiological functions and affect type 2 DM risk [25, 26]. It has also been reported that the toxic metals affect various substances, including GLUT4 (glucose transporter type 4), NF- κB (nuclear factor kappa B), MAPK (mitogen-activated protein kinases), and PI3K (phosphoinositide 3-kinase) involved in insulin signaling, thereby increasing the risk of DM [27-31]. The last hypothesis is that exposure to metals, especially heavy metals, increases body weight, as reported by population base studies. Because weight gain is a known risk factor for DM, exposure to heavy metals may be associated with DM [32-36]. Many studies on the relationship between heavy-metal exposure and DM, performed based on these findings, have shown inconsistent results [3-9]. It can be inferred that the direct association between heavy metals and DM has not been confirmed until now and, even if relevant, is very weak. Prior epidemiologic studies that could explain the reported inconsistent results connecting heavy metals to DM had limitations. Most previous studies were based on cross-sectional designs [3-5, 7-9]. A cross-sectional study is characterized by analysis carried out at a specific point in time and does not reflect the change over time. In the case of heavy-metal exposure, chronic long-time exposure is more common than is acute exposure. Therefore, the time of exposure to heavy metals is important, and the elapsed time since the first exposure should be also considered. A Chinese study reported that insulin secretion decreased more in the group exposed to cadmium for more than 10 years than in the group

exposed to cadmium for less than 10 years [37]. Next, previous studies were conducted with a case-control design [3, 9, 38, 39]. As is well known, a small case-control study tends to be less expensive and is shorter in duration, but it is placed low in the hierarchy of evidence.

This study investigated the relationship between serum ferritin, exposure to heavy metals, and DM during health screening in subjects who worked in battery, paint, and bullet manufacturing facilities, shipyards, or workplaces requiring welding. Although this study included data from a single institution, it was designed as a retrospective longitudinal study using a large number of health screening subjects and overcomes the limitations of prior studies. The following results are reported in the study. (1) Simple exposure to heavy metals did not increase the risk of developing DM over time, but the concentration of lead at the time of initial lead exposure was higher in subjects diagnosed with DM later on;

(2) Serum ferritin was a predictor of DM as previously reported [40], but serum ferritin was not a predictor of DM in subjects exposed to lead or cadmium; (3) The high blood lead concentration at the beginning of lead exposure was proportional to the rate of increase in FBS per year. It is noteworthy that when the blood lead concentration measured within a year after exposure is high, the rate of FBS rises gradually with time. A high blood lead concentration means that the lead exposure intensity is strong, and so the exposure intensity of lead may be a risk factor for DM. This aligns with our other study result, in which simple exposure to heavy metals is not related to the incidence of DM or the elevation of FBS/HbA1c. Concentration of heavy metals in our cohort was slightly higher than that of normal Korean adults in the demographic study on environmental exposure of heavy metals by Kim et al. [41]. This suggests that our cohort was occupationally exposed to heavy metals, but the intensity was not high enough to significantly affect the incidence of DM. Similar to our results, a Korean study demonstrated that low-dose lifetime environmental exposures to lead and cadmium may not affect the incidence of DM. Another interesting aspect of this study can be observed in Table 1. In the lead- or cadmium-exposed group, serum ferritin levels in the diabetic group were significantly higher than in the non-diabetic group, but not in the subjects exposed to lead or cadmium, serum ferritin was lower in the diabetic group. The reason for these results cannot be explained exactly, but we think that oxidative stress through the formation of free radicals [12-16,18], which is a mechanism by which heavy metals

cause DM, may be the same mechanism behind iron causing DM [42, 43]. Some large-scale U.S studies have shown that persistent organic pollutants (POPs), which are not heavy metals but are bio-accumulating as heavy metals are, with chronic environmental exposure becoming a global problem, pose an increased risk for DM in terms of blood levels [44]. The mechanism by which POPs induce DM is similar to that in heavy metals [45, 46], and just as for heavy metals, studies on the association of POPs with DM are discrepant [47-49].

The current findings should be interpreted with caution because of several limitations. Since the study was based on data from subjects undergoing health checkups, we could not identify and analyze risk factors of DM, including hypertension, family history, and dyslipidemia. The second limitation is that the concentration of heavy metals in the blood is measures only once at the beginning of exposure. Follow-up observation, such as diagnosis of DM was done longitudinally, but it did not reflect changes in serum heavy-metal concentrations as in the cross-sectional study. The limited population of our study cohort is the next limitation. Because of the possibility of iron deficiency during menstruation, female subjects were excluded, and young subjects who had low incidence of DM were included, mainly because of the occupational characteristics of a workplace with metal exposure. In conclusion, our findings demonstrated that simple exposure to lead or cadmium is not associated with prevalence of DM, but the blood lead concentration at the beginning of exposure may be an indicator of DM prevalence and glucose elevation. We suggest that low-dose, chronic occupational exposures to lead or cadmium may not affect the incidence of DM, but if the exposure intensity is high, screening for DM should be done.

A competing interests statement: The authors have no conflicts of interest to disclose.

Authors' contribution:

Conception or design: JHJ

Acquisition, analysis, or interpretation of data: JHJ

Drafting the work or revising: JHJ,MHJ,JHK,SIL,SL,SHK,SYO

Final approval of the manuscript: JHJ,MHJ,JHK,SIL,SL,SHK,SYO

A funding statement: This study was supported by Dong-A University Research fund 2020.

= Figure legends =

Figure 1. Schematic flow diagram

Figure 2. Mixed models were used to evaluate the effects of lead exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort A

B – Changes in fasting blood glucose according to lead exposure in cohort A

C – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort A

D – Changes in HbA1c according to serum ferritin levels in cohort A

E – Changes in HbA1c according to lead exposure in cohort A

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort A

Figure 3. Mixed models were used to evaluate the effects of cadmium exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort B

B – Changes in fasting blood glucose according to lead exposure in cohort B

C – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort B

D – Changes in HbA1c according to serum ferritin levels in cohort B

E – Changes in HbA1c according to lead exposure in cohort B

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort B

Figure 4. Scatter plot showing the annual changes of FBS by lead concentration (R=0.072, p = 0.032)

= Table legends =

Table1. Baseline characteristics

Table2. Cox regression models: Crude and adjusted HRs of baseline characteristics predicting the development of type 2 diabetes mellitus

Table1. Baseline characteristics

	Lead (Cohort A)					
	No exposure (n=33,779)			Exposure (n=1,035)		
	Non diabetes (n=32,778)	Diabetes (n=1,001)	P-value	Non diabetes (n=1,002)	Diabetes (n=33)	P-value
Age	34.99 ± 7.99	37.96 ± 8.16	<0.001	32.18 ± 8.36	34.19 ± 7.92	0.174
HbA1c (%)	5.32 ± 0.30	5.76 ± 0.59	<0.001	5.33 ± 0.29	5.88 ± 0.81	<0.001
Fasting blood sugar (mg/dL)	89.98 ± 8.65	103.49 ± 18.90	<0.001	91.37 ± 9.11	114.36 ± 32.71	<0.001
Ferritin (ng/mL)	145.71 ± 93.76	165.55 ± 119.90	<0.001	152.51 ± 99.86	139.77 ± 89.57	0.470
Body mass index (Kg/m²)	24.04 ± 3.03	25.89 ± 3.54	<0.001	24.04 ± 3.17	26.04 ± 2.87	<0.001
Lead concentration (mg/dL)	-	-	-	2.81 ± 2.03	3.94 ± 2.92	0.002
Follow-up duration (year)	5.65 ± 3.48	5.09 ± 3.67	<0.001	4.78 ± 2.77	3.18 ± 3.63	0.001
	Cadmium (Cohort B)					
	No exposure (n=34,614)			Exposure (n=200)		
	Non diabetes (n=33,591)	Diabetes (n=1,023)	P-value	Non diabetes (n=189)	Diabetes (n=11)	P-value
Age	34.91 ± 8.02	37.84 ± 8.19	<0.001	34.77 ± 8.28	38.04 ± 7.22	0.203
HbA1c (%)	5.32 ± 0.30	5.76 ± 0.60	<0.001	5.31 ± 0.31	5.65 ± 0.89	0.002
Fasting blood sugar (mg/dL)	90.01 ± 8.66	103.83 ± 19.55	<0.001	91.52 ± 9.43	104.00 ± 22.05	<0.001
Ferritin (ng/mL)	146.03 ± 94.00	165.27 ± 119.32	<0.001	124.24 ± 81.76	113.93 ± 85.87	0.686
Body mass index (Kg/m²)	24.04 ± 3.04	25.88 ± 3.53	<0.001	24.00 ± 3.20	26.78 ± 2.67	0.005
Cadmium concentration (mg/dL)	-	-	-	0.20 ± 0.26	0.17 ± 0.11	0.731
Follow-up duration (year)	5.61 ± 3.46	5.02 ± 3.67	<0.001	6.96 ± 3.77	5.45 ± 4.76	0.207

Table2. Cox regression models: Crude and adjusted HRs of baseline characteristics predicting the development of type 2 diabetes mellitus

	Crude		Adjusted	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Age	1.05 (1.04-1.06)	<0.001	1.02 (1.01-1.03)	0.001
HbA1c × 10	1.54 (1.51-1.57)	<0.001	1.38 (1.34-1.41)	<0.001
Fasting blood sugar	1.12 (1.11-1.12)	<0.001	1.07 (1.06-1.08)	<0.001
Body mass index	1.21 (1.19-1.22)	<0.001	1.11 (1.08-1.13)	<0.001
Ferritin (reference: <200)	2.25 (1.94-2.62)	<0.001	1.51 (1.30-1.77)	<0.001
Lead exposure	1.05 (0.68-1.63)	0.812	1.02 (0.60-1.76)	0.930
Cadmium exposure	1.08 (0.54-2.17)	0.828	1.23 (0.51-2.93)	0.646

References

1. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC: **Endocrine-disrupting chemicals: an Endocrine Society scientific statement**. *Endocr Rev* 2009, **30**(4):293-342.
2. Hanna-Attisha M, LaChance J, Sadler RC, Champney Schnepp A: **Elevated Blood Lead Levels in Children Associated With the Flint Drinking Water Crisis: A Spatial Analysis of Risk and Public Health Response**. *Am J Public Health* 2016, **106**(2):283-290.
3. Feng W, Cui X, Liu B, Liu C, Xiao Y, Lu W, Guo H, He M, Zhang X, Yuan J *et al*: **Association of urinary metal profiles with altered glucose levels and diabetes risk: a population-based study in China**. *PloS one* 2015, **10**(4):e0123742.
4. Menke A, Guallar E, Cowie CC: **Metals in Urine and Diabetes in U.S. Adults**. *Diabetes* 2016, **65**(1):164-171.
5. Barregard L, Bergstrom G, Fagerberg B: **Cadmium exposure in relation to insulin production, insulin sensitivity and type 2 diabetes: a cross-sectional and prospective study in women**. *Environmental research* 2013, **121**:104-109.
6. Hansen AF, Simic A, Asvold BO, Romundstad PR, Midthjell K, Syversen T, Flaten TP: **Trace elements in early phase type 2 diabetes mellitus-A population-based study. The HUNT study in Norway**. *J Trace Elem Med Biol* 2017, **40**:46-53.
7. Moon SS: **Association of lead, mercury and cadmium with diabetes in the Korean population: the Korea National Health and Nutrition Examination Survey (KNHANES) 2009-2010**. *Diabet Med* 2013, **30**(4):e143-148.
8. Borne Y, Fagerberg B, Persson M, Sallsten G, Forsgard N, Hedblad B, Barregard L, Engstrom G: **Cadmium exposure and incidence of diabetes mellitus--results from the Malmo Diet and Cancer study**. *PloS one* 2014, **9**(11):e112277.
9. Forte G, Bocca B, Peruzzu A, Tolu F, Asara Y, Farace C, Oggiano R, Madeddu R: **Blood metals concentration in type 1 and type 2 diabetics**. *Biol Trace Elem Res* 2013, **156**(1-3):79-90.
10. Gerber PA, Rutter GA: **The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus**. *Antioxid Redox Signal* 2017, **26**(10):501-518.
11. Kaneto H, Katakami N, Kawamori D, Miyatsuka T, Sakamoto K, Matsuoka TA, Matsuhisa M, Yamasaki Y: **Involvement of oxidative stress in the pathogenesis of diabetes**. *Antioxid Redox Signal* 2007, **9**(3):355-366.
12. Kubisch HM, Wang J, Bray TM, Phillips JP: **Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic beta-cells against oxidative stress**. *Diabetes* 1997, **46**(10):1563-1566.
13. Yen CC, Lu FJ, Huang CF, Chen WK, Liu SH, Lin-Shiau SY: **The diabetogenic effects of the combination of humic acid and arsenic: in vitro and in vivo studies**. *Toxicol Lett* 2007,

172(3):91-105.

14. Das KK, Das SN, Dhundasi SA: **Nickel, its adverse health effects & oxidative stress**. *Indian J Med Res* 2008, **128**(4):412-425.
15. Izquierdo-Vega JA, Soto CA, Sanchez-Pena LC, De Vizcaya-Ruiz A, Del Razo LM: **Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite**. *Toxicol Lett* 2006, **160**(2):135-142.
16. Valko M, Morris H, Cronin MT: **Metals, toxicity and oxidative stress**. *Curr Med Chem* 2005, **12**(10):1161-1208.
17. Kurata Y, Katsuta O, Doi T, Kawasuso T, Hiratsuka H, Tsuchitani M, Umemura T: **Chronic cadmium treatment induces islet B cell injury in ovariectomized cynomolgus monkeys**. *Jpn J Vet Res* 2003, **50**(4):175-183.
18. Chen YW, Yang CY, Huang CF, Hung DZ, Leung YM, Liu SH: **Heavy metals, islet function and diabetes development**. *Islets* 2009, **1**(3):169-176.
19. Khan AR, Awan FR: **Metals in the pathogenesis of type 2 diabetes**. *J Diabetes Metab Disord* 2014, **13**(1):16.
20. Sharma B, Singh S, Siddiqi NJ: **Biomedical implications of heavy metals induced imbalances in redox systems**. *Biomed Res Int* 2014, **2014**:640754.
21. Beyersmann D, Hartwig A: **Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms**. *Arch Toxicol* 2008, **82**(8):493-512.
22. Kajimoto Y, Matsuoka T, Kaneto H, Watada H, Fujitani Y, Kishimoto M, Sakamoto K, Matsuhisa M, Kawamori R, Yamasaki Y *et al*: **Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells**. *Diabetologia* 1999, **42**(12):1417-1424.
23. Kaur B, Henry J: **Micronutrient status in type 2 diabetes: a review**. *Adv Food Nutr Res* 2014, **71**:55-100.
24. Siddiqui K, Bawazeer N, Joy SS: **Variation in macro and trace elements in progression of type 2 diabetes**. *ScientificWorldJournal* 2014, **2014**:461591.
25. Ahamed M, Siddiqui MK: **Environmental lead toxicity and nutritional factors**. *Clin Nutr* 2007, **26**(4):400-408.
26. Flora SJ: **Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure**. *Oxid Med Cell Longev* 2009, **2**(4):191-206.
27. Walton FS, Harmon AW, Paul DS, Drobna Z, Patel YM, Styblo M: **Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes**. *Toxicol Appl Pharmacol* 2004, **198**(3):424-433.
28. Han JC, Park SY, Hah BG, Choi GH, Kim YK, Kwon TH, Kim EK, Lachaal M, Jung CY, Lee W: **Cadmium induces impaired glucose tolerance in rat by down-regulating GLUT4 expression in adipocytes**. *Arch Biochem Biophys* 2003, **413**(2):213-220.
29. Somwar R, Koterski S, Sweeney G, Sciotti R, Djuric S, Berg C, Trevillyan J, Scherer PE,

Rondinone CM, Klip A: **A dominant-negative p38 MAPK mutant and novel selective inhibitors of p38 MAPK reduce insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting GLUT4 translocation**. *The Journal of biological chemistry* 2002, **277**(52):50386-50395.

30. Souza K, Maddock DA, Zhang Q, Chen J, Chiu C, Mehta S, Wan Y: **Arsenite activation of P13K/AKT cell survival pathway is mediated by p38 in cultured human keratinocytes**. *Molecular medicine* 2001, **7**(11):767-772.
31. Zawalich WS, Zawalich KC: **A link between insulin resistance and hyperinsulinemia: inhibitors of phosphatidylinositol 3-kinase augment glucose-induced insulin secretion from islets of lean, but not obese, rats**. *Endocrinology* 2000, **141**(9):3287-3295.
32. Leasure JL, Giddabasappa A, Chaney S, Johnson JE, Jr., Pothakos K, Lau YS, Fox DA: **Low-level human equivalent gestational lead exposure produces sex-specific motor and coordination abnormalities and late-onset obesity in year-old mice**. *Environmental health perspectives* 2008, **116**(3):355-361.
33. Faulk C, Barks A, Sanchez BN, Zhang Z, Anderson OS, Peterson KE, Dolinoy DC: **Perinatal lead (Pb) exposure results in sex-specific effects on food intake, fat, weight, and insulin response across the murine life-course**. *PloS one* 2014, **9**(8):e104273.
34. Nie X, Wang N, Chen Y, Chen C, Han B, Zhu C, Chen Y, Xia F, Cang Z, Lu M *et al*: **Blood cadmium in Chinese adults and its relationships with diabetes and obesity**. *Environmental science and pollution research international* 2016, **23**(18):18714-18723.
35. Rothenberg SE, Korrick SA, Fayad R: **The influence of obesity on blood mercury levels for U.S. non-pregnant adults and children: NHANES 2007-2010**. *Environmental research* 2015, **138**:173-180.
36. Padilla MA, Elobeid M, Ruden DM, Allison DB: **An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02**. *International journal of environmental research and public health* 2010, **7**(9):3332-3347.
37. Lei LJ, Jin TY, Zhou YF: **[The effects of cadmium on the levels of insulin in smelters]**. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2006, **24**(1):3-6.
38. Serdar MA, Bakir F, Hasimi A, Celik T, Akin O, Kenar L, Aykut O, Yildirimkaya M: **Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose**. *Int J Diabetes Dev Ctries* 2009, **29**(1):35-40.
39. Afridi HI, Kazi TG, Brabazon D, Naher S, Talpur FN: **Comparative metal distribution in scalp hair of Pakistani and Irish referents and diabetes mellitus patients**. *Clin Chim Acta* 2013, **415**:207-214.
40. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB: **Body iron stores in relation to risk of type 2 diabetes in apparently healthy women**. *JAMA* 2004, **291**(6):711-717.

41. Kim NS, Lee BK: **National estimates of blood lead, cadmium, and mercury levels in the Korean general adult population**. *Int Arch Occup Environ Health* 2011, **84**(1):53-63.

42. Andrews PA: **Disorders of iron metabolism**. *N Engl J Med* 2000, **342**(17):1293; author reply 1294.

43. Oberley LW: **Free radicals and diabetes**. *Free Radic Biol Med* 1988, **5**(2):113-124.

44. Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr.: **A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002**. *Diabetes Care* 2006, **29**(7):1638-1644.

45. Hectors TL, Vanparys C, van der Ven K, Martens GA, Jorens PG, Van Gaal LF, Covaci A, De Coen W, Blust R: **Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function**. *Diabetologia* 2011, **54**(6):1273-1290.

46. Enan E, Liu PC, Matsumura F: **2,3,7,8-Tetrachlorodibenzo-p-dioxin causes reduction of glucose transporting activities in the plasma membranes of adipose tissue and pancreas from the guinea pig**. *The Journal of biological chemistry* 1992, **267**(28):19785-19791.

47. Longnecker MP, Michalek JE: **Serum dioxin level in relation to diabetes mellitus among Air Force veterans with background levels of exposure**. *Epidemiology* 2000, **11**(1):44-48.

48. Henriksen GL, Ketchum NS, Michalek JE, Swaby JA: **Serum dioxin and diabetes mellitus in veterans of Operation Ranch Hand**. *Epidemiology* 1997, **8**(3):252-258.

49. Steenland K, Piacitelli L, Deddens J, Fingerhut M, Chang LI: **Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin**. *J Natl Cancer Inst* 1999, **91**(9):779-786.

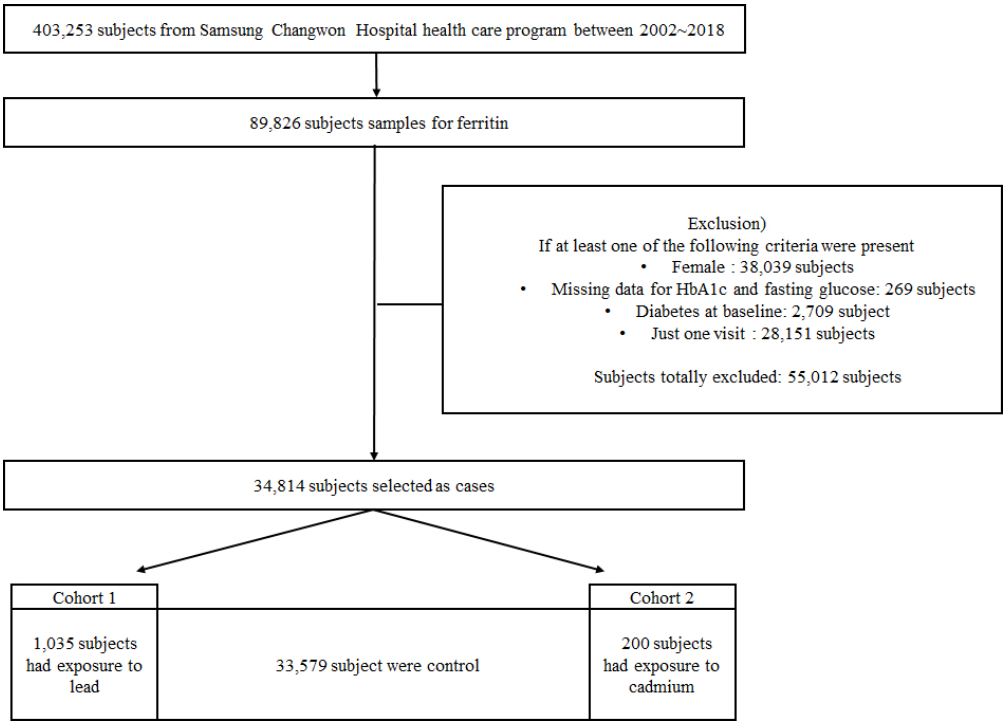
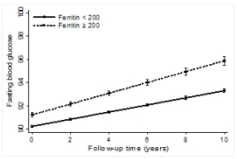


Figure 1. Schematic flow diagram

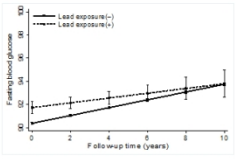
200x143mm (120 x 120 DPI)

A



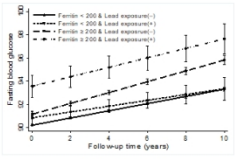
Follow-up time	Ferritin <200	Ferritin ≥200
Baseline	90.25 (90.15-90.34)	91.24 (91.05-91.43)
2 years	90.86 (90.77-90.95)	92.17 (91.99-92.34)
4 years	91.48 (91.38-91.57)	93.09 (92.90-93.29)
6 years	92.09 (91.98-92.20)	94.02 (93.78-94.27)
8 years	92.71 (92.58-92.83)	94.95 (94.64-95.26)
10 years	93.32 (93.17-93.47)	95.88 (95.50-96.26)

B



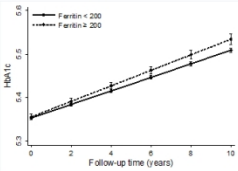
Follow-up time	Lead exposure Negative	Positive
Baseline	90.39 (90.30-90.48)	91.32 (90.82-91.82)
2 years	91.06 (90.98-91.14)	91.88 (91.42-92.34)
4 years	91.73 (91.65-91.82)	92.43 (91.92-92.94)
6 years	92.40 (92.30-92.50)	92.98 (92.36-93.61)
8 years	93.07 (92.95-93.19)	93.54 (92.76-94.31)
10 years	93.74 (93.60-93.89)	94.09 (93.14-95.04)

C



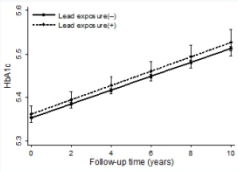
Follow-up time	Ferritin<200 Lead Negative	Ferritin<200 Lead Positive	Ferritin≥200 Lead Negative	Ferritin≥200 Lead Positive
Baseline	90.23 (90.13-90.33)	90.86 (90.31-91.42)	91.17 (90.98-91.36)	93.58 (92.63-94.52)
2 years	90.85 (90.76-90.94)	91.36 (90.84-91.88)	92.10 (91.92-92.28)	94.39 (93.46-95.32)
4 years	91.47 (91.37-91.56)	91.86 (91.30-92.42)	93.03 (92.83-93.23)	95.21 (94.24-96.17)
6 years	92.08 (91.97-92.19)	92.36 (91.70-93.03)	93.96 (93.71-94.21)	96.02 (94.98-97.06)
8 years	92.70 (92.57-92.83)	92.86 (92.05-93.68)	94.89 (94.58-95.21)	96.83 (95.68-97.99)
10 years	93.32 (93.16-93.47)	93.37 (92.39-94.34)	95.83 (95.44-96.21)	97.65 (96.35-98.95)

D



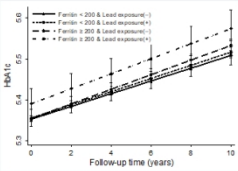
Follow-up time	Ferritin <200	Ferritin ≥200
Baseline	5.35 (5.35-5.36)	5.36 (5.35-5.36)
2 years	5.38 (5.38-5.39)	5.39 (5.39-5.40)
4 years	5.42 (5.41-5.42)	5.43 (5.42-5.43)
6 years	5.45 (5.44-5.45)	5.46 (5.45-5.47)
8 years	5.48 (5.47-5.48)	5.50 (5.49-5.51)
10 years	5.51 (5.50-5.51)	5.53 (5.52-5.55)

E



Follow-up time	Lead exposure Negative	Positive
Baseline	5.35 (5.35-5.36)	5.36 (5.34-5.38)
2 years	5.39 (5.38-5.39)	5.40 (5.38-5.41)
4 years	5.42 (5.41-5.42)	5.43 (5.41-5.45)
6 years	5.45 (5.45-5.45)	5.46 (5.44-5.48)
8 years	5.48 (5.48-5.49)	5.49 (5.47-5.52)
10 years	5.51 (5.51-5.52)	5.53 (5.50-5.56)

F

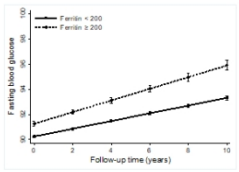


Follow-up time	Ferritin<200 Lead Negative	Ferritin<200 Lead Positive	Ferritin≥200 Lead Negative	Ferritin≥200 Lead Positive
Baseline	5.35 (5.35-5.36)	5.36 (5.34-5.38)	5.36 (5.35-5.36)	5.39 (5.35-5.43)
2 years	5.38 (5.38-5.39)	5.39 (5.37-5.41)	5.39 (5.38-5.40)	5.43 (5.39-5.46)
4 years	5.42 (5.41-5.42)	5.42 (5.40-5.44)	5.43 (5.42-5.430	5.46 (5.43-5.50)
6 years	5.45 (5.44-5.45)	5.45 (5.43-5.48)	5.46 (5.45-5.47)	5.50 (5.46-5.54)
8 years	5.48 (5.47-5.48)	5.48 (5.46-5.51)	5.50 (5.49-5.51)	5.54 (5.50-5.58)
10 years	5.51 (5.50-5.51)	5.52 (5.49-5.55)	5.53 (0.52-5.55)	5.57 (5.53-5.62)

Figure 2. Mixed models were used to evaluate the effects of lead exposure and ferritin on fasting blood glucose and HbA1c in cohort A. (A) Changes in fasting blood glucose according to serum ferritin levels (B) Changes in fasting blood glucose according to lead exposure (C) Changes in fasting blood glucose according to serum ferritin levels and lead exposure (D) Changes in HbA1c according to serum ferritin levels (E) Changes in HbA1c according to lead exposure (F) Changes in HbA1c according to serum ferritin levels and lead exposure

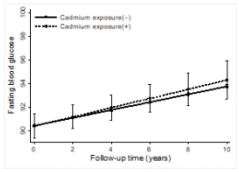
265x355mm (120 x 120 DPI)

A



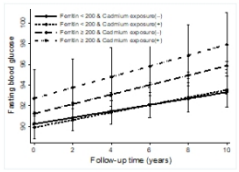
Follow-up time	Ferritin <200	Ferritin ≥200
Baseline	90.25 (90.15-90.34)	91.26 (91.07-91.45)
2 years	90.86 (90.77-90.95)	92.18 (92.01-92.36)
4 years	91.48 (91.38-91.57)	93.11 (92.92-93.31)
6 years	92.09 (91.98-92.20)	94.04 (93.80-94.29)
8 years	92.71 (92.58-92.84)	94.97 (94.66-95.28)
10 years	93.32 (93.17-93.48)	95.90 (95.51-96.28)

B



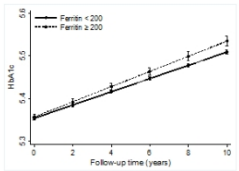
Follow-up time	Cadmium Negative	Cadmium Positive
Baseline	90.42 (90.33-90.50)	90.39 (89.32-91.45)
2 years	91.09 (91.01-91.17)	91.17 (90.16-92.18)
4 years	91.75 (91.67-91.84)	91.95 (90.90-93.00)
6 years	92.42 (92.32-92.52)	92.73 (91.55-93.91)
8 years	93.09 (92.97-93.21)	93.51 (92.14-94.89)
10 years	93.75 (93.61-93.90)	94.29 (92.69-95.90)

C



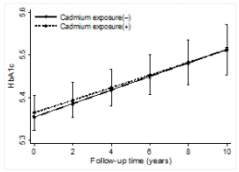
Follow-up time	Ferritin<200 Cadmium Negative	Ferritin<200 Cadmium Positive	Ferritin≥200 Cadmium Negative	Ferritin≥200 Cadmium Positive
Baseline	90.25 (90.15-90.35)	89.91 (88.78-91.04)	91.25 (91.06-91.44)	92.72 (89.91-95.53)
2 years	90.86 (90.78-90.95)	90.64 (89.57-91.71)	92.17 (92.00-92.35)	93.76 (90.97-96.56)
4 years	91.48 (91.38-91.57)	91.37 (90.25-92.48)	93.10 (92.90-93.30)	94.80 (91.99-97.62)
6 years	92.09 (91.98-92.20)	92.10 (90.86-93.33)	94.03 (93.78-94.27)	95.84 (92.97-98.72)
8 years	92.71 (92.58-92.84)	92.82 (91.40-94.24)	94.95 (94.64-95.26)	96.89 (93.92-99.85)
10 years	93.32 (93.16-93.47)	93.55 (91.91-95.20)	95.88 (95.50-96.26)	97.93 (94.84-101-02)

D



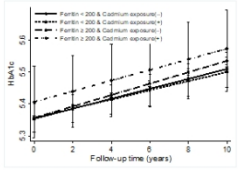
Follow-up time	Ferritin <200	Ferritin ≥200
Baseline	5.35 (5.35-5.36)	5.36 (5.35-5.36)
2 years	5.38 (5.38-5.39)	5.39 (5.39-5.40)
4 years	5.42 (5.41-5.42)	5.43 (5.42-5.44)
6 years	5.45 (5.44-5.45)	5.46 (5.45-5.47)
8 years	5.48 (5.47-5.48)	5.50 (5.49-5.51)
10 years	5.51 (5.50-5.51)	5.53 (5.52-5.55)

E



Follow-up time	Cadmium Negative	Cadmium Positive
Baseline	5.35 (5.35-5.36)	5.36 (5.32-5.41)
2 years	5.39 (5.38-5.39)	5.39 (5.35-5.43)
4 years	5.42 (5.41-5.42)	5.42 (5.38-5.47)
6 years	5.45 (5.45-5.45)	5.45 (5.41-5.50)
8 years	5.48 (5.48-5.49)	5.48 (5.43-5.54)
10 years	5.51 (5.51-5.52)	5.51 (5.45-5.57)

F



Follow-up time	Ferritin<200 Cadmium Negative	Ferritin<200 Cadmium Positive	Ferritin≥200 Cadmium Negative	Ferritin≥200 Cadmium Positive
Baseline	5.35 (5.35-5.36)	5.36 (5.31-5.40)	5.36 (5.35-5.36)	5.41 95.29-5.52)
2 years	5.38 (5.38-5.39)	5.38 (5.34-5.43)	5.39 (5.39-5.40)	5.44 (5.33-5.55)
4 years	5.42 (5.41-5.42)	5.41 (5.37-5.46)	5.43 (5.42-5.43)	5.47 (5.36-5.59)
6 years	5.45 (5.44-5.45)	5.44 (5.39-5.49)	5.46 (5.45-5.47)	5.51 (5.39-5.62)
8 years	5.48 (5.47-5.48)	5.47 (5.42-5.53)	5.50 (5.49-5.51)	5.54 (5.42-5.66)
10 years	5.51 (5.50-5.51)	5.50 (5.44-5.56)	5.53 (5.52-5.55)	5.57 (5.45-5.69)

Figure 3. Mixed models were used to evaluate the effects of cadmium exposure and ferritin on fasting blood glucose and HbA1c in cohort B. (A) Changes in fasting blood glucose according to serum ferritin levels (B) Changes in fasting blood glucose according to lead exposure (C) Changes in fasting blood glucose according to serum ferritin levels and lead exposure (D) Changes in HbA1c according to serum ferritin levels (E) Changes in HbA1c according to lead exposure (F) Changes in HbA1c according to serum ferritin levels and lead exposure

238x312mm (120 x 120 DPI)

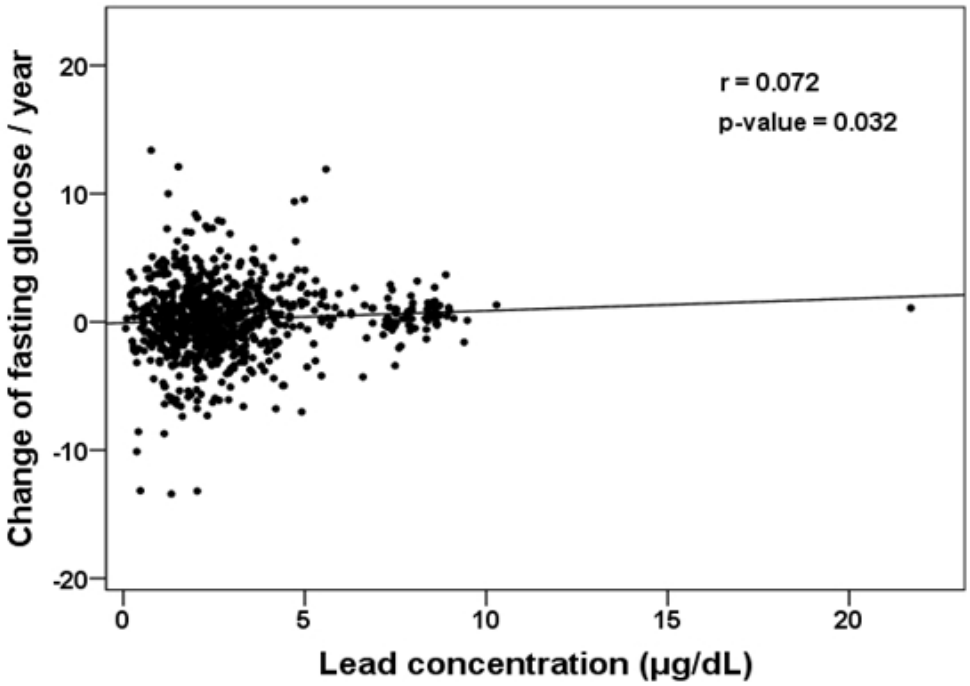


Figure 4. Scatter plot showing the annual changes of FBS by lead concentration (R=0.072, p = 0.032)

118x83mm (120 x 120 DPI)

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1

1Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	#4	Present key elements of study design early in the paper	7
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	7
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	7
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
8Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than	7

		one group. Give information separately for for exposed and unexposed groups if applicable.	
Bias	#9	Describe any efforts to address potential sources of bias	7
Study size	#10	Explain how the study size was arrived at	7
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	8
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	8
Statistical methods	#12c	Explain how missing data were addressed8	NA
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	NA
Statistical methods	#12e	Describe any sensitivity analyses	8
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-	9

		up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	
Participants	#13b	Give reasons for non-participation at each stage	NA
Participants	#13c	Consider use of a flow diagram	9
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	NA
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	9
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
Main results	#16b	Report category boundaries when continuous variables were categorized	9
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9

Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	#18	Summarise key results with reference to study objectives	10
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13
Generalisability	#21	Discuss the generalisability (external validity) of the study results	10-13
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

BMJ Open

The relationship between heavy metal exposure and type 2 diabetes: A large-scale cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039541.R1
Article Type:	Original research
Date Submitted by the Author:	15-Sep-2020
Complete List of Authors:	Ji, Jun Ho ; Samsung Changwon Hospital, Internal medicine Jin, Mi Hyeon ; Samsung Changwon Hospital Kang, Jung-Hun ; Gyeongsang National University College of Medicine, Internal Medicine Lee, Soon Il ; Dankook University College of Medicine, Internal Medicine Lee, Suee ; Dong-A University Medical Center, Internal medicine Kim, Sung-Hyun ; Dong-A University Medical Center, Internal Medicine Oh, Sung Yong; Dong-A University Medical Center
Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	Diabetes & endocrinology < INTERNAL MEDICINE, SOCIAL MEDICINE, EPIDEMIOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The relationship between heavy metal exposure and type 2 diabetes: A large-scale cohort study

Jun Ho Ji[1], Mi Hyeon Jin[2], Jung-Hun Kang[3], Soon Il Lee[4], Suee Lee[5], Sung-Hyun Kim[5], Sung Yong Oh[5#]

[1]Department of Internal Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea;

[2]Department of Biostatistics, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea;

[3]Department of Internal Medicine, Gyeongsang National University Scholl of Medicine, Jinju, Korea;

[4]Department of Internal Medicine, Dankook University College of Medicine, Cheonan;

[5]Department of Internal Medicine, Dong-A University College of Medicine, Busan

Running title: Relationship between heavy metal exposure and diabetes

Word count: 3489

Corresponding author: Sung Yong Oh

Address: Department of Internal Medicine, Dong-A University College of Medicine, 26 Daesingongwon-Ro, Seo-Gu, Busan 49201, Korea

Mobile: +82-10-8624-9818

E-mail:drosy@dau.ac.kr

Abstract

Objectives: To investigate associations of heavy-metal exposure with serum ferritin level, physical measurements, and type 2 diabetes mellitus (DM).

Design: A retrospective longitudinal cohort study.

Setting: Changwon, the location of this study, is a representative industrial city in Korea. Data were obtained from medical checkups between 2002 and 2018.

Participants: A total of 34,814 male subjects were included. Of them, 1,035 subjects with lead exposure, 200 subjects with cadmium exposure, and the remaining 33,579 were assigned into cohort A, cohort B, and control cohort, respectively. Data including personal history of alcohol and smoking, age, HbA1c, fasting glucose, ferritin, height, weight, follow-up duration, and blood levels of lead and cadmium within one year after exposure were collected.

Primary outcome measure: In subjects without diabetes, changes in FBS and HbA1c were analyzed through repeated tests at intervals of one year or longer after occupational exposure to heavy metals.

Results: In cohort A, DM was diagnosed in 33 subjects. There was a significant difference in lead concentration between subjects diagnosed with DM and those without DM during the follow-up period (3.94 ± 2.92 mg/dL versus 2.81 ± 2.03 mg/dL, $p = 0.002$). Simple exposure to heavy metals (lead and cadmium) was not found to be associated with DM in Cox regression models (lead exposure hazard ratio [HR]: 1.01, 95% CI: 0.58-1.77, $p = 0.971$; cadmium exposure HR: 1.48, 95% CI: 0.61-3.55, $p = 0.385$). Annual changes in fasting blood glucose according to lead concentration at the beginning of exposure showed a weak positive correlation ($r = 0.072$, $p = 0.032$).

Conclusion: Our findings demonstrate that simple occupational exposure to heavy metals of lead and cadmium is not associated with incidence of DM. However, lead concentration at the beginning of exposure might be an indicator of DM and glucose elevation.

Keywords: diabetes, heavy-metal exposure, HbA1c, body mass index, ferritin

Strengths and limitations of this study

- Limited by single institute data obtained from occupational medical checkup.
- This study was a large-scale study to determine blood concentrations of heavy metals (initial exposure to occupational heavy metal and exposure over a long period of time) and changes in fasting glucose and HbA1c levels.
- The remarkable point of this study is that females are not included. Ferritin is a known risk factor for diabetes and a chronic inflammatory marker. However, due to the demographic nature of occupational health checkup for most women of childbearing age, iron deficiency caused by menstruation can act as a confounding variable.

Introduction

Diabetes mellitus (DM), a common and rising global problem, is one of leading causes of death, blindness, and chronic renal failure. It is also a major risk factor for vascular diseases such as myocardial infarction, stroke, and peripheral vascular disease. The increase in social cost because of DM-related morbidity or mortality has intensified efforts to reduce the incidence of DM. The rising incidence of DM is considered to be associated with alterations in lifestyles and other contributing factors, including exposure to several environmental pollutants and industrial chemicals.

With rapid industrial development, exposure levels to various environmental toxic materials have risen along with DM incidence. Environmental substances that cause endocrine disruptions have been defined as endocrine-disrupting chemicals (EDC) by the U.S. Environmental Protection Agency (EPA) [1]. Metals are naturally existing inorganic elements that are present in very small amounts in the body. They are essential for vital processes. Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. Heavy metals and metalloids (e.g., lead, mercury, cadmium, and metalloid arsenic) might affect hormonal activity, suggesting that these compounds are EDCs generalized considered as toxicants. These heavy metals have negative effects on physiology. They might be associated with the incidence of DM in some populations. In this study, we particularly focused on the association between exposure to heavy metals and DM. In recent decades, environmental exposure to heavy metals has been declining because many countries have begun to pay attention to environmental problems rather than to the development of industry. However, unintended exposure to heavy metals in the environment such as older households and drinking water as in the case of Flint, Michigan, USA [2], is still possible. Such exposure can be due to illegal, unauthorized disposal of toxic materials including heavy metals from industries. In Korea, occupational exposure to heavy metals is more common than random environmental exposure.

For occupational exposure to heavy metals, relatively few studies have reported whether the degree of exposure has direct or indirect effects on the body or specific diseases. A few population-based studies have focused on the association between metal exposure and diabetes, showing inconsistent results [3-9]. Most of previous studies have examined the association of DM with heavy-metal

concentrations in blood or urine at one specific moment [6, 7].

Intense exposure to heavy metals can result in high levels of heavy metals in blood or urine, whereas light exposure results in extremely low levels. Although long-term light exposure to heavy metals might only lead to low levels of heavy metals in blood or urine, heavy metals deposited in organs may be harmful. Deposition of heavy metals in the liver and pancreas can alter gluconeogenesis in the liver and affect insulin secretion, eventually influencing the incidence of DM. Although this study was designed as a retrospective study of long-term occupational exposure to heavy metals (lead and cadmium) instead of measuring concentration of heavy metals in organs such as the liver, bone, and pancreas, we measured blood concentrations of heavy metals at the beginning of exposure (within one year) and compared changes in fasting glucose, HbA1c, and incidence of DM with those of the general population who were not exposed to heavy metals during the same period.

Material and Methods

1) Study population

Changwon, the location of this study, is a representative industrial city in Korea. It has many occupations involving heavy-metal exposure, including battery-manufacturing plants. This cohort study was based on data of the general population (n = 403,253) who underwent medical checkups at Samsung Changwon Hospital between 2002 and 2018. A schematic flow chart for the selection of subjects is shown in Fig 1. All participants underwent a physical exam with blood sample taken in the morning following an overnight fast. They also filled out a questionnaire. Among these 403,253 subjects, 89,826 who had taken a blood test for ferritin were included while 38,039 women were excluded. In occupational screening, most women were fertile. Results of ferritin might be inaccurate because of menstruation. A total of 269 subjects were excluded because of unavailability of HbA1c or fasting blood glucose (FBS) data. Furthermore, 2709 subjects who were already diagnosed with DM were excluded (DM was defined as FBS ≥ 126 mg/dl, HbA1c ≥ 6.5%, or history of DM in the questionnaire). Additionally, 28,151 subjects were excluded because they only had one screening result without follow-up data. Finally, 34,814 subjects were included in the analysis. Of these, 1,035 subjects with lead exposure, 200 subjects with cadmium exposure, and the remaining 33,579 subjects were assigned to cohort A, cohort B, and control cohort, respectively. This study collected subjects' data including age, HbA1c, FBS, ferritin, height, body weight, follow-up duration, and concentrations of heavy metals (lead and cadmium). The study protocol was approved by the Institutional Review Board (IRB) of Samsung Changwon Medical Center (SCMC-2019-04-014). All participants provided written informed consent for using their data.

2) Data collection

This study was based on data from occupational health checkups already carried out. Such health checkup data included numerical objective data such as blood test, imaging test, and physical exam as well as questionnaire of subjects. The authors used a questionnaire that included several items such as personal history, physical activity, systemic symptoms, sleep pattern, stress, anxiety, depression,

gambling, and job stress. All data were computerized. After obtaining IRB approval, two authors (JHJ and MHJ) independently analyzed these data.

3) Measuring blood levels of lead and cadmium

To measure blood levels of lead and cadmium, 3 ml of blood was collected from each subject into vacuum bottles using heparin as an anticoagulant in the morning following an overnight fast. Blood samples were diluted 1:15 and 1:10 to measurement of lead and cadmium concentrations, respectively, with 2.5 ml of 10% Triton X-100, 0.1 ml of concentrated nitric acid, and 1 ml of 10% ammonium di-hydrogen phosphate as a modifier. Graphite-furnace atomic absorption spectrometry with Zeeman background correction (PinAAcle 9i00z Atomic absorption spectrometer, PerkinElmer, USA) was used to measure lead and cadmium levels in all subjects within the first year of heavy-metal exposure.

4) Statistical analyses

Continuous variables are presented as means ± standard deviation. Categorical variables are presented as the number of cases and percentage. An independent t-test was used to evaluate the significance of mean differences between continuous variables for demographical factors such as age and body mass index (BMI). Cox proportional hazard model was used to identify potential predictors among baseline characteristics for type 2 DM in subjects who were not diagnosed with DM. Exposure levels of lead and cadmium in consecutive blood tests were set as independent variables while FBS and HbA1c levels were set as dependent variables. A mixed model was used to assess effects of heavy-metal exposure and ferritin on FBS and HbA1c, respectively. Annual changes of FBS and HbA1c with concentrations of lead are shown in a scatter plot. Stata 14.0 software (Stata Corporation, College Station, TX, USA) was used for all statistical analyses.

5) Operational definitions

1. Type 2 DM – Those who had a history of diabetes diagnosis with anti-diabetic medication or satisfied ADA (American Diabetes Association) criteria: HbA1c ≥ 6.5% or fasting plasma glucose ≥ 126 mg/dl in a blood test after 8-hour fast.

2. Newly diagnosed diabetes – Among subjects without a history of diabetes who had HbA1c < 6.5% and fasting plasma glucose < 100 mg/dl in the first health checkup after joining the company, diabetes was newly diagnosed (HbA1c ≥ 6.5% or fasting plasma glucose ≥ 126 mg/dl) in the follow-up health checkup conducted at least one year apart.

3. Heavy metal exposure subjects – Subjects with exposure to heavy metals were those who were working in the lead industry, those who were in charge of lead welding and mounting in shipyard, subjects who were working in Ni-Cd battery manufacturing factories.

6) Patient and Public involvement

Patient and public were not involved in the development of the research question or the design of the study. No patient and public involved in the recruitment to and conduct of the study. As this study used de-identified results, the authors do not plan to disseminate the study results to study participants separately, but we plan to publish the paper with open access.

Results

1) Baseline characteristics of the study subjects

Baseline characteristics of subjects in each cohort are shown in Table 1. Of 34,818 subjects, 1,034 were diagnosed with DM during the follow-up while 33,780 were not diagnosed with DM. In cohort A (1,035 lead-exposed subjects), 1,034 were confirmed to have DM. Of these 1,034 subjects, 33 were exposed to lead. In the control group without heavy-metal exposure, age, HbA1c, FBS, and ferritin level were associated with DM as expected. In heavy-metal exposed subjects, only HbA1c, FBS, and BMI were significantly associated with DM. An interesting aspect in cohort A was that the concentration of lead initially (within one year) was significantly higher in subjects who were later diagnosed with DM (2.81 ± 2.03 mg/dL in non-diabetes and 3.94 ± 2.92 mg/dL in diabetes, p = 0.002). In contrast, early blood levels of cadmium did not differ between the group of subjects progressing to have DM and those not progressing to have DM. Drinking and smoking were observed similar to the previous results in diabetes incidence. Overall, the incidence of diabetes was higher in drinkers than in non-drinkers and higher in smokers than in ex-smokers or never smokers. However, the total number of subjects exposed to heavy metals was small, resulting in no statistical significance. The follow-up period was shorter while the mean age was higher in subjects progressing to have DM in both cohorts. In the lead-exposed group, the mean follow-up duration was 3.18 ± 3.63 years for the group with DM and 4.78 ± 2. 77 years (p = 0.001) for the non-diabetic group. In the cadmium-exposed group, the mean follow-up duration was 5.45 ± 4.76 years for the DM group and 6.96 ± 3.77 years (p = 0.207) for the non-diabetic group.

2) Risk of developing DM from lead/cadmium exposure and serum ferritin

Cox-regression models showed crude and adjusted hazard ratios of variables for predicting the development of DM (Table 2). Age, HbA1c, FBS, BMI, current smoking, and ferritin were predictors for developing DM in both crude and adjusted models. However, simple exposure to lead or cadmium was not associated with DM. Ferritin level had a positive relationship with FBS and HbA1c elevation during the follow-up period in both cohorts A and B (Figures 2-A, 2-B, 3-A, 3-B). FBS elevation in subjects with simple lead exposure showed a slower pattern than that in those without lead exposure (Figure 2-C).

However, simple lead exposure did not have a significant effect on HbA1c elevation (Figure 2-D). The result of early exposure to cadmium did not differ from that of early exposure to lead. In cohort B, ferritin also had a significant effect on rates of elevation of FBS and HbA1c (Figure 3-A, Figure 3-B). Early exposure to cadmium was positively correlated with the rate of FBS change, but negatively correlated with HbA1c change (Figures 3-C, 3-D). The unusual finding in both cohorts was that all subjects were healthy without DM at the time of enrollment. However, subjects with elevated ferritin and heavy-metal exposure had higher baseline values of FBS and HbA1c than those who did not (Figures 2-E, 2-F, 3-E, 3-F). Regarding concentrations of heavy metals, annual variations of FBS according to initial concentrations of lead showed a weak but positive correlation (r = 0.072, p = 0.032, Figure 4).

Discussion

Many studies have attempted to explain the relationship between heavy-metal exposure and hyperglycemia. There are several plausible hypotheses as background of such research. First, oxidative stress caused by heavy metals can directly damage beta cells of the pancreas, leading to elevated serum glucose levels [10-17]. Such oxidative stress may also increase blood glucose levels by decreasing insulin release, impairing insulin receptors, disrupting glucose uptake, increasing hepatic gluconeogenesis and pancreatic glucagon secretion, and decreasing peripheral glucose use [16, 18-22]. Another hypothesis is about competitive inhibition of the toxic metals. It states that essential trace metals at normal levels play a key role in glucose homeostasis because these metals are essential cofactors for glucose metabolism, pancreatic beta cell function, and insulin signaling cascade [18, 19, 23, 24]. Toxic metals compete with these essential metals for various physiological functions and affect type 2 DM risk [25, 26]. It has also been reported that toxic metals can affect various substances, including glucose transporter type 4, nuclear factor kappa B, mitogen-activated protein kinases, and phosphoinositide 3-kinase involved in insulin signaling, thereby increasing the risk of DM [27-31]. The last hypothesis is that exposure to metals, especially heavy metals, can increase body weight based on population studies. Because weight gain is a known risk factor for DM, exposure to heavy metals might be associated with DM [32-36]. Many studies on the relationship between heavy-metal exposure and DM have been performed based on these findings. However, they show inconsistent results [3-9]. It can be inferred that a direct association between heavy metals and DM has not been confirmed yet. Even if such association is relevant, it is very weak. Prior epidemiologic studies that explain reported inconsistent results connecting heavy metals to DM have limitations. Most previous studies had cross-sectional designs [3-5, 7-9]. A cross-sectional study is characterized by analysis carried out at a specific point in time. It does not reflect changes over time. In the case of heavy-metal exposure, chronic long-time exposure is more common than acute exposure. Therefore, the time of exposure to heavy metals is important. The elapsed time since the first exposure should be also considered. A Chinese study has reported that insulin secretion is decreased more in the group exposed to cadmium for more than 10 years than in the group exposed to cadmium for less

than 10 years [37]. Previous studies have also been conducted with a case-control design [3, 9, 38, 39]. It is well-known that a small case-control study tends to be less expensive and shorter in duration. However, it has a low level of evidence.

This study investigated relationships of serum ferritin level, exposure to heavy metals, and DM during health screening in subjects who worked in battery, paint, and bullet manufacturing facilities, shipyards, or workplaces requiring welding. Although this study included data from a single institution, it was designed as a retrospective longitudinal study using a large number of health screening subjects, thus overcoming limitations of prior studies. The following results were obtained: (1) Simple exposure to heavy metals did not increase the risk of developing DM over time. However, the concentration of lead at the time of initial lead exposure was higher in subjects diagnosed with DM later on; (2) Serum ferritin was a predictor of DM as previously reported [40], However, serum ferritin was not a predictor of DM in subjects exposed to lead or cadmium; (3) High blood concentration of lead at the beginning of lead exposure was proportional to the rate of increase in FBS per year. It was noteworthy that when the blood lead concentration measured within a year after exposure was high, the rate of FBS increased gradually with time. A high blood lead concentration means that lead exposure intensity is strong. Thus, lead exposure intensity might be a risk factor for DM. This aligns with our other study results, in which simple exposure to heavy metals is not related to the incidence of DM or the elevation of FBS/HbA1c. Concentrations of heavy metals in our cohort were slightly higher than those in normal Korean adults based on a demographic study on environmental exposure to heavy metals by Kim et al. [41]. This suggests that our cohort was occupationally exposed to heavy metals. However, their exposure intensity was not high enough to significantly affect the incidence of DM. Similar to our results, a Korean study has demonstrated that low-dose lifetime environmental exposure to lead and cadmium might not affect the incidence of DM. Another interesting aspect of this study can be observed in Table 1. In lead- or cadmium-exposed group, serum ferritin levels in the diabetic group were significantly higher than those in the non-diabetic group, but not in subjects exposed to lead or cadmium (serum ferritin was lower in the diabetic group). The reason for these results cannot be explained exactly. Oxidative stress through the formation of free radicals [12-16,18], a mechanism by which heavy metals cause DM, might be the mechanism involved in the development of DM [42, 43]. Some large-scale US studies have

shown that high blood levels of persistent organic pollutants (POPs) that are not heavy metals but are bio-accumulating as heavy metals with chronic environmental exposure problem globally, pose an increased risk for DM [44]. The mechanism by which POPs induce DM is similar to that for DM induced by heavy metals [45, 46]. Similar to studies on associations of heavy metals and DM, studies on associations of POPs with DM also show discrepant results [47-49].

Current findings should be interpreted with caution because of several limitations. Since this study was based on data from subjects undergoing health checkups, we could not identify or analyze risk factors of DM, including hypertension, family history, and dyslipidemia. The second limitation was that blood concentrations of heavy metals were measured only once at the beginning of exposure. Follow-up observation such as diagnosis of DM was done longitudinally without reflecting changes in serum concentrations of heavy metals as in a cross-sectional study. The limited population of our study cohort was another limitation. Because of possible iron deficiency during menstruation, female subjects were excluded. Young subjects who had low incidence of DM were also included mainly because of occupational characteristics of a workplace with metal exposure. Although this study excluded female subjects, it would be interesting to investigate the relationship between occupational heavy metal exposure and diabetes in women. Despite menstruation of iron deficiency, it is a known that serum ferritin is associated with the risk of developing diabetes in fertile women. Thus, further study with female subjects is warranted.

In conclusion, our findings demonstrate that simple exposure to lead or cadmium is not associated with the prevalence of DM. On the other hand, blood concentration of lead at the beginning of exposure might be an indicator of DM prevalence and glucose elevation. Our results suggest that low-dose, chronic occupational exposure to lead or cadmium may not affect the incidence of DM. However, if the exposure intensity is high, screening for DM should be done.

A competing interests statement: The authors have no conflicts of interest to disclose.

Authors' contribution:

Conception or design: JHJ

Acquisition, analysis, or interpretation of data: JHJ

Drafting the work or revising: JHJ,MHJ,JHK,SIL,SL,SHK,SYO

Final approval of the manuscript: JHJ,MHJ,JHK,SIL,SL,SHK,SYO

A funding statement: This study was supported by Dong-A University Research fund 2020.

Acknowledgements: Thanks to all the patients who participated in this study.

Data availability: drosy@dau.ac.kr / junofanclub@hanmail.net. We will response to request including raw data form of excel file.

= Figure legends =

Figure 1. Schematic flow diagram

Figure 2. Mixed models were used to evaluate the effects of lead exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort A

B – Changes in HbA1c according to serum ferritin levels in cohort A

C – Changes in fasting blood glucose according to lead exposure in cohort A

D – Changes in HbA1c according to lead exposure in cohort A

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort A

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort A

Figure 3. Mixed models were used to evaluate the effects of cadmium exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort B

B – Changes in HbA1c according to serum ferritin levels in cohort B

C– Changes in fasting blood glucose according to lead exposure in cohort B

D – Changes in HbA1c according to lead exposure in cohort B

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort B

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort B

Figure 4. Scatter plot showing the annual changes of fasting blood glucose by lead concentration (r=0.072, p = 0.032)

= Table legends =

Table1. Baseline characteristics

Table2. Cox regression models: Crude and adjusted HRs of baseline characteristics predicting the development of type 2 diabetes mellitus

Table1. Baseline characteristics

	Lead (Cohort A)					
	No exposure (n=33,779)			Exposure (n=1,035)		
	Non diabetes (n=32,778)	Diabetes (n=1,001)	P-value	Non diabetes (n=1,002)	Diabetes (n=33)	P-value
Age	34.99 ± 7.99	37.96 ± 8.16	<0.001	32.18 ± 8.36	34.19 ± 7.92	0.174
HbA1c (%)	5.32 ± 0.30	5.76 ± 0.59	<0.001	5.33 ± 0.29	5.88 ± 0.81	<0.001
Fasting blood sugar (mg/dL)	89.98 ± 8.65	103.49 ± 18.90	<0.001	91.37 ± 9.11	114.36 ± 32.71	<0.001
Ferritin (ng/mL)	145.71 ± 93.76	165.55 ± 119.90	<0.001	152.51 ± 99.86	139.77 ± 89.57	0.470
Smoking (n=3.727)			<0.001			0.511
Never smoker	9,716	212		367	8	
Ex-smoker	6,210	173		183	8	
Current smoker	12,958	460		416	46	
Alcohol (n=34,814)			0.003			0.620
No	3,515	137		30	0	
Yes	29,263	864		972	33	
Body mass index (Kg/m²)	24.04 ± 3.03	25.89 ± 3.54	<0.001	24.04 ± 3.17	26.04 ± 2.87	<0.001
Lead concentration (mg/dL)	-	-	-	2.81 ± 2.03	3.94 ± 2.92	0.002
Follow-up duration (year)	5.65 ± 3.48	5.09 ± 3.67	<0.001	4.78 ± 2.77	3.18 ± 3.63	0.001
	Cadmium (Cohort B)					
	No exposure (n=34,614)			**Exposure (n=200)**		
	Non diabetes (n=33,591)	**Diabetes (n=1,023)**	**P-value**	**Non diabetes (n=189)**	**Diabetes (n=11)**	**P-value**
Age	34.91 ± 8.02	37.84 ± 8.19	<0.001	34.77 ± 8.28	38.04 ± 7.22	0.203
HbA1c (%)	5.32 ± 0.30	5.76 ± 0.60	<0.001	5.31 ± 0.31	5.65 ± 0.89	0.002
Fasting blood sugar (mg/dL)	90.01 ± 8.66	103.83 ± 19.55	<0.001	91.52 ± 9.43	104.00 ± 22.05	<0.001
Ferritin (ng/mL)	146.03 ± 94.00	165.27 ± 119.32	<0.001	124.24 ± 81.76	113.93 ± 85.87	0.686
Smoking (n=3.727)			<0.001			0.033
Never smoker	10,002	219		81	1	
Ex-smoker	6,359	177		34	4	
Current smoker	13,308	470		66	6	
Alcohol (n=34,814)			0.004			>0.999
No	3,540	137		5	0	
Yes	30,051	886		184	11	
Body mass index (Kg/m²)	24.04 ± 3.04	25.88 ± 3.53	<0.001	24.00 ± 3.20	26.78 ± 2.67	0.005
Cadmium concentration (mg/dL)	-	-	-	0.20 ± 0.26	0.17 ± 0.11	0.731
Follow-up duration (year)	5.61 ± 3.46	5.02 ± 3.67	<0.001	6.96 ± 3.77	5.45 ± 4.76	0.207

Table2. Cox regression models: Crude and adjusted HRs of baseline characteristics predicting the development of type 2 diabetes mellitus

	Crude		Adjusted (N=30,589)	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Age (year)	1.05 (1.04-1.06)	<0.001	1.01 (1.00-1.03)	0.012
HbA1c (%) × 10	1.54 (1.51-1.57)	<0.001	1.35 (1.32-1.39)	<0.001
Fasting blood sugar (mg/dL)	1.12 (1.11-1.12)	<0.001	1.07 (1.06-1.08)	<0.001
Body mass index (Kg/m²)	1.21 (1.19-1.22)	<0.001	1.10 (0.078-1.12)	<0.001
Ferritin (ng/mL, reference: <200)	2.25 (1.94-2.62)	<0.001	1.51 (1.28-1.79)	<0.001
Lead exposure	1.05 (0.68-1.63)	0.812	1.01 (0.58-1.77)	0.971
Cadmium exposure	1.08 (0.54-2.17)	0.828	1.48 (0.61-3.55)	0.385
Smoking				
Ex-smoker	1.22 (0.98-1.51)	0.071	1.05 (0.85-1.31)	0.634
Current smoker	1.61 (1.35-1.92)	<0.001	1.45 (1.22-1.73)	<0.01
Drinking	0.83 (0.68-1.01)	0.062	1.07 (0.53-2.17)	0.842

References

1. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC: **Endocrine-disrupting chemicals: an Endocrine Society scientific statement**. *Endocr Rev* 2009, **30**(4):293-342.
2. Hanna-Attisha M, LaChance J, Sadler RC, Champney Schnepp A: **Elevated Blood Lead Levels in Children Associated With the Flint Drinking Water Crisis: A Spatial Analysis of Risk and Public Health Response**. *Am J Public Health* 2016, **106**(2):283-290.
3. Feng W, Cui X, Liu B, Liu C, Xiao Y, Lu W, Guo H, He M, Zhang X, Yuan J *et al*: **Association of urinary metal profiles with altered glucose levels and diabetes risk: a population-based study in China**. *PloS one* 2015, **10**(4):e0123742.
4. Menke A, Guallar E, Cowie CC: **Metals in Urine and Diabetes in U.S. Adults**. *Diabetes* 2016, **65**(1):164-171.
5. Barregard L, Bergstrom G, Fagerberg B: **Cadmium exposure in relation to insulin production, insulin sensitivity and type 2 diabetes: a cross-sectional and prospective study in women**. *Environmental research* 2013, **121**:104-109.
6. Hansen AF, Simic A, Asvold BO, Romundstad PR, Midthjell K, Syversen T, Flaten TP: **Trace elements in early phase type 2 diabetes mellitus-A population-based study. The HUNT study in Norway**. *J Trace Elem Med Biol* 2017, **40**:46-53.
7. Moon SS: **Association of lead, mercury and cadmium with diabetes in the Korean population: the Korea National Health and Nutrition Examination Survey (KNHANES) 2009-2010**. *Diabet Med* 2013, **30**(4):e143-148.
8. Borne Y, Fagerberg B, Persson M, Sallsten G, Forsgard N, Hedblad B, Barregard L, Engstrom G: **Cadmium exposure and incidence of diabetes mellitus--results from the Malmo Diet and Cancer study**. *PloS one* 2014, **9**(11):e112277.
9. Forte G, Bocca B, Peruzzu A, Tolu F, Asara Y, Farace C, Oggiano R, Madeddu R: **Blood metals concentration in type 1 and type 2 diabetics**. *Biol Trace Elem Res* 2013, **156**(1-3):79-90.
10. Gerber PA, Rutter GA: **The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus**. *Antioxid Redox Signal* 2017, **26**(10):501-518.
11. Kaneto H, Katakami N, Kawamori D, Miyatsuka T, Sakamoto K, Matsuoka TA, Matsuhisa M, Yamasaki Y: **Involvement of oxidative stress in the pathogenesis of diabetes**. *Antioxid Redox Signal* 2007, **9**(3):355-366.
12. Kubisch HM, Wang J, Bray TM, Phillips JP: **Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic beta-cells against oxidative stress**. *Diabetes* 1997, **46**(10):1563-1566.
13. Yen CC, Lu FJ, Huang CF, Chen WK, Liu SH, Lin-Shiau SY: **The diabetogenic effects of the combination of humic acid and arsenic: in vitro and in vivo studies**. *Toxicol Lett* 2007,

172(3):91-105.

14. Das KK, Das SN, Dhundasi SA: **Nickel, its adverse health effects & oxidative stress**. *Indian J Med Res* 2008, **128**(4):412-425.
15. Izquierdo-Vega JA, Soto CA, Sanchez-Pena LC, De Vizcaya-Ruiz A, Del Razo LM: **Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite**. *Toxicol Lett* 2006, **160**(2):135-142.
16. Valko M, Morris H, Cronin MT: **Metals, toxicity and oxidative stress**. *Curr Med Chem* 2005, **12**(10):1161-1208.
17. Kurata Y, Katsuta O, Doi T, Kawasuso T, Hiratsuka H, Tsuchitani M, Umemura T: **Chronic cadmium treatment induces islet B cell injury in ovariectomized cynomolgus monkeys**. *Jpn J Vet Res* 2003, **50**(4):175-183.
18. Chen YW, Yang CY, Huang CF, Hung DZ, Leung YM, Liu SH: **Heavy metals, islet function and diabetes development**. *Islets* 2009, **1**(3):169-176.
19. Khan AR, Awan FR: **Metals in the pathogenesis of type 2 diabetes**. *J Diabetes Metab Disord* 2014, **13**(1):16.
20. Sharma B, Singh S, Siddiqi NJ: **Biomedical implications of heavy metals induced imbalances in redox systems**. *Biomed Res Int* 2014, **2014**:640754.
21. Beyersmann D, Hartwig A: **Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms**. *Arch Toxicol* 2008, **82**(8):493-512.
22. Kajimoto Y, Matsuoka T, Kaneto H, Watada H, Fujitani Y, Kishimoto M, Sakamoto K, Matsuhisa M, Kawamori R, Yamasaki Y *et al*: **Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells**. *Diabetologia* 1999, **42**(12):1417-1424.
23. Kaur B, Henry J: **Micronutrient status in type 2 diabetes: a review**. *Adv Food Nutr Res* 2014, **71**:55-100.
24. Siddiqui K, Bawazeer N, Joy SS: **Variation in macro and trace elements in progression of type 2 diabetes**. *ScientificWorldJournal* 2014, **2014**:461591.
25. Ahamed M, Siddiqui MK: **Environmental lead toxicity and nutritional factors**. *Clin Nutr* 2007, **26**(4):400-408.
26. Flora SJ: **Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure**. *Oxid Med Cell Longev* 2009, **2**(4):191-206.
27. Walton FS, Harmon AW, Paul DS, Drobna Z, Patel YM, Styblo M: **Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes**. *Toxicol Appl Pharmacol* 2004, **198**(3):424-433.
28. Han JC, Park SY, Hah BG, Choi GH, Kim YK, Kwon TH, Kim EK, Lachaal M, Jung CY, Lee W: **Cadmium induces impaired glucose tolerance in rat by down-regulating GLUT4 expression in adipocytes**. *Arch Biochem Biophys* 2003, **413**(2):213-220.
29. Somwar R, Koterski S, Sweeney G, Sciotti R, Djuric S, Berg C, Trevillyan J, Scherer PE,

Rondinone CM, Klip A: **A dominant-negative p38 MAPK mutant and novel selective inhibitors of p38 MAPK reduce insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting GLUT4 translocation**. *The Journal of biological chemistry* 2002, **277**(52):50386-50395.

30. Souza K, Maddock DA, Zhang Q, Chen J, Chiu C, Mehta S, Wan Y: **Arsenite activation of P13K/AKT cell survival pathway is mediated by p38 in cultured human keratinocytes**. *Molecular medicine* 2001, **7**(11):767-772.

31. Zawalich WS, Zawalich KC: **A link between insulin resistance and hyperinsulinemia: inhibitors of phosphatidylinositol 3-kinase augment glucose-induced insulin secretion from islets of lean, but not obese, rats**. *Endocrinology* 2000, **141**(9):3287-3295.

32. Leasure JL, Giddabasappa A, Chaney S, Johnson JE, Jr., Pothakos K, Lau YS, Fox DA: **Low-level human equivalent gestational lead exposure produces sex-specific motor and coordination abnormalities and late-onset obesity in year-old mice**. *Environmental health perspectives* 2008, **116**(3):355-361.

33. Faulk C, Barks A, Sanchez BN, Zhang Z, Anderson OS, Peterson KE, Dolinoy DC: **Perinatal lead (Pb) exposure results in sex-specific effects on food intake, fat, weight, and insulin response across the murine life-course**. *PloS one* 2014, **9**(8):e104273.

34. Nie X, Wang N, Chen Y, Chen C, Han B, Zhu C, Chen Y, Xia F, Cang Z, Lu M *et al*: **Blood cadmium in Chinese adults and its relationships with diabetes and obesity**. *Environmental science and pollution research international* 2016, **23**(18):18714-18723.

35. Rothenberg SE, Korrick SA, Fayad R: **The influence of obesity on blood mercury levels for U.S. non-pregnant adults and children: NHANES 2007-2010**. *Environmental research* 2015, **138**:173-180.

36. Padilla MA, Elobeid M, Ruden DM, Allison DB: **An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02**. *International journal of environmental research and public health* 2010, **7**(9):3332-3347.

37. Lei LJ, Jin TY, Zhou YF: **[The effects of cadmium on the levels of insulin in smelters]**. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2006, **24**(1):3-6.

38. Serdar MA, Bakir F, Hasimi A, Celik T, Akin O, Kenar L, Aykut O, Yildirimkaya M: **Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose**. *Int J Diabetes Dev Ctries* 2009, **29**(1):35-40.

39. Afridi HI, Kazi TG, Brabazon D, Naher S, Talpur FN: **Comparative metal distribution in scalp hair of Pakistani and Irish referents and diabetes mellitus patients**. *Clin Chim Acta* 2013, **415**:207-214.

40. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB: **Body iron stores in relation to risk of type 2 diabetes in apparently healthy women**. *JAMA* 2004, **291**(6):711-717.

41. Kim NS, Lee BK: **National estimates of blood lead, cadmium, and mercury levels in the Korean general adult population**. *Int Arch Occup Environ Health* 2011, **84**(1):53-63.
42. Andrews PA: **Disorders of iron metabolism**. *N Engl J Med* 2000, **342**(17):1293; author reply 1294.
43. Oberley LW: **Free radicals and diabetes**. *Free Radic Biol Med* 1988, **5**(2):113-124.
44. Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr.: **A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002**. *Diabetes Care* 2006, **29**(7):1638-1644.
45. Hectors TL, Vanparys C, van der Ven K, Martens GA, Jorens PG, Van Gaal LF, Covaci A, De Coen W, Blust R: **Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function**. *Diabetologia* 2011, **54**(6):1273-1290.
46. Enan E, Liu PC, Matsumura F: **2,3,7,8-Tetrachlorodibenzo-p-dioxin causes reduction of glucose transporting activities in the plasma membranes of adipose tissue and pancreas from the guinea pig**. *The Journal of biological chemistry* 1992, **267**(28):19785-19791.
47. Longnecker MP, Michalek JE: **Serum dioxin level in relation to diabetes mellitus among Air Force veterans with background levels of exposure**. *Epidemiology* 2000, **11**(1):44-48.
48. Henriksen GL, Ketchum NS, Michalek JE, Swaby JA: **Serum dioxin and diabetes mellitus in veterans of Operation Ranch Hand**. *Epidemiology* 1997, **8**(3):252-258.
49. Steenland K, Piacitelli L, Deddens J, Fingerhut M, Chang LI: **Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin**. *J Natl Cancer Inst* 1999, **91**(9):779-786.

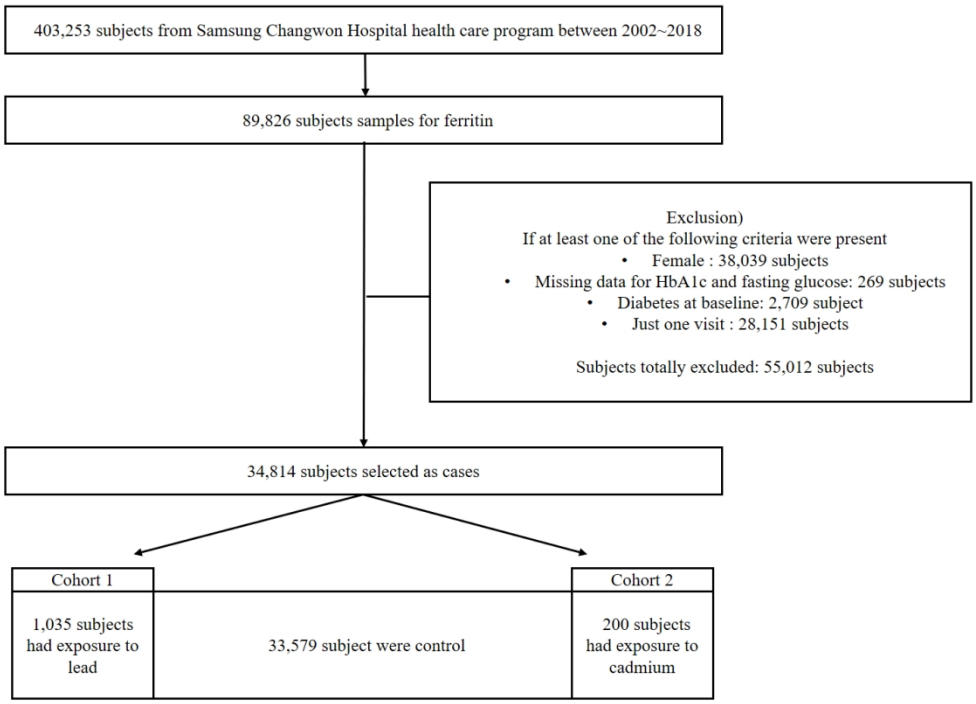
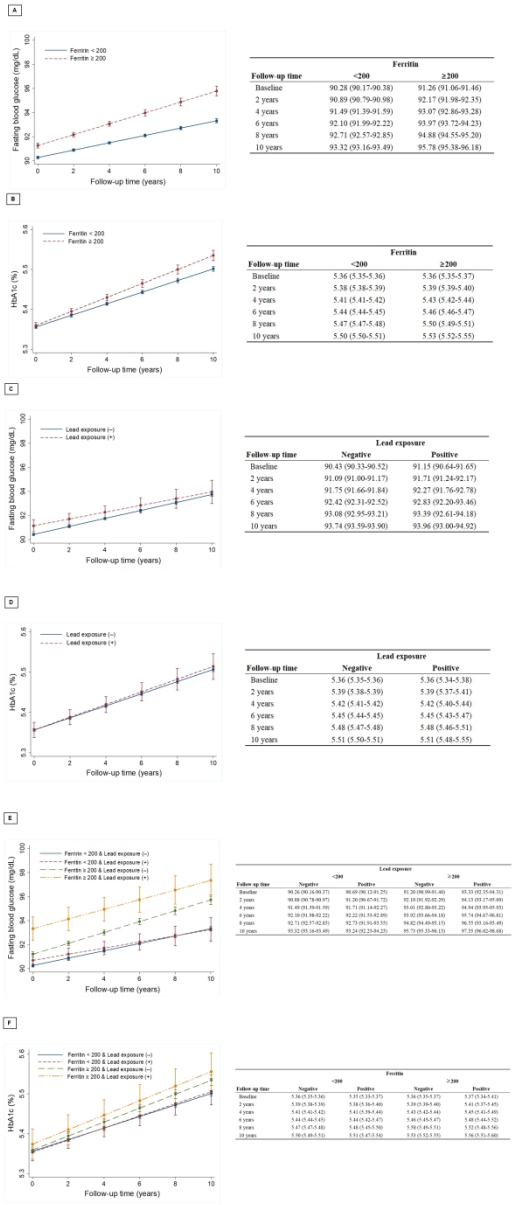


Figure 1. Schematic flow diagram

124x89mm (300 x 300 DPI)



A

	Ferritin	
Follow-up time	<200	≥200
Baseline	90.28 (90.17-90.38)	91.26 (91.06-91.46)
2 years	90.89 (90.79-90.98)	92.17 (91.98-92.35)
4 years	91.49 (91.39-91.59)	93.07 (92.86-93.28)
6 years	92.10 (91.99-92.22)	93.97 (93.72-94.23)
8 years	92.71 (92.57-92.85)	94.88 (94.55-95.20)
10 years	93.32 (93.16-93.49)	95.78 (95.38-96.18)

B

	Ferritin	
Follow-up time	<200	≥200
Baseline	5.36 (5.35-5.36)	5.36 (5.35-5.37)
2 years	5.38 (5.38-5.39)	5.39 (5.39-5.40)
4 years	5.41 (5.41-5.42)	5.43 (5.42-5.44)
6 years	5.44 (5.44-5.45)	5.46 (5.46-5.47)
8 years	5.47 (5.47-5.48)	5.50 (5.49-5.51)
10 years	5.50 (5.50-5.51)	5.53 (5.52-5.55)

C

	Lead exposure	
Follow-up time	Negative	Positive
Baseline	90.43 (90.33-90.52)	91.15 (90.64-91.65)
2 years	91.09 (91.00-91.17)	91.71 (91.24-92.17)
4 years	91.75 (91.66-91.84)	92.27 (91.76-92.78)
6 years	92.42 (92.31-92.52)	92.83 (92.20-93.46)
8 years	93.08 (92.95-93.21)	93.39 (92.61-94.18)
10 years	93.74 (93.59-93.90)	93.96 (93.00-94.92)

D

	Lead exposure	
Follow-up time	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.36 (5.34-5.38)
2 years	5.39 (5.38-5.39)	5.39 (5.37-5.41)
4 years	5.42 (5.41-5.42)	5.42 (5.40-5.44)
6 years	5.45 (5.44-5.45)	5.45 (5.43-5.47)
8 years	5.48 (5.47-5.48)	5.48 (5.46-5.51)
10 years	5.51 (5.50-5.51)	5.51 (5.48-5.55)

E

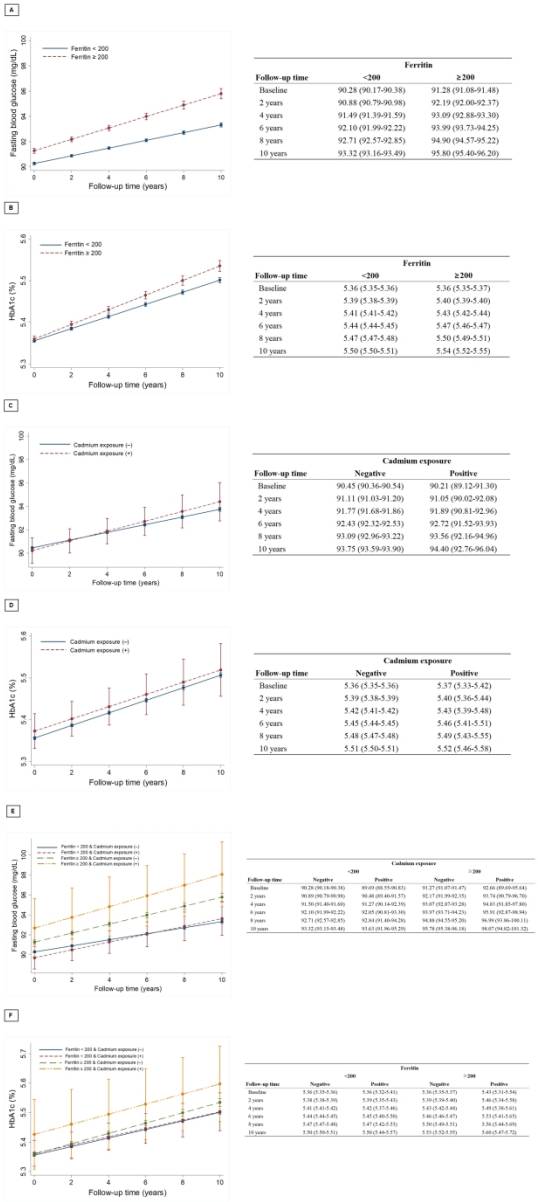
	Lead exposure			
	<200		≥200	
Follow-up time	Negative	Positive	Negative	Positive
Baseline	90.26 (90.16-90.37)	90.69 (90.12-91.25)	91.26 (90.99-91.48)	91.33 (92.35-94.31)
2 years	90.88 (90.78-90.97)	91.29 (90.67-91.72)	92.16 (91.92-92.29)	94.13 (93.17-95.09)
4 years	91.49 (91.39-91.59)	91.71 (91.14-92.27)	93.01 (92.80-93.22)	94.94 (93.95-95.93)
6 years	92.10 (91.98-92.22)	92.22 (91.55-92.89)	93.92 (93.66-94.18)	95.74 (94.67-96.81)
8 years	92.71 (92.57-92.85)	92.73 (91.91-93.55)	94.82 (94.49-95.15)	96.55 (95.16-97.49)
10 years	93.32 (93.16-93.49)	93.24 (92.25-94.23)	95.73 (95.33-96.13)	97.35 (96.02-98.68)

F

	Ferritin			
	<200		≥200	
Follow-up time	Negative	Positive	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.35 (5.33-5.37)	5.36 (5.35-5.37)	5.37 (5.34-5.41)
2 years	5.38 (5.38-5.38)	5.38 (5.36-5.40)	5.39 (5.39-5.40)	5.41 (5.37-5.45)
4 years	5.41 (5.41-5.42)	5.41 (5.39-5.44)	5.43 (5.42-5.44)	5.45 (5.41-5.49)
6 years	5.44 (5.44-5.45)	5.44 (5.42-5.47)	5.46 (5.45-5.47)	5.48 (5.44-5.52)
8 years	5.47 (5.47-5.48)	5.48 (5.45-5.50)	5.50 (5.49-5.51)	5.52 (5.48-5.56)
10 years	5.50 (5.49-5.51)	5.51 (5.47-5.54)	5.53 (5.52-5.55)	5.56 (5.51-5.60)

Figure 2. Mixed models were used to evaluate the effects of lead exposure and ferritin on FBS and HbA1c
A – Changes in fasting blood glucose according to serum ferritin levels in cohort A
B – Changes in HbA1c according to serum ferritin levels in cohort A
C – Changes in fasting blood glucose according to lead exposure in cohort A
D – Changes in HbA1c according to lead exposure in cohort A
E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort A
F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort A

154x360mm (300 x 300 DPI)



Ferritin

Follow-up time	<200	≥200
Baseline	90.28 (90.17-90.38)	91.28 (91.08-91.48)
2 years	90.88 (90.79-90.98)	92.19 (92.00-92.37)
4 years	91.49 (91.39-91.59)	93.09 (92.88-93.30)
6 years	92.10 (91.99-92.22)	93.99 (93.73-94.25)
8 years	92.71 (92.57-92.85)	94.90 (94.57-95.22)
10 years	93.32 (93.16-93.49)	95.80 (95.40-96.20)

Ferritin

Follow-up time	<200	≥200
Baseline	5.36 (5.35-5.36)	5.36 (5.35-5.37)
2 years	5.39 (5.38-5.39)	5.40 (5.39-5.40)
4 years	5.41 (5.41-5.42)	5.43 (5.42-5.44)
6 years	5.44 (5.44-5.45)	5.47 (5.46-5.47)
8 years	5.47 (5.47-5.48)	5.50 (5.49-5.51)
10 years	5.50 (5.50-5.51)	5.54 (5.52-5.55)

Cadmium exposure

Follow-up time	Negative	Positive
Baseline	90.45 (90.36-90.54)	90.21 (89.12-91.30)
2 years	91.11 (91.03-91.20)	91.05 (90.02-92.08)
4 years	91.77 (91.68-91.86)	91.89 (90.81-92.96)
6 years	92.43 (92.32-92.53)	92.72 (91.52-93.93)
8 years	93.09 (92.96-93.22)	93.56 (92.16-94.96)
10 years	93.75 (93.59-93.90)	94.40 (92.76-96.04)

Cadmium exposure

Follow-up time	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.37 (5.33-5.42)
2 years	5.39 (5.38-5.39)	5.40 (5.36-5.44)
4 years	5.42 (5.41-5.42)	5.43 (5.39-5.48)
6 years	5.45 (5.44-5.45)	5.46 (5.41-5.51)
8 years	5.48 (5.47-5.48)	5.49 (5.43-5.55)
10 years	5.51 (5.50-5.51)	5.52 (5.46-5.58)

Follow-up time	<200 Negative	<200 Positive	≥200 Negative	≥200 Positive
Baseline	90.28 (90.18-90.38)	89.69 (88.55-90.83)	91.27 (91.07-91.47)	92.66 (89.69-95.64)
2 years	90.89 (90.79-90.98)	90.48 (89.40-91.57)	92.17 (91.99-92.35)	93.74 (90.79-96.70)
4 years	91.50 (91.40-91.60)	91.27 (90.14-92.39)	93.07 (92.87-93.28)	94.83 (91.85-97.80)
6 years	92.10 (91.99-92.22)	92.05 (90.81-93.30)	93.97 (93.71-94.23)	95.91 (92.87-98.94)
8 years	92.71 (92.57-92.85)	92.84 (91.49-94.20)	94.86 (94.55-95.20)	96.99 (93.86-100.11)
10 years	93.32 (93.15-93.48)	93.63 (91.96-95.29)	95.78 (95.38-96.18)	98.07 (94.82-101.32)

Follow-up time	<200 Negative	<200 Positive	≥200 Negative	≥200 Positive
Baseline	5.36 (5.35-5.36)	5.36 (5.32-5.41)	5.36 (5.35-5.37)	5.43 (5.31-5.54)
2 years	5.38 (5.38-5.39)	5.39 (5.35-5.43)	5.39 (5.39-5.40)	5.46 (5.34-5.58)
4 years	5.41 (5.41-5.42)	5.42 (5.37-5.46)	5.43 (5.42-5.44)	5.49 (5.38-5.61)
6 years	5.44 (5.44-5.45)	5.45 (5.40-5.50)	5.46 (5.46-5.47)	5.53 (5.41-5.65)
8 years	5.47 (5.47-5.48)	5.47 (5.42-5.53)	5.50 (5.49-5.51)	5.56 (5.44-5.69)
10 years	5.50 (5.50-5.51)	5.50 (5.44-5.57)	5.53 (5.52-5.55)	5.60 (5.47-5.72)

Figure 3. Mixed models were used to evaluate the effects of cadmium exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort B

B – Changes in HbA1c according to serum ferritin levels in cohort B

C– Changes in fasting blood glucose according to lead exposure in cohort B

D – Changes in HbA1c according to lead exposure in cohort B

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort B

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort B

155x347mm (300 x 300 DPI)

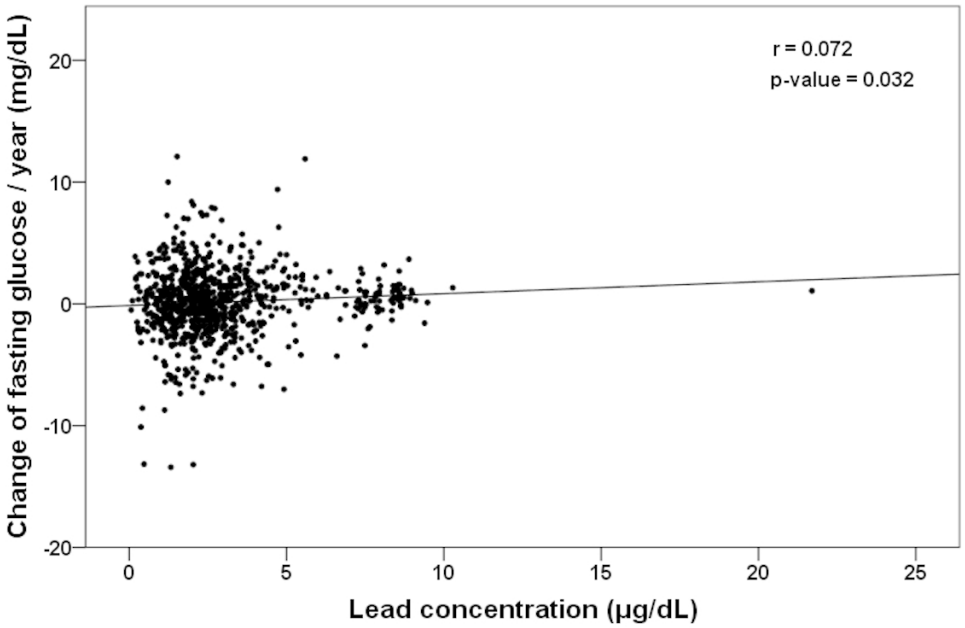


Figure 4. Scatter plot showing the annual changes of fasting blood glucose by lead concentration (r=0.072, p = 0.032)

99x67mm (300 x 300 DPI)

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1

1Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	6
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
8Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than	6

		one group. Give information separately for for exposed and unexposed groups if applicable.	
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	7
Statistical methods	#12c	Explain how missing data were addressed8	NA
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	NA
Statistical methods	#12e	Describe any sensitivity analyses	7
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-	9

		up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	
Participants	#13b	Give reasons for non-participation at each stage	9
Participants	#13c	Consider use of a flow diagram	6,9 (fig.1)
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	9
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	9
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
Main results	#16b	Report category boundaries when continuous variables were categorized	9
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9

Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	#18	Summarise key results with reference to study objectives	11
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13
Generalisability	#21	Discuss the generalisability (external validity) of the study results	10-13
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

BMJ Open

The relationship between heavy metal exposure and type 2 diabetes: A large-scale retrospective cohort study using occupational health examinations

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039541.R2
Article Type:	Original research
Date Submitted by the Author:	24-Nov-2020
Complete List of Authors:	Ji, Jun Ho ; Samsung Changwon Hospital, Internal medicine Jin, Mi Hyeon ; Samsung Changwon Hospital Kang, Jung-Hun ; Gyeongsang National University College of Medicine, Internal Medicine Lee, Soon Il ; Dankook University College of Medicine, Internal Medicine Lee, Suee ; Dong-A University Medical Center, Internal medicine Kim, Sung-Hyun ; Dong-A University Medical Center, Internal Medicine Oh, Sung Yong; Dong-A University Medical Center
Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	Diabetes & endocrinology < INTERNAL MEDICINE, SOCIAL MEDICINE, EPIDEMIOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The relationship between heavy metal exposure and type 2 diabetes: A large-scale retrospective cohort study using occupational health examinations

Jun Ho Ji[1], Mi Hyeon Jin[2], Jung-Hun Kang[3], Soon Il Lee[4], Suee Lee[5], Sung-Hyun Kim[5], Sung Yong Oh[5#]

[1]Department of Internal Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea;

[2]Department of Biostatistics, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea;

[3]Department of Internal Medicine, Gyeongsang National University Scholl of Medicine, Jinju, Korea;

[4]Department of Internal Medicine, Dankook University College of Medicine, Cheonan;

[5]Department of Internal Medicine, Dong-A University College of Medicine, Busan

Running title: Relationship between heavy metal exposure and diabetes

Word count: 3489

Corresponding author: Sung Yong Oh

Address: Department of Internal Medicine, Dong-A University College of Medicine, 26 Daesingongwon-Ro, Seo-Gu, Busan 49201, Korea

Mobile: +82-10-8624-9818

E-mail:drosy@dau.ac.kr

Abstract

Objectives: To investigate the associations between heavy metal exposure and serum ferritin levels, physical measurements, and type 2 diabetes mellitus (DM).

Design: A retrospective cohort study.

Setting: Changwon, the location of this study, is a Korean representative industrial city. Data were obtained from medical check-ups between 2002 and 2018.

Participants: A total of 34,814 male subjects were included. Of them, 1,035 subjects with lead exposure, 200 subjects with cadmium exposure, and the 33,579 remaining were assigned to cohort A, cohort B, and the control cohort, respectively. Data including personal history of alcohol and smoking, age, height, weight, the follow-up duration, HbA1c, fasting blood sugar (FBS), ferritin levels, and lead and cadmium levels within one year after exposure were collected.

Primary outcome measure: In subjects without diabetes, changes in FBS and HbA1c were analyzed through repeated tests at intervals of one year or longer after the occupational exposure to heavy metals.

Results: In cohort A, DM was diagnosed in 33 subjects. There was a significant difference in lead concentrations between the subjects diagnosed with DM and those without DM during the follow-up period (3.94 ± 2.92 mg/dL versus 2.81 ± 2.03 mg/dL, $p = 0.002$). Simple exposure to heavy metals (lead and cadmium) was not associated with DM in Cox regression models (lead exposure hazard ratio [HR] 1.01, 95% CI 0.58 – 1.77, p 0.971; cadmium exposure HR 1.48, 95% CI: 0.61 – 3.55, $p = 0.385$). Annual changes in FBS according to lead concentration at the beginning of exposure showed a positive correlation ($r = 0.072$, $p = 0.032$).

Conclusion: Our findings demonstrated that simple occupational exposure to heavy metals lead and cadmium was not associated with the incidence of DM. However, lead concentrations at the beginning of the exposure might be an indicator of DM and glucose elevations.

Keywords: diabetes, heavy metal exposure, HbA1c, body mass index, ferritin

Strengths and limitations of this study

- This study was limited by the single institute data obtained from occupational medical evaluations.
- Another important limitation of the study was the exclusion of females of childbearing age who have decreased serum ferritin due to menstruation.
- This study was a large-scale study to determine the blood concentrations of heavy metals (initial exposure to occupational heavy metal and exposure over a long period of time) and changes in FBS and HbA1c levels.
- This study showed changes in blood glucose and HbA1c over time after exposure to heavy metals.

- Introduction

Diabetes mellitus (DM), a common and rising global problem, is one of the leading causes of death, blindness, and chronic renal failure. It is also a major risk factor for vascular diseases such as myocardial infarction, stroke, and peripheral vascular disease. The increase in social cost due to DM-related morbidity or mortality has intensified efforts to reduce the incidence of DM. The rising incidence of DM is considered to be associated with alterations in lifestyles and other contributing factors, including exposure to several environmental pollutants and industrial chemicals.

With rapid industrial development, exposure to various environmental toxic materials has risen along with DM incidence. Environmental substances that cause endocrine disruption have been defined as endocrine-disrupting chemicals (EDC) by the U.S. Environmental Protection Agency (EPA) [1]. Metals are naturally existing inorganic elements that are present in very small amounts in the body. They are essential for vital processes. Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. Heavy metals and metalloids (e.g., lead, mercury, cadmium, and metalloid arsenic) can affect hormonal activity, suggesting that these compounds are EDCs generally considered to be toxicants. These heavy metals have negative effects on physiology. They might be associated with the incidence of DM in some populations. In this study, we particularly focused on the association between exposure to heavy metals and DM. In recent decades, environmental exposure to heavy metals has declined because many countries have begun to pay attention to environmental problems rather than industrial development. However, the unintended exposure to heavy metals in the environment such as older household structures and in drinking water in Flint, MI, USA [2], is still possible. Such exposure can be due to the illegal, unauthorized disposal of toxic materials including heavy metals from industries. In Korea, occupational exposure to heavy metals is more common than random environmental exposure.

In occupational exposure to heavy metals, relatively few studies have reported whether the degree of exposure has direct or indirect effects on the body or specific diseases. A few population-based studies have focused on the association between metal exposure and diabetes, showing inconsistent results [3-9]. Most previous studies have examined the association of DM with heavy metal

concentrations in the blood or urine at one specific time [6, 7].

Intense exposure to heavy metals can result in high levels of heavy metals in the blood or urine, whereas light exposure results in extremely low levels. Although long-term, light exposure to heavy metals might only lead to low levels of heavy metals in the blood or urine, heavy metals deposited in organs may be harmful. The deposition of heavy metals in the liver and pancreas can alter gluconeogenesis in the liver and affect insulin secretion, eventually influencing the incidence of DM. Although this study was designed as a retrospective study of long-term occupational exposure to heavy metals (lead and cadmium), instead of measuring the concentration of heavy metals in organs such as the liver, bone, and pancreas, the blood concentrations of heavy metals at the beginning of the exposure (within one year) were measured and compared to changes in FBS, HbA1c, and the incidence of DM in the general population who were not exposed to heavy metals during the same period.

Material and Methods

1) Study population

Changwon, the location of this study, is a representative industrial city in Korea. Many occupations involve heavy metal exposure, including employees of battery-manufacturing plants. This cohort study was based on the data from occupational health examinations (n = 403,253) conducted from 2002 to 2018 in subjects with jobs related to heavy metals. A schematic flow chart for the selection of subjects is shown in Figure 1. All participants underwent a physical examination with a blood sample taken in the morning following an overnight fast. They also filled out a questionnaire. Among these 403,253 subjects, 89,826 who had ferritin blood levels measured were included and 38,039 women were excluded. In occupational screening, most women were fertile. The ferritin results might be low because of menstruation. A total of 269 subjects were excluded because of the unavailability of HbA1c or FBS data. Furthermore, 2709 subjects who were already diagnosed with DM were excluded (DM was defined as FBS $\geq$ 126 mg/dl, HbA1c $\geq$ 6.5%, or a history of DM reported in the questionnaire). Additionally, 28,151 subjects were excluded because they only had only one screening result without follow-up data. Finally, 34,814 subjects were included in the analysis. Of these, 1,035 subjects with lead exposure, 200 subjects with cadmium exposure, and the 33,579 remaining subjects were assigned to cohort A, cohort B, and the control cohort, respectively. This study collected subject data including age, HbA1c, FBS, ferritin levels, height, body weight, the follow-up duration, and the concentrations of heavy metals (lead and cadmium). The study protocol was approved by the Institutional Review Board (IRB) of Samsung Changwon Medical Center (SCMC-2019-04-014). All participants provided written informed consent for the use of their data.

2) Data collection

This study was based on data from occupational health examinations already conducted. The health check-up data included objective numerical data such as blood tests, imaging tests, and physical examinations, as well as the questionnaire responses of the subjects. The questionnaire included items on personal history, physical activity, systemic symptoms, sleep patterns, stress, anxiety, depression,

gambling, and job stress. All data were computerized. The authors analysed the demographic information, physical examination results, past history, and laboratory results (HbA1c, blood glucose, ferritin, lead, and cadmium levels). After obtaining IRB approval, two authors (JHJ and MHJ) independently analysed the data.

3) Measuring blood levels of lead and cadmium

To measure the blood levels of lead and cadmium, 3 ml of blood was collected from each subject into vacuum bottles using heparin as an anticoagulant in the morning following an overnight fast. Blood samples were diluted 1:15 and 1:10 to measure the lead and cadmium concentrations, respectively, with 2.5 ml of 10% Triton X-100, 0.1 ml of concentrated nitric acid, and 1 ml of 10% ammonium di-hydrogen phosphate as a modifier. Graphite-furnace atomic absorption spectrometry with Zeeman background correction (PinAAcle 9i00z Atomic absorption spectrometer, PerkinElmer, Norwalk, Connecticut, USA) was used to measure the lead and cadmium levels in all subjects within the first year of heavy metal exposure. The minimum detectable limits of lead and cadmium were measured to the third decimal place (0.001mg/dl), and concentrations below that were considered to be zero.

4) Statistical analyses

The continuous variables are presented as means ± standard deviation. The categorical variables are presented as the number of cases and percentages. An independent t-test was used to evaluate the significance of the mean differences between the continuous variables for demographical factors such as age and body mass index (BMI). The Cox proportional hazard model was used to identify potential predictors in the baseline characteristics for type 2 DM in subjects who were not diagnosed with DM. In the Cox hazard model, the independent variables were set to the exposure levels of lead and cadmium and the known risk factors (age, BMI, smoking, drinking, HbA1c, FBS, and ferritin) of diabetes were set as dependent variables. A mixed model was used to assess the effects of heavy metal exposure and ferritin on FBS and HbA1c, respectively. The annual changes in FBS and HbA1c with lead concentrations are shown in a scatter plot. Stata 14.0 software (Stata Corporation, College Station, TX, USA) was used for all statistical analyses.

5) Operational definitions

1. Type 2 DM was defined in patients with a diabetes diagnosis history taking anti-diabetic medication or satisfying the American Diabetes Association (ADA) criteria of HbA1c ≥ 6.5% or FBS ≥ 126 mg/dl in a blood test after an 8-hour fast.

2. Newly diagnosed diabetes was defined in subjects without a history of diabetes who had an HbA1c of < 6.5% and an FBS of < 100 mg/dl in the first health check-up after joining the company and were newly diagnosed with diabetes (HbA1c ≥ 6.5% or FBS ≥ 126 mg/dl) in a follow-up health check-up conducted at least one year later.

3. The heavy metal exposure subjects were those who worked in the lead industry, those who were in charge of lead welding and mounting in shipyards, and subjects who worked in Ni-Cd battery manufacturing factories.

4. Simple occupational exposure to lead or cadmium, called simple exposure, referred to subjects who worked on-site at the workplace regardless of the intensity of the exposure.

5. The beginning of exposure referred to the first occupational health examination conducted within a year of working in the workplace related to heavy metal exposure.

6) Patient and public involvement

The patients and the public were not involved in the development of the research question or the design of the study. No patients or public members were involved in the recruitment or conduct of the study. Since this study used de-identified results, the authors do not plan to disseminate the study results to the study participants individually but plan to publish the paper with open access.

Results

1) Baseline characteristics of the study subjects

The baseline characteristics of the subjects in each cohort are shown in Table 1. Of 34,818 subjects, 1,034 were diagnosed with DM during the follow-up and 33,780 were not diagnosed with DM. In cohort A (1,035 subjects with lead-exposure and 33,779 subjects with no lead exposure), 1,034 were confirmed to have DM. Of these 1,034 subjects, 33 were exposed to lead. In the control group without heavy metal exposure, age, HbA1c, FBS, and ferritin levels were associated with DM, as expected. In heavy metal-exposed subjects, only HbA1c, FBS, and BMI were significantly associated with DM. An interesting aspect in cohort A was that the initial concentration of lead (within one year) was significantly higher in subjects who were later diagnosed with DM (2.81 ± 2.03 mg/dL in patients not diagnosed with diabetes and 3.94 ± 2.92 mg/dL in patients diagnosed with diabetes, p = 0.002). In contrast, the early cadmium blood levels did not differ between the group of subjects progressing to DM and those who did not progress to DM. The rates of drinking and smoking in patients with diabetes was similar to that in previous studies. Overall, the incidence of diabetes was higher in drinkers than in non-drinkers and higher in smokers than in ex-smokers or never smokers. However, the total number of subjects exposed to heavy metals was small, resulting in no statistical significance. The follow-up period was shorter and the mean age was higher in subjects progressing to DM in both cohorts. In the lead-exposed group, the mean follow-up duration was 3.18 ± 3.63 years for the group with DM and 4.78 ± 2. 77 years (p = 0.001) for the non-diabetes group. In the cadmium-exposed group, the mean follow-up duration was 5.45 ± 4.76 years for the DM group and 6.96 ± 3.77 years (p = 0.207) for the non-diabetes group.

2) Risk of developing DM from lead/cadmium exposure and serum ferritin levels

The Cox-regression models showed the crude and adjusted hazard ratios of the variables predicting the development of DM (Table 2). Age, HbA1c, FBS, BMI, current smoking, and ferritin were predictors for developing DM in both the crude and adjusted models. However, simple exposure to lead or cadmium was not associated with DM. Ferritin levels had a positive relationship with FBS and HbA1c elevations during the follow-up period in both cohorts A and B (Figures 2-A, 2-B, 3-A, 3-B). FBS elevations in subjects with simple lead exposure were slower than in those without lead exposure

(Figure 2-C). However, simple lead exposure did not have a significant effect on HbA1c elevation (Figure 2-D). The association of early cadmium exposure on the FBS/HbA1c change was not different from that of lead. In cohort B, ferritin also had significant effects on the elevation of FBS and HbA1c (Figure 3-A, Figure 3-B). Early exposure to cadmium was positively correlated with the rate of FBS change but negatively correlated with HbA1c change (Figures 3-C, 3-D). The unusual finding in both cohorts was that all subjects were healthy, without DM at the time of enrolment. However, subjects with elevated ferritin and heavy metal exposure had higher baseline FBS and HbA1c values than those who did not (Figures 2-E, 2-F, 3-E, 3-F). Regarding the concentrations of heavy metals, annual variations in FBS according to the initial lead concentrations showed weak but positive correlations (r = 0.072, p = 0.032, Figure 4).

Discussion

Many studies have attempted to explain the relationship between heavy metal exposure and hyperglycaemia. Several plausible hypotheses have resulted from such research. First, oxidative stress caused by heavy metals can directly damage beta cells of the pancreas, leading to elevated serum glucose levels [10-17]. Such oxidative stress may also increase blood glucose levels by decreasing insulin release, impairing insulin receptors, disrupting glucose uptake, increasing hepatic gluconeogenesis and pancreatic glucagon secretion, and decreasing peripheral glucose use [16, 18-22]. Another hypothesis is related to the competitive inhibition of toxic metals. It states that essential trace metals at normal levels play a key role in glucose homeostasis because these metals are essential cofactors for glucose metabolism, pancreatic beta-cell function, and the insulin signalling cascade [18, 19, 23, 24]. Toxic metals compete with these essential metals for various physiological functions and affect type 2 DM risk [25, 26]. It has also been reported that toxic metals can affect various substances, including glucose transporter type 4, nuclear factor kappa B, mitogen-activated protein kinases, and phosphoinositide 3-kinase involved in insulin signalling, thereby increasing the risk of DM [27-31]. The last hypothesis is that exposure to metals, especially heavy metals, can increase body weight, a theory based on population studies. Because weight gain is a known risk factor for DM, exposure to heavy metals might be associated with DM [32-36]. Many studies on the relationship between heavy metal exposure and DM have been performed based on these findings. However, they showed inconsistent results [3-9]. Thus, it can be inferred that a direct association between heavy metals and DM has not yet been confirmed. Even if such association is relevant, it is very weak. The prior epidemiologic studies reporting inconsistent results connecting heavy metals to DM have limitations. Most previous studies had cross-sectional designs [3-5, 7-9]. A cross-sectional study is characterized by an analysis conducted at a specific point in time. It does not reflect changes over time. In the case of heavy metal exposure, chronic long-time exposure is more common than acute exposure. Therefore, the time of exposure to heavy metals is important. The time elapsed since the first exposure should be also considered. A Chinese study reported that insulin secretion was decreased more in the group exposed to cadmium for more than 10 years than in the group exposed to cadmium for less than 10 years [37]. Previous studies have also been conducted with a case-control design [3, 9, 38, 39]. It is well-known

that a small case-control study tends to be less expensive and shorter in duration. However, it has a low level of evidence.

This study investigated the relationships between serum ferritin levels, exposure to heavy metals, and DM during the health screening of subjects who worked in battery, paint, and bullet manufacturing facilities, shipyards, or workplaces requiring welding. Although this study included data from a single institution, it was designed as a retrospective longitudinal study using a large number of health screening subjects, thus overcoming the limitations of prior studies. The following results were obtained. (1) Simple exposure to heavy metals did not increase the risk of developing DM over time. However, the concentration of lead at the time of initial lead exposure was higher in subjects later diagnosed with DM. (2) Serum ferritin was a predictor of DM, as previously reported [40]. However, serum ferritin was not a predictor of DM in subjects exposed to lead or cadmium. (3) A high blood lead concentration at the beginning of the lead exposure was proportional to the rate of increase in FBS per year. It was noteworthy that when the blood lead concentration measured within a year after exposure was high, the rate of FBS increased gradually with time. A high blood lead concentration means that the lead exposure intensity is strong in a short time. Thus, lead exposure intensity might be a risk factor for DM. This aligns with our other study results, in which simple exposure to heavy metals was not related to the incidence of DM or elevations in FBS and HbA1c. The concentrations of heavy metals in our cohort were slightly higher than those in the Korean general adult population in a demographic study on environmental exposure to heavy metals by Kim et al. [41]. This suggests that our cohort was occupationally exposed to heavy metals. However, their exposure intensity was not high enough to significantly affect the incidence of DM. Similar to our results, a Korean study demonstrated that low-dose lifetime environmental exposure to lead and cadmium might not affect the incidence of DM. Another interesting aspect of this study is shown in Table 1. In the lead- and cadmium-exposed groups, serum ferritin levels in the group with diabetes were significantly higher than those in the non-diabetes group, but not in subjects exposed to lead or cadmium (serum ferritin was lower in the diabetes group). The reason for these results cannot be precisely explained. Oxidative stress caused by the production of free radicals [12-16,18], a mechanism by which heavy metals cause DM, might be the mechanism involved in the development of DM [42, 43]. Some large-scale US studies have shown that high blood

levels of persistent organic pollutants (POPs), which are not heavy metals but bio-accumulate as heavy metals with chronic environmental exposure globally, pose an increased risk for DM [44]. The mechanism by which POPs induce DM is similar to that for DM induced by heavy metals [45, 46]. Similar to studies on the associations of heavy metals and DM, studies on the associations of POPs with DM also showed discrepant results [47-49].

The current findings should be interpreted with caution because of several limitations. Since this study was based on data from subjects undergoing health check-ups, we could not identify or analyse the risk factors of DM, including hypertension, family history, and dyslipidaemia. The second limitation was that the blood concentrations of heavy metals were measured only once at the beginning of the exposure. Follow-up observations such as the diagnosis of DM were done longitudinally without reflecting changes in the serum concentrations of heavy metals as in a cross-sectional study. The limited study cohort population was another limitation. Because of possible iron deficiency during menstruation, female subjects were excluded. Due to the nature of the industry dealing with heavy metals, it is a limited study cohort to include only young subjects in the study. Although this study excluded female subjects, it would be interesting to investigate the relationship between occupational heavy metal exposure and diabetes in women. Although menstruation can cause iron deficiency, serum ferritin is associated with the risk of developing diabetes in fertile women. Thus, further studies with female subjects are warranted.

In conclusion, our findings demonstrated that simple exposure to lead or cadmium was not associated with the prevalence of DM. However, blood lead concentrations at the beginning of exposure might be a predictor of DM development and glucose elevations. Our results suggest that low-dose, chronic occupational exposure to lead or cadmium may not affect the incidence of DM. However, if the exposure intensity is high, screening for DM should be performed.

1

2

3

4 1 **A competing interests statement:** Non declared

5

6

7 2

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

Authors' contribution:

Conception or design: JHJ

Acquisition, analysis, or interpretation of data: JHJ

Drafting the work or revising: JHJ,MHJ,JHK,SIL,SL,SHK,SYO

Final approval of the manuscript: JHJ,MHJ,JHK,SIL,SL,SHK,SYO

A funding statement: This study was supported by Dong-A University Research fund 2020.

Acknowledgements: Thanks to all the patients who participated in this study.

Data availability: drosy@dau.ac.kr / junofanclub@hanmail.net. We will response to request including raw data form of excel file.

= Figure legends =

Figure 1. Schematic flow diagram

Figure 2. Mixed models were used to evaluate the effects of lead exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort A

B – Changes in HbA1c according to serum ferritin levels in cohort A

C – Changes in fasting blood glucose according to lead exposure in cohort A

D – Changes in HbA1c according to lead exposure in cohort A

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort A

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort A

Figure 3. Mixed models were used to evaluate the effects of cadmium exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort B

B – Changes in HbA1c according to serum ferritin levels in cohort B

C– Changes in fasting blood glucose according to lead exposure in cohort B

D – Changes in HbA1c according to lead exposure in cohort B

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort B

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort B

Figure 4. Scatter plot showing the annual changes of fasting blood glucose by lead concentration (r=0.072, p = 0.032)

= Table legends =

Table1. Baseline characteristics

Table2. Cox regression models: Crude and adjusted HRs of baseline characteristics predicting the development of type 2 diabetes mellitus

Table1. Baseline characteristics

	Lead (Cohort A)					
	No exposure (n=33,779)			Exposure (n=1,035)		
	Non diabetes (n=32,778)	Diabetes (n=1,001)	P-value	Non diabetes (n=1,002)	Diabetes (n=33)	P-value
Age	34.99 ± 7.99	37.96 ± 8.16	<0.001	32.18 ± 8.36	34.19 ± 7.92	0.174
HbA1c (%)	5.32 ± 0.30	5.76 ± 0.59	<0.001	5.33 ± 0.29	5.88 ± 0.81	<0.001
Fasting blood sugar (mg/dL)	89.98 ± 8.65	103.49 ± 18.90	<0.001	91.37 ± 9.11	114.36 ± 32.71	<0.001
Ferritin (ng/mL)	145.71 ± 93.76	165.55 ± 119.90	<0.001	152.51 ± 99.86	139.77 ± 89.57	0.470
Smoking (n=3.727)			<0.001			0.511
Never smoker	9,716	212		367	8	
Ex-smoker	6,210	173		183	8	
Current smoker	12,958	460		416	46	
Alcohol (n=34,814)			0.003			0.620
No	3,515	137		30	0	
Yes	29,263	864		972	33	
Body mass index (Kg/m²)	24.04 ± 3.03	25.89 ± 3.54	<0.001	24.04 ± 3.17	26.04 ± 2.87	<0.001
Lead concentration (mg/dL)	-	-	-	2.81 ± 2.03	3.94 ± 2.92	0.002
Follow-up duration (year)	5.65 ± 3.48	5.09 ± 3.67	<0.001	4.78 ± 2.77	3.18 ± 3.63	0.001
	Cadmium (Cohort B)					
	No exposure (n=34,614)			**Exposure (n=200)**		
	Non diabetes (n=33,591)	**Diabetes (n=1,023)**	**P-value**	**Non diabetes (n=189)**	**Diabetes (n=11)**	**P-value**
Age	34.91 ± 8.02	37.84 ± 8.19	<0.001	34.77 ± 8.28	38.04 ± 7.22	0.203
HbA1c (%)	5.32 ± 0.30	5.76 ± 0.60	<0.001	5.31 ± 0.31	5.65 ± 0.89	0.002
Fasting blood sugar (mg/dL)	90.01 ± 8.66	103.83 ± 19.55	<0.001	91.52 ± 9.43	104.00 ± 22.05	<0.001
Ferritin (ng/mL)	146.03 ± 94.00	165.27 ± 119.32	<0.001	124.24 ± 81.76	113.93 ± 85.87	0.686
Smoking (n=3.727)			<0.001			0.033
Never smoker	10,002	219		81	1	
Ex-smoker	6,359	177		34	4	
Current smoker	13,308	470		66	6	
Alcohol (n=34,814)			0.004			>0.999
No	3,540	137		5	0	
Yes	30,051	886		184	11	
Body mass index (Kg/m²)	24.04 ± 3.04	25.88 ± 3.53	<0.001	24.00 ± 3.20	26.78 ± 2.67	0.005
Cadmium concentration (mg/dL)	-	-	-	0.20 ± 0.26	0.17 ± 0.11	0.731
Follow-up duration (year)	5.61 ± 3.46	5.02 ± 3.67	<0.001	6.96 ± 3.77	5.45 ± 4.76	0.207

Table2. Cox regression models: Crude and adjusted HRs of baseline characteristics predicting the development of type 2 diabetes mellitus

	Crude		Adjusted (N=30,589)	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Age (year)	1.05 (1.04-1.06)	<0.001	1.01 (1.00-1.03)	0.012
HbA1c (%) × 10	1.54 (1.51-1.57)	<0.001	1.35 (1.32-1.39)	<0.001
Fasting blood sugar (mg/dL)	1.12 (1.11-1.12)	<0.001	1.07 (1.06-1.08)	<0.001
Body mass index (Kg/m²)	1.21 (1.19-1.22)	<0.001	1.10 (0.078-1.12)	<0.001
Ferritin (ng/mL, reference: <200)	2.25 (1.94-2.62)	<0.001	1.51 (1.28-1.79)	<0.001
Lead exposure	1.05 (0.68-1.63)	0.812	1.01 (0.58-1.77)	0.971
Cadmium exposure	1.08 (0.54-2.17)	0.828	1.48 (0.61-3.55)	0.385
Smoking				
Ex-smoker	1.22 (0.98-1.51)	0.071	1.05 (0.85-1.31)	0.634
Current smoker	1.61 (1.35-1.92)	<0.001	1.45 (1.22-1.73)	<0.01
Drinking	0.83 (0.68-1.01)	0.062	1.07 (0.53-2.17)	0.842

References

1. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC: **Endocrine-disrupting chemicals: an Endocrine Society scientific statement**. *Endocr Rev* 2009, **30**(4):293-342.
2. Hanna-Attisha M, LaChance J, Sadler RC, Champney Schnepp A: **Elevated Blood Lead Levels in Children Associated With the Flint Drinking Water Crisis: A Spatial Analysis of Risk and Public Health Response**. *Am J Public Health* 2016, **106**(2):283-290.
3. Feng W, Cui X, Liu B, Liu C, Xiao Y, Lu W, Guo H, He M, Zhang X, Yuan J *et al*: **Association of urinary metal profiles with altered glucose levels and diabetes risk: a population-based study in China**. *PloS one* 2015, **10**(4):e0123742.
4. Menke A, Guallar E, Cowie CC: **Metals in Urine and Diabetes in U.S. Adults**. *Diabetes* 2016, **65**(1):164-171.
5. Barregard L, Bergstrom G, Fagerberg B: **Cadmium exposure in relation to insulin production, insulin sensitivity and type 2 diabetes: a cross-sectional and prospective study in women**. *Environmental research* 2013, **121**:104-109.
6. Hansen AF, Simic A, Asvold BO, Romundstad PR, Midthjell K, Syversen T, Flaten TP: **Trace elements in early phase type 2 diabetes mellitus-A population-based study. The HUNT study in Norway**. *J Trace Elem Med Biol* 2017, **40**:46-53.
7. Moon SS: **Association of lead, mercury and cadmium with diabetes in the Korean population: the Korea National Health and Nutrition Examination Survey (KNHANES) 2009-2010**. *Diabet Med* 2013, **30**(4):e143-148.
8. Borne Y, Fagerberg B, Persson M, Sallsten G, Forsgard N, Hedblad B, Barregard L, Engstrom G: **Cadmium exposure and incidence of diabetes mellitus--results from the Malmo Diet and Cancer study**. *PloS one* 2014, **9**(11):e112277.
9. Forte G, Bocca B, Peruzzu A, Tolu F, Asara Y, Farace C, Oggiano R, Madeddu R: **Blood metals concentration in type 1 and type 2 diabetics**. *Biol Trace Elem Res* 2013, **156**(1-3):79-90.
10. Gerber PA, Rutter GA: **The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus**. *Antioxid Redox Signal* 2017, **26**(10):501-518.
11. Kaneto H, Katakami N, Kawamori D, Miyatsuka T, Sakamoto K, Matsuoka TA, Matsuhisa M, Yamasaki Y: **Involvement of oxidative stress in the pathogenesis of diabetes**. *Antioxid Redox Signal* 2007, **9**(3):355-366.
12. Kubisch HM, Wang J, Bray TM, Phillips JP: **Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic beta-cells against oxidative stress**. *Diabetes* 1997, **46**(10):1563-1566.
13. Yen CC, Lu FJ, Huang CF, Chen WK, Liu SH, Lin-Shiau SY: **The diabetogenic effects of the combination of humic acid and arsenic: in vitro and in vivo studies**. *Toxicol Lett* 2007,

172(3):91-105.

14. Das KK, Das SN, Dhundasi SA: **Nickel, its adverse health effects & oxidative stress**. *Indian J Med Res* 2008, **128**(4):412-425.
15. Izquierdo-Vega JA, Soto CA, Sanchez-Pena LC, De Vizcaya-Ruiz A, Del Razo LM: **Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite**. *Toxicol Lett* 2006, **160**(2):135-142.
16. Valko M, Morris H, Cronin MT: **Metals, toxicity and oxidative stress**. *Curr Med Chem* 2005, **12**(10):1161-1208.
17. Kurata Y, Katsuta O, Doi T, Kawasuso T, Hiratsuka H, Tsuchitani M, Umemura T: **Chronic cadmium treatment induces islet B cell injury in ovariectomized cynomolgus monkeys**. *Jpn J Vet Res* 2003, **50**(4):175-183.
18. Chen YW, Yang CY, Huang CF, Hung DZ, Leung YM, Liu SH: **Heavy metals, islet function and diabetes development**. *Islets* 2009, **1**(3):169-176.
19. Khan AR, Awan FR: **Metals in the pathogenesis of type 2 diabetes**. *J Diabetes Metab Disord* 2014, **13**(1):16.
20. Sharma B, Singh S, Siddiqi NJ: **Biomedical implications of heavy metals induced imbalances in redox systems**. *Biomed Res Int* 2014, **2014**:640754.
21. Beyersmann D, Hartwig A: **Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms**. *Arch Toxicol* 2008, **82**(8):493-512.
22. Kajimoto Y, Matsuoka T, Kaneto H, Watada H, Fujitani Y, Kishimoto M, Sakamoto K, Matsuhisa M, Kawamori R, Yamasaki Y *et al*: **Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells**. *Diabetologia* 1999, **42**(12):1417-1424.
23. Kaur B, Henry J: **Micronutrient status in type 2 diabetes: a review**. *Adv Food Nutr Res* 2014, **71**:55-100.
24. Siddiqui K, Bawazeer N, Joy SS: **Variation in macro and trace elements in progression of type 2 diabetes**. *ScientificWorldJournal* 2014, **2014**:461591.
25. Ahamed M, Siddiqui MK: **Environmental lead toxicity and nutritional factors**. *Clin Nutr* 2007, **26**(4):400-408.
26. Flora SJ: **Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure**. *Oxid Med Cell Longev* 2009, **2**(4):191-206.
27. Walton FS, Harmon AW, Paul DS, Drobna Z, Patel YM, Styblo M: **Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes**. *Toxicol Appl Pharmacol* 2004, **198**(3):424-433.
28. Han JC, Park SY, Hah BG, Choi GH, Kim YK, Kwon TH, Kim EK, Lachaal M, Jung CY, Lee W: **Cadmium induces impaired glucose tolerance in rat by down-regulating GLUT4 expression in adipocytes**. *Arch Biochem Biophys* 2003, **413**(2):213-220.
29. Somwar R, Koterski S, Sweeney G, Sciotti R, Djuric S, Berg C, Trevillyan J, Scherer PE,

Rondinone CM, Klip A: **A dominant-negative p38 MAPK mutant and novel selective inhibitors of p38 MAPK reduce insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting GLUT4 translocation**. *The Journal of biological chemistry* 2002, **277**(52):50386-50395.

30. Souza K, Maddock DA, Zhang Q, Chen J, Chiu C, Mehta S, Wan Y: **Arsenite activation of P13K/AKT cell survival pathway is mediated by p38 in cultured human keratinocytes**. *Molecular medicine* 2001, **7**(11):767-772.

31. Zawalich WS, Zawalich KC: **A link between insulin resistance and hyperinsulinemia: inhibitors of phosphatidylinositol 3-kinase augment glucose-induced insulin secretion from islets of lean, but not obese, rats**. *Endocrinology* 2000, **141**(9):3287-3295.

32. Leasure JL, Giddabasappa A, Chaney S, Johnson JE, Jr., Pothakos K, Lau YS, Fox DA: **Low-level human equivalent gestational lead exposure produces sex-specific motor and coordination abnormalities and late-onset obesity in year-old mice**. *Environmental health perspectives* 2008, **116**(3):355-361.

33. Faulk C, Barks A, Sanchez BN, Zhang Z, Anderson OS, Peterson KE, Dolinoy DC: **Perinatal lead (Pb) exposure results in sex-specific effects on food intake, fat, weight, and insulin response across the murine life-course**. *PloS one* 2014, **9**(8):e104273.

34. Nie X, Wang N, Chen Y, Chen C, Han B, Zhu C, Chen Y, Xia F, Cang Z, Lu M *et al*: **Blood cadmium in Chinese adults and its relationships with diabetes and obesity**. *Environmental science and pollution research international* 2016, **23**(18):18714-18723.

35. Rothenberg SE, Korrick SA, Fayad R: **The influence of obesity on blood mercury levels for U.S. non-pregnant adults and children: NHANES 2007-2010**. *Environmental research* 2015, **138**:173-180.

36. Padilla MA, Elobeid M, Ruden DM, Allison DB: **An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02**. *International journal of environmental research and public health* 2010, **7**(9):3332-3347.

37. Lei LJ, Jin TY, Zhou YF: **[The effects of cadmium on the levels of insulin in smelters]**. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2006, **24**(1):3-6.

38. Serdar MA, Bakir F, Hasimi A, Celik T, Akin O, Kenar L, Aykut O, Yildirimkaya M: **Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose**. *Int J Diabetes Dev Ctries* 2009, **29**(1):35-40.

39. Afridi HI, Kazi TG, Brabazon D, Naher S, Talpur FN: **Comparative metal distribution in scalp hair of Pakistani and Irish referents and diabetes mellitus patients**. *Clin Chim Acta* 2013, **415**:207-214.

40. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB: **Body iron stores in relation to risk of type 2 diabetes in apparently healthy women**. *JAMA* 2004, **291**(6):711-717.

41. Kim NS, Lee BK: **National estimates of blood lead, cadmium, and mercury levels in the Korean general adult population**. *Int Arch Occup Environ Health* 2011, **84**(1):53-63.
42. Andrews PA: **Disorders of iron metabolism**. *N Engl J Med* 2000, **342**(17):1293; author reply 1294.
43. Oberley LW: **Free radicals and diabetes**. *Free Radic Biol Med* 1988, **5**(2):113-124.
44. Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr.: **A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002**. *Diabetes Care* 2006, **29**(7):1638-1644.
45. Hectors TL, Vanparys C, van der Ven K, Martens GA, Jorens PG, Van Gaal LF, Covaci A, De Coen W, Blust R: **Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function**. *Diabetologia* 2011, **54**(6):1273-1290.
46. Enan E, Liu PC, Matsumura F: **2,3,7,8-Tetrachlorodibenzo-p-dioxin causes reduction of glucose transporting activities in the plasma membranes of adipose tissue and pancreas from the guinea pig**. *The Journal of biological chemistry* 1992, **267**(28):19785-19791.
47. Longnecker MP, Michalek JE: **Serum dioxin level in relation to diabetes mellitus among Air Force veterans with background levels of exposure**. *Epidemiology* 2000, **11**(1):44-48.
48. Henriksen GL, Ketchum NS, Michalek JE, Swaby JA: **Serum dioxin and diabetes mellitus in veterans of Operation Ranch Hand**. *Epidemiology* 1997, **8**(3):252-258.
49. Steenland K, Piacitelli L, Deddens J, Fingerhut M, Chang LI: **Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin**. *J Natl Cancer Inst* 1999, **91**(9):779-786.

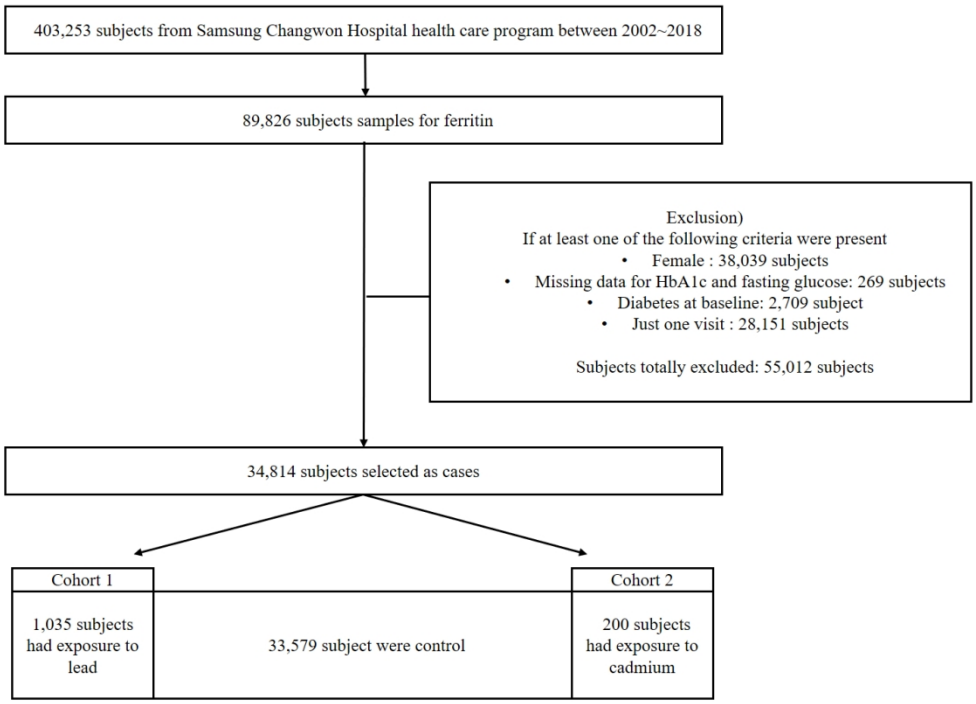
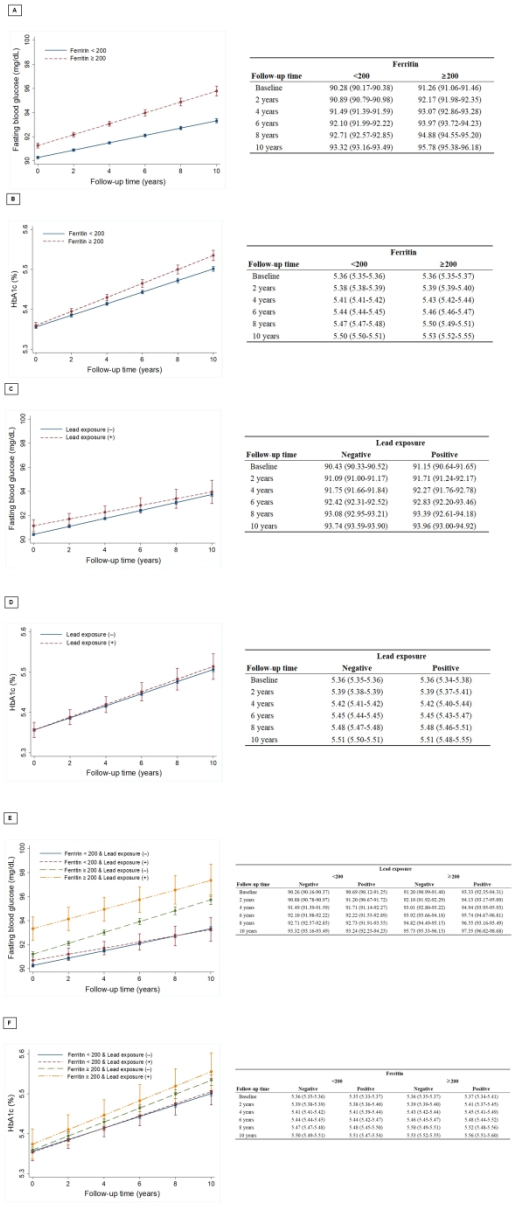


Figure 1. Schematic flow diagram

124x89mm (300 x 300 DPI)



A

	Ferritin	
Follow-up time	<200	≥200
Baseline	90.28 (90.17-90.38)	91.26 (91.06-91.46)
2 years	90.89 (90.79-90.98)	92.17 (91.98-92.35)
4 years	91.49 (91.39-91.59)	93.07 (92.86-93.28)
6 years	92.10 (91.99-92.22)	93.97 (93.72-94.23)
8 years	92.71 (92.57-92.85)	94.88 (94.55-95.20)
10 years	93.32 (93.16-93.49)	95.78 (95.38-96.18)

B

	Ferritin	
Follow-up time	<200	≥200
Baseline	5.36 (5.35-5.36)	5.36 (5.35-5.37)
2 years	5.38 (5.38-5.39)	5.39 (5.39-5.40)
4 years	5.41 (5.41-5.42)	5.43 (5.42-5.44)
6 years	5.44 (5.44-5.45)	5.46 (5.46-5.47)
8 years	5.47 (5.47-5.48)	5.50 (5.49-5.51)
10 years	5.50 (5.50-5.51)	5.53 (5.52-5.55)

C

	Lead exposure	
Follow-up time	Negative	Positive
Baseline	90.43 (90.33-90.52)	91.15 (90.64-91.65)
2 years	91.09 (91.00-91.17)	91.71 (91.24-92.17)
4 years	91.75 (91.66-91.84)	92.27 (91.76-92.78)
6 years	92.42 (92.31-92.52)	92.83 (92.20-93.46)
8 years	93.08 (92.95-93.21)	93.39 (92.61-94.18)
10 years	93.74 (93.59-93.90)	93.96 (93.00-94.92)

D

	Lead exposure	
Follow-up time	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.36 (5.34-5.38)
2 years	5.39 (5.38-5.39)	5.39 (5.37-5.41)
4 years	5.42 (5.41-5.42)	5.42 (5.40-5.44)
6 years	5.45 (5.44-5.45)	5.45 (5.43-5.47)
8 years	5.48 (5.47-5.48)	5.48 (5.46-5.51)
10 years	5.51 (5.50-5.51)	5.51 (5.48-5.55)

E

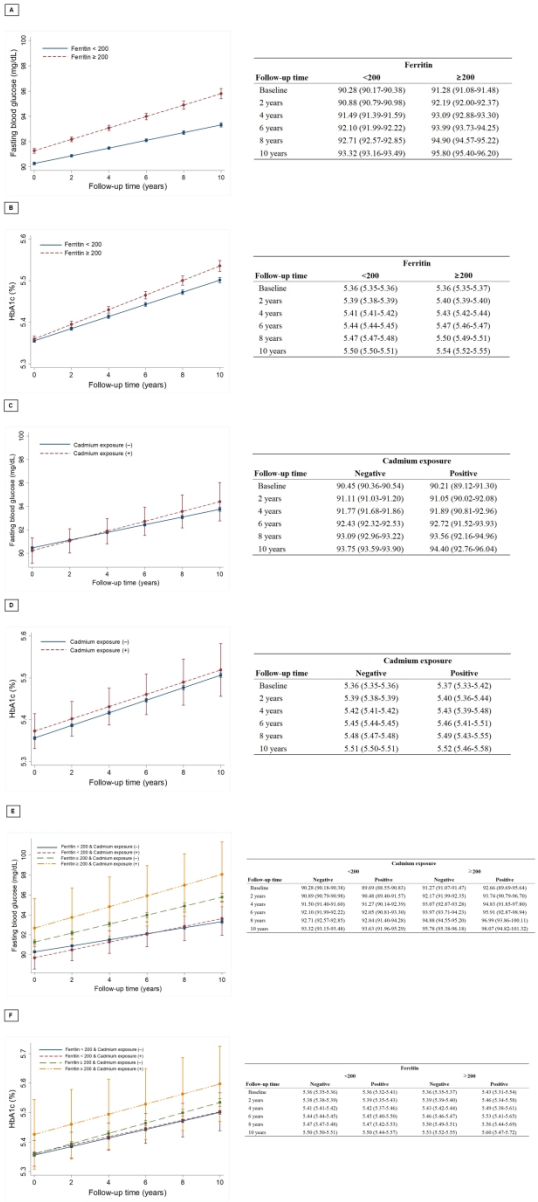
	Lead exposure			
	<200		≥200	
Follow-up time	Negative	Positive	Negative	Positive
Baseline	90.26 (90.16-90.37)	90.69 (90.12-91.25)	91.26 (90.99-91.48)	91.33 (92.35-94.31)
2 years	90.88 (90.78-90.97)	91.29 (90.67-91.72)	92.16 (91.92-92.29)	94.13 (93.17-95.09)
4 years	91.49 (91.39-91.59)	91.71 (91.14-92.27)	93.01 (92.80-93.22)	94.94 (93.95-95.93)
6 years	92.10 (91.98-92.22)	92.22 (91.55-92.89)	93.92 (93.66-94.18)	95.74 (94.67-96.81)
8 years	92.71 (92.57-92.85)	92.73 (91.91-93.55)	94.82 (94.49-95.15)	96.55 (95.16-97.49)
10 years	93.32 (93.16-93.49)	93.24 (92.25-94.23)	95.73 (95.33-96.13)	97.35 (96.02-98.68)

F

	Ferritin			
	<200		≥200	
Follow-up time	Negative	Positive	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.35 (5.33-5.37)	5.36 (5.35-5.37)	5.37 (5.34-5.41)
2 years	5.38 (5.38-5.38)	5.38 (5.36-5.40)	5.39 (5.39-5.40)	5.41 (5.37-5.45)
4 years	5.41 (5.41-5.42)	5.41 (5.39-5.44)	5.43 (5.42-5.44)	5.45 (5.41-5.49)
6 years	5.44 (5.44-5.45)	5.44 (5.42-5.47)	5.46 (5.45-5.47)	5.48 (5.44-5.52)
8 years	5.47 (5.47-5.48)	5.48 (5.45-5.50)	5.50 (5.49-5.51)	5.52 (5.48-5.56)
10 years	5.50 (5.49-5.51)	5.51 (5.47-5.54)	5.53 (5.52-5.55)	5.56 (5.51-5.60)

Figure 2. Mixed models were used to evaluate the effects of lead exposure and ferritin on FBS and HbA1c
A – Changes in fasting blood glucose according to serum ferritin levels in cohort A
B – Changes in HbA1c according to serum ferritin levels in cohort A
C – Changes in fasting blood glucose according to lead exposure in cohort A
D – Changes in HbA1c according to lead exposure in cohort A
E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort A
F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort A

154x360mm (300 x 300 DPI)



Ferritin

Follow-up time	<200	≥200
Baseline	90.28 (90.17-90.38)	91.28 (91.08-91.48)
2 years	90.88 (90.79-90.98)	92.19 (92.00-92.37)
4 years	91.49 (91.39-91.59)	93.09 (92.88-93.30)
6 years	92.10 (91.99-92.22)	93.99 (93.73-94.25)
8 years	92.71 (92.57-92.85)	94.90 (94.57-95.22)
10 years	93.32 (93.16-93.49)	95.80 (95.40-96.20)

Ferritin

Follow-up time	<200	≥200
Baseline	5.36 (5.35-5.36)	5.36 (5.35-5.37)
2 years	5.39 (5.38-5.39)	5.40 (5.39-5.40)
4 years	5.41 (5.41-5.42)	5.43 (5.42-5.44)
6 years	5.44 (5.44-5.45)	5.47 (5.46-5.47)
8 years	5.47 (5.47-5.48)	5.50 (5.49-5.51)
10 years	5.50 (5.50-5.51)	5.54 (5.52-5.55)

Cadmium exposure

Follow-up time	Negative	Positive
Baseline	90.45 (90.36-90.54)	90.21 (89.12-91.30)
2 years	91.11 (91.03-91.20)	91.05 (90.02-92.08)
4 years	91.77 (91.68-91.86)	91.89 (90.81-92.96)
6 years	92.43 (92.32-92.53)	92.72 (91.52-93.93)
8 years	93.09 (92.96-93.22)	93.56 (92.16-94.96)
10 years	93.75 (93.59-93.90)	94.40 (92.76-96.04)

Cadmium exposure

Follow-up time	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.37 (5.33-5.42)
2 years	5.39 (5.38-5.39)	5.40 (5.36-5.44)
4 years	5.42 (5.41-5.42)	5.43 (5.39-5.48)
6 years	5.45 (5.44-5.45)	5.46 (5.41-5.51)
8 years	5.48 (5.47-5.48)	5.49 (5.43-5.55)
10 years	5.51 (5.50-5.51)	5.52 (5.46-5.58)

Follow-up time	<200 Negative	<200 Positive	≥200 Negative	≥200 Positive
Baseline	90.28 (90.18-90.38)	89.69 (88.55-90.83)	91.27 (91.07-91.47)	92.66 (89.69-95.64)
2 years	90.89 (90.79-90.98)	90.48 (89.40-91.57)	92.17 (91.99-92.35)	93.74 (90.79-96.70)
4 years	91.50 (91.40-91.60)	91.27 (90.14-92.39)	93.07 (92.87-93.28)	94.83 (91.85-97.80)
6 years	92.10 (91.99-92.22)	92.05 (90.81-93.30)	93.97 (93.71-94.23)	95.91 (92.87-98.94)
8 years	92.71 (92.57-92.85)	92.84 (91.49-94.20)	94.86 (94.55-95.20)	96.99 (93.86-100.11)
10 years	93.32 (93.15-93.48)	93.63 (91.96-95.29)	95.78 (95.38-96.18)	98.07 (94.82-101.32)

Follow-up time	<200 Negative	<200 Positive	≥200 Negative	≥200 Positive
Baseline	5.36 (5.35-5.36)	5.36 (5.32-5.41)	5.36 (5.35-5.37)	5.43 (5.31-5.54)
2 years	5.38 (5.38-5.39)	5.39 (5.35-5.43)	5.39 (5.39-5.40)	5.46 (5.34-5.58)
4 years	5.41 (5.41-5.42)	5.42 (5.37-5.46)	5.43 (5.42-5.44)	5.49 (5.38-5.61)
6 years	5.44 (5.44-5.45)	5.45 (5.40-5.50)	5.46 (5.46-5.47)	5.53 (5.41-5.65)
8 years	5.47 (5.47-5.48)	5.47 (5.42-5.53)	5.50 (5.49-5.51)	5.56 (5.44-5.69)
10 years	5.50 (5.50-5.51)	5.50 (5.44-5.57)	5.53 (5.52-5.55)	5.60 (5.47-5.72)

Figure 3. Mixed models were used to evaluate the effects of cadmium exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort B

B – Changes in HbA1c according to serum ferritin levels in cohort B

C– Changes in fasting blood glucose according to lead exposure in cohort B

D – Changes in HbA1c according to lead exposure in cohort B

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort B

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort B

155x347mm (300 x 300 DPI)

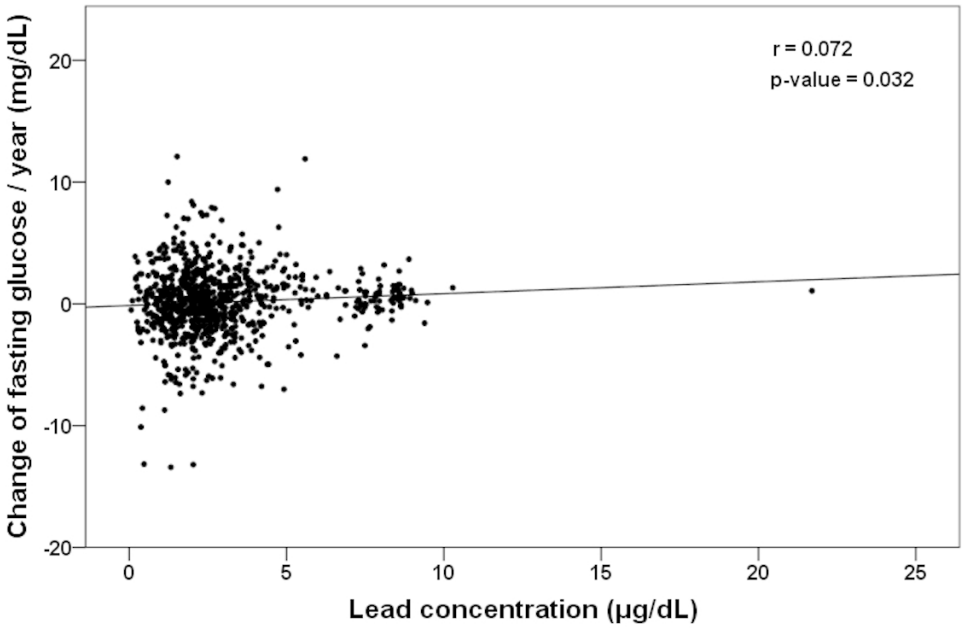


Figure 4. Scatter plot showing the annual changes of fasting blood glucose by lead concentration (r=0.072, p = 0.032)

99x67mm (300 x 300 DPI)

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1

1Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	6
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
8Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than	6

		one group. Give information separately for for exposed and unexposed groups if applicable.	
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	7
Statistical methods	#12c	Explain how missing data were addressed8	NA
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	NA
Statistical methods	#12e	Describe any sensitivity analyses	7
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-	9

		up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	
Participants	#13b	Give reasons for non-participation at each stage	9
Participants	#13c	Consider use of a flow diagram	6,9 (fig.1)
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	9
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	9
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
Main results	#16b	Report category boundaries when continuous variables were categorized	9
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9

Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	#18	Summarise key results with reference to study objectives	11
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13
Generalisability	#21	Discuss the generalisability (external validity) of the study results	10-13
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

BMJ Open

The relationship between heavy metal exposure and type 2 diabetes: A large-scale retrospective cohort study using occupational health examinations

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039541.R3
Article Type:	Original research
Date Submitted by the Author:	23-Jan-2021
Complete List of Authors:	Ji, Jun Ho ; Samsung Changwon Hospital, Internal medicine Jin, Mi Hyeon ; Samsung Changwon Hospital Kang, Jung-Hun ; Gyeongsang National University College of Medicine, Internal Medicine Lee, Soon Il ; Dankook University College of Medicine, Internal Medicine Lee, Suee ; Dong-A University Medical Center, Internal medicine Kim, Sung-Hyun ; Dong-A University Medical Center, Internal Medicine Oh, Sung Yong; Dong-A University Medical Center
Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	Diabetes & endocrinology < INTERNAL MEDICINE, SOCIAL MEDICINE, EPIDEMIOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The relationship between heavy metal exposure and type 2 diabetes: A large-scale retrospective cohort study using occupational health examinations

Jun Ho Ji[1], Mi Hyeon Jin[2], Jung-Hun Kang[3], Soon Il Lee[4], Suee Lee[5], Sung-Hyun Kim[5], Sung Yong Oh[5#]

[1]Department of Internal Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea;

[2]Department of Biostatistics, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea;

[3]Department of Internal Medicine, Gyeongsang National University Scholl of Medicine, Jinju, Korea;

[4]Department of Internal Medicine, Dankook University College of Medicine, Cheonan;

[5]Department of Internal Medicine, Dong-A University College of Medicine, Busan

Running title: Relationship between heavy metal exposure and diabetes

Word count: 3424

Corresponding author: Sung Yong Oh

Address: Department of Internal Medicine, Dong-A University College of Medicine, 26 Daesingongwon-Ro, Seo-Gu, Busan 49201, Korea

Mobile: +82-10-8624-9818

E-mail:drosy@dau.ac.kr

Abstract

Objectives: To investigate the associations between heavy metal exposure and serum ferritin levels, physical measurements, and type 2 diabetes mellitus (DM).

Design: A retrospective cohort study.

Setting: Changwon, the location of this study, is a Korean representative industrial city. Data were obtained from medical check-ups between 2002 and 2018.

Participants: A total of 34,814 male subjects were included. Of them, 1,035 subjects with lead exposure, 200 subjects with cadmium exposure, and the 33,579 remaining were assigned to cohort A, cohort B, and the control cohort, respectively. Data including personal history of alcohol and smoking, age, height, weight, the follow-up duration, HbA1c, fasting blood sugar (FBS), ferritin levels, and lead and cadmium levels within one year after exposure were collected.

Primary outcome measure: In subjects without diabetes, changes in FBS and HbA1c were analyzed through repeated tests at intervals of one year or longer after the occupational exposure to heavy metals.

Results: In cohort A, DM was diagnosed in 33 subjects. There was a significant difference in lead concentrations between the subjects diagnosed with DM and those without DM during the follow-up period (3.94 ± 2.92 mg/dL versus 2.81 ± 2.03 mg/dL, $p = 0.002$). Simple exposure to heavy metals (lead and cadmium) was not associated with DM in Cox regression models (lead exposure hazard ratio [HR] 1.01, 95% CI 0.58 – 1.77, p 0.971; cadmium exposure HR 1.48, 95% CI: 0.61 – 3.55, $p = 0.385$). Annual changes in FBS according to lead concentration at the beginning of exposure showed a positive correlation ($r = 0.072$, $p = 0.032$).

Conclusion: Our findings demonstrated that simple occupational exposure to heavy metals lead and cadmium was not associated with the incidence of DM. However, lead concentrations at the beginning of the exposure might be an indicator of DM and glucose elevations.

Keywords: diabetes, heavy metal exposure, HbA1c, body mass index, ferritin

Strengths and limitations of this study

- This study was limited by the single institute data obtained from occupational medical evaluations.
- Another important limitation of the study was the exclusion of females of childbearing age who have decreased serum ferritin due to menstruation.
- This study was a large-scale study to determine the blood concentrations of heavy metals (initial exposure to occupational heavy metal and exposure over a long period of time) and changes in FBS and HbA1c levels.
- This study showed changes in blood glucose and HbA1c over time after exposure to heavy metals.

- Introduction

Diabetes mellitus (DM), a common and rising global problem, is one of the leading causes of death, blindness, and chronic renal failure. It is also a major risk factor for vascular diseases such as myocardial infarction, stroke, and peripheral vascular disease. The increase in social cost due to DM-related morbidity or mortality has intensified efforts to reduce the incidence of DM. The rising incidence of DM is considered to be associated with alterations in lifestyles and other contributing factors, including exposure to several environmental pollutants and industrial chemicals.

With rapid industrial development, exposure to various environmental toxic materials has risen along with DM incidence. Environmental substances that cause endocrine disruption have been defined as endocrine-disrupting chemicals (EDC) by the U.S. Environmental Protection Agency (EPA) [1]. Metals are naturally existing inorganic elements that are present in very small amounts in the body. They are essential for vital processes. Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. Heavy metals and metalloids (e.g., lead, mercury, cadmium, and metalloid arsenic) can affect hormonal activity, suggesting that these compounds are EDCs generally considered to be toxicants. These heavy metals have negative effects on physiology. They might be associated with the incidence of DM in some populations. In this study, we particularly focused on the association between exposure to heavy metals and DM. In recent decades, environmental exposure to heavy metals has declined because many countries have begun to pay attention to environmental problems rather than industrial development. However, the unintended exposure to heavy metals in the environment such as older household structures and in drinking water in Flint, MI, USA [2], is still possible. Such exposure can be due to the illegal, unauthorized disposal of toxic materials including heavy metals from industries. In Korea, occupational exposure to heavy metals is more common than random environmental exposure.

In occupational exposure to heavy metals, relatively few studies have reported whether the degree of exposure has direct or indirect effects on the body or specific diseases. A few population-based studies have focused on the association between metal exposure and diabetes, showing inconsistent results [3-9]. Most previous studies have examined the association of DM with heavy metal

concentrations in the blood or urine at one specific time [6, 7].

Intense exposure to heavy metals can result in high levels of heavy metals in the blood or urine, whereas light exposure results in extremely low levels. Although long-term, light exposure to heavy metals might only lead to low levels of heavy metals in the blood or urine, heavy metals deposited in organs may be harmful. The deposition of heavy metals in the liver and pancreas can alter gluconeogenesis in the liver and affect insulin secretion, eventually influencing the incidence of DM. Although this study was designed as a retrospective study of long-term occupational exposure to heavy metals (lead and cadmium), instead of measuring the concentration of heavy metals in organs such as the liver, bone, and pancreas, the blood concentrations of heavy metals at the beginning of the exposure (within one year) were measured and compared to changes in FBS, HbA1c, and the incidence of DM in the general population who were not exposed to heavy metals during the same period.

Material and Methods

1) Study population

Changwon, the location of this study, is a representative industrial city in Korea. Many occupations involve heavy metal exposure, including employees of battery-manufacturing plants. This cohort study was based on the data from occupational health examinations (n = 403,253) conducted from 2002 to 2018 in subjects with jobs related to heavy metals. A schematic flow chart for the selection of subjects is shown in Figure 1. All participants underwent a physical examination with a blood sample taken in the morning following an overnight fast. They also filled out a questionnaire. Among these 403,253 subjects, 89,826 who had ferritin blood levels measured were included and 38,039 women were excluded. In occupational screening, most women were fertile. The ferritin results might be low because of menstruation. A total of 269 subjects were excluded because of the unavailability of HbA1c or FBS data. Furthermore, 2709 subjects who were already diagnosed with DM were excluded (DM was defined as FBS $\geq$ 126 mg/dl, HbA1c $\geq$ 6.5%, or a history of DM reported in the questionnaire). Additionally, 28,151 subjects were excluded because they only had one screening result without follow-up data. Finally, 34,814 subjects were included in the analysis. Of these, 1,035 subjects with lead exposure, 200 subjects with cadmium exposure, and the 33,579 remaining subjects were assigned to cohort A, cohort B, and the control cohort, respectively. This study collected subject data including age, HbA1c, FBS, ferritin levels, height, body weight, the follow-up duration, and the concentrations of heavy metals (lead and cadmium). The study protocol was approved by the Institutional Review Board (IRB) of Samsung Changwon Medical Center (SCMC-2019-04-014). All participants provided written informed consent for the use of their data.

2) Data collection

This study was based on data from occupational health examinations already conducted. The health check-up data included objective numerical data such as blood tests, imaging tests, and physical examinations, as well as the questionnaire responses of the subjects. The questionnaire included items on personal history, physical activity, systemic symptoms, sleep patterns, stress, anxiety, depression,

gambling, and job stress. All data were computerized. The authors analysed the demographic information, physical examination results, past history, and laboratory results (HbA1c, blood glucose, ferritin, lead, and cadmium levels). After obtaining IRB approval, two authors (JHJ and MHJ) independently analysed the data.

3) Measuring blood levels of lead and cadmium

To measure the blood levels of lead and cadmium, 3 ml of blood was collected from each subject into vacuum bottles using heparin as an anticoagulant in the morning following an overnight fast. Blood samples were diluted 1:15 and 1:10 to measure the lead and cadmium concentrations, respectively, with 2.5 ml of 10% Triton X-100, 0.1 ml of concentrated nitric acid, and 1 ml of 10% ammonium di-hydrogen phosphate as a modifier. Graphite-furnace atomic absorption spectrometry with Zeeman background correction (PinAAcle 9i00z Atomic absorption spectrometer, PerkinElmer, Norwalk, Connecticut, USA) was used to measure the lead and cadmium levels in all subjects within the first year of heavy metal exposure. The minimum detectable limits of lead and cadmium were measured to the third decimal place (0.001mg/dl), and concentrations below that were considered to be zero.

4) Statistical analyses

The continuous variables are presented as means ± standard deviation. The categorical variables are presented as the number of cases and percentages. An independent t-test was used to evaluate the significance of the mean differences between the continuous variables for demographical factors such as age and body mass index (BMI). The Cox proportional hazard model was used to identify potential predictors in the baseline characteristics for type 2 DM in subjects who were not diagnosed with DM. In the Cox hazard model, the development of type 2 DM was considered a dependent variable and as independent variables were set to the exposure levels of lead and cadmium and the known risk factors (age, BMI, smoking, drinking, HbA1c, FBS, and ferritin). A mixed model was used to assess the effects of heavy metal exposure and ferritin on FBS and HbA1c, respectively. The annual changes in FBS and HbA1c with lead concentrations are shown in a scatter plot. Stata 14.0 software (Stata Corporation, College Station, TX, USA) was used for all statistical analyses.

5) Operational definitions

1. Type 2 DM was defined in patients with a diabetes diagnosis history taking anti-diabetic medication or satisfying the American Diabetes Association (ADA) criteria of HbA1c ≥ 6.5% or FBS ≥ 126 mg/dl in a blood test after an 8-hour fast.

2. Newly diagnosed diabetes was defined in subjects without a history of diabetes who had an HbA1c of < 6.5% and an FBS of < 100 mg/dl in the first health check-up after joining the company and were newly diagnosed with diabetes (HbA1c ≥ 6.5% or FBS ≥ 126 mg/dl) in a follow-up health check-up conducted at least one year later.

3. The heavy metal exposure subjects were those who worked in the lead industry, those who were in charge of lead welding and mounting in shipyards, and subjects who worked in Ni-Cd battery manufacturing factories.

4. Simple occupational exposure to lead or cadmium, called simple exposure, referred to subjects who worked on-site at the workplace regardless of the intensity of the exposure.

5. The beginning of exposure referred to the first occupational health examination conducted within a year of working in the workplace related to heavy metal exposure.

6) Patient and public involvement

The patients and the public were not involved in the development of the research question or the design of the study. No patients or public members were involved in the recruitment or conduct of the study. Since this study used de-identified results, the authors do not plan to disseminate the study results to the study participants individually but plan to publish the paper with open access.

Results

1) Baseline characteristics of the study subjects

The baseline characteristics of the subjects in each cohort are shown in Table 1. Of 34,818 subjects, 1,034 were diagnosed with DM during the follow-up and 33,780 were not diagnosed with DM. In cohort A (1,035 subjects with lead-exposure and 33,779 subjects with no lead exposure), 1,034 were confirmed to have DM. Of these 1,034 subjects, 33 were exposed to lead. In the control group without heavy metal exposure, age, HbA1c, FBS, and ferritin levels were associated with DM, as expected. In heavy metal-exposed subjects, only HbA1c, FBS, and BMI were significantly associated with DM. An interesting aspect in cohort A was that the initial concentration of lead (within one year) was significantly higher in subjects who were later diagnosed with DM (2.81 ± 2.03 mg/dL in patients not diagnosed with diabetes and 3.94 ± 2.92 mg/dL in patients diagnosed with diabetes, p = 0.002). In contrast, the early cadmium blood levels did not differ between the group of subjects progressing to DM and those who did not progress to DM. The rates of drinking and smoking in patients with diabetes was similar to that in previous studies. Overall, the incidence of diabetes was higher in drinkers than in non-drinkers and higher in smokers than in ex-smokers or never smokers. However, the total number of subjects exposed to heavy metals was small, resulting in no statistical significance. The follow-up period was shorter and the mean age was higher in subjects progressing to DM in both cohorts. In the lead-exposed group, the mean follow-up duration was 3.18 ± 3.63 years for the group with DM and 4.78 ± 2. 77 years (p = 0.001) for the non-diabetes group. In the cadmium-exposed group, the mean follow-up duration was 5.45 ± 4.76 years for the DM group and 6.96 ± 3.77 years (p = 0.207) for the non-diabetes group.

2) Risk of developing DM from lead/cadmium exposure and serum ferritin levels

The Cox-regression models showed the crude and adjusted hazard ratios of the variables predicting the development of DM (Table 2). Age, HbA1c, FBS, BMI, current smoking, and ferritin were predictors for developing DM in both the crude and adjusted models. However, simple exposure to lead or cadmium was not associated with DM. Ferritin levels had a positive relationship with FBS and HbA1c elevations during the follow-up period in both cohorts A and B (Figures 2-A, 2-B, 3-A, 3-B). FBS elevations in subjects with simple lead exposure were slower than in those without lead exposure

(Figure 2-C). However, simple lead exposure did not have a significant effect on HbA1c elevation (Figure 2-D). The association of early cadmium exposure on the FBS/HbA1c change was not different from that of lead. In cohort B, ferritin also had significant effects on the elevation of FBS and HbA1c (Figure 3-A, Figure 3-B). Early exposure to cadmium was positively correlated with the rate of FBS change but negatively correlated with HbA1c change (Figures 3-C, 3-D). The unusual finding in both cohorts was that all subjects were healthy, without DM at the time of enrolment. However, subjects with elevated ferritin and heavy metal exposure had higher baseline FBS and HbA1c values than those who did not (Figures 2-E, 2-F, 3-E, 3-F). Regarding the concentrations of heavy metals, annual variations in FBS according to the initial lead concentrations showed weak but positive correlations (r = 0.072, p = 0.032, Figure 4).

Discussion

Many studies have attempted to explain the relationship between heavy metal exposure and hyperglycaemia. Several plausible hypotheses have resulted from such research. First, oxidative stress caused by heavy metals can directly damage beta cells of the pancreas, leading to elevated serum glucose levels [10-17]. Such oxidative stress may also increase blood glucose levels by decreasing insulin release, impairing insulin receptors, disrupting glucose uptake, increasing hepatic gluconeogenesis and pancreatic glucagon secretion, and decreasing peripheral glucose use [16, 18-22]. Another hypothesis is related to the competitive inhibition of toxic metals. It states that essential trace metals at normal levels play a key role in glucose homeostasis because these metals are essential cofactors for glucose metabolism, pancreatic beta-cell function, and the insulin signalling cascade [18, 19, 23, 24]. Toxic metals compete with these essential metals for various physiological functions and affect type 2 DM risk [25, 26]. It has also been reported that toxic metals can affect various substances, including glucose transporter type 4, nuclear factor kappa B, mitogen-activated protein kinases, and phosphoinositide 3-kinase involved in insulin signalling, thereby increasing the risk of DM [27-31]. The last hypothesis is that exposure to metals, especially heavy metals, can increase body weight, a theory based on population studies. Because weight gain is a known risk factor for DM, exposure to heavy metals might be associated with DM [32-36]. Many studies on the relationship between heavy metal exposure and DM have been performed based on these findings. However, they showed inconsistent results [3-9]. Thus, it can be inferred that a direct association between heavy metals and DM has not yet been confirmed. Even if such association is relevant, it is very weak. The prior epidemiologic studies reporting inconsistent results connecting heavy metals to DM have limitations. Most previous studies had cross-sectional designs [3-5, 7-9]. A cross-sectional study is characterized by an analysis conducted at a specific point in time. It does not reflect changes over time. In the case of heavy metal exposure, chronic long-time exposure is more common than acute exposure. Therefore, the time of exposure to heavy metals is important. The time elapsed since the first exposure should be also considered. A Chinese study reported that insulin secretion was decreased more in the group exposed to cadmium for more than 10 years than in the group exposed to cadmium for less than 10 years [37]. Previous studies have also been conducted with a case-control design [3, 9, 38, 39]. It is well-known

that a small case-control study tends to be less expensive and shorter in duration. However, it has a low level of evidence.

This study investigated the relationships between serum ferritin levels, exposure to heavy metals, and DM during the health screening of subjects who worked in battery, paint, and bullet manufacturing facilities, shipyards, or workplaces requiring welding. Although this study included data from a single institution, it was designed as a retrospective longitudinal study using a large number of health screening subjects, thus overcoming the limitations of prior studies. The following results were obtained. (1) Simple exposure to heavy metals did not increase the risk of developing DM over time. However, the concentration of lead at the time of initial lead exposure was higher in subjects later diagnosed with DM. (2) Serum ferritin was a predictor of DM, as previously reported [40]. However, serum ferritin was not a predictor of DM in subjects exposed to lead or cadmium. (3) A high blood lead concentration at the beginning of the lead exposure was proportional to the rate of increase in FBS per year. It was noteworthy that when the blood lead concentration measured within a year after exposure was high, the rate of FBS increased gradually with time. A high blood lead concentration means that the lead exposure intensity is strong in a short time. Thus, lead exposure intensity might be a risk factor for DM. This aligns with our other study results, in which simple exposure to heavy metals was not related to the incidence of DM or elevations in FBS and HbA1c. The concentrations of heavy metals in our cohort were slightly higher than those in the Korean general adult population in a demographic study on environmental exposure to heavy metals by Kim et al. [41]. This suggests that our cohort was occupationally exposed to heavy metals. However, their exposure intensity was not high enough to significantly affect the incidence of DM. Similar to our results, a Korean study demonstrated that low-dose lifetime environmental exposure to lead and cadmium might not affect the incidence of DM. Another interesting aspect of this study is shown in Table 1. In the lead- and cadmium-exposed groups, serum ferritin levels in the group with diabetes were significantly higher than those in the non-diabetes group, but not in subjects exposed to lead or cadmium (serum ferritin was lower in the diabetes group). The reason for these results cannot be precisely explained. Oxidative stress caused by the production of free radicals [12-16,18], a mechanism by which heavy metals cause DM, might be the mechanism involved in the development of DM [42, 43]. Some large-scale US studies have shown that high blood

levels of persistent organic pollutants (POPs), which are not heavy metals but bio-accumulate as heavy metals with chronic environmental exposure globally, pose an increased risk for DM [44]. The mechanism by which POPs induce DM is similar to that for DM induced by heavy metals [45, 46]. Similar to studies on the associations of heavy metals and DM, studies on the associations of POPs with DM also showed discrepant results [47-49].

The current findings should be interpreted with caution because of several limitations. Since this study was based on data from subjects undergoing health check-ups, we could not identify or analyse the risk factors of DM, including hypertension, family history, and dyslipidaemia. The second limitation was that the blood concentrations of heavy metals were measured only once at the beginning of the exposure. Follow-up observations such as the diagnosis of DM were done longitudinally without reflecting changes in the serum concentrations of heavy metals as in a cross-sectional study. The limited study cohort population was another limitation. Because of possible iron deficiency during menstruation, female subjects were excluded. Due to the nature of the industry dealing with heavy metals, it is a limited study cohort to include only young subjects in the study. Although this study excluded female subjects, it would be interesting to investigate the relationship between occupational heavy metal exposure and diabetes in women. Although menstruation can cause iron deficiency, serum ferritin is associated with the risk of developing diabetes in fertile women [40]. Thus, further studies with female subjects are warranted.

In conclusion, our findings demonstrated that simple exposure to lead or cadmium was not associated with the prevalence of DM. However, blood lead concentrations at the beginning of exposure might be a predictor of DM development and glucose elevations. Our results suggest that low-dose, chronic occupational exposure to lead or cadmium may not affect the incidence of DM. However, if the exposure intensity is high, screening for DM should be performed.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **A competing interests statement:** Non declared

2

Authors' contribution:

Conception or design: JHJ

Acquisition, analysis, or interpretation of data: JHJ

Drafting the work or revising: JHJ,MHJ,JHK,SIL,SL,SHK,SYO

Final approval of the manuscript: JHJ,MHJ,JHK,SIL,SL,SHK,SYO

A funding statement: This study was supported by Dong-A University Research fund 2020.

Acknowledgements: Thanks to all the patients who participated in this study.

Data availability: Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi: 10.5061/dryad.tht76hdz4

= Figure legends =

Figure 1. Schematic flow diagram

Figure 2. Mixed models were used to evaluate the effects of lead exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort A

B – Changes in HbA1c according to serum ferritin levels in cohort A

C – Changes in fasting blood glucose according to lead exposure in cohort A

D – Changes in HbA1c according to lead exposure in cohort A

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort A

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort A

Figure 3. Mixed models were used to evaluate the effects of cadmium exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort B

B – Changes in HbA1c according to serum ferritin levels in cohort B

C– Changes in fasting blood glucose according to lead exposure in cohort B

D – Changes in HbA1c according to lead exposure in cohort B

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort B

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort B

Figure 4. Scatter plot showing the annual changes of fasting blood glucose by lead concentration (r=0.072, p = 0.032)

= Table legends =

Table1. Baseline characteristics

Table2. Cox regression models: Crude and adjusted HRs of baseline characteristics predicting the development of type 2 diabetes mellitus

Table1. Baseline characteristics

	Lead (Cohort A)					
	No exposure (n=33,779)			Exposure (n=1,035)		
	Non diabetes (n=32,778)	Diabetes (n=1,001)	P-value	Non diabetes (n=1,002)	Diabetes (n=33)	P-value
Age	34.99 ± 7.99	37.96 ± 8.16	<0.001	32.18 ± 8.36	34.19 ± 7.92	0.174
HbA1c (%)	5.32 ± 0.30	5.76 ± 0.59	<0.001	5.33 ± 0.29	5.88 ± 0.81	<0.001
Fasting blood sugar (mg/dL)	89.98 ± 8.65	103.49 ± 18.90	<0.001	91.37 ± 9.11	114.36 ± 32.71	<0.001
Ferritin (ng/mL)	145.71 ± 93.76	165.55 ± 119.90	<0.001	152.51 ± 99.86	139.77 ± 89.57	0.470
Smoking (n=3.727)			<0.001			0.511
Never smoker	9,716	212		367	8	
Ex-smoker	6,210	173		183	8	
Current smoker	12,958	460		416	46	
Alcohol (n=34,814)			0.003			0.620
No	3,515	137		30	0	
Yes	29,263	864		972	33	
Body mass index (Kg/m²)	24.04 ± 3.03	25.89 ± 3.54	<0.001	24.04 ± 3.17	26.04 ± 2.87	<0.001
Lead concentration (mg/dL)	-	-	-	2.81 ± 2.03	3.94 ± 2.92	0.002
Follow-up duration (year)	5.65 ± 3.48	5.09 ± 3.67	<0.001	4.78 ± 2.77	3.18 ± 3.63	0.001
	Cadmium (Cohort B)					
	No exposure (n=34,614)			**Exposure (n=200)**		
	Non diabetes (n=33,591)	**Diabetes (n=1,023)**	**P-value**	**Non diabetes (n=189)**	**Diabetes (n=11)**	**P-value**
Age	34.91 ± 8.02	37.84 ± 8.19	<0.001	34.77 ± 8.28	38.04 ± 7.22	0.203
HbA1c (%)	5.32 ± 0.30	5.76 ± 0.60	<0.001	5.31 ± 0.31	5.65 ± 0.89	0.002
Fasting blood sugar (mg/dL)	90.01 ± 8.66	103.83 ± 19.55	<0.001	91.52 ± 9.43	104.00 ± 22.05	<0.001
Ferritin (ng/mL)	146.03 ± 94.00	165.27 ± 119.32	<0.001	124.24 ± 81.76	113.93 ± 85.87	0.686
Smoking (n=3.727)			<0.001			0.033
Never smoker	10,002	219		81	1	
Ex-smoker	6,359	177		34	4	
Current smoker	13,308	470		66	6	
Alcohol (n=34,814)			0.004			>0.999
No	3,540	137		5	0	
Yes	30,051	886		184	11	
Body mass index (Kg/m²)	24.04 ± 3.04	25.88 ± 3.53	<0.001	24.00 ± 3.20	26.78 ± 2.67	0.005
Cadmium concentration (mg/dL)	-	-	-	0.20 ± 0.26	0.17 ± 0.11	0.731
Follow-up duration (year)	5.61 ± 3.46	5.02 ± 3.67	<0.001	6.96 ± 3.77	5.45 ± 4.76	0.207

Table2. Cox regression models: Crude and adjusted HRs of baseline characteristics predicting the development of type 2 diabetes mellitus

	Crude		Adjusted (N=30,589)	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Age (year)	1.05 (1.04-1.06)	<0.001	1.01 (1.00-1.03)	0.012
HbA1c (%) × 10	1.54 (1.51-1.57)	<0.001	1.35 (1.32-1.39)	<0.001
Fasting blood sugar (mg/dL)	1.12 (1.11-1.12)	<0.001	1.07 (1.06-1.08)	<0.001
Body mass index (Kg/m²)	1.21 (1.19-1.22)	<0.001	1.10 (0.078-1.12)	<0.001
Ferritin (ng/mL, reference: <200)	2.25 (1.94-2.62)	<0.001	1.51 (1.28-1.79)	<0.001
Lead exposure	1.05 (0.68-1.63)	0.812	1.01 (0.58-1.77)	0.971
Cadmium exposure	1.08 (0.54-2.17)	0.828	1.48 (0.61-3.55)	0.385
Smoking				
Ex-smoker	1.22 (0.98-1.51)	0.071	1.05 (0.85-1.31)	0.634
Current smoker	1.61 (1.35-1.92)	<0.001	1.45 (1.22-1.73)	<0.01
Drinking	0.83 (0.68-1.01)	0.062	1.07 (0.53-2.17)	0.842

References

1. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC: **Endocrine-disrupting chemicals: an Endocrine Society scientific statement**. *Endocr Rev* 2009, **30**(4):293-342.
2. Hanna-Attisha M, LaChance J, Sadler RC, Champney Schnepp A: **Elevated Blood Lead Levels in Children Associated With the Flint Drinking Water Crisis: A Spatial Analysis of Risk and Public Health Response**. *Am J Public Health* 2016, **106**(2):283-290.
3. Feng W, Cui X, Liu B, Liu C, Xiao Y, Lu W, Guo H, He M, Zhang X, Yuan J *et al*: **Association of urinary metal profiles with altered glucose levels and diabetes risk: a population-based study in China**. *PloS one* 2015, **10**(4):e0123742.
4. Menke A, Guallar E, Cowie CC: **Metals in Urine and Diabetes in U.S. Adults**. *Diabetes* 2016, **65**(1):164-171.
5. Barregard L, Bergstrom G, Fagerberg B: **Cadmium exposure in relation to insulin production, insulin sensitivity and type 2 diabetes: a cross-sectional and prospective study in women**. *Environmental research* 2013, **121**:104-109.
6. Hansen AF, Simic A, Asvold BO, Romundstad PR, Midthjell K, Syversen T, Flaten TP: **Trace elements in early phase type 2 diabetes mellitus-A population-based study. The HUNT study in Norway**. *J Trace Elem Med Biol* 2017, **40**:46-53.
7. Moon SS: **Association of lead, mercury and cadmium with diabetes in the Korean population: the Korea National Health and Nutrition Examination Survey (KNHANES) 2009-2010**. *Diabet Med* 2013, **30**(4):e143-148.
8. Borne Y, Fagerberg B, Persson M, Sallsten G, Forsgard N, Hedblad B, Barregard L, Engstrom G: **Cadmium exposure and incidence of diabetes mellitus--results from the Malmo Diet and Cancer study**. *PloS one* 2014, **9**(11):e112277.
9. Forte G, Bocca B, Peruzzu A, Tolu F, Asara Y, Farace C, Oggiano R, Madeddu R: **Blood metals concentration in type 1 and type 2 diabetics**. *Biol Trace Elem Res* 2013, **156**(1-3):79-90.
10. Gerber PA, Rutter GA: **The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus**. *Antioxid Redox Signal* 2017, **26**(10):501-518.
11. Kaneto H, Katakami N, Kawamori D, Miyatsuka T, Sakamoto K, Matsuoka TA, Matsuhisa M, Yamasaki Y: **Involvement of oxidative stress in the pathogenesis of diabetes**. *Antioxid Redox Signal* 2007, **9**(3):355-366.
12. Kubisch HM, Wang J, Bray TM, Phillips JP: **Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic beta-cells against oxidative stress**. *Diabetes* 1997, **46**(10):1563-1566.
13. Yen CC, Lu FJ, Huang CF, Chen WK, Liu SH, Lin-Shiau SY: **The diabetogenic effects of the combination of humic acid and arsenic: in vitro and in vivo studies**. *Toxicol Lett* 2007,

172(3):91-105.

14. Das KK, Das SN, Dhundasi SA: **Nickel, its adverse health effects & oxidative stress**. *Indian J Med Res* 2008, **128**(4):412-425.
15. Izquierdo-Vega JA, Soto CA, Sanchez-Pena LC, De Vizcaya-Ruiz A, Del Razo LM: **Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite**. *Toxicol Lett* 2006, **160**(2):135-142.
16. Valko M, Morris H, Cronin MT: **Metals, toxicity and oxidative stress**. *Curr Med Chem* 2005, **12**(10):1161-1208.
17. Kurata Y, Katsuta O, Doi T, Kawasuso T, Hiratsuka H, Tsuchitani M, Umemura T: **Chronic cadmium treatment induces islet B cell injury in ovariectomized cynomolgus monkeys**. *Jpn J Vet Res* 2003, **50**(4):175-183.
18. Chen YW, Yang CY, Huang CF, Hung DZ, Leung YM, Liu SH: **Heavy metals, islet function and diabetes development**. *Islets* 2009, **1**(3):169-176.
19. Khan AR, Awan FR: **Metals in the pathogenesis of type 2 diabetes**. *J Diabetes Metab Disord* 2014, **13**(1):16.
20. Sharma B, Singh S, Siddiqi NJ: **Biomedical implications of heavy metals induced imbalances in redox systems**. *Biomed Res Int* 2014, **2014**:640754.
21. Beyersmann D, Hartwig A: **Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms**. *Arch Toxicol* 2008, **82**(8):493-512.
22. Kajimoto Y, Matsuoka T, Kaneto H, Watada H, Fujitani Y, Kishimoto M, Sakamoto K, Matsuhisa M, Kawamori R, Yamasaki Y *et al*: **Induction of glycation suppresses glucokinase gene expression in HIT-T15 cells**. *Diabetologia* 1999, **42**(12):1417-1424.
23. Kaur B, Henry J: **Micronutrient status in type 2 diabetes: a review**. *Adv Food Nutr Res* 2014, **71**:55-100.
24. Siddiqui K, Bawazeer N, Joy SS: **Variation in macro and trace elements in progression of type 2 diabetes**. *ScientificWorldJournal* 2014, **2014**:461591.
25. Ahamed M, Siddiqui MK: **Environmental lead toxicity and nutritional factors**. *Clin Nutr* 2007, **26**(4):400-408.
26. Flora SJ: **Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure**. *Oxid Med Cell Longev* 2009, **2**(4):191-206.
27. Walton FS, Harmon AW, Paul DS, Drobna Z, Patel YM, Styblo M: **Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes**. *Toxicol Appl Pharmacol* 2004, **198**(3):424-433.
28. Han JC, Park SY, Hah BG, Choi GH, Kim YK, Kwon TH, Kim EK, Lachaal M, Jung CY, Lee W: **Cadmium induces impaired glucose tolerance in rat by down-regulating GLUT4 expression in adipocytes**. *Arch Biochem Biophys* 2003, **413**(2):213-220.
29. Somwar R, Koterski S, Sweeney G, Sciotti R, Djuric S, Berg C, Trevillyan J, Scherer PE,

Rondinone CM, Klip A: **A dominant-negative p38 MAPK mutant and novel selective inhibitors of p38 MAPK reduce insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting GLUT4 translocation**. *The Journal of biological chemistry* 2002, **277**(52):50386-50395.

30. Souza K, Maddock DA, Zhang Q, Chen J, Chiu C, Mehta S, Wan Y: **Arsenite activation of P13K/AKT cell survival pathway is mediated by p38 in cultured human keratinocytes**. *Molecular medicine* 2001, **7**(11):767-772.

31. Zawalich WS, Zawalich KC: **A link between insulin resistance and hyperinsulinemia: inhibitors of phosphatidylinositol 3-kinase augment glucose-induced insulin secretion from islets of lean, but not obese, rats**. *Endocrinology* 2000, **141**(9):3287-3295.

32. Leasure JL, Giddabasappa A, Chaney S, Johnson JE, Jr., Pothakos K, Lau YS, Fox DA: **Low-level human equivalent gestational lead exposure produces sex-specific motor and coordination abnormalities and late-onset obesity in year-old mice**. *Environmental health perspectives* 2008, **116**(3):355-361.

33. Faulk C, Barks A, Sanchez BN, Zhang Z, Anderson OS, Peterson KE, Dolinoy DC: **Perinatal lead (Pb) exposure results in sex-specific effects on food intake, fat, weight, and insulin response across the murine life-course**. *PloS one* 2014, **9**(8):e104273.

34. Nie X, Wang N, Chen Y, Chen C, Han B, Zhu C, Chen Y, Xia F, Cang Z, Lu M *et al*: **Blood cadmium in Chinese adults and its relationships with diabetes and obesity**. *Environmental science and pollution research international* 2016, **23**(18):18714-18723.

35. Rothenberg SE, Korrick SA, Fayad R: **The influence of obesity on blood mercury levels for U.S. non-pregnant adults and children: NHANES 2007-2010**. *Environmental research* 2015, **138**:173-180.

36. Padilla MA, Elobeid M, Ruden DM, Allison DB: **An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02**. *International journal of environmental research and public health* 2010, **7**(9):3332-3347.

37. Lei LJ, Jin TY, Zhou YF: **[The effects of cadmium on the levels of insulin in smelters]**. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2006, **24**(1):3-6.

38. Serdar MA, Bakir F, Hasimi A, Celik T, Akin O, Kenar L, Aykut O, Yildirimkaya M: **Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose**. *Int J Diabetes Dev Ctries* 2009, **29**(1):35-40.

39. Afridi HI, Kazi TG, Brabazon D, Naher S, Talpur FN: **Comparative metal distribution in scalp hair of Pakistani and Irish referents and diabetes mellitus patients**. *Clin Chim Acta* 2013, **415**:207-214.

40. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB: **Body iron stores in relation to risk of type 2 diabetes in apparently healthy women**. *JAMA* 2004, **291**(6):711-717.

41. Kim NS, Lee BK: **National estimates of blood lead, cadmium, and mercury levels in the Korean general adult population**. *Int Arch Occup Environ Health* 2011, **84**(1):53-63.
42. Andrews PA: **Disorders of iron metabolism**. *N Engl J Med* 2000, **342**(17):1293; author reply 1294.
43. Oberley LW: **Free radicals and diabetes**. *Free Radic Biol Med* 1988, **5**(2):113-124.
44. Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr.: **A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002**. *Diabetes Care* 2006, **29**(7):1638-1644.
45. Hectors TL, Vanparys C, van der Ven K, Martens GA, Jorens PG, Van Gaal LF, Covaci A, De Coen W, Blust R: **Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function**. *Diabetologia* 2011, **54**(6):1273-1290.
46. Enan E, Liu PC, Matsumura F: **2,3,7,8-Tetrachlorodibenzo-p-dioxin causes reduction of glucose transporting activities in the plasma membranes of adipose tissue and pancreas from the guinea pig**. *The Journal of biological chemistry* 1992, **267**(28):19785-19791.
47. Longnecker MP, Michalek JE: **Serum dioxin level in relation to diabetes mellitus among Air Force veterans with background levels of exposure**. *Epidemiology* 2000, **11**(1):44-48.
48. Henriksen GL, Ketchum NS, Michalek JE, Swaby JA: **Serum dioxin and diabetes mellitus in veterans of Operation Ranch Hand**. *Epidemiology* 1997, **8**(3):252-258.
49. Steenland K, Piacitelli L, Deddens J, Fingerhut M, Chang LI: **Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin**. *J Natl Cancer Inst* 1999, **91**(9):779-786.

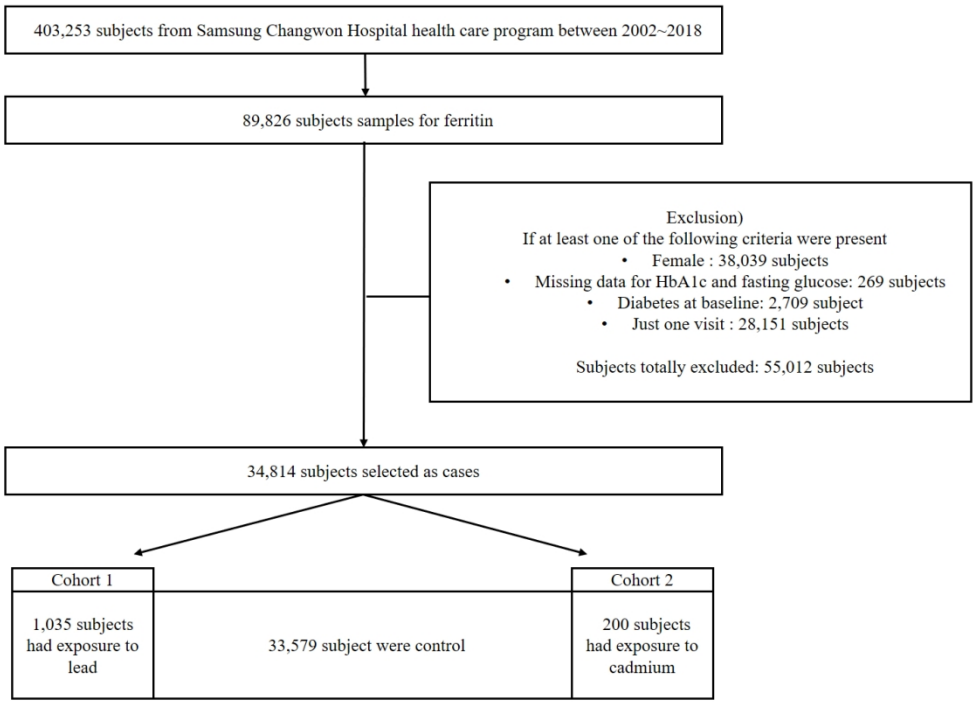
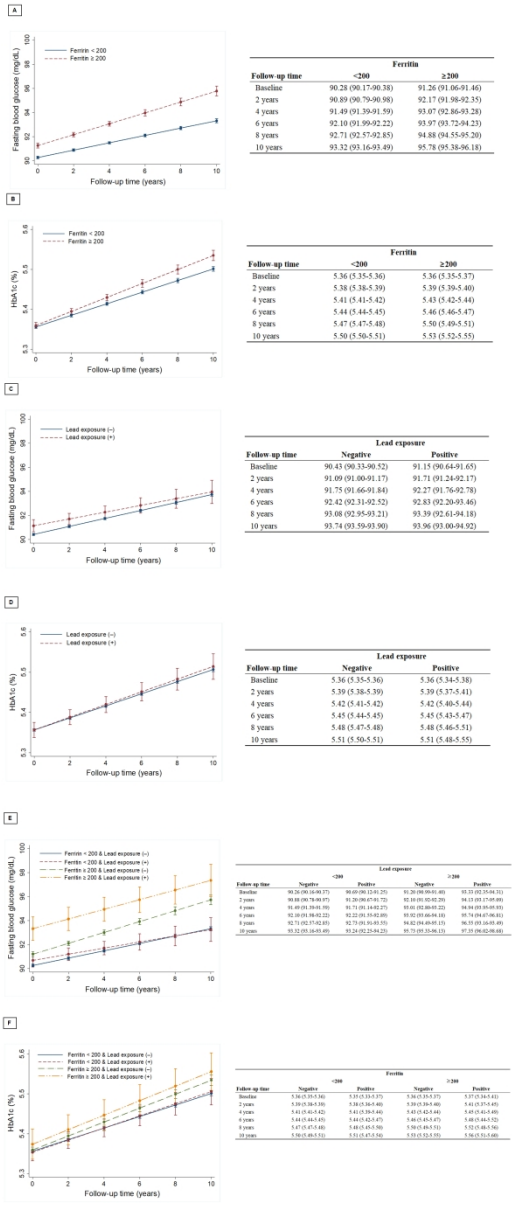


Figure 1. Schematic flow diagram

124x89mm (300 x 300 DPI)



A

	Ferritin	
Follow-up time	<200	≥200
Baseline	90.28 (90.17-90.38)	91.26 (91.06-91.46)
2 years	90.89 (90.79-90.98)	92.17 (91.98-92.35)
4 years	91.49 (91.39-91.59)	93.07 (92.86-93.28)
6 years	92.10 (91.99-92.22)	93.97 (93.72-94.23)
8 years	92.71 (92.57-92.85)	94.88 (94.55-95.20)
10 years	93.32 (93.16-93.49)	95.78 (95.38-96.18)

B

	Ferritin	
Follow-up time	<200	≥200
Baseline	5.36 (5.35-5.36)	5.36 (5.35-5.37)
2 years	5.38 (5.38-5.39)	5.39 (5.39-5.40)
4 years	5.41 (5.41-5.42)	5.43 (5.42-5.44)
6 years	5.44 (5.44-5.45)	5.46 (5.46-5.47)
8 years	5.47 (5.47-5.48)	5.50 (5.49-5.51)
10 years	5.50 (5.50-5.51)	5.53 (5.52-5.55)

C

	Lead exposure	
Follow-up time	Negative	Positive
Baseline	90.43 (90.33-90.52)	91.15 (90.64-91.65)
2 years	91.09 (91.00-91.17)	91.71 (91.24-92.17)
4 years	91.75 (91.66-91.84)	92.27 (91.76-92.78)
6 years	92.42 (92.31-92.52)	92.83 (92.20-93.46)
8 years	93.08 (92.95-93.21)	93.39 (92.61-94.18)
10 years	93.74 (93.59-93.90)	93.96 (93.00-94.92)

D

	Lead exposure	
Follow-up time	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.36 (5.34-5.38)
2 years	5.39 (5.38-5.39)	5.39 (5.37-5.41)
4 years	5.42 (5.41-5.42)	5.42 (5.40-5.44)
6 years	5.45 (5.44-5.45)	5.45 (5.43-5.47)
8 years	5.48 (5.47-5.48)	5.48 (5.46-5.51)
10 years	5.51 (5.50-5.51)	5.51 (5.48-5.55)

E

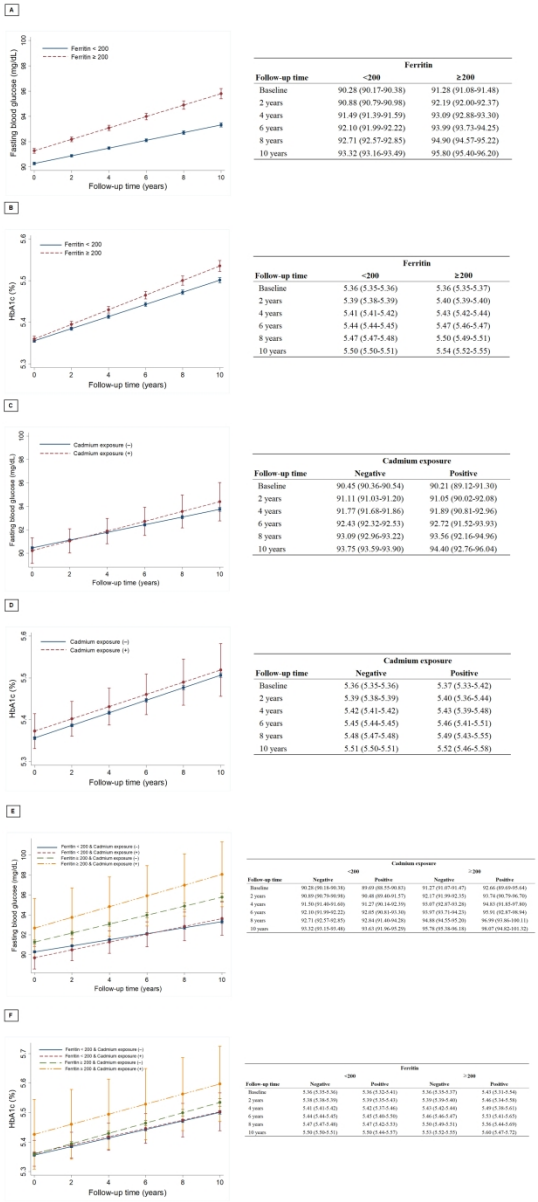
	Lead exposure			
	<200		≥200	
Follow-up time	Negative	Positive	Negative	Positive
Baseline	90.26 (90.16-90.37)	90.69 (90.12-91.25)	91.26 (90.99-91.48)	91.33 (92.35-94.31)
2 years	90.88 (90.78-90.97)	91.29 (90.67-91.72)	92.16 (91.92-92.29)	94.13 (93.17-95.09)
4 years	91.49 (91.39-91.59)	91.71 (91.14-92.27)	93.01 (92.80-93.22)	94.94 (93.95-95.93)
6 years	92.10 (91.98-92.22)	92.22 (91.55-92.89)	93.92 (93.66-94.18)	95.74 (94.67-96.81)
8 years	92.71 (92.57-92.85)	92.73 (91.91-93.55)	94.82 (94.49-95.15)	96.55 (95.16-97.49)
10 years	93.32 (93.16-93.49)	93.24 (92.25-94.23)	95.73 (95.33-96.13)	97.35 (96.02-98.68)

F

	Ferritin			
	<200		≥200	
Follow-up time	Negative	Positive	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.35 (5.33-5.37)	5.36 (5.35-5.37)	5.37 (5.34-5.41)
2 years	5.38 (5.38-5.38)	5.38 (5.36-5.40)	5.39 (5.39-5.40)	5.41 (5.37-5.45)
4 years	5.41 (5.41-5.42)	5.41 (5.39-5.44)	5.43 (5.42-5.44)	5.45 (5.41-5.49)
6 years	5.44 (5.44-5.45)	5.44 (5.42-5.47)	5.46 (5.45-5.47)	5.48 (5.44-5.52)
8 years	5.47 (5.47-5.48)	5.48 (5.45-5.50)	5.50 (5.49-5.51)	5.52 (5.48-5.56)
10 years	5.50 (5.49-5.51)	5.51 (5.47-5.54)	5.53 (5.52-5.55)	5.56 (5.51-5.60)

Figure 2. Mixed models were used to evaluate the effects of lead exposure and ferritin on FBS and HbA1c
A – Changes in fasting blood glucose according to serum ferritin levels in cohort A
B – Changes in HbA1c according to serum ferritin levels in cohort A
C – Changes in fasting blood glucose according to lead exposure in cohort A
D – Changes in HbA1c according to lead exposure in cohort A
E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort A
F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort A

154x360mm (300 x 300 DPI)



Ferritin

Follow-up time	<200	≥200
Baseline	90.28 (90.17-90.38)	91.28 (91.08-91.48)
2 years	90.88 (90.79-90.98)	92.19 (92.00-92.37)
4 years	91.49 (91.39-91.59)	93.09 (92.88-93.30)
6 years	92.10 (91.99-92.22)	93.99 (93.73-94.25)
8 years	92.71 (92.57-92.85)	94.90 (94.57-95.22)
10 years	93.32 (93.16-93.49)	95.80 (95.40-96.20)

Ferritin

Follow-up time	<200	≥200
Baseline	5.36 (5.35-5.36)	5.36 (5.35-5.37)
2 years	5.39 (5.38-5.39)	5.40 (5.39-5.40)
4 years	5.41 (5.41-5.42)	5.43 (5.42-5.44)
6 years	5.44 (5.44-5.45)	5.47 (5.46-5.47)
8 years	5.47 (5.47-5.48)	5.50 (5.49-5.51)
10 years	5.50 (5.50-5.51)	5.54 (5.52-5.55)

Cadmium exposure

Follow-up time	Negative	Positive
Baseline	90.45 (90.36-90.54)	90.21 (89.12-91.30)
2 years	91.11 (91.03-91.20)	91.05 (90.02-92.08)
4 years	91.77 (91.68-91.86)	91.89 (90.81-92.96)
6 years	92.43 (92.32-92.53)	92.72 (91.52-93.93)
8 years	93.09 (92.96-93.22)	93.56 (92.16-94.96)
10 years	93.75 (93.59-93.90)	94.40 (92.76-96.04)

Cadmium exposure

Follow-up time	Negative	Positive
Baseline	5.36 (5.35-5.36)	5.37 (5.33-5.42)
2 years	5.39 (5.38-5.39)	5.40 (5.36-5.44)
4 years	5.42 (5.41-5.42)	5.43 (5.39-5.48)
6 years	5.45 (5.44-5.45)	5.46 (5.41-5.51)
8 years	5.48 (5.47-5.48)	5.49 (5.43-5.55)
10 years	5.51 (5.50-5.51)	5.52 (5.46-5.58)

Follow-up time	<200 Negative	<200 Positive	≥200 Negative	≥200 Positive
Baseline	90.28 (90.18-90.38)	89.69 (88.55-90.83)	91.27 (91.07-91.47)	92.66 (89.69-95.64)
2 years	90.89 (90.79-90.98)	90.48 (89.40-91.57)	92.17 (91.99-92.35)	93.74 (90.79-96.70)
4 years	91.50 (91.40-91.60)	91.27 (90.14-92.39)	93.07 (92.87-93.28)	94.83 (91.85-97.80)
6 years	92.10 (91.99-92.22)	92.05 (90.81-93.30)	93.97 (93.71-94.23)	95.91 (92.87-98.94)
8 years	92.71 (92.57-92.85)	92.84 (91.49-94.20)	94.86 (94.55-95.20)	96.99 (93.86-100.11)
10 years	93.32 (93.15-93.48)	93.63 (91.96-95.29)	95.78 (95.38-96.18)	98.07 (94.82-101.32)

Follow-up time	<200 Negative	<200 Positive	≥200 Negative	≥200 Positive
Baseline	5.36 (5.35-5.36)	5.36 (5.32-5.41)	5.36 (5.35-5.37)	5.43 (5.31-5.54)
2 years	5.38 (5.38-5.39)	5.39 (5.35-5.43)	5.39 (5.39-5.40)	5.46 (5.34-5.58)
4 years	5.41 (5.41-5.42)	5.42 (5.37-5.46)	5.43 (5.42-5.44)	5.49 (5.38-5.61)
6 years	5.44 (5.44-5.45)	5.45 (5.40-5.50)	5.46 (5.46-5.47)	5.53 (5.41-5.65)
8 years	5.47 (5.47-5.48)	5.47 (5.42-5.53)	5.50 (5.49-5.51)	5.56 (5.44-5.69)
10 years	5.50 (5.50-5.51)	5.50 (5.44-5.57)	5.53 (5.52-5.55)	5.60 (5.47-5.72)

Figure 3. Mixed models were used to evaluate the effects of cadmium exposure and ferritin on FBS and HbA1c

A – Changes in fasting blood glucose according to serum ferritin levels in cohort B

B – Changes in HbA1c according to serum ferritin levels in cohort B

C– Changes in fasting blood glucose according to lead exposure in cohort B

D – Changes in HbA1c according to lead exposure in cohort B

E – Changes in fasting blood glucose according to serum ferritin levels and lead exposure in cohort B

F – Changes in HbA1c according to serum ferritin levels and lead exposure in cohort B

155x347mm (300 x 300 DPI)

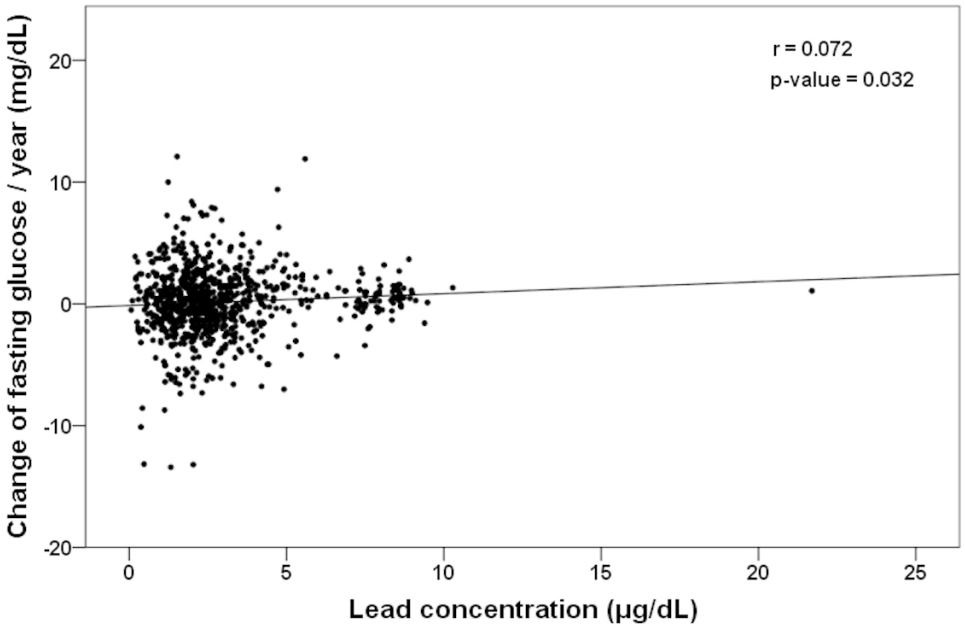


Figure 4. Scatter plot showing the annual changes of fasting blood glucose by lead concentration (r=0.072, p = 0.032)

99x67mm (300 x 300 DPI)

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1

1Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	6
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
8Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than	6

		one group. Give information separately for for exposed and unexposed groups if applicable.	
Bias	#9	Describe any efforts to address potential sources of bias	6
Study size	#10	Explain how the study size was arrived at	6
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	6
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	7
Statistical methods	#12c	Explain how missing data were addressed8	NA
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	NA
Statistical methods	#12e	Describe any sensitivity analyses	7
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-	9

		up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	
Participants	#13b	Give reasons for non-participation at each stage	9
Participants	#13c	Consider use of a flow diagram	6,9 (fig.1)
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	9
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	9
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
Main results	#16b	Report category boundaries when continuous variables were categorized	9
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9

Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	#18	Summarise key results with reference to study objectives	11
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13
Generalisability	#21	Discuss the generalisability (external validity) of the study results	10-13
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16