Original Article

Endothelial dysfunction in subjects with chronic cadmium exposure

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ABSTRACT — Vascular endothelium is a target of cadmium (Cd) toxicity. Cd exposure has been reported to be associated with vascular disorders. In this study, we aimed to investigate the effects of Cd exposure on markers of endothelial function in human subjects chronically exposed to Cd. Based on blood Cd levels, seventy-five women were categorized into non-exposed, Cd-exposed and severely Cd-exposed groups. Nitrite, L-arginine, asymmetric dimethylarginine (ADMA), and soluble thrombomodulin levels in blood were measured. Nitrite levels were lower in Cd-exposed subjects than non-exposed subjects. Plasma L-arginine decreased while ADMA, an endogenous endothelial nitric oxide synthase (eNOS) inhibitor, increased in Cd-exposed subjects. Soluble thrombomodulin also increased in Cd-exposed subjects. In Cd-exposed subjects, plasma malondialdehyde and protein carbonyl groups increased while the erythrocytic glutathione decreased. Multiple linear regression analysis revealed a negative association between urinary Cd and nitrite levels in erythrocytes. Our research suggests that subjects with chronic Cd exposure have endothelial dysfunction.

Key words: Cadmium, Nitrite, Soluble thrombomodulin, ADMA, Endothelial dysfunction

INTRODUCTION

Environmental Cd contamination causes a significant health problem. In addition to renal tubular dysfunction and osteoporosis, Cd exposure contributes to the development of cardiovascular diseases. The increased prevalence of hypertension and kidney diseases has been reported in the areas contaminated with Cd such as Mae Sot District, Tak Province in the northwestern part of Thailand (Swaddiwudhipong *et al.*, 2012). High urinary Cd was found to be associated with mortality and incidence of cardiovascular diseases such as coronary artery disease, heart failure and stroke (Tellez-Plaza *et al.*, 2013).

Evidence suggests that the vascular wall is also a target of Cd deposition. Cd deposited in vascular wall of smokers at concentration up to 20 μM as compared to the blood concentration of low nM range (Abu-Hayyeh

et al., 2001). *In vitro* exposure of endothelial cells to Cd (15 μM) resulted in the increased permeability, inhibition of cell proliferation and induction of cell death (Messner *et al.*, 2009). Cd induced hypertension in rats, which involved oxidative stress and suppression of eNOS expression in blood vessels leading to impaired acetylcholine-induced vasorelaxation (Yoopan *et al.*, 2008; Donpunha *et al.*, 2011). Rats treated with Cd had increased response to vasopressor phenylephrine, and increased expression of NADPH oxidase in endothelial cells (Almenara *et al.*, 2013). However, the change in endothelial function markers by Cd had never been reported in human.

In this study, we hypothesized that subjects with chronic Cd exposure would have impaired endothelial function. Blood nitrite was measured as an index of nitric oxide (NO) production by endothelial cells. Stimulation of eNOS in human forearm vessels resulted in increased

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nitrite concentrations in plasma (Lauer *et al.*, 2001) and whole blood (Dejam *et al.*, 2005). eNOS inhibition resulted in a decrease in plasma nitrite concentration, and eNOS knockout mice had lower plasma nitrite compared to wild-type mice (Kleinbongard *et al.*, 2003). Soluble thrombomodulin was measured as a marker of endothelial damage in which its elevated levels were associated with endothelial dysfunction and thrombosis (Ishii *et al.*, 1991; Bouman *et al.*, 2014; Sharma *et al.*, 2014). ADMA is an endogenous eNOS inhibitors and its high levels are associated with vascular disorders (Böger *et al.*, 1998; Tripepi *et al.*, 2011). Moreover, oxidative stress was determined by the levels of lipid and protein oxidation in plasma, and reduced glutathione in erythrocytes.

MATERIALS AND METHODS

Subjects and baseline clinical parameters

This study was approved by the Ethical Clearance Committee on Human Rights to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, based on the Declaration of Helsinki. Cd concentrations in whole blood and urine were measured by atomic absorption spectrometry (AAnalyst 600, PerkinElmer, Waltham, MA, USA). Baseline clinical laboratory parameters were determined at Laboratory of Mae Sot General Hospital. Seventy-five nonsmoker women living in Mae Sot District, Tak Province, Thailand were enrolled in the study and categorized according to their previously recorded blood Cd levels into non-exposed ($n = 25$, blood Cd < 0.5 µg/L), Cd-exposed ($n = 25$, blood Cd 1-5 µg/L) and severely Cd-exposed ($n = 25$, blood Cd > 5 µg/L) groups (Prozialeck and Edwards, 2010). All subjects were in clinically stable state. Subjects with hypertension, heart diseases and diabetes mellitus were excluded from the study.

Measurement of nitrite, nitrate and soluble thrombomodulin

Fasting venous blood samples were collected using heparin (143 units/10 mL) as an anticoagulant. Whole blood samples were mixed immediately with the nitrite-preserving solution containing 0.8 M ferricyanide, 10 mM N-ethylmaleimide and 1% NP-40 in a 5:1 dilution (Dejam *et al.*, 2005; Pelletier *et al.*, 2006). Meanwhile, whole blood samples in other tubes were quickly centrifuged at 2000 g for 1 min at 4°C to separate erythrocytes and plasma. Within 2 min, the erythrocytes were mixed with the nitrite-preserving solution. All samples were stored at -80°C. Nitrite levels in whole blood, erythrocytes and plasma were measured by triiodide-based chemilumi-

nescence (Yang *et al.*, 2003). Nitrite and S-nitrosothiols reacted with triiodide reagent to release NO gas that was detected by a chemiluminescence NO analyzer (Eco Medics Analyzer CLD88, Duernten, Switzerland). Nitrite was distinguished from S-nitrosothiols by pre-treating samples with 5% acid sulfanilamide as acid sulfanilamide formed a complex with nitrite that was not reduced to NO in triiodide reagent. Addition of acid sulfanilamide to samples abolished the chemiluminescence signal completely, indicating that the majority of signal represented nitrite levels. Plasma nitrate concentrations were measured by vanadium chloride-based chemiluminescence (Yang *et al.*, 2003). Area under the curve was calculated using Origin 7 (Microcal Software, Inc., Northampton, MA, USA).

To confirm the presence of endothelial dysfunction, soluble thrombomodulin in plasma was measured by ELISA (Abcam, Cambridge, UK). Elevated soluble thrombomodulin in plasma is indicative of endothelial injury (Tohda *et al.*, 1998; Sharma *et al.*, 2014).

Determination of L-arginine, ADMA and SDMA by high-performance liquid chromatography

In order to assess the endothelial activity regarding NO synthesis, we also determined the plasma levels of L-arginine (a substrate for eNOS), ADMA (asymmetric dimethylarginine or N^G , N^G -dimethyl-L-arginine, an eNOS inhibitor and maker of endothelial dysfunction) and SDMA (symmetric dimethylarginine or N^G , N^G -dimethyl-L-arginine, an inhibitor of L-arginine uptake). L-arginine, ADMA and SDMA in plasma were measured by high-performance liquid chromatography (Teerlink *et al.*, 2002) consisting of Water Alliance 2695 Separation Module (Waters Corporation, Milford, MA, USA) and a FP-2020 fluorescence detector (Jasco, Easton, MD, USA). Chromatographic separation was achieved at 35°C on AccQ-Taq column (4 µm, 3.9×150 mm; Waters). Monomethylarginine was used as an internal standard. L-arginine, ADMA and SDMA were extracted from plasma by solid phase extraction using Oasis MCX SPE columns (Waters). Then, the samples were washed with 1 mL of 100 mM HCl and methanol. L-arginine, ADMA and SDMA were eluted with concentrated ammonia/water/methanol (10/40/50) and evaporated with N_2 at 60-80°C. The samples were re-dissolved in 0.1 mL of water and derivatization reagent. Separation was performed with mobile phase consisting of 93% 50 mM potassium phosphate buffer and 7% acetonitrile at flow rate of 0.8 mL/min for 19 min. The column was washed with 50% acetonitrile in water for 20 min before injecting the next sample. Fluorescence was detected at 340 nm excitation and

455 nm emission.

Measurement of lipid peroxidation, protein carbonyl groups and reduced glutathione

Plasma malondialdehyde, a lipid peroxidation marker, was measured by spectrofluometric method using thio-barbituric acid assay (Ohkawa *et al.*, 1979). Plasma protein carbonyl groups, markers of protein oxidation, were measured by commercial ELISA kit (BioVision, Milpitas, CA, USA). Reduced glutathione in erythrocytes was measured by colorimetric method (Beutler *et al.*, 1963).

Statistical analysis

PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 4 (GraphPad software Inc., San Diego, CA, USA) were used for statistical analysis. Comparison analysis was performed by Analysis of Variance (ANOVA) with Tukey's multiple comparison test. Multiple linear regression analysis was used to explore the relationship

between endothelial function markers and the potential independent variables. *p* value < 0.05 was considered significantly different.

RESULTS

Baseline subjects' characteristics

Seventy-five women were enrolled in the study; 25 subjects in each group. There was no difference in age and systemic blood pressure among the groups (Table 1). Cd-exposed and severely exposed subjects had no difference in serum ferritin, calcium, zinc, creatinine, blood urea nitrogen, hemoglobin, white blood cell count, platelet count, and urinary β_2 -microglobulin. Both exposed groups had higher levels of serum creatinine, blood Cd, urinary Cd, and urinary β_2 -microglobulin than the non-exposed group. Severely exposed subjects had higher triglyceride levels than non-exposed subjects. The high-density lipoprotein cholesterol (HDL-C) and serum zinc levels were lower in both Cd-exposed groups than those in non-

Table 1. Subjects' characteristics.

Parameters	Non-exposed	Cd-exposed	Severely Cd-exposed
<i>n</i>	25	25	25
Age (years)	48.4 ± 1.2	50.9 ± 2.3	51.4 ± 1.7
Systolic blood pressure (mmHg)	123.7 ± 2.6	118.4 ± 2.9	127.0 ± 3.7
Diastolic blood pressure (mmHg)	74.3 ± 2.3	75.2 ± 1.3	78.1 ± 1.3
Mean arterial blood pressure (mmHg)	90.8 ± 2.3	89.6 ± 1.6	94.4 ± 1.9
Serum ferritin (ng/mL)	100.2 ± 20.9	59.2 ± 7.4	84.7 ± 14.7
Blood urea nitrogen (mg%)	10.8 ± 0.6	11.3 ± 0.7	11.1 ± 0.6
Serum creatinine (mg%)	0.67 ± 0.02	0.96 ± 0.04 ^a	0.90 ± 0.02 ^a
Serum calcium (mg%)	8.63 ± 0.11	9.62 ± 0.06 ^a	9.60 ± 0.08 ^a
Serum zinc (µg/dL)	91.1 ± 1.81	77.8 ± 2.1 ^a	75.6 ± 1.6 ^a
Total cholesterol (mg/dL)	204.3 ± 11.7	240.8 ± 14.0	183.5 ± 11.5
HDL-C (mg/dL)	57.6 ± 3.7	47.2 ± 2.1 ^a	42.1 ± 2.3 ^a
LDL-C (mg/dL)	124.8 ± 9.8	159.6 ± 15.5	121.6 ± 6.8
Triglycerides (mg/dL)	136.2 ± 15.0	177.4 ± 20.3	204.8 ± 20.1 ^a
Hemoglobin (g/dL)	13.5 ± 0.4	12.5 ± 0.2	12.4 ± 0.3 ^a
White blood cell (10 ³ /µL)	6.43 ± 0.42	6.78 ± 0.28	7.14 ± 0.40
Platelet count (10 ³ /µL)	248.1 ± 14.8	236.7 ± 14.8	242.8 ± 12.7
Blood Cd (µg/L) ^c	0.31 ± 0.25	3.97 ± 2.05 ^a	8.48 ± 3.85 ^{a,b}
Urinary Cd (µg/g creatinine) ^c	0.32 ± 0.30	3.40 ± 1.58 ^a	9.56 ± 3.93 ^{a,b}
Urinary β_2 -microglobulin (µg/g creatinine) ^c	34.0 ± 36.0	119.6 ± 148.7 ^a	140.5 ± 197.2 ^a

Values are mean ± S.E. of subjects with blood Cd < 0.5 (non-exposed), 1-5 (Cd-exposed) and > 5 µg/L (severely Cd-exposed).

^a*p* < 0.05 vs. non-exposed group. ^b*p* < 0.05 vs. Cd-exposed group (ANOVA with Tukey's multiple comparison test). ^cData are expressed as geometric mean ± geometric S.D. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

exposed group. The blood and urinary Cd levels showed significant correlation (Pearson $r = 0.65$, $p < 0.0001$), and were higher in severely exposed than exposed subjects. For unclear reason, Cd-exposed subjects had higher serum calcium levels than non-exposed subjects.

Nitrite and nitrate

The baseline nitrite levels in plasma, erythrocytes and whole blood were lower in severely exposed subjects than non-exposed subjects (mean $\pm$ S.E.; 31.8 ± 4.0 vs. 69.1 ± 7.3 nM, 47.0 ± 3.4 vs. 195.0 ± 11.6 nM and 93.6 ± 5.9 vs. 155.5 ± 12.7 nM, respectively; $p < 0.05$) (Fig. 1). In Cd-exposed group, only the erythrocytic nitrite showed a significant reduction compared to non-exposed group (mean $\pm$ S.E.; 96.7 ± 13.1 vs. 195.0 ± 11.6 nM; $p < 0.05$) (Fig. 1C). There was no difference in plasma nitrate levels among the three groups. The plasma nitrate levels (mean $\pm$ S.E.) in non-exposed, exposed and severely exposed groups were 11.7 ± 0.8 , 13.5 ± 1.2 and 12.8 ± 0.8 μ M, respectively.

L-arginine, ADMA, SDMA, and soluble thrombomodulin

Further, we examined L-arginine availability and levels of ADMA and SDMA. Both Cd-exposed groups had lower L-arginine plasma levels than non-exposed group (Fig. 2A). L-arginine levels (mean $\pm$ S.E.) in non-exposed, exposed and severely exposed groups were 280.3 ± 30.6 , 167.9 ± 8.4 and 185.1 ± 9.0 nM, respectively. Plasma ADMA concentrations were higher in severely exposed groups than non-exposed group (mean $\pm$ S.E.; 0.57 ± 0.08 vs. 0.30 ± 0.05 μ M; $p < 0.05$) (Fig. 2B). There was no difference in plasma SDMA concentrations (Fig. 2C). Raised levels of soluble thrombomodulin in both Cd-exposed groups were observed (Fig. 2D).

Oxidative stress

Because oxidative stress could lead to a decrease in NO availability, we measured malondialdehyde and protein carbonyl as markers of lipid and protein oxidation, respectively. Plasma concentrations of malondialdehyde

Fig. 1. Baseline nitrite levels in blood. (A) Representative traces of chemiluminescence signals of the erythrocytic nitrite. Nitrite levels in plasma (B), erythrocytes (C) and whole blood (D) of non-exposed, Cd-exposed and severely Cd-exposed subjects. Data are presented as mean $\pm$ S.E. * $p < 0.05$ vs. non-exposed group (ANOVA with Tukey's multiple comparison test).

Endothelial dysfunction in Cd-exposed subjects

Fig. 2. Levels of L-arginine (A), ADMA (B), SDMA (C), and soluble thrombomodulin (D) in plasma of non-exposed, Cd-exposed and severely Cd-exposed subjects. Data are presented as mean $\pm$ S.E. * $p < 0.05$ vs. non-exposed group (ANOVA with Tukey's multiple comparison test).

were elevated in both Cd-exposed groups (exposed and severely exposed) than non-exposed group (mean $\pm$ S.E.; 1.95 ± 0.09 , 1.75 ± 0.11 vs. 0.29 ± 0.01 μ M, respectively; $p < 0.05$) (Fig. 3A). Similarly, the protein carbonyl groups in plasma of exposed and severely exposed were higher than non-exposed group (mean $\pm$ S.E.; 1.36 ± 0.10 , 1.15 ± 0.10 vs. 0.74 ± 0.08 nmol/mg protein, respectively; $p < 0.05$) (Fig. 3B). Reduced glutathione concentrations in erythrocytes decreased in exposed and severely exposed compared with non-exposed group (mean $\pm$ S.E.; 2277.3 ± 126.9 , 2260.3 ± 111.8 vs. 3151.9 ± 397.7 μ M,

respectively; $p < 0.05$) (Fig. 3C).

Multiple regression analysis

Multiple linear regression analysis was used to examine a correlation between endothelial function markers and Cd after adjusting other co-variables. Only urinary Cd was significantly related to erythrocytic nitrite levels (Table 2). Erythrocytic nitrite decreased 99.212 nM for 1 μ g/g creatinine increase in urinary Cd. Age, blood pressure, serum lipid levels, blood Cd, and urinary β_2 -microglobulin showed no statistical relationship with

Fig. 3. Levels of plasma malondialdehyde (A) and protein carbonyl groups (B), and reduced glutathione in erythrocytes (C) of non-exposed, Cd-exposed and severely Cd-exposed subjects. Data are presented as mean $\pm$ S.E. * $p < 0.05$ vs. non-exposed group (ANOVA with Tukey's multiple comparison test).

any markers of endothelial function.

DISCUSSION

Our study demonstrates a decrease in nitrite and increase in soluble thrombomodulin and ADMA in subjects with chronic Cd exposure. Plasma L-arginine decreased while ADMA increased in Cd-exposed subjects. The elevated levels of plasma malondialdehyde and protein carbonyl groups and a decrease in erythrocytic glutathione suggest the presence of oxidative stress. From multiple linear regression adjusting for age, blood pressure, lipid profile, blood Cd, and urinary β_2 -microglobulin, erythrocytic nitrite levels inversely correlated with urinary Cd, but not with blood Cd. Kidneys are the critical

target organs of cadmium toxicity. Urinary Cd excretion is a good marker of long-term Cd exposure and body burden while blood Cd may represent recent exposure (Jarup *et al.*, 1998; Gallagher and Meliker, 2010). Collectively, our findings suggest the endothelial dysfunction in Cd-exposed subjects.

From our study, Cd-exposed subjects also show decreased HDL-C and increased triglyceride levels compared with non-exposed subjects. These results are consistent with the report that cadmium exposure is associated with the increased risk of low HDL-C and high triglycerides to HDL-C ratio in adults (Kim, 2012). However, in multiple regression analysis, HDL-C, triglycerides, age, and blood pressure were not related with erythrocytic nitrite and any endothelial markers. We speculate

Table 2. Multiple linear regression analysis of the determinants of erythrocytic nitrite.

Independent variables ^a	Erythrocytic nitrite (nM)		
	β^b	S.E. of β	p
Age	-0.042	0.701	0.953
Mean arterial blood pressure	0.214	0.764	0.781
Total cholesterol	148.661	113.422	0.198
HDL-C	0.201	0.880	0.820
LDL-C	0.343	0.185	0.072
Triglycerides	0.092	0.077	0.239
Blood Cd ^c	35.134	31.060	0.266
Urinary Cd ^c	-99.212	31.914	0.004
Urinary β_2 -microglobulin ^c	-6.788	15.305	0.660

^aAge (years), mean arterial blood pressure (mmHg), total cholesterol (mg/dL), HDL-C (high-density lipoprotein cholesterol, mg/dL), LDL-C (low-density lipoprotein cholesterol, mg/dL), triglycerides (mg/dL), blood Cd (μ g/L), urinary Cd (μ g/g creatinine), and urinary β_2 -microglobulin (μ g/g creatinine)

^bRegression coefficient.

^cLog-transformed before analysis.

Adjusted $R^2 = 0.42$

that the exclusion of subjects with diabetes, hypertension and heart diseases may account for these findings.

Nitrite anion is a bioactive NO product circulating in blood. Although the source of nitrite is incompletely understood, its blood levels are believed to represent endothelial NO production (Lauer *et al.*, 2001; Kleinbongard *et al.*, 2003). In blood, nitrite is contained mostly in erythrocytes as unbound form in cytosol (Dejam *et al.*, 2005). Thereby, the nitrite levels in erythrocytes may be an endothelial function marker that exhibit correlation with urinary cadmium. Shear stress and acetylcholine-induced eNOS activation increased blood nitrite and forearm blood flow in humans (Dejam *et al.*, 2005). The biological effects of nitrite are NO-dependent vasodilation and platelet inhibition (Srihirun *et al.*, 2012), which require bioactivation catalyzed by deoxygenated heme proteins or molybdopterin-containing proteins (Kim-Shapiro and Gladwin, 2014). Although lower nitrite concentrations suggest impaired vascular function in Cd-exposed subjects, further study on vascular reactivity such as measurement of forearm blood flow remains to be investigated. In contrast to nitrite, plasma nitrate was unchanged in Cd-exposed subjects. Nitrate is biologically inert and exists at high μ M range in blood (Lauer *et al.*, 2001); thus, its levels are unlikely to reflect endothelial activity.

Plasma L-arginine was lower in Cd-exposed subjects than non-exposed subjects. The availability of L-arginine is essential for eNOS activity (Rochette *et al.*, 2013).

Endothelial cells take up L-arginine via cationic amino acid transporters and may accumulate L-arginine within cells up to 0.8-2.0 mM (Pollock *et al.*, 1991; Rochette *et al.*, 2013). Despite high intracellular concentration of L-arginine, its transport from plasma is required for NO synthesis. Nonetheless, the relationship of L-arginine and nitrite levels remains unclear.

NO is synthesized from L-arginine in endothelial cells by eNOS which is inhibited by ADMA. ADMA is a byproduct of protein degradation in cells and competes with L-arginine at the active site of eNOS (Vallance *et al.*, 1992; Rochette *et al.*, 2013). SDMA is not an eNOS inhibitor, but its high levels may compete with L-arginine for cellular uptake (Closs *et al.*, 1997), thus indirectly reducing NO production. During the intracellular protein breakdown, ADMA and SDMA are produced by protein arginine N-methyltransferases (PRMTs) and released into the cytosol. ADMA may be accumulated in cells or exported into circulation via cationic amino acid transporter (Rochette *et al.*, 2013). ADMA is taken up into cells and metabolized into citrulline by dimethylarginine dimethylaminohydrolases (DDAH) in liver and kidneys as the major route of elimination. Some amount of ADMA is excreted in urine. Oxidative stress increases PRMTs gene expression (Böger *et al.*, 2000) but decreases DDAH activity (Ito *et al.*, 1999), leading to ADMA accumulation and suppression of NO synthesis. Moreover, ADMA causes eNOS uncoupling, resulting in production of superoxide rather than NO (Antoniades *et al.*, 2009).

The raised ADMA levels are reported in diseases associated with endothelial dysfunction such as atherosclerosis, hypertension, diabetes mellitus, hyperlipidemia, ischemic heart disease, and chronic kidney disease (Valkonen *et al.*, 2001; Lu *et al.*, 2011).

Thrombomodulin is a transmembrane glycoprotein located on surface of endothelial cells, and functions as an anticoagulant. Thrombomodulin binds to thrombin, forming complex that inhibits fibrin formation, protein S inactivation and platelet activation (Conway, 2012). The complex also activates protein C, leading to inactivation of clotting factor Va and VIIIa. Thrombomodulin is released from endothelial cells following cell injury (Ishii *et al.*, 1991). Levels of soluble thrombomodulin are elevated in diseases associated with vascular injury such as vasculitis (Boehme *et al.*, 1996), diabetes mellitus (Gabat *et al.*, 1996) or nephrotic syndrome (Sharma *et al.*, 2014). A rise in soluble thrombomodulin in Cd-exposed subjects suggests endothelial injury despite the lack of correlation between thrombomodulin and Cd.

Cd has adverse effects on endothelial cells by several possible mechanisms. For example, Cd was shown to attenuate NO production in endothelial hybrid cell line by decreasing eNOS phosphorylation at serine 1177 (Majumder *et al.*, 2008). Rats orally exposed to Cd exhibited hypertension which was associated with decreased eNOS protein levels (Yoopan *et al.*, 2008). Cd altered endothelial cell integrity by disruption of cadherin-cadherin bonds, resulting in the increased permeability of vascular endothelium (Pearson *et al.*, 2003). Cd exposure promoted NADPH oxidase expression in rat aorta, inducing oxidative stress in endothelial cells and impairing relaxation response to acetylcholine (Almenara *et al.*, 2013). Oxidation of tetrahydrobiopterin (BH₄), a NOS cofactor, to BH₂ uncouples eNOS (Vásquez-Vivar *et al.*, 2003), leading to production of superoxide. Cd also induces endothelial cell death by several death pathways, thus promoting atherosclerosis (Messner *et al.*, 2009; Messner and Bernhard, 2010).

Taken together, our results suggest that subjects with long-term Cd exposure exhibit endothelial dysfunction, which reinforces previous *in vitro* and *in vivo* (animal) findings that Cd induces endothelial damage. Further studies are needed to understand the pathway by which Cd affects endothelial cell function.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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