Associations of urinary and dietary cadmium with urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine and blood biochemical parameters

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Associations of urinary and dietary cadmium with oxidative stress and hematological parameters

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	exposure. Overall, these findings suggest that even low levels of cadmium exposure may adversely alter hematological and biochemical variables and induce oxidative stress.
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To Editorial Office of
Environmental Research

Re: manuscript submission

Dear Editorial Office,

also on behalf of the coauthors, I would like to submit our manuscript titled 'Associations of urinary and dietary cadmium with oxidative stress and hematological parameters' for possible publication in *Environmental Research*.

In this study, we assessed cadmium exposure, through urinary excretion and dietary intake, in a population of healthy non-smoking blood donors. We found a positive correlation between cadmium and a biomarker of oxidative stress and DNA damage, 8-oxo-7,8-dihydro-2'-deoxyguanosine. Urinary cadmium levels were also positively associated with some hematological parameters, especially triglyceride levels. Our results indicate that cadmium may exert adverse effects even at very low levels of exposure.

All of the authors have read and approved the paper and it has not been published previously nor is it being considered by any other peer-reviewed journal.

Please address all editorial correspondence to Marco Vinceti, Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università di Modena e Reggio Emilia, Via Campi, 287, 41125 Modena – Italy, tel. +39 059 2055 481, Fax +39 059 2055 483, email marco.vinceti@unimore.it.

I sign this letter also on behalf of the remaining coauthors, and I wish to thank you in advance for reviewing our manuscript.

Sincerely yours

(Marco Vinceti)

Highlights

- Urinary and dietary cadmium were positively correlated in a non-smoking population
- Cadmium positively correlated with 8-oxo-7,8-dihydro-2’-deoxyguanosine
- Urinary cadmium was strongly positively associated with triglyceride levels
- Cadmium may exert adverse effects even at low levels of exposure

Title: Associations of urinary and dietary cadmium with oxidative stress and hematological parameters

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Abstract:

Cadmium is a heavy metal with established adverse effects on human health, namely on bone, liver and kidney function and the cardiovascular system. In this study, we assessed cadmium exposure and its correlation with biomarkers of toxicity. We recruited 137 non-smoking blood donors without a history of chronic disease or cancer who resided in the Northern Italy province of Reggio Emilia (mean age 47 years, range 30-60 years) in the 2017-2019 period. We used a semi-quantitative food frequency questionnaire to estimate dietary cadmium intake and urine samples to assess concentrations of urinary cadmium and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG). Median urinary cadmium and 8-oxodG concentrations were 0.21 µg/L (interquartile range (IQR): 0.11–0.34 µg/L) and 3.21 µg/g creatinine (IQR: 2.21-4.80 µg/g creatinine), respectively, while median dietary cadmium intake was 6.16 µg/day (IQR: 5.22-7.93 µg/day). We used multivariable linear and spline regression models to estimate mean differences exposure concentrations. Dietary and urinary cadmium were positively correlated, and both were positively and linearly correlated with 8-oxodG. We found a positive association of urinary cadmium with blood alanine aminotransferase (ALT), total cholesterol, low-density lipoprotein (LDL)-cholesterol and thyroid-stimulating hormone (TSH) concentrations. We also observed a positive association with triglycerides, in both linear (beta regression coefficient=77.03 (95% confidence interval 32.27-121.78) and non-linear spline regression analyses. Despite the positive correlation between dietary and urinary cadmium estimates, dietary cadmium intake showed inconsistent results with the study endpoints and generally weaker associations, suggesting a decreased capacity to reflect actual cadmium exposure. Overall, these findings suggest that even low levels of cadmium exposure may adversely alter hematological and biochemical variables and induce oxidative stress.

Keywords: Biomarkers of exposure; Cadmium; Environmental epidemiology; Heavy metals; Toxicology; 8-Hydroxy-2'-Deoxyguanosine

Abbreviations:

8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine;

β, beta regression coefficient;

ALT, alanine aminotransferase;

BMI, body mass index;

CI, confidence interval;

EFSA, European Food Safety Authority;

EPIC, European Prospective Investigation into Cancer and nutrition;

EV, extreme value;

FFQ, food frequency questionnaire;

HDL, high-density lipoprotein;

IQR, interquartile range;

LDL, low-density lipoprotein;

QC, quality control;

ROS, reactive oxygen species;

TSH, thyroid-stimulating hormone;

WHO, World Health Organization.

1. Introduction

Cadmium is a toxic heavy metal with no known physiological or essential functions in the human body. Exposure to cadmium occurs through different environmental sources: natural and agricultural and industrial processes. A major source of cadmium in non-occupationally exposed populations is tobacco smoking, while for non-smokers its presence in food, air, and water represents the main source of exposure. Cadmium can enter the human body through inhalation, ingestion, and dermal contact (Wu et al., 2016) and may accumulate in all organs and tissues, although it exerts its genotoxic effects especially in kidney, bone, liver, and cardiovascular system (Maret and Moulis, 2013; Messner and Bernhard, 2010; Tinkov et al., 2018). Cadmium has been classified as a human carcinogen (IARC, 2012; NTP, 2016).

To quantify cadmium exposure, investigators generally rely on assessments of dietary intake (via food frequency questionnaire) and urinary concentrations (via excretion) (Filippini et al., 2016; Satarug et al., 2019). For both, the overall dietary intake and biomarker parameters, different standards have been set from risk assessments performed by international agencies. In 2010 the World Health Organization (WHO) revised the tolerable intake of cadmium (FAO/WHO, 2010) setting it at 25 μg of cadmium/Kg body weight per month and a urinary threshold of 5.24 μg/g creatinine. One year later the European Food Safety Authority (EFSA) established lower safe levels of both intake and urinary concentration as 2.5 μg of cadmium/Kg body weight per week and 1.0 μg/g creatinine, respectively (EFSA, 2011).

Cadmium, together with other heavy metals such as arsenic and lead, is known to induce oxidative stress and produce reactive oxygen species (ROS) (Beyersmann and Hartwig, 2008), which along with other mechanisms, might underlie its carcinogenicity (IARC, 1993). In humans and other living organisms, one of the most sensitive biomarkers of oxidative stress and DNA damage is 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), an oxidized derivative of deoxyguanosine (Cadet et al., 2014; Koppen et al., 2020), which showed to increase also after cadmium exposure (PMID: 32489905 e 30529889). The 8-oxodG compound is not absorbed through the gut, therefore its levels in urine are independent from food sources and gut bacteria, and they exclusively reflect oxidative stress derived from the body's cells (Poulsen et al., 2014).

In a population of non-smoking Italian blood donors, we estimated cadmium exposure via urinary excretion and dietary intake, and then evaluated their correlation. We also assessed the association between cadmium exposure and blood concentrations of 8-oxodG, a biomarker of oxidative stress. Finally, we assessed associations between cadmium exposure and selected

metabolic parameters, including blood concentrations of glucose, alanine aminotransferase (ALT), lipids, and thyroid-stimulating hormone (TSH).

2. Materials and Methods

2.1 Study participants

The population investigated in this study has been previously described (Urbano et al., 2021a). Briefly, we recruited healthy blood donors without a history of chronic disease or cancer from the Transfusion Medicine Center 'Casa del Dono' of AUSL-IRCCS of Reggio Emilia from April 2017 to April 2019; 148 individuals agreed to participate in the study. Of these, four participants withdrew before completing the study and seven were excluded because of excessive cotinine levels (>30 µg/L) that we deemed being inconsistent with the self-declared non-smoker status. The final cohort comprised 137 subjects, for which we obtained written informed consent, as required by the Ethics Committee of the Reggio Emilia province (protocol approval no. 2016/0022799). Each participant was asked to complete a questionnaire concerning medical history and personal information (Urbano et al., 2021b). During the first visit, participants were also asked to provide fasting blood and urine samples and if it was not feasible, samples were collected later, once the questionnaire on the food habits was returned.

2.2 Analytical determinations

We collected blood and urine samples in polyethylene tubes. Fasting blood samples were collected in the morning, centrifuged for 10 minutes at room temperature and then stored at -20°C until analysis. For urine samples, we followed the same storage conditions.

2.2.1 Hematological and biochemical parameters measurement

Automatized laboratory procedures were used to quantify the total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride, glucose and TSH levels. For the determination of ALT concentration, we used an automatic enzymatic colorimetric assay.

2.2.2. Urinary cadmium, 8oxo-dG and cotinine measurements

Before analysis, urine samples, were thawed at room temperature for 2 hours. Each sample was mixed and heated at 37°C for 30 minutes to dissolve the sediment. An aliquot of 600 µL was transferred into a 10 mL polyethylene tube and added to 2.4 mL of an aqueous solution of nitric acid 0.05% v/v prepared by diluting ultrapure nitric acid (69% TraceSelect, Fluka, France), containing Indium-111 (111In) at 7.5 µg/L as internal standard (Inorganic Ventures, Inc., Lakewood, NJ, USA). All solutions were prepared using Milli-Q® ultrapure water (conductivity 0.056 µS/cm) (Merck, Darmstadt, Germany).

The urine samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) X Series II (Thermo Electron Corporation, Rodano, Italy). The instrument was operated in collision cell mode (CCT-Ked), with 2.0 mL/minutes of helium used to reduce interference. For each sample, three replicates were run. The calibration curves were obtained by dilution of the multi-element standard stock solution 71A, containing Cd at 10 mg/ml (Inorganic Ventures, Inc., Lakewood, NJ, USA). The calibration curve was linear in the range of 0.01–30 µg/L with a correlation coefficient of ≥0.999. The limits of quantification (LOQs), calculated as ten times the standard deviation of the blank, was 0.01 µg/L. Internal quality assurance was performed using quality controls (QCs) for metals in urine: Lyphocheck Urine Metals Control (Bio-Rad Laboratories, Anaheim, CA, USA), and Seronorm® (Sero AS, Billingstad, Norway) both at Level-1 and Level-2. Before analysis, QCs were reconstituted in accordance with manufacturers' instructions. QC accuracy was between 98% and 103% and precision ranged between 9% and 3%.

Urinary 8oxo-dG and urinary cotinine were analyzed simultaneously by liquid chromatography coupled with triple quadrupole mass spectrometry, as previously described (Hanchi et al., 2017). Briefly, 500 µl of urine was mixed in a polyethylene tube with 500 µl of MilliQ-purified water and 5 µl of internal standards ([15N5]-2'-deoxyguanosine 1 mg/L and cotinine-d310 mg/L). The solution was centrifuged for 10 min and the supernatant was filtered directly in the vial for the analysis. An aliquot of 10 µl was injected into the liquid chromatography equipped with a reverse phase column Gemini-NX C18 110A (100-mm length, 2.00-mm internal diameter, 3-µm particle size; Phenomenex, Bologna, Italy). The chromatographic separation was performed at room temperature using a gradient mixture of 1.5 mM ammonium formate with 0.1% HCOOH in methanol and 1.5 mM ammonium formate with 0.1% HCOOH in water at 0.25 ml/min, for a total run time of 10 min. Detection were performed using a triple quadrupole mass spectrometer (TSQ Quantum Access, Thermo Scientific, Rodano, Italy) equipped with a heated electrospray ionization source operating in positive ion mode. Quantification was based on selective reaction monitoring.

A linear calibration curve for both analytes was obtained in urine by adding aqueous solutions of 8oxo-dG and cotinine at different concentrations. The limit of quantification, calculated as the lowest point in the calibration curve that gave a reproducible analytical response with a precision value, were 0.12 µg/l for 8oxo-dG and 0.1 µg/l for cotinine. The recovery rate of the analytical procedure was 98% for both analytes, and the analytical precision was <10%.

2.3 Data collection and analysis

We conducted standard medical in-person interviews to collect data on medical history, sociodemographic characteristics, and lifestyle factors for each participant. To assess the daily intake of cadmium, participants completed a validated semi-quantitative food frequency questionnaire (FFQ) specifically developed for the Northern Italian population. Using the self-administered European Prospective Investigation into Cancer and Nutrition (EPIC)-FFQ all subjects were asked to answer to questions about frequency and quantity of 188 food items consumption over the previous year. We then calculated dietary intake of cadmium (total daily exposure) using previously-described methods based on combination of analytic results of trace elements from foods and dietary assessment from the EPIC-FFQ (Filippini et al., 2018).

We computed associations between urinary and dietary cadmium concentrations and estimated them between each of these indicators of cadmium exposure and blood glucose, ALT, LDL- cholesterol, HDL-cholesterol and total-cholesterol, triglyceride and TSH levels, as well as 8-oxodG and 8-oxodG/g creatinine, using both linear and spline regression analyses in crude and multivariable adjusted models. In our regression models, we adjusted for sex as discrete variable, along with age, body mass index (BMI), cotinine, alcohol and fiber intake as continuous variables. All spline regression analyses were performed using linear regression fitted on a restricted cubic spline model and based on three knots at fixed percentiles of cadmium exposure levels (10^{th}, 50^{th}, and 90^{th}). For regression analyses, we computed beta regression coefficient (β) to compare the strength of the effect of each individual independent variable, i.e., biomarkers of exposure, to the dependent variable, i.e., outcome variables, and 95% confidence intervals (CI).

In the study some extreme values (EV) occurred, therefore we considered them as outliers and winsorized at 99^{th} percentile the following variables: urinary cadmium concentration (EV: 1.32 µg/L); triglyceride levels (EV: 574 µg/L); TSH levels (EV: 15.64 µg/L).

Data analyses were performed using the following 'mkspline', 'regress', 'winsor', and 'xbcrsplinei' routines of the Stata statistical software (version 17.1, Stata Corp., College Station, TX 2021).

3. Results

Our analytic population comprised 137 healthy blood donors, 62 males and 75 females aged 30-60 (mean age 47.4), whose main characteristics including sex, age, BMI, smoking habits, marital status, educational attainment, and occupation are summarized in Table 1. The two indicators of cadmium exposure were urinary excretion concentrations expressed in micrograms per liter (μg/L) and dietary intake expressed in micrograms per day (μg/day). The creatinine-adjusted concentrations of urinary cadmium were substantially similar to unadjusted ones with slightly lower median value of 0.18 μg/g creatinine (interquartile range (IQR): 0.12–0.27 μg/g creatinine). Urinary cadmium levels were higher among females (median 0.22 μg/L) than males (median 0.21 μg/L), while dietary cadmium intake was higher among males (median 6.34 μg/day) than females (6.01 μg/day). Table 2 shows the percentile distribution of cadmium, blood parameters, urinary cotinine, creatinine, 8-oxodG and 8-oxodG/creatinine levels in the study population and divided by sex. Almost all values were higher in males than females, except for urinary cotinine and 8-oxodG/creatinine levels.

In multivariable linear regression models, urinary cadmium concentrations were strongly associated with 8-oxodG levels, even when adjusted for creatinine (β= 1.76, 95% CI 0.10-3.42). Dietary intake was also positively associated with blood 8-oxodG concentrations, although less strongly compared to urinary excretion and especially when adjusted for creatinine (β=0.25). Spline regression analyses indicated a positive monotonic association between urinary and dietary cadmium concentrations with one another (Figure 1).

We then used multivariable adjusted spline regression analyses to assess the association between the two biomarkers of cadmium exposure used in our study. In linear regression analysis, they were slightly and positively associated (β=0.02). In spline regression, results were substantially similar (Figure 2).

Using linear regression analysis, we assessed the associations of urinary cadmium levels and dietary cadmium intake with the metabolic endpoints in crude and multivariable models adjusted for age, sex, urinary cotinine levels, BMI, and alcohol intake. Concerning urinary cadmium, we found positive associations with ALT, total and LDL-cholesterol, TSH and a strong positive relation with

triglyceride levels. On the contrary, inverse associations emerged with glycemia and HDL-cholesterol. Dietary cadmium intake levels mirrored the positive association with ALT, total and LDL-cholesterol, as well as the inverse association with HDL-cholesterol, although regression coefficients were smaller. In addition, dietary cadmium intake showed almost null associations with glycemia, triglycerides and TSH levels. Both urinary and dietary cadmium estimates showed positive associations with 8-oxodG, in models with and without adjustment for creatinine (Table 3).

In spline regression analysis, we obtained conflicting findings when using urinary concentrations compared with dietary intake to assess cadmium exposure. Urinary cadmium concentrations were positively associated with triglyceride levels and above 0.3 μg/L the relation was almost monotonic. Conversely, we observed an inverted U-shaped association with other endpoints, i.e., glycemia, ALT, total and LDL-cholesterol, with positive direction under 0.3 μg/L and then became inverse. We observed an inverse association with HDL-cholesterol and a slight positive association with TSH concentrations (Figure 3). In contrast, dietary cadmium intake was almost linearly associated with ALT and LDL-cholesterol, while no relation emerged with total cholesterol. Non-linear associations were observed with glycemia, with the curve showing a positive association up to 7 μg of daily intake, and then a downward trend. In addition, we observed U-shaped associations for HDL-cholesterol and TSH levels, where we observed positive associations above the same value. It appeared that 7 μg of daily cadmium intake was also threshold for a decreasing of the curve in relation with triglycerides, while below that dose, there was a slightly positive association (Figure 4).

When analyzing the differences according to sex, for urinary cadmium the results in males were substantially similar to that of the overall population, except for LDL-cholesterol for which the association was negative. In females, negative associations emerged between urinary cadmium versus glucose, ALT, HDL-and, LDL-cholesterol levels. Dietary cadmium intake levels were positively associated only with ALT, and LDL-cholesterol in males, while in females this biomarker of exposure showed positive associations with all the metabolic endpoints, except triglyceride levels (Supplemental eTables S1 and S2). In both females and males an almost entirely linear association with 8-oxodG/g creatinine was observed. For dietary intake there were some differences between males and females. In the first subgroup there was a non-linear and entirely positive association with 8-oxodG/g creatinine, while in females we observed a U-shaped association. In fact, no association emerged until 7 μg of daily cadmium intake and a slight positive association above that amount (Supplemental eFigures S1 and S2).

4. Discussion

Cadmium is a non-essential toxic metal that is ubiquitous in the environment. Exposure to cadmium occurs primarily through industrial occupations and tobacco smoke, while for non-occupationally exposed and non-smokers subjects, food represents the main route of exposure (EFSA, 2012). Since it is naturally present in soil and added to phosphate fertilizers, a major part of cadmium intake comes from rice, wheat, and vegetables (Fagerberg and Barregard, 2021; Filippini et al., 2018; Gonzalez et al., 2019).

Cadmium concentrations vary considerably through the world. One recent European study conducted among mother-child dyads reported that the Polish females had the highest urinary cadmium concentrations (> 0.4 μg/g creatinine for smokers; > 0.35 μg/g creatinine for non-smokers), while Danish non-smokers had the lowest levels (0.1 μg/g creatinine) among the 16 countries analyzed (Berglund et al., 2015). In Europe and North American countries, the average cadmium intake in the general population ranged between 10 and 20 μg/day. The corresponding average urinary excretion was about 0.5/1.0 μg/day (EFSA, 2009; Nordberg et al., 2007). In our cohort, the median urinary excretion concentrations and dietary intake of cadmium were 0.18 μg/g creatinine (interquartile range (IQR): 0.12–0.27 μg/g creatinine) and 6.16 μg/day (IQR: 5.22-7.93 μg/day), respectively, indicating that they were lower than those observed in many Western populations and below the thresholds defined by most international agencies. In fact, the tolerable intake of cadmium is today defined according to kidney damage and primary sign of overexposure, including proteinuria. According to EFSA, cadmium limits are set at 2.5 μg of cadmium/Kg body weight per week and 1.0 μg/g creatinine of urinary excretion levels, while the WHO set the tolerable exposure at 25 μg of cadmium/Kg body weight per month and a urinary threshold of 5.24 μg/g creatinine (EFSA, 2011; FAO/WHO, 2010).

In our cohort, the two biomarkers of exposure used to assess cadmium exposure were positively and linearly correlated. Some studies have reported correlations between dietary cadmium, as assessed via food frequency questionnaire, and urinary cadmium concentrations (Ikeda et al., 2015; Julin et al., 2011), while other studies have reported weak correlations (Vacchi-Suzzi et al., 2015). Another biomarker frequently used to assess cadmium exposure is cadmium concentrations in whole blood or in serum. However, in the previous studies conducted by our group in the population of a city nearby of this study, no association was observed between dietary cadmium intake and its serum cadmium concentrations (Filippini et al., 2020b). Although serum

cadmium is considerably stabler than other biomarkers, it is rarely adopted in exposure biomonitoring studies because of its low content and uncertain correlation with cadmium erythrocyte content, to which it is largely bound (Kjellström and Nordberg, 1978; Nordberg et al., 1971).

We acknowledge that urinary cadmium is considered a better parameter to estimate exposure rather than the dietary intake. In fact, as outlined in literature, urinary cadmium reflects accumulation even after several decades, since its half-life is estimated to be around 10-30 years in humans (Casarett et al., 2001; Klaassen et al., 2013). For this reason, we consider more reliable the exposure estimate based on urinary cadmium, than that yielded by the dietary intake assessment. In particular, the associations with the endpoints may be masked by recent consumption of whole grain and vegetables.

Adverse effects on human health induced by cadmium have been identified, including Itai-itai disease in the Toyama Prefecture (Aoshima, 2016), and bone disease like osteomalacia and osteoporosis, as well as anemia and kidney damage and failure (Akesson et al., 2014; Buha et al., 2019; Genchi et al., 2020). High urinary cadmium is associated with increased overall mortality (Liu et al., 2021). Other effects involve increased risks of prediabetes and diabetes (Filippini et al., 2022; Tinkov et al., 2017; Xiao et al., 2021). Cadmium is classified as a carcinogenic agent with sufficient evidence for lung cancer and limited evidence for prostate and kidney cancers (IARC, 2020; Vinceti et al., 2007). Involvement of cadmium in other types of cancer, such as breast cancer and melanoma, has been suggested but consistent evidence is still lacking (Filippini et al., 2019; Filippini et al., 2020a; Satarug et al., 2010).

In our study, urinary cadmium concentrations were slightly positively associated with blood glucose concentrations up to 0.3 μg/L of urinary excretion levels, but at higher levels we observed an inverse association, in line with the conflicting results obtained in other studies (Borne et al., 2014; Li et al., 2019; Xiao et al., 2021). For what concerns ALT, urinary cadmium showed an inverted U-shaped association, with a positive association observed up to 0.3 μg/L of urinary cadmium. Previous epidemiologic studies identified positive associations between cadmium and ALT, suggesting that cadmium may exert adverse effects on liver function and play a role in liver disease (Kang et al., 2013; Kim et al., 2021; Park et al., 2021). Cardiovascular disease has also been linked to cadmium exposure, particularly hypertension and atherosclerosis (Fagerberg and Barregard, 2021; Zhong et al., 2021). In this cohort, and in line with recent evidence (Obeng-Gyasi, 2020; Tinkov et al., 2018), we found an indication of adverse effects by cadmium on markers of cardiovascular

disease risk, since urinary cadmium was inversely associated with HDL-cholesterol and directly (though non linearly) with LDL and total cholesterol, and with triglyceride levels in particular, the latter being the strongest association identified in our study. These results corroborate the adverse metabolic effects induced by cadmium in toxicological studies (Chagas et al., 2020; Ige and Akhigbe, 2013; Khan et al., 2019). Cadmium may induce hypertriglyceridemia by decreasing plasma lipoprotein lipase, a key enzyme involved in triglycerides hydrolysis (Larregle et al., 2008). Thus, it may increase LDL-cholesterol concentrations by inducing upregulation of ATP-binding cassette transporter A1, a protein responsible for cholesterol export and metabolism (Wang et al., 2018).

Likewise, we detected a slight positive association emerged between urinary cadmium concentrations and TSH levels. Indeed, cadmium is considered an endocrine disruptor affecting different pituitary hormones secretion, thus including TSH, prolactin, adrenocorticotropic, luteinizing, follicle-stimulating, and growth hormones (Lafuente et al., 2003). As reported in previous studies, cadmium induces thyroid dysfunction, disturbances of the hypothalamic-pituitary axis and its accumulation may result in higher TSH concentrations in rats and humans (Caride et al., 2010; Hammouda et al., 2008; Iijima et al., 2007; Nie et al., 2017).

Our results showed positive linear associations between cadmium exposure and 8-oxodG in urine adjusted for creatinine, with a marked dose-effect relationship. These findings are in line with previous epidemiological studies (Engstrom et al., 2010; Ketelslegers et al., 2008; Kippler et al., 2012), but add new insights regarding the effects of cadmium exposure on 8oxo-dG since the positive associations were observed at lower levels than previous studies. Two cross-sectional studies were conducted among a chronically-exposed population (via elevated levels in rice, the main staple food) (Engstrom et al., 2010; Kippler et al., 2012). Our results corroborate the hypothesis derived from several *in vitro* and animal experiments of higher ROS production after cadmium exposure (Cuypers et al., 2010; Joseph, 2009; Liu et al., 2009) alongside inhibition of DNA repair systems (Engstrom et al., 2010; Hartwig and Schwerdtle, 2002). In the third US National Health and Nutrition Survey a positive association between urinary cadmium and an oxidative stress marker (serum γ-glutamyltransferase) emerged in a population with levels of exposure similar to ours, suggesting that oxidative stress should accounted for even in the general population with low environmental exposure to cadmium (Lee et al., 2006), and that such a mechanisms could contribute to the increased mortality associated with cadmium exposure.

The occurrence of adverse effects in non-occupationally exposed populations of non-smokers is not well established, given the relevance of smoking to exposure to cadmium as well as many

other contaminants (Fagerberg and Barregard, 2021). Thus, one of the major strengths of our study is that urinary cadmium concentrations in our cohort was not influenced by smoking, thereby decreasing the risk of confounding and allowing an examination of lower ranges of cadmium exposure. A history of smoking and passive smoking were also accounted for, and our results were adjusted for urinary cotinine levels, a well-established biomarker of smoke exposure.

Study limitations include statistical imprecision of associations due to the relatively small sample size. In addition, we could not rule out unmeasured confounding resulting from additional dietary and non-dietary factors. Finally, the cross-sectional study design precluded our ability to clarify the temporality of associations. Thus, the results from this study may not have a causal interpretation.

In conclusion, this study provides additional suggestive evidence of pro-oxidant effects of cadmium in humans even at low levels of exposure, and of potentially adverse effects of cadmium on hematological and biochemical parameters. These associations were observed even at very low levels of cadmium exposure, generally considered not to pose a meaningful risk for the general population. In particular, the strong positive association of urinary cadmium with triglyceride levels may be considered the most relevant finding alongside the positive associations observed with 8-oxodG, a biomarker of oxidative stress.

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Table 1. Characteristics of the study population and mean urinary and dietary cadmium (Cd) levels for each subgroup of the cohort (n=137).

Characteristics	All				Males				Females			
	N	%	Urinary Cd (µg/L)	Dietary Cd (µg/day)	N	%	Urinary Cd (µg/L)	Dietary Cd (µg/day)	N	%	Urinary Cd (µg/L)	Dietary Cd (µg/day)
All subjects	137	100	0.25	6.88	62	45.3	0.24	7.26	75	54.7	0.25	6.56
Age (years)												
<50	80	58.4	0.22	6.72	39	62.9	0.21	7.33	41	54.7	0.23	6.14
≥50	57	41.6	0.29	7.10	23	37.1	0.30	7.14	34	45.3	0.29	7.07
BMI (kg/m²)												
<25	74	54.0	0.24	6.68	32	51.6	0.25	6.99	42	56.0	0.23	6.45
≥25 - <30	50	36.5	0.28	6.82	27	43.6	0.24	7.31	23	30.7	0.33	6.26
≥30	13	9.5	0.19	8.19	3	4.8	0.22	9.65	10	13.3	0.18	7.75
Smoking habits												
Never	101	73.7	0.23	6.77	45	72.6	0.23	7.21	56	74.7	0.24	6.43
Former	36	26.3	0.29	7.17	17	27.4	0.28	7.39	19	25.3	0.31	6.97
Marital status												
Married/unmarried partner	97	70.8	0.24	6.83	44	71.0	0.23	7.22	53	70.7	0.24	6.50
Single	26	19.0	0.25	7.45	12	19.4	0.21	8.34	14	18.7	0.29	6.68
Separated/divorced	14	10.2	0.32	6.17	6	9.6	0.39	5.38	8	10.7	0.26	6.77
Educational level												
Elementary school	2	1.5	0.40	12.12	2	3.2	0.40	12.12	-	-	-	-
Middle school	20	14.6	0.25	7.45	8	12.9	0.25	7.00	12	16.0	0.25	7.75
High school	66	48.2	0.21	6.60	28	45.2	0.20	7.38	38	50.7	0.22	6.03
College or more	49	35.8	0.29	6.79	24	38.7	0.27	6.79	25	33.3	0.31	6.80
Occupation (ISCO)												
Managers	9	6.6	0.21	6.68	6	9.7	0.15	6.57	3	4.0	0.32	6.90
Professionals	26	19.0	0.27	7.43	12	19.4	0.24	7.91	14	18.7	0.29	7.01
Technicians/associate professionals	21	15.3	0.27	7.14	11	17.7	0.23	7.79	10	13.3	0.30	6.43
Clerical support workers	43	31.4	0.21	5.95	12	19.4	0.20	5.75	31	41.3	0.22	6.02
Service and sales workers	11	8.0	0.43	7.29	2	3.2	0.83	8.10	9	12.0	0.34	7.11
Craft and related trade workers	10	7.3	0.23	7.00	8	12.9	0.27	7.29	2	2.7	0.10	5.83
Plant and machine operators	11	8.0	0.23	8.59	8	12.9	0.25	8.34	3	4.0	0.20	9.25
Elementary occupations	6	4.4	0.15	6.40	3	4.8	0.19	6.51	3	4.0	0.11	6.30

Abbreviations: BMI, body mass index; ISCO, International Standard Classification of Occupations; N, number.

Table 2. Median (50^{th}) and interquartile range (IQR of urinary and dietary cadmium distribution and fasting blood parameters and urinary parameters in the overall study population (n=137) and divided by sex (n males/females=62/75)

	All		Males		Females	
	50^{th}	**IQR**	**50^{th}**	**IQR**	**50^{th}**	**IQR**
Cadmium						
Dietary intake (μg/day)	6.16	(5.22 - 7.93)	6.34	(5.39 - 8.48)	6.01	(5.05 - 7.44)
Urinary concentration (μg/L)	0.21	(0.11 - 0.34)	0.21	(0.11 - 0.35)	0.21	(0.11 - 0.35)
Blood parameters						
Glycemia (mg/dL)	86	(81 - 91)	88	(82 - 94)	85	(79 - 89)
ALT (U/L)	27	(22 - 35)	30	(26 - 43)	24	(20 - 29)
Total cholesterol (mg/dL)	204	(184 - 224)	192	(177 - 219)	210	(192 - 227)
HDL-cholesterol (mg/dL)	59	(51 - 69)	52	(46 - 59)	67	(57 - 73)
LDL-cholesterol (mg/dL)	124	(109 - 144)	120	(101 - 142)	125	(112 - 146)
Triglycerides (mg/dL)	78	(62 - 112)	85	(67 - 135)	73	(58 - 106)
TSH (mU/mL)	1.59	(1.18 - 2.21)	1.75	(1.16 - 2.34)	1.54	(1.18 - 2.16)
Urinary parameters						
Urinary cotinine levels (μg/L)	0.27	(0.05 - 0.86)	0.2	(0.05 - 0.77)	0.32	(0.05 - 0.94)
Urinary creatinine (g/L)	1.13	(0.73 - 1.53)	1.27	(0.88 - 1.87)	1.04	(0.66 - 1.34)
8-oxodG (μg/L)	3.39	(1.76 - 6.32)	3.48	(2.1 - 7.61)	3.17	(1.6 - 5.77)
8-oxodG/crea. (μg/g creatinine)	3.21	(2.21 - 4.8)	2.78	(2.09 - 4.3)	3.55	(2.33 - 5.09)

Abbreviations: ALT, alanine-aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; TSH, thyroid-stimulating hormone.

Table 3. Linear regression analysis of glycemia, lipid profile variables and thyroid-stimulating hormone (TSH) versus urinary Cadmium (Cd) concentration and dietary Cd intake biomarkers as independent variables. Crude model and adjusted for age, sex, body mass index (BMI), cotinine levels, and alcohol intake along with their 95% confidence interval (CI).

		Crude		**Adjusted**
Urinary Cd concentration[a]	**β**	**(95% CI)**	**β**	**(95% CI)**
Glycemia (mg/dL)	−5.66	(−14.47, 3.15)	−3.77	(−12.00, 4.46)
ALT (U/L)	4.04	(−7.07, 15.15)	6.42	(−3.69, 16.53)
Total cholesterol (mg/dL)	20.74	(−10.40, 51.89)	16.28	(−14.41, 46.98)
HDL-cholesterol (mg/dL)	−7.75	(−22.27, 6.77)	−12.68	(−24.75, -0.61)
LDL-cholesterol (mg/dL)	3.42	(−24.21, 31.22)	3.34	(−24.54, 31.22)
Triglycerides (mg/dL)[a]	67.32	(19.40, 115.23)	77.03	(32.27, 121.78)
TSH (mU/mL) [a]	0.39	(−0.54, 1.32)	0.43	(−0.53, 1.40)
8-oxodG (µg/L)	11.54	(8.29, 14.79)	11.38	(8.16, 14.61)
8-oxodG/creatinine (µg/g creatinine)	2.71	(0.88, 4.53)	2.27	(0.46, 4.09)
Dietary Cd intake	**β**	**(95% CI)**	**β**	**(95% CI)**
Glycemia (mg/dL)	0.27	(−0.28, 0.83)	0.005	(−0.52, 0.53)
ALT (U/L)	0.75	(0.06, 1.45)	0.58	(−0.06, 1.22)
Total cholesterol (mg/dL)	0.27	(−1.70, 2.24)	0.12	(−1.83, 2.07)
HDL-cholesterol (mg/dL)	−0.61	(−1.52, 0.29)	−0.19	(−0.97, 0.58)
LDL-cholesterol (mg/dL)	0.93	(−0.79, 2.65)	0.69	(−1.07, 2.44)
Triglycerides (mg/dL) [a]	−0.33	(−3.42, 2.76)	−1.83	(−4.76, 1.11)
TSH (mU/mL)[a]	0.02	(−0.04, 0.08)	0.02	(-0.04, 0.08)
8-oxodG (µg/L)	0.16	(-0.08, 0.41)	0.22	(−0.02, 0.47)
8-oxodG/creatinine (µg/g creatinine)	0.02	(−0.11, 0.14)	0.06	(−0.06, 0.19)

Abbreviations: ALT, alanine-aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; β, beta regression estimate of linear regression analysis.

Note: [a]Estimates calculated using variables winsorized at 99th percentile.

Figure 1. Spline regression analyses for the association between urinary cadmium (Cd) concentration and dietary Cd intake versus the urinary biomarkers of oxidative stress 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxodG adjusted for creatinine. Results adjusted for age, sex, body mass index, cotinine levels, alcohol and fiber intake.

Figure 2. Spline regression analysis for the association between urinary cadmium (Cd) concentration and dietary Cd intake. Results adjusted for age, sex, body mass index, cotinine levels, alcohol and fiber intake.

Figure 3. Spline regression analyses for the association between urinary cadmium (Cd) versus blood glucose, alanine aminotransferase (ALT), total, high- (HDL) and low- (LDL) density lipoprotein cholesterol, triglyceride, and thyroid-stimulating hormone (TSH) levels. Results adjusted for age, sex, body mass index, cotinine levels, alcohol and fiber intake.

Figure 4. Spline regression analyses for the association between dietary cadmium (Cd) intake versus blood glucose, alanine aminotransferase (ALT), total, high- (HDL) and low-(LDL) density lipoprotein cholesterol, triglyceride, and thyroid-stimulating hormone (TSH) levels. Results adjusted for age, sex, body mass index, cotinine levels, alcohol and fiber intake.

Figure 1

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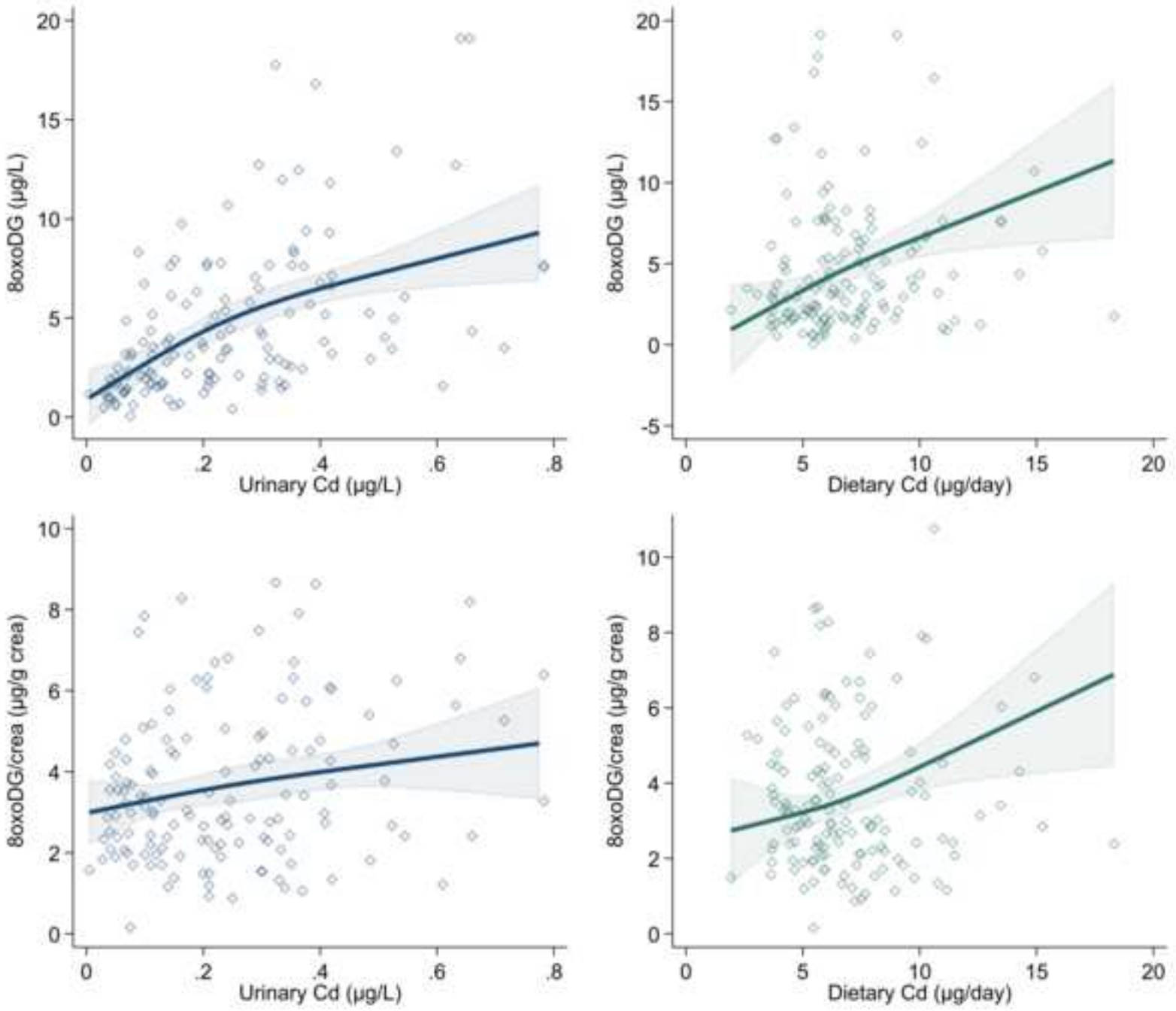

Figure 2

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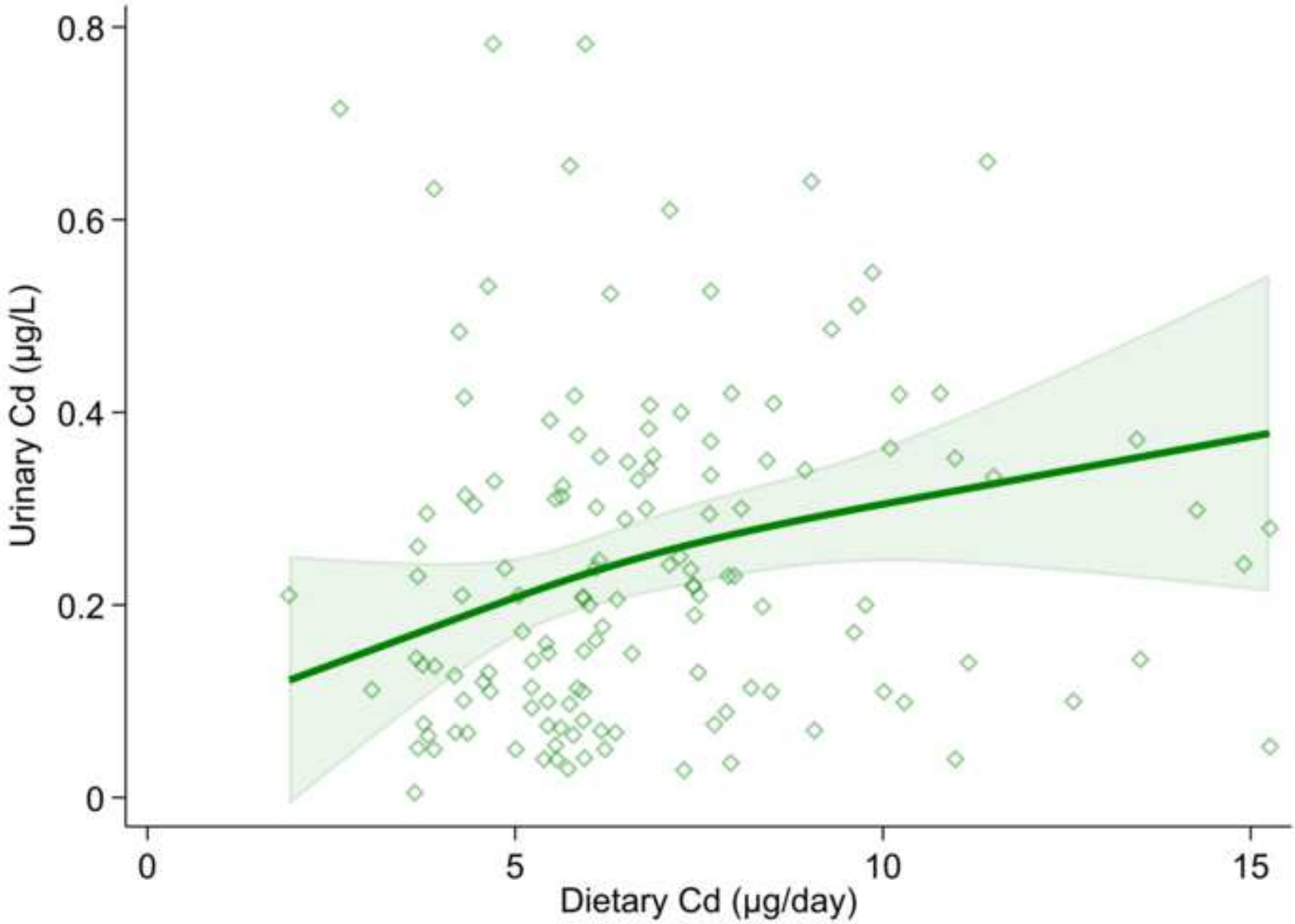

Figure 3

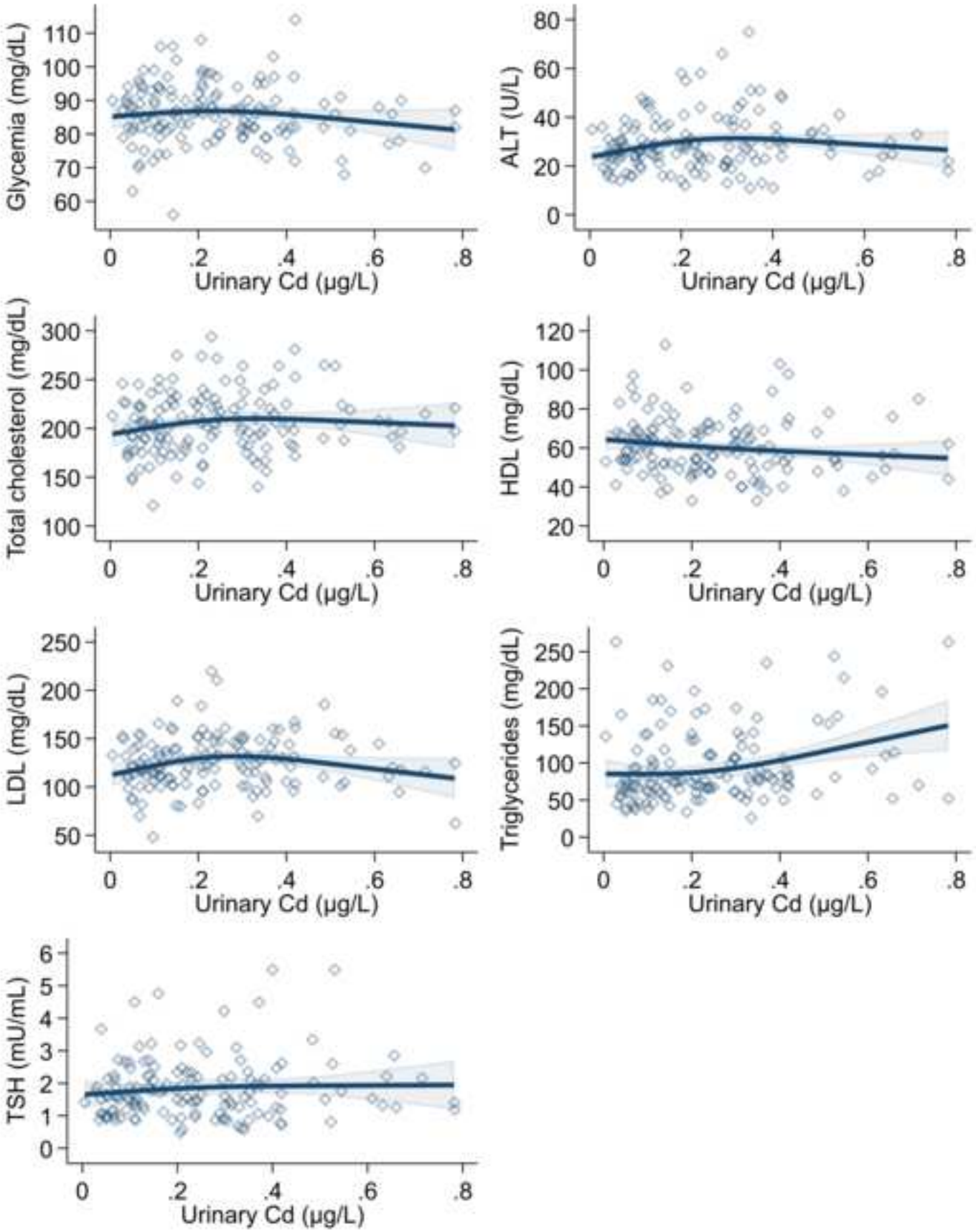

Figure 4

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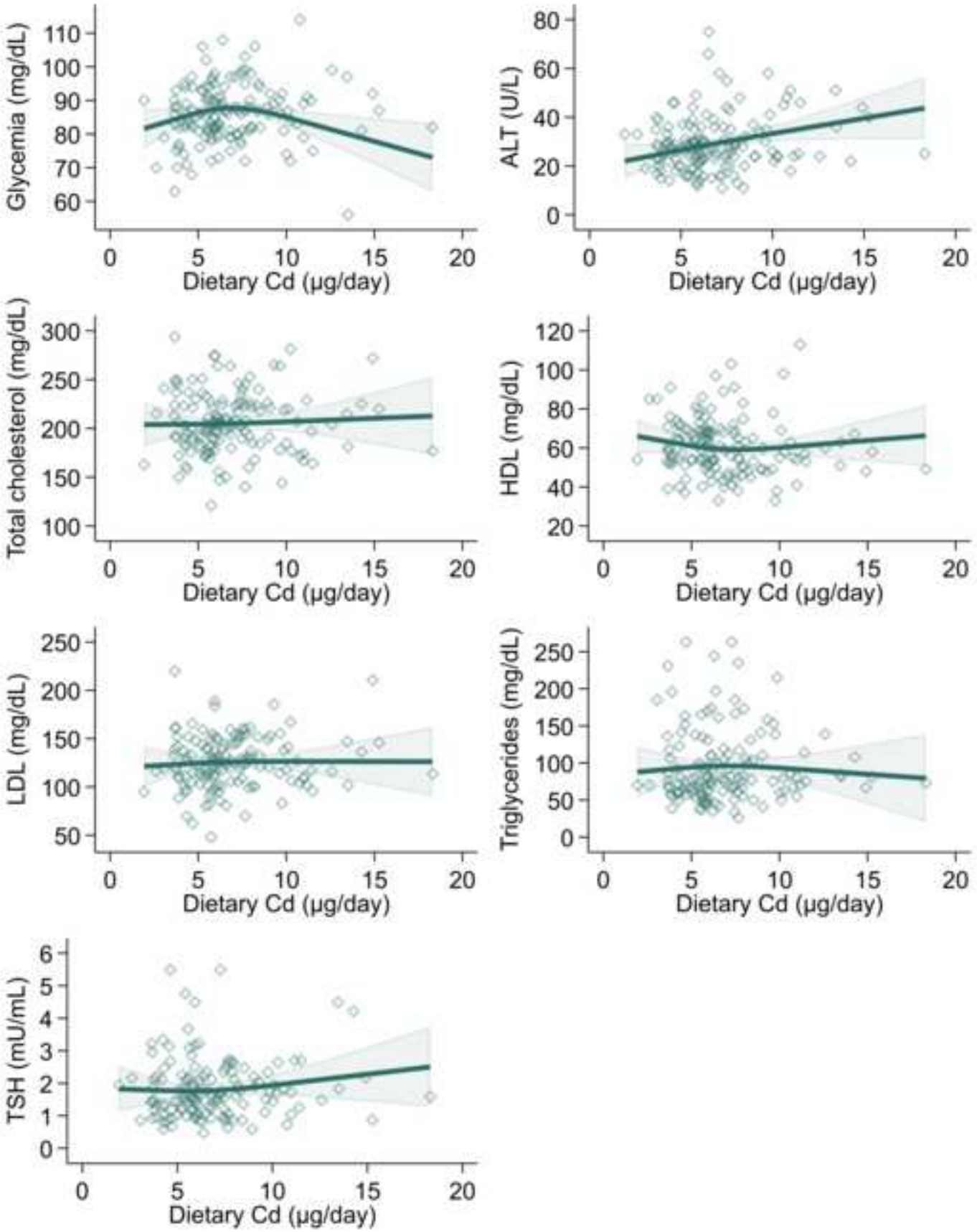

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: