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## The association between blood cadmium and glycated haemoglobin among never-, former, and current smokers: A cross-sectional study in France

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1 **The association between blood cadmium and glycated haemoglobin among never-,**  
2 **former, and current smokers: a cross-sectional study in France**

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12

13 Highlights

14

- 15 - Blood cadmium was 2.5-fold higher in current smokers than in nonsmokers  
16 - Smoking is a major confounding factor in the cadmium-diabetes relationship  
17 - Blood cadmium is associated with HbA1c levels, notably in never smokers  
18 - The cadmium-diabetes association appears not to be explained by smoking

19

## 20 **Abstract**

### 21 **Introduction**

22 The association between cadmium levels in the body and diabetes has been extensively studied,  
23 with sometimes contrasting results. Smoking is the primary non-occupational source of cadmium,  
24 and constitutes a risk factor for diabetes. One can therefore hypothesize that the putative  
25 association with cadmium is actually explained by tobacco. To fully control for this confounding  
26 factor, we studied the relationship between blood cadmium and glycated haemoglobin (HbA1c)  
27 levels separately in never-, former and current smokers.

### 28 **Methods**

29 We studied a sample of 2749 middle-aged adults from the cross-sectional ELISABET survey in and  
30 around the cities of Lille and Dunkirk; none had chronic kidney disease or a history of  
31 haematological disorders, and none were taking antidiabetic medication. The blood cadmium level-  
32 HbA1c associations in never-, former and current smokers were studied in separate multivariate  
33 models. The covariables included age, sex, city, educational level, tobacco consumption (or passive  
34 smoking, for the never-smokers), body mass index, estimated glomerular filtration rate, and (to take  
35 account of the within-batch effect) the cadmium batch number.

### 36 **Results**

37 In the multivariate analysis, a significant association between cadmium and HbA1c levels was found  
38 in all three smoking status subgroups. A 0.1 µg/L increment in blood cadmium was associated with  
39 an HbA1c increase [95% confidence interval] of 0.016% [0.003; 0.029] among never-smokers,  
40 0.024% [0.010; 0.037] among former smokers, and 0.020% [0.012; 0.029] among current smokers.

### 41 **Conclusions**

42 The observation of a significant association between the blood cadmium concentration and HbA1c  
43 levels in a group of never-smokers strengthens the hypothesis whereby diabetes is associated with  
44 cadmium *per se* and not solely with tobacco use. The small effect size observed in our population of  
45 never smokers with low levels of exposure to cadmium suggested that the risk attributable to this  
46 metal is not high. However, the impact of exposure to high cadmium levels (such as occupational  
47 exposure) on the risk of diabetes might be of concern.

48

49 **Introduction**

50 Several modifiable risk factors for type 2 diabetes have now been identified; they include obesity,  
51 physical activity, diet, and smoking (1–4). However, etiological research on diabetes is still ongoing,  
52 and is focusing on the role of pollutants in general (5–7) and heavy metals (such as cadmium) in  
53 particular. Given the widespread use of cadmium in agriculture and industry, large quantities of this  
54 metal can be found in the soil and water compartments in areas where these products are  
55 processed or disposed, or where cadmium itself is extracted. Along with water and food, sources of  
56 cadmium include smoking, occupational exposure, and – to a much lesser extent – air pollution (8).  
57 Renal excretion of cadmium is low, as so the metal accumulates in the kidneys, liver, pancreas, and  
58 adipose tissue (8,9).

59 The association between cadmium levels in the body and diabetes has been extensively studied,  
60 with sometimes contrasting results. Three meta-analyses found a significant association between  
61 cadmium and the incidence of diabetes (10–12) but a third did not (13). The available data from  
62 animal studies suggest that the pancreatic toxicity of low doses of cadmium has an influence on  
63 carbohydrate metabolism (14).

64 Smoking is an important source of toxic elements such as arsenic, cadmium, mercury, nickel and  
65 lead. Smoking is associated to higher concentration of cadmium and mercury in hair and blood  
66 sample and cigarette contains from 1.70 to 2.12 µg of cadmium (15). Given that smoking is the first  
67 non-occupational source of cadmium (16) and is strongly linked to both cadmium levels and the  
68 incidence of diabetes (3), smoking status is a major confounding factor in this relationship. Some of  
69 the studies evidencing a significant association between blood or urine cadmium and diabetes may  
70 have been biased by a residual confounding effect of smoking status even after adjustment for  
71 tobacco consumption - as highlighted in a recent meta-analysis (10). Indeed, given that tobacco  
72 smoking is the main source of cadmium in the general population, there is very little overlap  
73 between blood cadmium concentrations in smokers and those in non-smokers. Therefore, the

74 association between cadmium and diabetes observed in epidemiological studies might be solely  
75 due to the fact that cadmium is a marker of tobacco consumption, rather than to a direct effect of the  
76 metal *per se*. Some researchers consider that the evidence of a cadmium- diabetes relationship is  
77 strongly linked to smoking and therefore not interpretable (17). Even though most of the earlier  
78 studies adjusted for current tobacco consumption, a residual confounding influence is possible in  
79 studies of former smokers. Tobacco consumption cannot be perfectly quantified by the  
80 questionnaires used in epidemiological studies. The best way to avoid the residual confounding  
81 influence of tobacco is to study associations in non-smokers and especially in never smokers.  
82 However, given that levels of exposure in these populations are low, the effect size of the  
83 association may be low, and studies of the prevalence of diabetes may not be sufficiently powered.  
84 Hence, we decided to focus on a biomarker of diabetes: glycated haemoglobin (HbA1c).

85 Therefore, the objective of the present study of general population sample was to assess the  
86 relationship between blood cadmium concentration and glycated haemoglobin separately in never-,  
87 former and current smokers.

88

## 89 **Methods**

### 90 **Participants and recruitment**

91 The procedures for recruitment and data collection in the Enquête Littoral Souffle Air Biologie  
92 Environnement (ELISABET) cross-sectional survey (ClinicalTrials.gov Identifier: NCT02490553)  
93 have been described previously (18). In brief, male and female participants aged from 40 to 64 were  
94 selected from electoral rolls by random sampling, with stratification for gender, age, and city (Lille or  
95 Dunkirk urban areas, in northern France) between 2011 and 2013. A registered nurse administered  
96 the study questionnaire in the participant's own home. During the visit, the nurse also took  
97 anthropometric measurement and collected a sample of peripheral venous blood. The study

98 protocol was approved by the local institutional review board (CPP Nord Ouest IV, Lille, France;  
99 reference number: 2010-A00065-34), in compliance with the French legislation on biomedical  
100 research. All participants provided their written informed consent to participation in the study.

#### 101 **Exclusion criteria**

102 Participants with chronic kidney disease (as a self-reported medical condition or an estimated  
103 glomerular filtration rate (eGFR)  $\leq$  60 mL/min/1.73 m<sup>2</sup>, using the CKD EPI equation) (19) were  
104 excluded from the present study, since kidney failure is associated with elevated blood cadmium  
105 levels. People who reported taking antidiabetic medication were also excluded because of the  
106 latter's impact on HbA1c levels. Lastly, participants with missing or incoherent data for HbA1c,  
107 cadmium or at least one of the covariates were excluded from our analyses.

#### 108 **Laboratory measurements**

109 The fasting HbA1c level in whole blood was measured using high-performance liquid  
110 chromatography (VARIANT II, Bio-Rad). To determine cadmium concentrations, venous blood  
111 samples were collected in a dedicated tube for trace elements (Vacutainer Trace elements K2EDTA  
112 10.8 mg, Ref. 368381 Blue cup, Becton Dickinson, Le Pont de Claix, France) and immediately  
113 stored at +4 °C. Whole blood samples were diluted 1:10 with a mixture of Triton 0.1% and ammonia  
114 0.05% in water. The samples were rapidly analysed in an inductively coupled plasma mass  
115 spectrometer equipped with a collision reaction interface system (Varian 820-MS, Bruker,  
116 Wissembourg, France). <sup>103</sup>Rhodium was used as an internal standard. The calibration range was  
117 prepared by adding known concentrations to control blood samples, so as to establish 5 to 6 points  
118 within the concentration ranges typically observed in the general population. The limit of detection  
119 (LOD; 0.001 µg/L) and limit of quantification (LOQ; 0.003 µg/L) were calculated as being three times  
120 and ten times the standard deviation of the concentrations of the blank samples, respectively. The  
121 repeatability (intra-day variation) and reproducibility (inter-day variation) were 7% and 15 %,

122 respectively. The quality of the analyses was monitored with an internal quality control program,  
123 comprising the use of calibration standards, laboratory blanks and reference materials (Seronom™  
124 trace elements Whole Blood, SERO, Billingstad, Norway), and participation in an external quality  
125 control program throughout the project (an interlab comparison program established by the Quebec  
126 Toxicology Centre, Quebec National Institute of Public Health, Quebec, Canada).

127 All the samples within a given batch were analyzed at the same time with the same calibration  
128 standard. The within-batch variability in the cadmium calibration (also known as the batch effect)  
129 was taken into account by using the batch number as a random effect in a mixed linear model. All  
130 biological samples were tested in the same laboratory.

### 131 **Definition of variables**

132 A participant was considered to be a current smoker if he/she was currently smoking at least one  
133 cigarette a day (20). A participant was considered to be a former smoker if he/she had previously  
134 smoked at least one cigarette a day for a year. Diabetes was defined current medication for  
135 diabetes, an HbA1C level  $\geq 6.5\%$ , fasting glycemia  $\geq 1.26\text{g/l}$ , or non-fasting glycemia  $\geq 2\text{g/l}$  (21). The  
136 EGFR were calculated as following with creatinine in mg/dl (19). for women with creatinin  
137 ( $\leq 0,7\text{mg/dl}$ )  $e\text{GFR} = 144 \times (\text{creatinine}/0,7)^{-0,329} \times (0,993)^{\text{age}}$ . for women with creatinin ( $>0,7\text{mg/dl}$ )  
138  $e\text{GFR} = 144 \times (\text{creatinine}/0,7)^{-1,209} \times (0,993)^{\text{age}}$ . for men with creatinin ( $\leq 0,9\text{mg/dl}$ )  $e\text{GFR} = 141 \times$   
139  $(\text{creatinine}/0,9)^{-0,411} \times (0,993)^{\text{age}}$ . for men with creatinin ( $>0,9\text{mg/dl}$ )  $e\text{GFR} = 141 \times (\text{creatinine}/0,9)^{-$   
140  $1,209} \times (0,993)^{\text{age}}$ .

141

### 142 **Statistical analysis**

143 We used chi-squared tests to analyze qualitative variables. An analysis of variance, Student's t test  
144 (when the data distribution was not skewed), and the Kruskal-Wallis test (when the distribution was



145 skewed) were used to describe and compare the sociodemographic characteristics and laboratory  
146 results in never-smokers, former smokers, and current smokers. Quantitative data were quoted as  
147 the mean  $\pm$  standard deviation or (when the distribution was skewed) the median [interquartile  
148 range]. For descriptive purposes, we calculated Spearman's coefficient for the correlations between  
149 the number of cigarettes per day, the HbA1c concentration, and the cadmium concentration in  
150 current smoker. We first applied the model to the whole study population (i.e. including smokers and  
151 non-smokers). Secondly, the association between blood cadmium and HbA1c was studied using a  
152 multivariate mixed linear model including age, sex, city, educational level, current pack-years (for  
153 current smokers), total pack-years (for the former smokers), passive smoking (for never-smokers),  
154 body mass index, cadmium batch, and eGFR as covariates. As mentioned above, the cadmium  
155 batch number was set as a random effect; the other variables were set as fixed effects. The  
156 distribution of the model's residuals was checked graphically. In order to deal with tobacco  
157 consumption as a confounding effect, the main analysis was stratified by smoking status. Therefore,  
158 we analyzed the associations with HbA1c and fasting blood glucose in three separate multivariate  
159 models, built for the samples of never-, former and current smokers, respectively. Lastly, we  
160 performed sensitivity analyses for the HbA1c outcome by adjusting for the number of glasses of  
161 alcohol per day or for the haemoglobin concentration, and by excluding participants with anaemia  
162 (haemoglobin <12 g/l for women or <13 g/l for men) or those with hypertriglyceridemia (triglycerides  
163 >1.5 g/l).

164 All statistical analyses were performed with R software (version 3.5.1, R Core Team, R Foundation  
165 for Statistical Computing, Vienna, Austria, 2014, <http://www.R-project.org>). The threshold for  
166 statistical significance was set to  $p < 0.05$ .

## 167 **Results**

168 A total of 3275 participants were included in the ELISABET survey. We excluded 174 participants  
169 with chronic kidney disease, 21 participants with an history of haematological disorders, 178

170 participants who reported taking antidiabetic medication, 152 participants with missing data for  
171 Hba1c, cadmium or at least one of the covariates, and one participant with an outlier value for .  
172 Hence, 2749 participants were analysed (Figure 1).

173 The characteristics of the 2749 eligible participants are summarized in Table 1. Current smokers  
174 (n=518) were younger, comprised a lower proportion of females, and had a lower educational level  
175 than never-smokers. The mean blood cadmium level were 2.5 times greater among current smokers  
176 than among former and never-smokers ( $p<0.001$ ). Similarly, HbA1c levels were higher in current  
177 smokers than in former smokers (by 0.14 percentage points) and in never smokers (by 0.18  
178 percentage points). The eGFR was significantly higher in current smokers than in former smokers  
179 (by 3.73 ml/min/1.73 m<sup>2</sup>) and in never smokers (by 3.94 ml/min/1.73 m<sup>2</sup>).

180 An analysis of the blood cadmium distribution by smoking status provided information on how  
181 tobacco consumption influences cadmium levels (Figure 2). While never-smokers and former  
182 smokers had similar blood cadmium levels, current smokers had a very different distribution of  
183 values, with a far greater mean and standard deviation. In current smokers, Spearman's coefficient  
184 was 0.32 for the correlation between the number of cigarettes per day and cadmium ( $p<0.0001$ ),  
185 0.07 for the correlation between the number of cigarettes per day and HbA1c ( $p=0.10$ ), and 0.20 for  
186 the correlation between cadmium and HbA1c ( $p<0.0001$ ).

187 The results of the model that included the whole study population are shown in Table 2. Tobacco  
188 status and cadmium were significantly associated with HbA1C. In the multivariate analysis, a  
189 significant association between the blood cadmium concentration and HbA1c levels was found in all  
190 three smoking status subgroups (Table 3). A 0.1 µg/L increment in blood cadmium concentration  
191 was associated with an increase in the HbA1c level of 0.016% among never-smokers, 0.024%  
192 among former smokers, and 0.020% among current smokers. Smoking did not significantly  
193 influence the interaction between cadmium and HbA1c (data not shown). In the sensitivity analyses  
194 (Supplemental Table 1), all associations remained statistically significant in all groups other than

195 non-smokers after the exclusion of participants with anaemia. The fasting blood glucose  
196 concentration was significantly associated with blood cadmium in current smokers only. A 0.1 µg/L  
197 increment in the blood cadmium concentration was associated with an increase [95%CI] in glycemia  
198 of 0.0004 g/L [-0.0033; 0.0041] (p=0.83) among never-smokers, -0.0021 g/L [-0.0063; 0.0021] (p=  
199 0.33) among former smokers, and 0.0032 g/L [0.0005; 0.006] (p=0.02) among current smokers.

## 200 **Discussion**

201 We found a significant association between the blood cadmium concentration and HbA1c levels in  
202 never-smokers. This suggests that cadmium is associated with blood glucose homeostasis and  
203 diabetes *per se* and not only with the consequences of smoking. Although some of the individual  
204 blood cadmium concentrations were very high in smokers (relative to never and former smokers)  
205 the overall overlap in the concentration distribution between smokers and non-smokers was  
206 minimal. Our multivariate analysis also evidenced associations between cadmium and HbA1c and  
207 between tobacco consumption and HbA1c. In addition, in current smoker, blood cadmium were  
208 correlated with number of cigarette per day and HbA1c - illustrating the three-way relationship  
209 between cadmium, tobacco consumption and HbA1c. This finding emphasizes the need to stratify  
210 analyses. Indeed, in a general population sample with low mean levels of environmental or  
211 occupational exposure to cadmium (as in the ELISABET survey), blood cadmium concentrations are  
212 first and foremost determined by the level of tobacco consumption. The non-significant correlation  
213 between the number of cigarettes per day and HbA1c, in current smoker, may be explain by the not  
214 sufficient accuracy of the questionnaire to quantify precisely the exposure to tobacco. The blood  
215 cadmium concentration is a proxy of recent exposure to cadmium; in the present cross-sectional  
216 study, we could not measure the decrease in cadmium after smoking cessation. The blood cadmium  
217 concentrations in smokers and non-smokers were similar; the level was significantly higher in non-  
218 smokers but the difference was very small. This suggest that former smoking has a low impact on  
219 the current blood cadmium concentration. Therefore, our results in never-smokers are strengthened

220 by the significant association observed in former smokers, where the impact of previous smoking on  
221 blood cadmium seems to be weak.

222 Cadmium has been linked to an increased risk of diabetes. In a recent meta-analysis, high levels of  
223 exposure to cadmium (as judged by the urine or blood cadmium concentration) were associated  
224 with a significant increase in the risk of diabetes (odds ratio [95%CI]: 1.27 [1.07;1.52]). In the dose  
225 response analysis, each 1 µg/g increment in the urine creatinine concentration was associated with  
226 a 16% increase in the risk of diabetes. This association were significant above 2.43 µg/g - far below  
227 the WHO's standard of 5 µg/g creatinine (12). Nevertheless, there was significant inter-study  
228 heterogeneity, and the analyses were not stratified for smoking status; hence, the strength of this  
229 association remains to be characterized. Another review of studies in human and animals  
230 suggested an association between cadmium exposure and the development of diabetes and  
231 diabetes-related kidney disease (14). Lastly, the urine cadmium concentration was associated with  
232 an abnormal fasting blood glucose concentration in the NHANES study(22) and with prediabetes in  
233 the NHANES 2005-2010 study (23). The latter association was not observed in non-smokers.

234 Data on the association between cadmium and diabetes biomarkers are scarce. In a Swedish study,  
235 HbA1c was significantly associated with blood cadmium (but not urine cadmium) in a sample of  
236 women with type 2 diabetes, impaired blood glucose, and normal glucose tolerance (24). No  
237 associations with insulin, proinsulin or the Homeostatic Model Assessment for Insulin Resistance  
238 score were observed. Consistently, blood cadmium was associated with HbA1c but not blood insulin  
239 or blood glucose in the Malmö Diet and Cancer Study (17). These two latter results were obtained in  
240 bivariate analyses. Lastly, no significant associations were observed for high glucose in the  
241 NHANES 2011 2014 study.

242 To the best of our knowledge, this study is the first to have clarified the impact of smoking on the  
243 relationship between cadmium and biomarker diabetes. Few previous analyses have been stratified  
244 by smoking status. In a study of never-smokers, the associations between HbA1c and the first and

245 fourth quartiles of blood cadmium levels were no longer significant after adjustment for age and  
246 waist circumference (17). These results should be considered with caution, however, because (i)  
247 they were not part of the principal analysis and (ii) the sample size in the fourth quartile was  
248 particularly small, leading to a lack of power.-(17,24)

249

250 Studies focusing on markers of diabetes-related morbidity (rather than HbA1c) among never-  
251 smokers have also led to contrasting conclusions. In line with our present results, a cross-sectional  
252 study showed that higher levels of cadmium in urine were associated with a greater likelihood of  
253 abnormal fasting glucose values and diabetes (22). In contrast, two more recent studies did not find  
254 a significant association between cadmium and morbidity indicators of diabetes in a population of  
255 never-smokers, after adjustment for covariates (5,17). However, these non-significant results in  
256 never-smokers might also be due to a lack of power after the exclusion of smokers and the choice  
257 of the incidence of diabetes as the main outcome. Indeed, a large number of cases and a wide  
258 range of cadmium levels would be necessary to show a significant association; this is rarely the  
259 case when a study only includes never-smokers with no specific occupational exposure to cadmium.

260

261 Using a marker of diabetes (HbA1c) as an outcome (rather than diabetes itself) can be considered  
262 as a study limitation. Nevertheless, the blood HbA1c reflects average plasma glucose over the  
263 previous 8 to 12 weeks(25–27). Elevated HbA1c levels are associated with both higher complication  
264 rates among diabetic participants and higher all-cause mortality in the general population (28,29).  
265 Moreover, our approach maximized the statistical power in the general population, which presented  
266 with a low range of occupational or environmental exposure to cadmium.

267 Despite our observation of a statistically significant association, the effect size was small, which  
268 suggests a moderate impact of cadmium on public health. Nevertheless, it would be interesting to

269 conduct similar analyses on the urine cadmium level, since the latter is considered to be a better  
270 marker than blood cadmium of chronic cadmium exposure (8,30,31). Hence, analyzing the urine  
271 cadmium level might provide a more accurate estimate of exposure, and might reveal a stronger  
272 correlation. Moreover, in studies of occupational and environmental exposure of cadmium,  
273 concentrations among at-risk populations are 70% to 600% higher than in the control groups (32–  
274 34). Thus, one can reasonably suppose that cadmium's diabetogenic effect would be considerably  
275 higher among exposed individuals.

276 Our study's cross-sectional design was not suitable for assessing the possible causal nature of the  
277 observed relationship between cadmium and HbA1c; in principle, concentration might be due to  
278 impaired renal function in diabetes(35). However, there are several ways in which cadmium may be  
279 involved in glucose homeostasis and the diabetes risk. Pancreatic islets accumulate cadmium to a  
280 greater extent than other tissues do, and cadmium may induce pancreatic islet dysfunction.  
281 Cadmium may also alter the activity of gluconeogenic enzymes. Furthermore, cadmium may reduce  
282 glucose transport in adipose and renal tissues. Consequently, acute and subchronic exposure to  
283 cadmium has been linked to diabetogenic effects in animal models(16). Data from animals studies  
284 (11) support the hypothesis whereby diabetes is a consequence (rather than a cause) of cadmium  
285 exposure, and demonstrate that cadmium-induced changes of adipose tissue physiology may  
286 predispose (at least to some extent) to insulin resistance and subsequent type 2 diabetes mellitus  
287 (36–38). Furthermore, direct cadmium-induced damage to the pancreas's beta cells cadmium  
288 exposure was highlighted in an animal study by Edwards and Prozialeck (14).

289 Another limitation is the absence of differentiation between type 1 and type 2 diabetes. However, we  
290 can assume fairly safely that the very great majority of the diabetic participants in our sample of the  
291 middle-aged general population had type 2 diabetes. In a French study, only 6% of the diabetic  
292 patients in this age group presented with type 1 diabetes (39). The association was still significant  
293 in a sensitivity analysis, except when never-smokers with anaemia were excluded. Anaemia due to

294 haemolysis might influence HbA1c levels and thus explain this result. Nevertheless, haemolysis was  
295 probably not the most frequent cause of anaemia in our population; iron deficiency is typically be  
296 more frequent, although this data was not collected in the present study. The cadmium  
297 concentrations in our study population were not high enough to induce anaemia. Conversely, the  
298 association still significant after adjustment for the haemoglobin concentration. Another hypothesis  
299 is that the association was no longer significant ( $p=0.096$ ) when participants with anaemia were  
300 excluded, given the smaller sample size. The association between blood cadmium and fasting blood  
301 glucose was not statistically significant in non-smokers or former smokers. This result is consistent  
302 with previous studies (17,24). Within-subject variability is higher for glycemia than for HbA1c, and so  
303 the classification bias may also be higher for glycemia than HbA1c (40)- weakening the strength of  
304 the association and decreasing the power of the analysis.

### 305 **Conclusion**

306 Our observation of a significant association between the blood cadmium concentration and HbA1c  
307 levels in groups of never-smokers and former smokers strengthens the hypothesis whereby  
308 cadmium is associated with diabetes *per se* and not solely as a marker of smoking. The small effect  
309 size observed in our population with low levels of exposure to cadmium suggested that the risk  
310 attributable to this toxic metal is not high. However, the impact of exposure to high cadmium levels  
311 (such as occupational exposure) on the risk of diabetes might be of concern.

312

313

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334 **Conflicts of interest**

335 The authors declare that they have no conflicts of interest.



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**Table 1. Characteristics of the population, by smoking status**

Variable	Never-smokers (n=1440)	Former smokers (n=791)	Current smokers (n=518)	<i>p</i>
Age, median [Q1-Q3]	53.08 [47.19; 59.59]	53.71 [47.59; 59.43]	49.85 [45.21; 56.50]	<0.001 <sup>b,c</sup>
Female gender, n (%)	924 (64.2)	301 (38.1)	233 (45.0)	<0.001 <sup>a,b,c</sup>
Educational level (years or more of full-time education), n (%)				<0.001 <sup>b,c</sup>
17 years or more	291 (20.2)	152 (19.2)	70 (13.5)	
15-16 years	279 (19.4)	160 (20.2)	85 (16.4)	
9-14 years	721 (50.1)	412 (52.1)	321 (62.0)	
Less than 9 years	149 (10.3)	67 ( 8.5)	42 ( 8.1)	
City : Dunkirk, n (%)	716 (49.7)	381 (48.2)	264 (51.0)	0.596
BMI (kg/m <sup>2</sup> ), median [Q1-Q3]	25.96 [23.15; 29.16]	26.93 [23.97; 29.92]	25.66 [22.52; 28.55]	<0.001 <sup>a,b,c</sup>
Pack-years, median [Q1-Q3]	-	10.00 [3.75; 20.00]	18.00 [10.00; 30.00]	<0.001 <sup>b,c</sup>
Number of cigarettes/day, median [Q1-Q3]	-	-	11.21 [7.00; 20.00]	
Blood cadmium (µg/L), median [Q1-Q3]	0.28 [0.18; 0.40]	0.30 [0.20; 0.43]	0.86 [0.51; 1.31]	<0.001 <sup>a,b,c</sup>
HbA1c (%), mean (SD)	5.58 (0.48)	5.62 (0.50)	5.76 (0.69)	<0.001 <sup>b,c</sup>
Fasting plasma glucose (mg/dL), mean (SD)	0.93 (0.13)	0.97 (0.15)	0.96 (0.21)	<0.001 <sup>a,b,c</sup>
Untreated diabetes (%)	53 ( 3.8)	45 ( 5.9)	34 ( 6.7)	0.013 <sup>a,c</sup>
eGFR, ml/min/1.73m <sup>2</sup> (mean (SD))	89.79 (13.18)	90.00 (12.83)	93.72 (12.99)	<0.001 <sup>b,c</sup>

**BMI: body mass index; HbA1c: haemoglobin A1c; eGFR: estimated glomerular filtration rate**

**\* p-value when comparing the three groups, Kruskal-Wallis test was performed for non-normal variables (i.e. when median [Q1-Q3] is indicated), ANOVA for the other variables**

**a p < 0.05 when comparing never-smokers with former smokers**

**b p < 0.05 when comparing former smokers with current smokers**

**c p < 0.05 when comparing never-smokers with current smokers**

**Table 2. Multivariate analysis among all subjects (N = 2749) : factors associated with HbA1c (mixed effects linear model)**

<b>Variable</b>	<b>Beta coefficient</b>	<b>95% CI</b>	<b>p-value</b>
Blood cadmium (variation in % HbA1c for 0.1 µg/L cadmium increase)	<b>0.017</b>	[0.012; 0.022]	<0.001
Age (variation in % HbA1c for a 10-years increase)	<b>0.17</b>	[0.144; 0.197]	<0.001
Gender (ref = male)	<b>-0.035</b>	[-0.069; -0.002]	0.048
Educational level (years or more of full-time education)			0.706
17 years or more	<b>0.005</b>	[-0.065; 0.076]	
15-16 years	<b>-0.021</b>	[-0.091; 0.048]	
9-14 years	<b>0.006</b>	[-0.055; 0.066]	
Less than 9 years	ref		
City (ref = Lille)	<b>-0.001</b>	[-0.034; 0.034]	0.979
BMI (kg/m <sup>2</sup> )	<b>0.017</b>	[0.013; 0.020]	<0.001
Smoking status			0.001
Never-smokers	ref		
Former smokers	<b>-0.008</b>	[-0.048; 0.032]	
Current smokers	<b>0.095</b>	[0.017; 0.172]	
eGFR (ml/min/1.73m <sup>2</sup> )	<b>0.001</b>	[-0.001; 0.002]	0.563

**95% CI: confidence interval and p-value for adjusted coefficient**

**BMI: body mass index; HbA1c: haemoglobin A1c; eGFR: estimated glomerular filtration rate**

**The mixed effect model includes all variable presented in the table (blood cadmium, age, gender, city, education level, Smoking status, BMI and eGFR) and Cadmium Batch set as a random effect variable.**

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**Table 3. Relationship between blood cadmium and HbA1C according to smoking status (mixed effects linear model)**

Smoking status	Coefficient beta for HbA1c (variation in % for 0,1 microg/L cadmium increase)				<i>p-value</i> ‡
	Crude [95% CI]*		Adjusted [95% CI]†		
Never	<b>0.023</b>	[0.010; 0.036]	<b>0.016</b>	[0.003; 0.029]	0.012
Former	<b>0.031</b>	[0.018; 0.045]	<b>0.024</b>	[0.010; 0.037]	<0.001
Current	<b>0.017</b>	[0.008; 0.025]	<b>0.020</b>	[0.012; 0.029]	<0.0001

\* mixed model with cadmium batch set as random effect variable. And no other covariables.

†mixed model adjusted for age, sex, city, educational level, current pack-years (for current smokers), total pack-years (for the former smokers), passive smoking (for never-smokers), body mass index, eGFR as covariates and cadmium batch. Cadmium batch was set as random effect.

‡ p-value for adjusted coefficient.

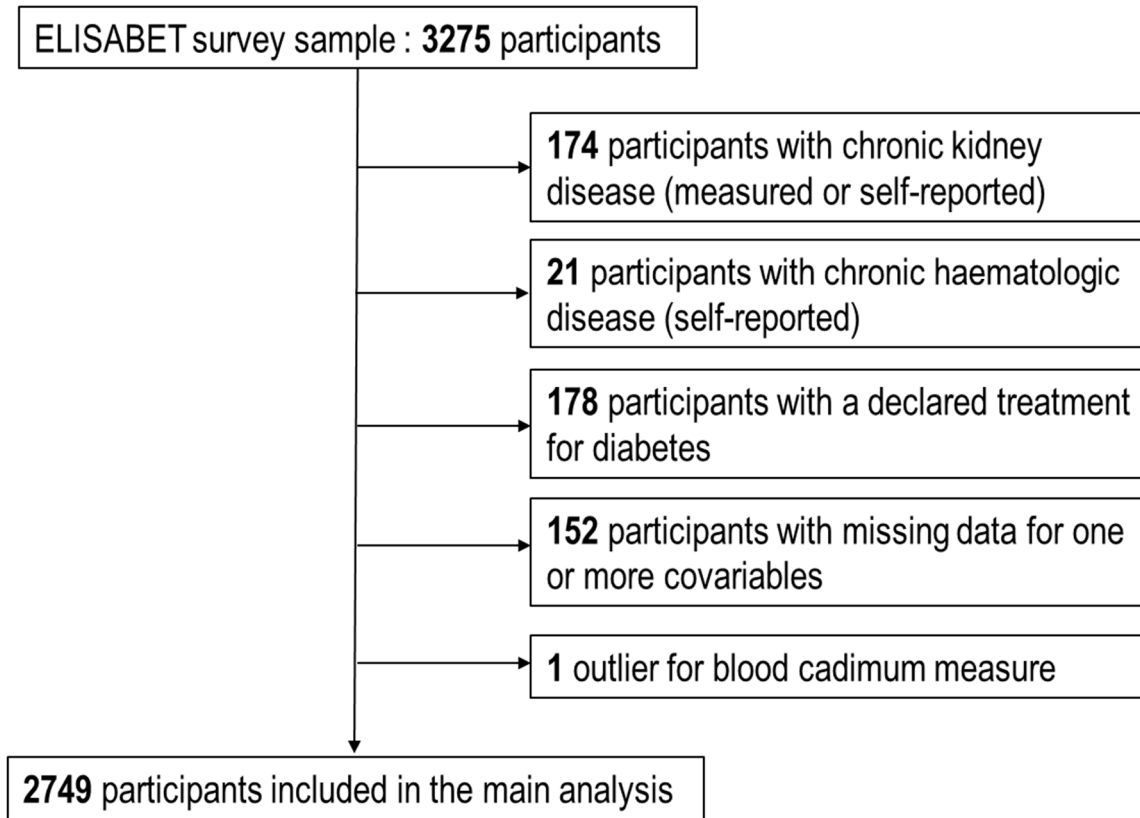
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465 Figure 1. Study flow chart

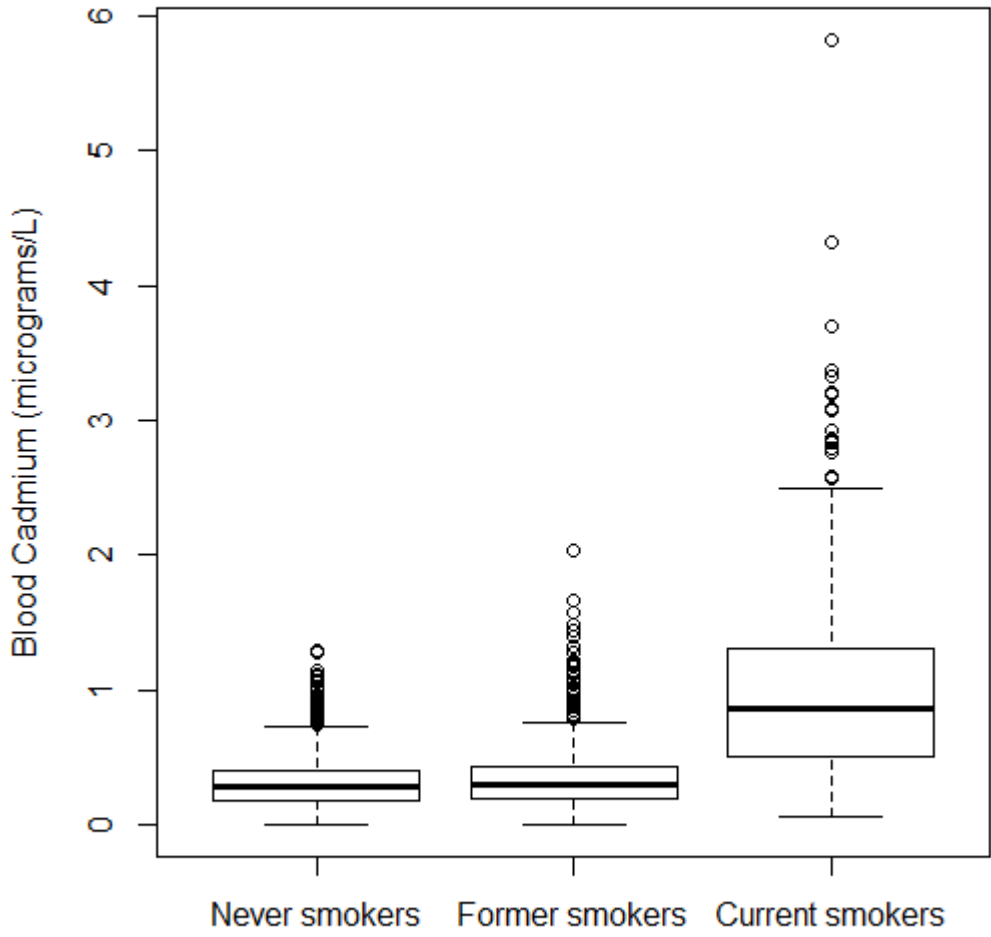


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468 Figure 2. Box plot distribution of cadmium distribution, by smoking status



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