

# Urinary Bisphenol: A Concentration and Risk of Future Coronary Artery Disease in Apparently Healthy Men and Women

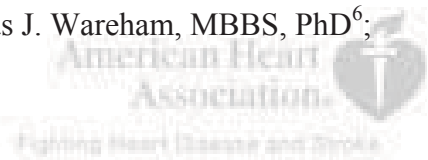
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**Abstract:**

**Background** - The endocrine disrupting chemical Bisphenol A (BPA) is widely used in food and beverage packaging. Higher urinary BPA concentrations (uBPA) were cross-sectionally associated with heart disease in NHANES 2003/04 and NHANES 2005/6, independent of traditional risk factors.

**Methods and Results** - We included 758 incident coronary artery disease (CAD) cases and 861 controls followed for 10.8 yrs from the European Prospective Investigation of Cancer – Norfolk UK. Respondents aged 40-74 yrs and free of CAD, stroke or diabetes provided baseline spot urine samples. uBPA concentrations (median value 1.3 ng/ml) were low. Per standard deviation (4.56ng/ml) increases in uBPA concentration were associated with incident CAD in age, sex and urinary creatinine adjusted models (n=1919, OR=1.13 95% CI 1.02 to 1.24, p=0.017). With CAD risk factor adjustment (including education, occupational social class, BMI category, systolic blood pressure, lipid concentrations and exercise) the estimate was similar but narrowly missed two-sided significance (n=1744 OR=1.11 95% CI: 1.00 to 1.23, p=0.058). Sensitivity analyses with the fully adjusted model, excluding early CAD (<3 year follow up); those with BMI>30; abnormal renal function; or adjusting additionally for vitamin C; C-reactive protein; or alcohol consumption, all produced similar estimates and all showed associations at  $p \leq 0.05$ .

**Conclusions** - Associations between higher BPA exposure (reflected in higher urinary concentrations) and incident CAD during over ten years of follow-up showed similar trends to previously reported cross-sectional findings in the more highly exposed NHANES respondents. Further work is needed to accurately estimate the prospective exposure response curve and to establish the underlying mechanisms.

**Key words:** Coronary Artery Disease, Bisphenol A, Endocrine Disruption, Body Mass Index, Blood Lipids

## Introduction

Bisphenol A (BPA) is one of the world's highest production volume chemicals,<sup>1</sup> used in polycarbonate plastics in many consumer products and epoxy resins lining food and beverage containers. BPA is an endocrine disrupting chemical (EDC) first synthesized with a novel estrogenic molecular structure in the 1930s.<sup>2</sup> The American Endocrine Society<sup>3</sup> have called for further research on EDCs including BPA, citing a strong basis for concern about possible links between EDCs, obesity and related disorders.

The global population is subject to repeated exposure to BPA, primarily through packaged food but also through drinking water, dental sealants, dermal exposure and inhalation of household dusts<sup>4</sup> with detectable concentrations of metabolites in the urine of > 90 % of the population worldwide.<sup>5,6</sup> In the first major epidemiological analysis of adult health effects associated with BPA, we studied 1455 adults aged 18 to 74 years with measured urinary BPA (uBPA) from the US National Health and Nutrition Survey (NHANES) 2003-2004.<sup>7-9</sup> We found higher BPA concentrations were associated with cardiovascular diagnoses (odds ratio (OR) per one standard deviation (SD) increase in BPA concentration 1.39, 95% CI 1.18-1.63; p=0.001 with full adjustment: the survey weighted standard deviation of uBPA was SD=6.68 ng/ml and the geometric mean 2.47ng/ml (data from authors). With the release of new (independent) data from NHANES 2005/06 (n=1493) we replicated the association of higher uBPA concentrations with coronary heart disease (OR per Z score increase in BPA 1.33, 95% CI: 1.01-1.75, p=0.043), despite a significant decrease in NHANES sample uBPA concentrations since the 2003/04 survey (NHANES 2005/6 geometric mean 1.79 ng/ml, 95% CI: 1.64 to 1.96). Initially reported associations with diabetes and some liver enzyme changes did not reach significance in the 2005-2006 data, but remained significant in pooled data.<sup>9</sup>

These analyses were cross-sectional and it is theoretically possible, for example, that participants with coronary artery disease (CAD) change their exposures to BPA (perhaps through change of diet) after diagnosis. Longitudinal data demonstrating temporality i.e. higher BPA concentrations predicting subsequent first diagnoses of disease would greatly strengthen the evidence for BPA playing a causal role.<sup>10, 11</sup>

Our aim was to estimate the prospective association between uBPA and incident CAD.

## **Methods**

### **Study design**

We undertook a nested case-control analysis, measuring uBPA in stored samples from a baseline clinical examination. We compared uBPA concentrations in a case group who later developed coronary artery disease to a control group who remained CAD free during follow-up.

### **Study cohort**

We studied respondents in a well-characterized nested coronary artery disease case-control set within the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk cohort study<sup>12</sup> EPIC-Norfolk is a prospective population study of 25,663 men and women aged between 45 and 79 years, resident in Norfolk, United Kingdom, who completed a baseline questionnaire and attended a clinic examination.<sup>13</sup> The sample was comparable to UK national population samples with respect to many characteristics.<sup>13</sup> Participants were recruited by post from age-sex registers of general practices. The baseline sample collection was completed between 1993 and 1997; participants completed a detailed health and lifestyle questionnaire, and additional data collection was performed by trained nurses at a clinic visit as described previously. The Norwich

District Health Authority Ethics Committee approved the study, and all participants gave signed informed consent.

## **Participants**

Boekholdt et al<sup>12</sup> selected a CAD case-control set within EPIC-Norfolk, originally with two controls matched to each case by sex, age (within 5 years), and date of clinic visit (within 3 months). We used the Boekholdt<sup>12</sup> cases and controls, but included only those aged 40 to 74, free of diabetes at baseline, with an available urine sample and valid uBPA measure. We selected equal numbers of incident CAD cases and controls, but the above constraints (especially urine sample availability) did not always allow selection within the Boekholdt original matching (see statistical analysis). Diabetes was excluded (n=84) as associations between uBPA and diabetes have been reported.<sup>9</sup>: we excluded those aged 75 plus to minimize biases caused by co-morbidity and non-representation of seniors in institutions, as with our previous NHANES analyses.

## **Coronary artery disease endpoints**

Participants were identified as having CAD during follow-up if they had a recorded hospital admission and/or died with CAD as an underlying cause during follow-up. All EPIC-Norfolk participants were flagged for death certification at the UK Office of National Statistics<sup>14</sup> and vital status is obtained for the whole cohort. Participants admitted to a hospital are identified by their unique National Health Service number, which a local health authority in Norfolk links to the Hospital Episode Statistics (including hospital contacts throughout the country). Coronary artery disease is classified according to the *International Classification of Diseases, Ninth Revision* codes 410 to 414 or *International Statistical Classification of Diseases, 10th Revision* codes I20 to I25. A case is considered if a participant had a hospital diagnosis and/or died of coronary heart disease during the follow-up. In 1996 the EPIC study conducted a validation study<sup>15</sup> of CAD

cases ascertained from death certificates and hospital admissions. Confirmation of the cause of death was sought in general practice and hospital notes or the post-mortem report. For CAD deaths identified from death certificates, the cause of death was coded as a definite CAD death, possible CAD death, or not a CAD death using standard WHO-MONICA criteria. Of 39 deaths, 38 were confirmed by inspection of the notes. For cases identified based on linkage with hospital admission databases, the admission diagnosis was evaluated by inspection of hospital notes. The event was then coded as a definite myocardial infarction, possible myocardial infarction, or not a myocardial infarction on the basis of the clinical history, electrocardiographic changes, and enzyme changes using standard criteria. All 26 patients with a hospital discharge diagnosis of myocardial infarction had either a definite or possible myocardial infarction by WHO-MONICA criteria. Follow-up occurred until first CAD onset or December 2003 (mean 6.8 years, SD 2.4, range 0.1 to 10.8 years).

### **Analysis of urinary BPA concentrations**

Study participants attended the research clinic and provided a urine sample between March 1993 and April 1998. We followed WHO guidelines over study design to evaluate exposure to BPA using biomonitoring.<sup>16</sup> Analysis of uBPA metabolites was undertaken in 2011 by Brixham Environmental Laboratory, Analytical Chemistry (a division of AstraZeneca PLC) in accordance with Good Laboratory Practice, EU Directive 88/32/EEC. Because orally administered BPA is considered to be rapidly and completely excreted, urine is the body fluid most appropriate for biomonitoring assessment of BPA exposure. We measured total (free and conjugated) urinary concentrations of BPA based on the methods employed by NHANES<sup>17</sup> and adopted by the Division of Environmental Health Laboratory Sciences, National Centre for Environmental Health, Centers for Disease Control and Prevention (CDC) i.e. sample preparation and on-line

solid-phase extraction (SPE) coupled with high performance liquid chromatography (HPLC)-isotope dilution tandem mass spectrometry (MS/MS) with peak focusing.

The GLP compliant quality control system included reagent blanks and we confirmed that EPIC stored samples contained almost exclusively metabolized compound, showing minimal leaching of BPA from collection or storage vessels. Total (free and conjugated) urinary concentrations of BPA were obtained using online, solid-phase extraction (SPE) coupled with high performance liquid chromatography (LC)-isotope dilution tandem mass spectrometry (MS/MS) with peak focusing.<sup>18</sup> Calibration was linear from 0.50-100 µg/L ( $R^2 > 0.996$ ), limit of detection was <0.50 ng/ml uBPA, limit of quantification, 0.50 ng/ml uBPA, lowest calibration standard gave a signal height:noise ratio >10 (relative standard deviations <20%, all other standards <15%).

### **Biochemical Analyses**

Non-fasting blood samples were taken by venepuncture into plain and citrate bottles.<sup>12</sup> Blood samples were processed soon after baseline collection at the Department of Clinical Biochemistry, University of Cambridge, by *Quotient* (<http://www.quotientbioresearch.com/>) using an Olympus AU640 chemistry analyzer or stored at -80°C. Serum levels of total cholesterol, HDL-C, and triglycerides were measured in fresh plasma samples with the RA 1000 (Bayer Diagnostics, Leverkusen, Germany), and LDL-C levels were calculated with the Friedewald formula.<sup>19</sup> C-reactive protein (CRP) concentrations were later measured on thawed baseline plasma from cases and controls. CRP levels were measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and biotinylated monoclonal antibodies against CRP (Sanquin Research, Amsterdam) were used as the detecting antibody.<sup>20</sup> Results were related to a standard that consisted of commercially

available CRP (Behringwerke AG, Marburg). Researchers and laboratory personnel had no access to identifiable information and could identify samples by unique identifier only.

### Statistical analysis

We applied a similar analysis approach to that we previously used in NHANES<sup>9</sup>: we assigned a value of 0.28 ng/mL to uBPA assays below the level of accurate detection (n=190 controls and 140 cases reassigned); respondents with ‘outlier’ BPA concentrations >80.1 ng/ml were excluded from the analyses; and we present per standard deviation of uBPA linear estimates of association with incident CAD, adjusted for markers of relative social privilege and conventional CAD risk factors.

Logistic regression models were used to estimate log-odds ratios of case status as a linear function of standardized uBPA concentrations (z-scores). The original EPIC age, sex and clinic date matched case control sets<sup>12</sup> were sometimes incomplete (mainly due to urine sample availability): there were 217/861 controls (25.2%) with no matched case and 251/758 cases (33.1%) had no matched control. Controls with no matched case had higher uBPA concentrations (OR=1.41 95% CI 1.17 to 1.70, p<0.000), and were less likely to be obese (compared to normal weight OR = 0.04 95% CI 0.21 to 0.75, p=0.005) compared to controls with matched cases. Amongst cases without controls, there were fewer women (OR for women vs men 0.58 95% CI 0.37 to 0.90, p=0.014). In our main analysis we therefore analyzed the case controls groups without matching, and provide a sub-analysis for the matched sets, using conditional logistic regression.

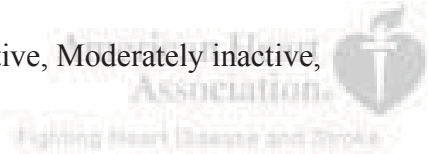
Regression models were adjusted for potential confounders, including socioeconomic markers which Calafat and colleagues<sup>17</sup> reported to be associated with BPA concentrations, and urinary creatinine to account for urine concentration.<sup>21</sup> Initial adjustment was for: age, sex,



education (categorized no qualifications, O level or equivalent (15 years), A level or equivalent (17 years) and post school or degree qualifications); occupational social class (grouped into uncoded (unemployed etc.), professional, managerial, skilled non-manual, skilled manual, semiskilled, non-skilled) and urinary creatinine concentration in mg/dl. Fully adjusted models were additionally adjusted for Body Mass Index (BMI, measured weight in kilograms divided by the square of measured height in meters, categorized into: underweight (BMI <18.5), recommended (BMI 18.5 to 24.9), overweight (BMI 25.0 to 29.9), obese I (BMI 30.0 to 34.9), obese II (BMI 35.0 or above), and unknown BMI); smoking (never smoked, former smoker, current smoker); systolic blood pressure (mm/hg), total cholesterol, HDL- and LDL- cholesterol, triglyceride concentrations, and level of physical activity (Inactive, Moderately inactive, Moderately active, Active).

Generalized additive models with penalized cubic regression splines<sup>22</sup> were used to explore the functional form of the relationships between presence of a cardiovascular disease diagnosis and BPA concentration. These models provide a method of identifying departures from linearity in exposure-response relationships. Linearity was assessed by visual inspection of the estimated spline functions and by consideration of the “estimated degrees of freedom” (edf) for the smoothed BPA term. Values of the edf close to 1 were taken as evidence of linearity. These models were fitted in the statistical software ‘R’ using the mgcv package for generalized additive modeling.

Throughout, we tested our *a-priori* hypothesis of a positive association between uBPA concentration and CAD. However, following convention we have presented two sided p-value estimates and 95% confidence intervals.



## Results

Data were available on 861 controls and 758 cases of incident CAD (total n=1619). The mean age of cases was 64.1±sd7.5 years and controls 63.8±sd7.3 years. There were marginally fewer males in the case group (62.0% vs 66.1% versus in controls) and fewer had never smoked (**Table 1**). As expected, CAD risk markers were associated with case status. Urinary BPA concentrations were relatively low. The median uBPA concentration in controls was 1.24 ng/ml and in cases 1.35 ng/ml (geometric means 1.23 and 1.39 ng/ml respectively: 1.304 ng/ml combined): The distributions were strongly skewed with, for example, 12.5% (108/861) of the controls having uBPA concentrations  $\geq 4$  ng/ml, compared to 16.6% (126/758) of the cases. Amongst controls (**Table 2**), those with higher uBPA concentrations (top 50% >1.243 ng/ml, vs bottom 50%) tended to be less likely to be from professional or managerial occupational backgrounds but there were no other differences on demographic or CAD risks.

In logistic models with case/control status as the dependent variable, per standard deviation (SD=4.56ng/ml uBPA) linear increases in uBPA concentration were associated with incident coronary artery disease in age, sex and urinary creatinine adjusted models (model B, **Table 3**: Odds ratio per z-score=1.13 95% CI 1.02 to 1.24, p=0.017). This association remained after additional adjustment for education and occupational groupings (model C, OR=1.14 95% CI 1.03 to 1.26, p=0.021). With additional adjustment for CAD risk factors (as in model D), the central estimate was similar but narrowly missed conventional two-sided significance (n=1477, OR=1.11 95% CI 1.00 to 1.23, p=0.058).

We fitted a generalized additive model with a cubic regression spline to explore the shape of the dose response curve. This provided marginal evidence of a linear relationship between standard deviation increases in BPA concentration and log-odds of cardiovascular disease (to 4

standard deviations above the mean BPA concentration as in our earlier work<sup>9</sup>, **Figure 1**; edf=1.001; p-value for smoothed term=0.068; 6 knots placed at -0.5, -0.37, -0.26, -0.08, 0.26, 3.92): a quadratic model did not provide a better fit (p=0.40) and inspection of residual plots for the linear and quadratic models did not suggest threshold effects.

### **Sensitivity analyses**

We undertook post-hoc sensitivity analyses, using separate variations of the fully (CAD risk factor adjusted) model (**Table 3**, models E to K). We excluded the earliest three years of follow-up (to remove those close to CAD onset at uBPA sample collection, model E); we excluded those with a BMI $\geq$ 30, given the suggestion that obesity may be a key factor (model F); we adjusted for serum creatinine and excluded those with elevated concentrations, mainly removing impaired renal function, which may result in biased uBPA measures (model G); we adjusted for vitamin C concentrations, a marker of dietary quality (particularly high fruit and vegetable intake, model H); adjusted for C reactive protein concentrations (reflecting inflammation) (model I); adjusted for liver enzymes to account for liver cell function effects (model J); adjusted for units of alcohol consumed at the time of baseline interview (model J). None of these analyses changed estimates materially, and all associations reached  $p\leq 0.05$ .

Finally, our cases and controls were originally drawn in matched sets on the basis of date of birth, sex and date of clinic visits categories. Due to the non-CAD risk based matching and limited availability of urine specimens, we ignored matching in the above: estimating a conditional (matched) logistic model on the subset with at least one matched pair, per SD increases in uBPA were associated with incident CAD (OR = 1.34 CI 1.12 to 1.62,  $p=0.0015$ ).

### **Discussion**

In NHANES 2003/04<sup>7</sup> and again in NHANES 2005/06, higher uBPA concentrations were associated with heart disease (pooled p-value<0.001).<sup>9</sup> A major limitation of the NHANES analyses is their cross-sectional nature, making it theoretically possible, for example, that CAD patients might have changed their behaviors and incidentally increased their BPA exposure. To strengthen the evidence for causal inference we conducted the longitudinal study presented here, which provides the first report of similar trends in associations between higher BPA exposure (evidenced as higher uBPA metabolite concentrations) and incident CAD. The prospective design adopted shows that such reverse causation cannot account for BPA - CAD associations.

The concentrations of uBPA seen in this sample are relatively low: the overall median value was 1.3 ng/ml, compared to 2.7 ng/ml (inter-quartile range 1.3 to 5.4 ng/ml) in the US NHANES 2003/4 study in which the uBPA association with cardiovascular disease was first identified.<sup>23</sup> The relative paucity of more highly exposed study subjects clearly reduces our power to detect true associations, which makes our results more noteworthy. This reduced power may explain the marginal loss of two sided significance for the fully adjusted unmatched linear model. In our NHANES 03/04 analysis, the standard deviation (SD) of uBPA was 6.68 ng/ml and produced a (per SD) OR=1.39 (95% CI 1.18-1.63; p=0.001) for cardiovascular diagnoses in fully adjusted models. To compare this estimate to the current EPIC-Norfolk results (SD=4.56ng/ml, per SD OR=1.11 CI: 1.00 to 1.23), we can re-scale raising the EPIC OR to the power of the result of dividing the NHANES SD by the EPIC SD. Using this approach the EPIC per 6.68 ng/ml uBPA Odds Ratio is OR=1.17 (95%CI 1.00 to 1.35, p=0.058) for incident CAD. Thus, associations between higher uBPA concentrations and incident CAD in EPIC-Norfolk showed similar although somewhat smaller trends compared to the cross-sectional results in NHANES 03/04.

The BPA measures in EPIC-Norfolk (as in NHANES) are from single spot urine specimens: ingested BPA in humans is rapidly excreted; hence the use of urine in biomonitoring.<sup>5</sup> We used urine samples taken at the same time of day for each respondent to minimize inter-individual variation. Regarding the use of single spot samples as measures of longer term exposure, a study of temporal variability found a single spot sample had moderate sensitivity for predicting an individual's tertiary BPA categorization.<sup>24</sup> Nepomnaschy et al<sup>25</sup> measured stability of BPA over 2 week intervals in first voided urine samples from 60 women and found a Spearman correlation of 0.5, indicating that within-individual BPA exposures were generally stable over periods of weeks. Ye et al<sup>26</sup> similarly reported changes between spot measures during each day and across 7 days, but concluded that spot samples may adequately reflect population average exposures.

Whilst humans can rapidly eliminate BPA when it is provided as a single bolus<sup>27</sup> continuous external BPA exposure through diet appears to lead to sustained concentrations that are detectable in serum or plasma. A recent study using deuterated BPA found the half life of BPA was six times longer for diet-fed mice than those who received a bolus, a phenomenon consistent with an inhibitory effect of food on first pass metabolism.<sup>28</sup> Stahlhut et al<sup>29</sup> reported the population half life of BPA to be considerably longer than six hours, based on NHANES data on fasting times. The supposition is that BPA, which is lipophilic, is redistributing to lipid rich tissues, from which slow release may occur. However, there is an absence of human pharmacokinetic data for BPA to fully explain these findings and extrapolations from animal studies have been hindered by species-specific differences in the metabolism and toxicity of BPA<sup>30</sup> and by the multiple potential routes by which humans may be exposed, including dermal exposure<sup>31</sup> and inhalation of dusts, which would avoid first-pass metabolism. Once ingested,

BPA is metabolized in the intestines and liver,<sup>32</sup> with the major metabolite BPA-monoglucuronide eliminated in humans via urine, but in rats via bile. Glucuronidation and enterohepatic recirculation also show differences between rodents, primates and humans, although the effect of this on pharmacokinetics is not yet clear.<sup>33</sup>

It should be noted that any misclassification of longer term BPA body burden is likely to have resulted in a smaller (diluted) estimate of the strength of association between BPA and CAD: the true association is likely to be stronger. Some<sup>34</sup> have suggested that BPA disease associations are driven by higher dietary intakes, which would result in obesity related risks and incidental higher BPA excretions. However, our sensitivity analyses show that exclusion of those with obesity and adjustment for blood lipid concentrations and levels of physical activity have little effect on the association, making such an explanation unlikely. Similarly the lack of effect of adjustment for vitamin C makes diets poor in fruit and vegetables an unlikely explanation.<sup>35</sup> Liver and kidney function changes, resulting in altered BPA metabolism or excretion, are also possible confounding factors, but excluding those with high blood creatinine concentrations or adjusting for liver enzymes sensitive to cell damage show these as unlikely explanations. In any observational study it is impossible to exclude the possibility that some unmeasured confounder is present. It is clear, however, that any such confounder must be independent of classical CAD risk factors.

There are several potential mechanisms by which BPA could plausibly raise CAD incidence rates. BPA and metabolites have well-documented estrogenic, anti-androgenic<sup>36</sup> and additional receptor-mediated modes of toxicity.<sup>36</sup> Given the known receptor-mediated effects of estrogen on cardiovascular tissues, it is biologically plausible that BPA might exert estrogenic

effects or antagonize endogenous estrogens in cardiovascular tissues by binding to soluble or membrane bound estrogen receptors.<sup>37</sup>

The mean uBPA concentration in our study was 3.65 ng/ml. Taking an average 24 hour urine volume for adults to be 1600ml, and assuming an 100% excretion rate and a total blood volume of 6 litres, this would give an estimated BPA blood concentration in the ng/ml range. BPA shows relatively weak estrogenic agonist activities against both human estrogen receptor alpha and beta subtypes ( $ER\alpha$ ,  $ER\beta$ ) that control many estrogen-mediated activities. The IC50 for receptor binding of BPA to human  $ER\alpha$  and  $ER\beta$  is in the low micromolar range when calculated *in vitro* and if extrapolated directly to the *in vivo* situation (without considering competitive binding to serum binding proteins, for instance), this would imply low ER receptor occupancy rates in blood and potential target tissues. However, BPA binds to other estrogen-related receptors with high affinity, including the estrogen related receptor gamma ( $ERR\gamma$ ), for which optimal receptor binding is in the nanomolar range.<sup>38</sup> A recent study has reported positive associations between increased BPA exposure and *in vivo* estrogenic gene expression in adults, including  $ER\beta$  and the estrogen related receptor alpha,  $ERR\alpha$ .<sup>39</sup>  $ERR\alpha$  is an orphan nuclear receptor involved in estrogenic signaling and energy homeostasis that is coordinately regulated with  $ERR\gamma$ . It is relevant to note that expression of  $ERR\alpha$  is highest in tissues that preferentially use fatty acids as energy sources, including adipose tissue, skeletal muscle and heart.

In addition to its estrogenic mode of action, BPA has been shown to possess anti-androgenic activity<sup>40</sup>, and uBPA levels have been associated with higher blood testosterone concentrations in Italian men.<sup>18</sup> Lee et al.<sup>40</sup> showed BPA to affect multiple steps in the activation and function of the androgen receptor. Conversely, the enzyme responsible for BPA conjugation in the intestine and liver, uridine diphosphate-glucuronosyl transferase (UGT) is itself

downregulated by androgens<sup>41</sup>, which could result in an increase in serum BPA concentration under hyperandrogenic conditions. It is unlikely that such metabolic change could alter 24-hr urinary BPA excretion in the context of repeated ingestion of BPA at the population level, although it has been suggested that a combination of hyperandrogenemia and insulin resistance may further enhance BPA levels in younger populations, especially in women with syndromes associated with increased CVD markers and CVD.<sup>42</sup> The relationship between androgen homeostasis and cardiovascular risk remains to be comprehensively established, although an increased risk of cardiovascular adverse events was recently reportedly in a trial of testosterone supplementation in older men.<sup>43</sup>

Because the pharmacokinetic behavior of BPA in humans is not comprehensively documented for practical and ethical reasons, it is not possible to rule out the conversion of BPA to metabolites that show enhanced estrogenic activity. The major metabolite of BPA, BPA-mono-glucuronide has no estrogenic activity, but oxidative cleavage of BPA to form the estrogenically active metabolite 4-methyl-2,4-bis (4-hydroxyphenyl)pent-1-ene (MBP) has been shown in rat liver. MBP was 500 fold more potent as an inducer of dose-dependent changes of estrogen receptor genes *in vivo* compared with BPA itself.<sup>44</sup> The extent to which MBP may be present in humans is not known, but the oxidation product BPA-catechol, which also shows estrogenic activity, is reported to be a minor (approximately 10%) metabolite in both human and rat microsomal models. Given these potential contributory factors, a comprehensive documentation of BPA phase 1 metabolism is clearly merited.

There are other potential mechanisms of BPA toxicity that may be relevant to the results presented here. Maxi-K channels and the  $\beta 1$  subunit in particular<sup>45</sup> play key roles in regulating smooth muscle excitability and are estrogen sensitive. BPA in the micromolar range activates



Maxi-K (KCa1.1) ion channels in human coronary smooth muscle cells in culture, sufficient to hyperpolarize the membrane potential.<sup>46</sup> Laboratory exposure studies have shown that BPA can induce liver and oxidative cellular damage,<sup>47</sup> disrupt pancreatic  $\beta$  cell function,<sup>48</sup> and have obesity-promoting effects,<sup>49</sup> all of which could plausibly contribute towards CAD risk. Certain BPA derivatives including bisphenol A diglycidyl ether (BADGE) are peroxisome proliferation activated receptor gamma (PPAR $\gamma$ ) antagonists.<sup>50</sup> PPAR $\gamma$  agonists may activate or inhibit ion channel activity in vessel walls directly,<sup>51</sup> providing an alternative mechanism worthy of further investigation.

Much remains unknown about the mechanisms involved in the BPA CAD association in humans. Future scientific work in humans is, of course, constrained by ethical limits and the practicality of repeated BPA exposure measures, long term and larger follow-up studies. Without these constraints, controlled trials would be needed to prove causation in humans, but such evidence is almost certainly beyond reach.

## Conclusions

Associations between higher BPA exposure (reflected in higher urinary concentrations) and incident CAD during over ten years of follow-up in the EPIC-Norfolk study showed similar trends to previously reported cross-sectional findings in the more highly exposed NHANES 03/04 and 05/06 study respondents. More work is needed to accurately estimate the shape of the dose-response relationship. Work is also needed to identify the mechanism underlying the association between higher BPA exposure and incident coronary artery disease.

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**Conflict of Interest Disclosures:** None

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**Table 1.** Socio-demographic and risk factor characteristics of the cases (with incident coronary artery disease) and controls

	Controls	(sd)	Cases	(sd)	p value
Number	861		758		
Age (years)	63.8	(7.3)	64.1	(7.5)	0.412
Male sex	62.0%		66.1%		0.088
Educational attainment*					9.4*10 <sup>-4</sup>
No qualifications (<15 years of schooling)	38.6%		44.0%		
‘O’ levels or equivalent (15 yrs)	8.1%		7.7%		
‘A’ level or equivalent (17yrs)	40.2%		41.0%		
Post school or degree qualification	13.1%		7.3%		
Occupational social class					0.010
Professional	8.1%		5.2%		
Managerial	36.2%		34.6%		
Skilled non-manual	16.6%		15.9%		
Skilled manual	23.4%		22.6%		
Semi-skilled	11.9%		15.3%		
Non-skilled	3.3%		5.7%		
uncoded	0.5%		0.7%		
Smoking status					2.3*10 <sup>-8</sup>
current	9.6%		17.1%		
past	46.4%		51.5%		
Never	43.9%		31.5%		
Physical activity					0.0029
Inactive	31.2%		39.5%		
Moderately inactive	27.9%		25.7%		
Moderately active	22.4%		21.8%		
Active	18.5%		14.1%		
BMI kg/m <sup>2</sup>	26.2	(3.4)	27.2	(3.8)	2.5*10 <sup>-9</sup>
LDL-C, mmol/l (n=1532)	4.1	(1.0)	4.3	(1.0)	5.1*10 <sup>-6</sup>
HDL-C, mmol/l (n=1532)	1.4	(0.4)	1.3	(0.4)	1.5*10 <sup>-9</sup>
Total cholesterol (n=1595)	6.33	(1.19)	6.56	(1.13)	5.5*10 <sup>-5</sup>
Triglycerides (n=1594)	1.91	(1.32)	2.18	(1.13)	6.0*10 <sup>-6</sup>
Systolic blood pressure, mm Hg (n=1615)	137.5	(17.8)	143	(18.7)	2.6*10 <sup>-10</sup>
Urinary Bisphenol A concentration (ng/mL) (n=1619)					
Median	1.24		1.35		0.042 <sup>+</sup>
(25 <sup>th</sup> to 75 <sup>th</sup> percentile)	(0.59 to 2.52)		(0.67 to 2.70)		
Geometric mean	1.23	(2.95)	1.39	(3.02)	0.027

Notes: Data are presented as arithmetic mean (SD), or %

Means, percentages may be based on marginally fewer observations than the indicated number of subjects; \* one case had unknown educational status. <sup>+</sup> Mann-Whitney rank sum test

**Table 2:** Socio-demographic and coronary artery disease risk factor status by lower and higher urinary Bisphenol A concentration (dichotomised at uBPA=1.243ng/ml) in controls.

	Lower uBPA concentration ≤1.243ng/ml	(sd)	Higher uBPA concentration >1.243ng/ml	(sd)	Unadjusted p-value*
N	842		842		
Age (years)	63.8	(7.19)	63.8	(7.38)	0.91
Male sex	62.9%		62.8%		0.99
<b>Smoking status</b>					0.42
Never	8.1%		10.5%		
Past	48.5%		45.9%		
current	43.5%		33.8%		
<b>Education</b>					0.072
No qualifications (<15 years of schooling)	36.9%		42.0%		
'O' levels or equivalent (15 yrs)	6.7%		9.6%		
'A' level or equivalent (17yrs)	41.9%		37.0%		
Post school or degree qualification	15.6%		11.5%		
<b>Occupational Social class</b>					0.034
Professional	8.3%		7.8%		
Managerial	40.3%		32.1%		
Skilled non-manual	15.0%		17.8%		
Skilled manual	21.6%		25.2%		
Semi-skilled	10.0%		14.0%		
Non-skilled	4.6%		2.5%		
Uncoded	0.2%		0.7%		
<b>Physical activity</b>					0.49
Inactive	30.5%		34.6%		
Moderately inactive	29.2%		26.3%		
Moderately active	23.0%		20.9%		
Active	17.4%		18.3%		
<b>Body Mass Index categories (kg/m<sup>2</sup>)</b>					0.21
<18.4	0.43%		0.0%		
18.4 to 24.9	38.2%		33.7%		
25.0 to 29.9	47.0%		52.8%		
30.0 to 34.9	12.9%		11.3%		
>35	1.5%		2.2%		
LDL-Cholesterol, mmol/l	4.06	(1.04)	4.11	(0.97)	0.45
HDL-Cholesterol, mmol/l	1.36	(0.39)	1.36	(0.41)	0.93
Total cholesterol, mmol/l	6.31	(1.30)	6.28	(1.08)	0.78
Triglycerides, mmol/l	2.03	(1.68)	1.87	(1.02)	0.097
Systolic blood pressure, mm Hg	138.4	(16.6)	137.8	(17.6)	0.59

Note Data are presented as arithmetic mean (SD), or %

\* Unadjusted chi squared or t-test estimate



**Table 3.** Logistic regression estimates of odds ratios (95% CI) per standard deviation increase in uBPA concentrations (SD=4.56ng/ml) with incident coronary artery disease

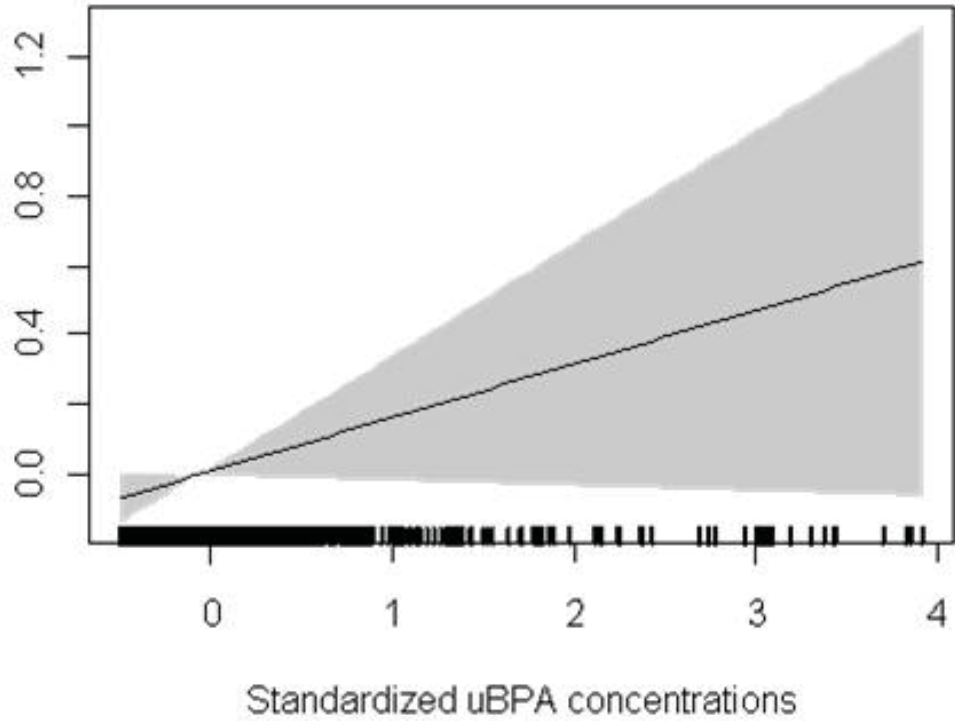
Model	model definition	OR	95% CI	p
<b>Hypothesis testing</b>				
A	Age, sex (n=1619)	1.13	1.02 to 1.24	0.018
B	age, sex, urinary creatinine (n=1619)	1.13	1.02 to 1.24	0.017
C	B plus education level and occupational group (n=1579)	1.14	1.03 to 1.26	0.012
D	C plus cardiovascular risk factors* (n=1477)	1.11	1.00 to 1.23	0.058
<b>Post-hoc sensitivity analyses</b>				
E	D excluding the earliest three years of follow-up (n=1350)	1.12	1.00 to 1.26	0.050
F	D excluding obesity (BMI>30, remaining n=1273)	1.14	1.01 to 1.29	0.040
G	D with additional adjustment for serum creatinine (excluding serum creatinine>120 nmol/l, n=963)	1.19	1.05 to 1.35	0.008
H	D with additional adjustment for serum vitamin C concentration (excluding <23 nmol/L, included n=1308)	1.17	1.03 to 1.33	0.017
I	D with additional adjustment for high sensitivity CRP concentration (n=1027)	1.16	1.03 to 1.30	0.017
J	D with additional adjustment for gamma-glutamyltransferase and alanine aminotransferase concentrations: (n=1055)	1.14	1.02 to 1.28	0.021
K	D with additional adjustment for alcohol intake at baseline (volume units) (n=1582)	1.13	1.01 to 1.25	0.027

See methods for coding of covariates. Numbers vary due to missing data on specific measures. \*adjusted as in C and with the additional variables: Body mass index, cigarette smoking, average of the two systolic BP readings in mmHg, total cholesterol, LDL and HDL cholesterol, triglycerides and level of physical activity.

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**Figure Legend:**

**Figure 1.** Dose response curves for the association of BPA concentration (per standard deviation increase (4.56 ng/ml) with logged odds of incident coronary artery disease. Generalized additive models with cubic regression splines in EPIC-Norfolk, for uBPA range to 4 standard deviations from mean.



## Urinary Bisphenol: A Concentration and Risk of Future Coronary Artery Disease in Apparently Healthy Men and Women

David Melzer, Nicholas J. Osborne, William E. Henley, Ricardo Cipelli, Anita Young, Cathryn Money, Paul McCormack, Robert Luben, Kay-Tee Khaw, Nicholas J. Wareham and Tamara S. Galloway

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