

# Daily diet containing canned products significantly increases serum concentrations of endocrine disruptor bisphenol A in young women

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**Introduction** Nowadays, exposure to environmental factors is considered to be one of the possible causes of several lifestyle diseases, such as obesity, type 2 diabetes, cardiovascular disease, and cancer. Particularly noteworthy are endocrine-disrupting chemicals (EDCs), which affect the metabolism of hormones and interact with their receptors, thus exerting adverse health effects. One of the most ubiquitous EDC in daily life is bisphenol A (BPA), an organic compound that, due to its phenolic structure, has an ability to interact with estrogen receptors and is a weak environmental estrogen. The affinity of BPA is lower than that of the endogenous 17 $\beta$ -estradiol; nevertheless, it has comparable estrogenic potency when mediated by nonnuclear estrogen receptors.<sup>1</sup> BPA is a precursor of polycarbonates used in everyday objects, such as food packaging, plastic bottles, toys, dental sealants and composites, thermal paper, and electronic and medical devices.<sup>2</sup> It is also a component of polyvinyl chloride and epoxy resins used as the inner layer of food cans, hence BPA is detected in a variety of canned products. Diet is the crucial source of human exposure to this EDC.<sup>3</sup> Its concentrations in alimentary products correspond with the duration of storage as well as the temperatures used during sterilization, pasteurization, or heating directly before consumption. Moreover, BPA may migrate to the content of a can as a consequence of mechanical factors such as denting and reshaping of cans.<sup>4</sup>

The presence of BPA has been shown in various human tissues and fluids, such as the adipose tissue, placenta, breast milk, urine, serum, and saliva.<sup>5</sup> A number of studies emphasized its potential role in the pathogenesis of several

endocrinopathies and fertility problems.<sup>6,7</sup> High serum BPA concentrations were also associated with obesity, type 2 diabetes, cardiovascular disease, and hormone-dependent neoplasms (ie, breast or prostate cancer).<sup>8</sup>

According to the European Food Safety Authority, high BPA exposure in women is 1.063  $\mu\text{g}/\text{kg}$  of body weight per day (bw/d) (0.388  $\mu\text{g}$  from dietary and 0.675  $\mu\text{g}$  from nondietary sources), whereas an average exposure is 0.216  $\mu\text{g}/\text{kg}$  of bw/d (0.132  $\mu\text{g}$  and 0.084  $\mu\text{g}$ , respectively). Only recently, the European Food Safety Authority has reduced the toxicological reference values and established a temporary tolerable daily intake of 4  $\mu\text{g}/\text{kg}$  of bw/d, which is far lower than the previous tolerable daily intake (50  $\mu\text{g}/\text{kg}$  of bw/d).<sup>9</sup>

The aim of this study was to evaluate serum BPA concentrations in young women after 7 days of dietary exposure to canned products that are a source of this EDC in daily life.

**Patients and methods** The study was approved by the Independent Bioethics Commission for Research of Medical University of Gdańsk (no. NKBBN/423/2014), and all participants signed a written informed consent form. A 7-day intervention study included 20 female volunteers (age, 22–25 years), of whom 19 were students of dietetics. They were nonsmokers, had regular menses, and did not take any medications. All participants filled a questionnaire to determine the potential coexposure to BPA from nondietary sources, such as occupational exposure through contact with carbonless copy paper, cans, and paints, having dental sealants and composites, and wearing contact lenses. Weight

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and body composition were measured on a Body Composition Analyzer BC-418 (Tanita Corporation, Tokyo, Japan). Then, the women were randomly assigned to 2 groups ( $n = 10$  in each group) and received a special 7-day meal plan that either included canned products (fish and fish products, meat and meat products, corn, beans, peas, tomatoes, fruits, and nonalcoholic beverages) or excluded those products (control group). The meal plan comprised products that contained different amount of BPA, and, according to the data from the literature,<sup>9</sup> the estimated intake of BPA through the consumption of canned foods was 1.28  $\mu\text{g}/\text{kg}$  of  $\text{bw}/\text{d}$  (range, 0.57–2.53  $\mu\text{g}/\text{kg}$  of  $\text{bw}/\text{d}$ ).

Venous blood samples for the analysis of BPA concentrations in serum were collected at baseline (the first day of dietary intervention), after 7 days of dietary intervention, and 7 days after the completed intervention. All analyses were conducted with precautions intended to minimize the risk of sample contamination. Blood was drawn into high-quality polyethylene terephthalate plastic tubes with a clot accelerator. Then, within a maximum of 30 minutes, the blood was centrifuged for 15 minutes (2500 rpm) and serum samples were collected into Eppendorf 1.5 ml tubes made of polypropylene, which is also devoid of BPA. Samples were then stored at  $-35^{\circ}\text{C}$  for further analyses.

Serum BPA concentrations were analyzed using high-performance liquid chromatography combined with tandem mass spectrometry at the Department of Analytical Chemistry at Gdańsk University of Technology. One milliliter of ultra-pure water, obtained with the use of an HLP5 system from Hydrolab (Wiślina, Poland), was added to 500  $\mu\text{l}$  of each serum sample together with the solution of 100  $\mu\text{l}$  of BPA-d16 in acetonitrile (20 ng/ml) and vortexed for 30 seconds. Then, 250 mg of anhydrous magnesium sulfate ( $\text{MgSO}_4$ ) was added into the solution, vortexed, and centrifuged (6000 rpm) for 2 minutes. Supernatants were collected and transferred to the glass test tubes, further placed in a  $42^{\circ}\text{C}$  water bath in order to evaporate, under a stream of nitrogen, to approximately 150  $\mu\text{l}$ . The residue was analyzed after mixing with 250  $\mu\text{l}$  of water and placing in a sample vial. The standards of BPA ( $\geq 99\%$ ) and the BPA-d16 (98 atom% D) were purchased from Sigma-Aldrich (Deisenhofen, Germany), acetonitrile from Merck (Darmstadt, Germany), and  $\text{MgSO}_4$  from Eurochem BGD (Tarnów, Poland). The high-performance liquid chromatography system (Shimadzu, Japan), consisting of a degasser, binary pump, autosampler, and a column oven, was used for the chromatographic separation. The analytes were separated on a LiChrospher C18 column (Merck, 250 mm  $\times$  4 mm, 5  $\mu\text{m}$ ). All analyses were performed using a Shimadzu LCMS-8050 triple quadrupole mass spectrometer (Shimadzu, Japan) equipped with an electrospray ionization source working in the negative multiple reaction monitoring mode. The limit

of detection (LOD) was 0.25 ng/ml. Concentrations below the LOD (defined as 30% of the limit of quantification) were discarded.

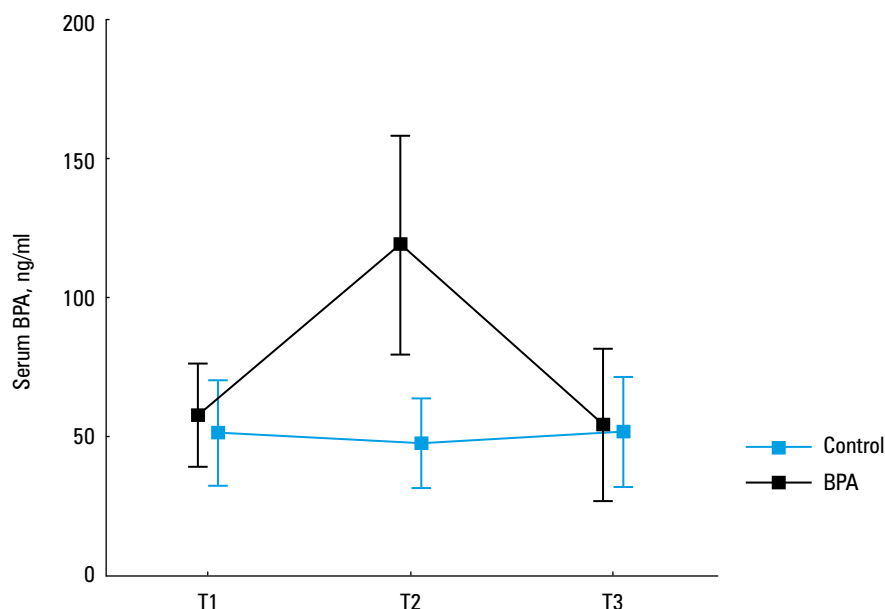
All statistical analyses were performed using the Prism 5.0 software for Mac OS X (GraphPad Software, San Diego, California, United States). The D'Agostino–Pearson test was used to determine the distribution of the measured variables. Before the analysis, variables that were not normally distributed were log transformed. Differences at baseline between the 2 study groups were compared using an unpaired  $t$  test or its nonparametric equivalent, the Mann–Whitney test. Differences in serum BPA concentrations during the intervention study were compared using a paired  $t$  test or its nonparametric equivalent, the Wilcoxon rank sum test. Due to multiple comparisons (6 in total), a Bonferroni correction was applied; therefore, differences were considered statistically significant with a  $P$  value of less than 0.008.

**Results** All participants were at a similar age (mean [SD] age, 23.9 [0.99] years for women consuming canned products vs 23.5 [1.08] years in the control group,  $P = 0.4$ ), had comparable body mass index (mean [SD] body mass index, 21.6 [1.27]  $\text{kg}/\text{m}^2$  vs 21.6 [1.65]  $\text{kg}/\text{m}^2$ ; range, 19.6–24.3  $\text{kg}/\text{m}^2$ ,  $P = 0.9$ ), and the percentage of total body fat (mean [SD] total body fat, 23.7% [4.1%] vs 25.9% [4.2%]; range, 19.2%–31.9%,  $P = 0.2$ ).

Baseline serum BPA concentrations were quantified in 18 of the 20 studied samples (90%); in 2 samples, BPA was below LOD and such results were treated as 0. The mean (SD) BPA concentration did not differ between the 2 groups; 58.3 (18.8) ng/ml compared to 51.7 (19.3) ng/ml; range, 0–185.5 ng/ml ( $P = 0.3$ ). After 7 days of dietary intervention, mean (SD) serum BPA concentrations increased significantly in women consuming canned products to 120.0 (39.9) ng/ml (range, 30–441 ng/ml;  $P = 0.0008$ ), whereas no change was observed in the control group, where the mean (SD) serum concentration of BPA was 47.9 (16.2) ng/ml (range, 0–98.55 ng/ml;  $P = 0.3$ ). Serum BPA concentrations in both groups were analyzed 7 days after completing the intervention study, when each woman was on her regular diet. In the group previously consuming canned products, the mean (SD) BPA concentration was 54.7 (27.9) ng/ml (range, 4.31–217 ng/ml). The decrease of the serum BPA concentration in comparison with the concentration after 7 days of dietary intervention was significant ( $P = 0.03$ ). In the control group, 7 days after completing the intervention study, the mean (SD) serum BPA concentration was 52.2 (20.2) ng/ml (range, 0–167 ng/ml;  $P = 0.4$ ). All results are shown in **FIGURE 1**.

Before dietary intervention, BPA was detected in the majority of the serum samples. This finding was consistent with the results of the study by Calafat et al<sup>10</sup> and suggested wide exposure to BPA in everyday life. Data from our 7-day

**FIGURE 1** Serum bisphenol A (BPA) concentrations during the dietary intervention study. T1, first day of dietary intervention; T2, after 7 days of dietary intervention; T3, 7 days after the intervention; BPA, group consuming products with BPA content; control, control group



intervention study showed that the consumption of canned products significantly increases serum BPA concentrations. It is worth noting that during the intervention none of the women had dental sealants placed, nor did they change their lifestyle in a way that could have been additional source of BPA exposure. Our results, supported also by other studies,<sup>11,12</sup> are alarming, especially considering the fact that BPA is an environmental estrogen, and it is likely that constant exposure even to low doses may disrupt the action of endogenous hormones.

**Conclusions** Dietary exposure to BPA from canned foods increases its serum concentrations in young women. In view of the numerous reports on the correlation between BPA concentrations in body fluids and the pathogenesis of lifestyle diseases, such as obesity, type 2 diabetes, and cardiovascular disease, it is crucial to conduct further studies, minimize human exposure to BPA and other EDCs, and reconsider toxicological reference values for this compound.

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