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Bisphenol A is related to circulating levels of adiponectin, leptin and ghrelin, but not to fat mass or fat distribution in humans

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HIGHLIGHTS

- Human serum levels of BPA did not relate to fat mass or fat distribution.
- Serum levels of BPA were associated with levels of adiponectin, leptin and ghrelin.
- BPA may interfere with hormonal control of hunger and satiety.

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ABSTRACT

Objective: Since bisphenol A (BPA) has been shown to induce obesity in experimental studies, we explored the associations between BPA and fat mass, fat distribution and circulating levels of adiponectin, leptin and ghrelin in humans.
Methods: In the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS), fat mass and fat distribution were determined in 70-year-old men and women ($n = 890$) by dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) ($n = 287$). Serum levels of BPA were analyzed using isotope liquid chromatography/tandem mass spectrometer (API4000LC–MS/MS). Hormone levels were analyzed with radioimmunoassays (RIA) or enzyme-linked immunosorbent assay (ELISA). Imaging was performed approximately two years following collection of other data.
Results: Serum concentrations of BPA were not related to adipose tissue measurements by DXA or MRI. BPA associated positively with adiponectin and leptin, but negatively with ghrelin, following adjustments for sex, height, fat mass, lean mass, smoking, alcohol consumption, physical activity, energy intake, and educational levels ($p < 0.001$, $p = 0.009$, $p < 0.001$, respectively). The relationship between BPA and ghrelin was stronger in women than in men.
Conclusion: Although no relationships between BPA levels and measures of fat mass were seen, BPA associated strongly with the adipokines adiponectin and leptin and with the gut-hormone ghrelin suggesting that BPA may interfere with hormonal control of hunger and satiety.

1. Introduction

Obesity is a major health concern in the Western world and is also increasing in developing countries. Obesity is most often blamed to be due to excess caloric intake in relation to a reduced physical activity (Sallis and Glanz, 2009; Westerterp, 2010; Mitchell et al., 2011). Although an imbalance between energy intake and output clearly is a determinant of body weight, other factors might also be of importance. During the last decade the hypothesis that some anthropogenic chemicals act as obesogens promoting fat accumulation has been put forward (Baillie-Hamilton, 2002; Grün and Blumberg, 2009). This hypothesis deserves further attention since obesity is considered a global issue of epidemic proportions. Regulation of hunger and satiety is complex and influenced

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by hormones (Perry and Wang, 2012; Yu and Kim, 2012), thus there are especial concerns regarding chemicals that may act as endocrine disrupters.

The estrogenic properties of bisphenol A (BPA) has been known since 1936 (Dodds, 1936), but recognized as an environmental endocrine disrupting compound (EDC), a xenoestrogen, in the late 1990s. Endocrine effects of BPA has been observed in both epidemiological and experimental studies (Alonso-Magdalena et al., 2012; Teppala et al., 2012; vom Saal et al., 2012), but yet without any solid proof of deleterious effects, and therefore more studies are required. BPA can act by the classical nuclear estrogen receptor α (ERα) and estrogen receptor β (ERβ), but the estrogenicity has been regarded as weak in relation to endogenous estrogen, and thus negligible. However, other mechanisms of the estrogen than the classic nuclear estrogen receptor α (ERα) and estrogen receptor β (ERβ) suggest that there are routes where BPA can act as a rather potent estrogen, e.g. via membrane-bound estrogen receptors, mERα, mERβ and G protein-coupled receptor 30 (GPR30) (Watson et al., 2007).

BPA is a high-volume chemical in the plastics industry. It is also found in cash receipts, can resins and sealants, etc. Most people worldwide have detectable levels of BPA in their blood or urine indicating a ubiquitous exposure (Vandenberg et al., 2007; Olsen et al., 2012). Furthermore, BPA has been shown to induce obesity in rodents in experimental settings by different groups (Miyawaki et al., 2007; Somm et al., 2009), and in humans. Lang et al. and Shankar et al. found urinary BPA to be related to obesity measured by BMI in the NHANES study performed in adults (Lang et al., 2008; Shankar et al., 2012). Similar findings were reported in a Chinese population (Wang et al., 2012b). In both American and Chinese school-children, urinary BPA levels were associated with BMI (Trasande et al., 2012; Wang et al., 2012a; Bhandari et al., 2013; Harley et al., 2013; Li et al., 2013). Valvi and co-workers reported that urinary BPA in pregnant women were related to BMI in their offspring at 4 years of age (Valvi et al., 2013). Similar results were found in another mother/child cohort at age 9 years, but in this case the association was only found in girls (Harley et al., 2013).

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (Lind et al., 2005) is unique in its kind since not only BMI is determined, but both fat mass and fat distribution have been measured using both dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI). In addition, some of the major hormones related to hunger and satiety, such as ghrelin, leptin and adoponectin have been measured together with major potential confounding variables, such as energy intake, alcohol intake, exercise habits and education level. We therefore used the PIVUS study to evaluate the relationships between circulating BPA levels and different indices of obesity, as well as hormones related to hunger and satiety.

2. Materials and methods

2.1. Study population

Eligible for the study were all men and women aged 70 living in the community of Uppsala, Sweden from the year 2001 to 2004. Using the Swedish population register, 2025 individuals were randomly chosen and invited in the way that they would perform the examination 1–2 months following their 70th birthday. The total background population turning 70 years in the years between 2001 and 2004 were approximately 7000 individuals. A total of 1016 participated, yielding a participation rate of 50.1% (51.5% women). They were all examined in the morning after an overnight fast, and no medication or smoking was allowed after midnight. Venous blood samples were stored at −70 °C until analysis. Fat mass and fat distribution were determined by dual-energy X-ray absorptiometry (DXA) in 890 individuals, and by magnetic resonance imaging (MRI) in 287 individuals.

The study was approved by the Ethics Committee of the University of Uppsala, and all the participants gave their informed consent before the study. As the participation rate in this cohort was only 50%, we carried out an evaluation of cardiovascular disorders and medications in 100 consecutive non-participants. The prevalence of cardiovascular drug intake, history of myocardial infarction, coronary revascularization, antihypertensive medication, statin use and insulin treatment were similar to those in the investigated sample, while the prevalence of diabetes, congestive heart failure and stroke tended to be higher among the non-participants The study design and the status in non-participants have been described in detail in Lind et al. (2005).

2.2. Baseline examination including questionnaire

The participants were asked to answer a questionnaire about their medical history, educational level, exercise, and smoking habits. Educational level was categorized into three groups: <9 years, 9–12 years and >12 years of education. The individuals were asked two questions: "How many times a week do you engage in light exercise for at least 30 min (activities like walking, cycling, golf, etc. usually not causing sweating)?" and "How many times a week do you engage in heavy exercise for at least 30 min (activities like running, swinging, football, etc. generally causing sweating)?" Based on these questions exercise habits were categorized into four groups: <2 times light exercise (no sweat) per week, ⩾2 times light exercise per week, 1–2 times heavy exercise (sweat) per week, >2 times heavy exercise (sweat) per week (Table 2). The dietary intakes were assessed by a 7-d food diary in which the participants were asked to indicate the intake meal by meal in a list of different predefined food and drink items, and also to write down eating between meals. These data were then coded into food items and processed by a custom-made software program (Mats^{R}, Västerås, Sweden) to obtain daily caloric and alcohol intake.

2.3. BPA analysis

The serum levels of BPA taken at baseline examination were analyzed at ALS Environmental Canada. Serum samples (0.5 ml) were analyzed for levels of BPA (sum of both free and the conjugated forms) using isotope liquid chromatography/tandem mass spectrometer (API 4000LC–MS/MS), as previously described in detail (Olsen et al., 2012). Quality control of the analysis was maintained by analyzing a method blank (calf serum) and two spiked calf serum samples (20 ng mL^{-1}, all analytes) along with every 17 samples. The detection limit (LOD) was 0.2 ng mL^{-1} based upon a lower calibration standard (0.5 ng mL^{-1}) which gave an instrument signal to noise response of 3:1. BPA in serum was detectable in 98 percent of both females and males. As previously published, the median serum concentration of BPA was 2.1 ng mL^{-1} (range of <LOD to 24 ng mL^{-1}) in females, and 3.9 ng mL^{-1} (range of <LOD to 27.3 ng mL^{-1}) in males.

2.4. Adipose tissue quantification by DXA and MRI

On average two years following the baseline investigation all participants in the sample were offered an examination with dual-energy X-ray absorptiometry (DXA), by which fat mass and lean mass in different body compartments were estimated (DPX, Lunar Prodigy, Lunar Corp., Madison, WI, USA). 890 (87%) individuals participated. The analyses included measures of trunk fat mass, defined as fat between the groins and the shoulders, and leg fat mass, defined as fat between the groins and the toes, and ra-

tios thereof. These calculations in fat depots were performed by software provided by the manufacturer of the hardware. The rational for subdivisions of these fat depots is that centrally deposited fat has been shown to relate more closely to cardiovascular disease than peripherally located fat.

By triple measurements in 15 subjects, the precision error of the DXA measurements was calculated to be 1.5% for total fat mass and 1.0% for total lean mass (Ingelsson et al., 2009). The bias associated with DXA fat measurement is systematic, with an underestimation of fat content for lean individuals and an overestimation of fat content among obese individuals, but these inaccuracies account for less than 2% of the variation (Wang et al., 2010).

In parallel with the DXA examination, MRI of the abdominal region was performed in 287 individuals invited in a random manner from the PIVUS cohort. For economic reasons the MRI investigation could not be offered to everybody. The majority of those investigated by MRI did also perform the DXA scanning ($n = 276$). Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) areas (cm^2) were manually segmented from a single 10 mm thick axial steady-state-free precession slice as previously described (Kullberg et al., 2007). Based on repeated measurements of VAT and SAT in 22 subjects, the reproducibility/coefficients of variation were found to be 5.9% and 3.4%, respectively. Imaging was performed on a 1.5 T MRI system (Gyroscan Intera, Philips Medical Systems, Best, the Netherlands), using the standard quadrature body coil. The rational for subdivisions of these fat depots is that visceral fat has been shown to relate more closely to cardio-metabolic disease, such as diabetes, disease than subcutaneously located fat. The DXA measurements of trunk fat cannot differentiate between VAT and SAT.

2.5. Adiponectin, leptin and ghrelin analyses

Adiponectin and leptin in plasma were analyzed at baseline with double-antibody radioimmunoassays (RIA) (Linco Research, St. Louis, MO, USA). Total coefficient of variation (CV) for leptin was 4.7% at both low (2–4 ng mL^{-1}) and high (10–15 ng mL^{-1}) levels, and for adiponectin the total CV was 15.2% at low (2–4 μg mL^{-1}) and 8.8% at high (26–54 μg mL^{-1}) levels.

Serum ghrelin was analyzed at baseline with an enzyme-linked immunosorbent assay (ELISA) (Ghrelin EIA kit Ek-031-30, Phoenix Pharmaceuticals, Burlingame, CA, USA). The total CV for the assay was 5%.

2.6. Statistical analyses

All variables were evaluated regarding non-normality and variables with a skewed distribution, such as BPA and the hormone levels, were ln-transformed. Since men and women normally have different distributions for leptin and adiponectin, with higher levels in women, the levels for those adipocytokines were transformed to the SD scale in men and women separately. This scale is also called *Z*-score, and expresses the distance from the mean value in terms of one standard deviation.

In linear regression models, BPA was related to indices of fat mass as well as to the three hormones evaluated.

To screen for non-linear relationships, the quadratic term of BPA was added to the regression models.

Three models were tested. The primary model was adjusted for sex only. In the second model, adjustments were performed for sex, height, and lean mass by DXA. In the third model, additional adjustment was performed for smoking, exercise habits, educational level, total daily energy intake, and alcohol consumption. Smoking, educational level, energy intake and alcohol consumption have all previously been linked to exposure to environmental contaminants and exercise habits are closely linked to obesity. Since data on dietary intake was missing in 155 individuals, missing data on total daily energy intake, and alcohol consumption were imputed by using a multiple imputations command (mi) generating 20 new observations for each missing value. Imputation was performed in order not to lose 155 individuals in the third model.

For all outcomes, interactions between the BPA level and sex were evaluated in the first model. If there was an improved fit of the model with an interaction term included ($p < 0.05$), separate sex specific analyses were performed.

In the present study BPA levels were related to 10 different adipose-tissue-related outcomes. Applying a strict Bonferroni adjustment will result in a critical p-value of 0.005.

STATA 12 (College Station, TX, USA) was used for calculations.

3. Results

Basic characteristics for height, weight, fat mass, and fat distribution are given in Table 1. Distributions of educational level, physical activity, smoking habits, energy intake, and alcohol consumption are presented in Table 2.

3.1. BPA vs. fat mass and fat distribution

No significant relationships between BPA levels and indices of fat mass or fat distribution were found at any level of adjustment (Table 3).

The interaction between BPA concentration and sex was not significant for any of the fat measures studied. Neither did we found any significant relationship between BPA and any of the indices of obesity evaluated when we stratified the analysis according to sex.

3.2. BPA vs. adiponectin and leptin levels

After adjustment for sex, serum concentrations of BPA were positively associated with adiponectin ($p < 0.001$) and leptin ($p = 0.018$) levels.

These results remained significant ($p < 0.001$ and $p = 0.009$, respectively) also in the fully adjusted models. The results for adiponectin and leptin levels were also independent of adjustment for fat mass ($p = 0.0001$ for both). Introducing a quadratic term suggested a non-linear relationship for BPA vs. adiponectin ($p < 0.001$), but not vs. leptin. The effect of BPA regarding adiponectin was seen even at rather low levels of circulating BPA.

No significant interactions were seen between BPA and sex regarding the relationships vs. adiponectin or leptin. Neither did a sex-stratified analysis reveal any major differences between men and women regarding the strength or the shape of the relationships ($p < 0.0001$ for both men and women regarding adiponectin with almost identical beta-coefficients. For leptin the beta-coefficient was similar in men and women, 0.076 and 0.087, respectively, but due to the lower power in the sex-stratified analysis the p-values did not reach significance, $p = 0.13$ and $p = 0.06$ respectively).

3.3. BPA vs. ghrelin level

Serum concentrations of BPA adjusted for sex were negatively related to serum concentrations of ghrelin also in the fully adjusted models ($p < 0.001$). Introducing a quadratic term for BPA in the models did not reveal any significant non-linear effects. The results were independent of adjustment for fat mass.

The interaction term between BPA and sex was significant for ghrelin ($p = 0.04$), revealing a stronger relationship between BPA and ghrelin levels for women, with a regression coefficient about

Table 1
Basic characteristics in males and females. Mean and standard deviation (SD) are given together with minimum (Min) and maximum (Max) values. Median is given together with the inter quartile rate (IQR) for bisphenol A and the hormones.

Variables	Males				Females			
	Mean	SD	Min–Max	*N*	Mean	SD	Min–Max	*N*
Total fat mass (kg)	23.5	8.2	3.2–51.8	431	27.7	9.2	3.4–55.8	459
Fat leg (kg)	6.3	2.6	1.6–25.1	431	9.7	3.4	1.3–22.9	459
Fat trunk (kg)	14.2	5.1	1.1–30.1	431	13.9	5.0	1.6–28.4	459
VAT[a] (cm^3)	119	63	11.3–368	148	94	49	7.1–268	139
SAT[b] (cm^3)	189	80.3	46–463	148	262	109	52–727	139
Height (cm)	176	6.4	155–198	431	162	5.7	148–184	459
Weight (kg)	83.8	12.6	53–132	431	70.7	12.4	42–115	459
BMI[c] ($kg\ m^{-2}$)	27.1	3.6	17.7–43.4	431	26.9	4.6	16.6–49.8	459
Energy ($kcal\ d^{-1}$)	2056	531	913–4116	431	1724	416	711–3363	430
Alcohol ($g\ d^{-1}$)	8.9	9.1	0–61	431	4.4	5.0	0–32	430
	Median		IQR	*N*	Median		IQR	*N*
Bisphenol A ($ng\ mL^{-1}$)	3.9		2.08–6.57	502[d]	2.1		1.97–6.51	501[e]
Adiponectin ($\mu g\ mL^{-1}$)	4.8		3.0–7.2	429	8.2		5.0–12.0	454
Leptin ($ng\ mL^{-1}$)	6.4		4.1–9.8	429	17.0		11.0–25.1	454
Ghrelin ($ng\ mL^{-1}$)	5.6		4.3–7.2	407	6.1		4.5–7.7	432

[a] Visceral adipose tissue.
[b] Subcutaneous adipose tissue.
[c] Body mass index.
[d] 6 < Level of detection.
[e] 6 < Level of detection.

Table 2
Distribution of educational levels, physical activity and smoking habits given as percentage of participants.

	Males ($n = 427$)	Females ($n = 453$)
Sex	48.5	51.5
Education: 1–9 years	54.8	57.6
Education: 9–12 years	18.5	18.3
Education: university level	26.7	24.1
Physical activity: very light[a]	13	10
Physical activity: light[b]	56	60
Physical activity: medium[c]	22	25
Physical activity: hard[d]	9	5
Current smokers	9.5	10.7

[a] Light exercise, no sweat <2 times $week^{-1}$.
[b] Light exercise, no sweat $\geqslant$2 times $week^{-1}$.
[c] Hard exercise, sweat 1–2 times $week^{-1}$.
[d] Hard exercise, sweat >2 times $week^{-1}$.

twice as high as for men (-0.12, $p = 0.002$ vs. -0.06, $p < 0.001$). The relationships between BPA and ghrelin levels were very similar in individuals with a fat mass above the median compared to those below the median (linear regression coefficient -0.09, $p < 0.001$ for both strata).

4. Discussion

4.1. BPA and obesity

Numerous studies, both experimental and epidemiological, have shown an association between BPA and obesity and other metabolic effects, such as insulin resistance and dyslipidemia (Miyawaki et al., 2007; Wei et al., 2011; Trasande et al., 2012; vom Saal et al., 2012; Wang et al., 2012b; Bhandari et al., 2013) In the present study, we could not detect any relationship between BPA and obesity indices despite the fact that we used both DXA and MRI to characterize fat mass. However, obesity is a multifactorial disease (Speakman and O'Rahilly, 2012) and weight gain often occur at a rather slow pace. These features regarding etiology and time-course of obesity development will make it difficult to establish a relationship between BPA in serum measured at one time point and fat volume in this elderly cohort, mainly exposed as adults. Hormone levels on the other hand generally respond relatively fast to changes in the environment and therefore a relationship between hormone levels and BPA in the circulation measured at the same time point is more likely to be revealed than a potential relationship between BPA levels and fat mass.

4.2. BPA as an endocrine disruptor

BPA was developed as a synthetic estrogen, a xenoestrogen (Dodds, 1936). BPA initiates rapid responses through membrane-bound estrogen receptors with about the same potency as 17β-estradiol (E2) (Welshons et al., 2006; Alonso-Magdalena et al., 2012), but the affinity to the classical nuclear ERα and ERβ receptors is much lower than that of the endogenous hormone.

In women decreased levels of estrogen after menopause increase the incidence of obesity and risk for the metabolic syndrome (Carr, 2003). In female rats ovariectomy (OVX) leads to increased food intake and weight gain. These effects are counteracted by E2 supplementation in therapeutic doses ($20\ \mu g\ kg^{-1}\ d^{-1}$) (Kafkas et al., 2012), demonstrating the importance of estrogen in the control of energy balance and fat accumulation.

Interactions between estrogens and adipokines and ghrelin have been described. Estradiol reduces the orexigenic potency of ghrelin as demonstrated in male rats and OVX female rats given exogenous ghrelin, resulting in increased food intake, which is reversed by E2 treatment. (Clegg et al., 2007). Leptin sensitivity in the brain is influenced by estrogen, and estradiol restores the central leptin sensitivity in OVX female rats. Such treatment also results in increases in subcutaneous fat deposition (Clegg et al., 2006).

The thyroid system is another hormonal pathway influencing metabolism that might be affected by BPA. An inverse association between urinary BPA and TSH and positive association with free T3, i.e. an increase in thyroid function, were shown in a cross-sectional study by Wang et al. (2013), but other studies have shown the opposite effect (Sriphrapradang et al., 2013). If BPA increases thyroid function this could possibly mask an obesogenic effect of BPA through an increased metabolic rate.

Table 3
Relationships between bisphenol A (BPA) concentration in serum and fat mass and hormone levels related to fat mass, hunger and satiety. Relationships are given for BPA as a continuous variable (regression coefficient using ln-transformed values).

Variable	N	Model 1: gender adjusted		Model 2: body stature adjusted		Model 3: multiple adjusted	
		Beta (95% CI)	p-Value[a]	Beta (95% CI)	p-Value[b]	Beta (95% CI)	p-Value[c]
Total fat	845	−113 (−744; 518)	0.73	−188 (−759; 383)	0.52	−130 (−703; 442)	0.66
Fat leg	845	−33.0 (−253; 187)	0.77	−54.2 (−267; 158)	0.62	−35.0 (−251; 181)	0.75
Fat trunk	845	−54.0 (−417; 309)	0.77	−96.0 (−422; 230)	0.56	−65.5 (−390; 259)	0.69
Fat trunk/leg ratio	845	−0.001 (−0.038; 0.035)	0.94	−0.002 (−0.037; 0.034)	0.93	−0.0003 (−0.037; 0.036)	0.99
SAT[d]	268	2.23 (−9.50; 14.0)	0.71	0.248 (−11.0; 11.5)	0.97	1.17 (−9.99; 12.3)	0.84
VAT[e]	268	0.756 (−6.18; 7.70)	0.83	−0.766 (−7.26; 5.73)	0.82	−0.279 (−6.70; 6.14)	0.93
VAT/SAT ratio	268	−0.017 (−0.044; 0.01)	0.22	−0.017 (−0.045; 0.01)	0.22	−0.0157 (−0.044; 0.012)	0.27
Adiponectin[f]	844	0.169 (0.104; 0.235)	<0.001	0.167 (0.096; 0.238)	<0.001	0.164 (0.093; 0.236)	<0.001
Leptin[g]	844	0.08 (0.014; 0.146)	0.018	0.09 (0.021; 0.159)	0.010	0.0918 (0.023; 0.160)	0.009
Ghrelin[h]	834	−0.09 ((−0.12; −0.064)	<0.001	−0.096 (−0.127; −0.065)	<0.001	−0.10 (−0.13; −0.07)	<0.001

[a] *p*-Value adjusted for sex.
[b] *p*-Value adjusted for sex, height and lean mass.
[c] *p*-Value adjusted for sex, height, lean mass, smoking, exercise habits, educational level, total daily energy intake, and alcohol consumption.
[d] Subcutaneous adipose tissue.
[e] Visceral adipose tissue.
[f] ln transformed adiponectin, SD scale.
[g] ln transformed leptin, SD scale.
[h] ln transformed ghrelin.

4.3. BPA and obesity-related hormones

The hormones adiponectin, leptin and ghrelin are known to be involved in the regulation of hunger and satiety (Klok et al., 2007; Swarbrick and Havel, 2008). Leptin and adiponectin regulate the energy intake in the long term and ghrelin also in the short term (Cummings, 2006). Circulating levels of leptin and adiponectin are generally higher in women, and sex steroids including estrogens are partly responsible for these differences (Sowers et al., 2008).

In the present study leptin levels were positively associated with the BPA concentration in serum, independent of adjustment for fat mass. This is consistent with results in an experimental study by Wei et al. in which leptin levels were increased in perinatal BPA-treated offspring (Wei et al., 2011). On the other hand, in a study by Anderson et al. serum leptin levels in mice were lower in the female offspring of dams exposed to BPA than in offspring of control dams (Anderson et al., 2013).

Adiponectin is also secreted from adipose tissue and, in contrast to leptin, inversely related to fat mass. In the present study adiponectin levels were positively associated with BPA in serum. The levels of adiponectin in the previously mentioned study by Anderson et al. were increased in BPA-exposed female offspring. In the male offspring, adiponectin levels were lower compared with the controls, indicating a sex-related difference. Another study showed inhibited adiponectin release from human adipose tissue explants in response to BPA exposure (Hugo et al., 2008).

Ghrelin is secreted from the gut and considered a hunger hormone with higher levels during fasting and lower levels after feeding. In the present study, the ghrelin level was negatively associated with serum concentrations of BPA. In an experimental study by Nieminen et al. where field voles (*Microtus agrestis*) were exposed subcutaneously to BPA, the opposite results were seen: increased ghrelin levels and decreased leptin levels (Nieminen et al., 2002). In an experimental study by Ferguson et al. no differences in ghrelin serum concentrations were seen after perinatal BPA exposure of Sprague–Dawley rats (Ferguson et al., 2011).

Thus, our data regarding the associations between BPA levels and obesity-related hormones are in line with some of these experimental results, but not with others.

4.4. Measurements of BPA levels

The serum levels of BPA in the present study were comparable with the levels in studies by others, as reviewed by Olsen et al. (2012). Both free BPA and its metabolites, mainly its glucorinated form, were included in the measured concentration. Although it is free BPA that has a biological activity, the measurement of both free BPA and its metabolites yields a more reliable measure of exposure, since free BPA is rapidly glucoronidated. However, it is more common to measure BPA in the urine. This approach has the advantage of obtaining higher levels, which are easier to detect, and the problem of contamination of BPA in the laboratory is avoided since it is mainly the glucorinated form of BPA being detected in the urine. However, several groups have shown that BPA is also measurable in the circulation (Takeuchi et al., 2004; Dirtu et al., 2008; Lee et al., 2008), and own previous data using this approach have shown serum BPA levels to be related to lipid infiltration in the vascular wall as well as being linked to high cholesterol levels (Lind and Lind, 2011; Olsén et al., 2012). Furthermore, the PIVUS study has shown associations between serum BPA levels and sex hormones and also thyroid hormones (yet unpublished data). Thus it is likely that the determinations of BPA are valid. If laboratory contamination or analytical problems had been encountered, no such relationships are likely to have been discovered. Additionally, data from contaminated samples will always drive associations, as investigated in the present study, towards the null hypothesis. If the data are highly corrupt, true associations would most likely be analyzed as being non-significant, while only lightly corrupt data could still show a significant finding. Thus, if the BPA measurements used in the present study would be inaccurate, this could only induce less significant results and not produce any false positive associations.

Contamination is a problem regarding BPA measurement. We used blanks in the measurement process to check for this, and in the present study contamination is not a general problem since there are 12 individuals with levels below the LOD, being around 10% of the mean value in the sample. Thus, if a general contamination had occurred it would influence the measurements by less than 10% compared to the mean value and therefore would not have any major impact on the statistical analysis performed between BPA level and different markers of obesity.

BPA has a rather short half-life and it would therefore have been desirable to have repeated measurements of this compound in the assessment of BPA exposure, since such approach probably have given a more stable measure of exposure. However, as in the case of contamination, less precise estimations of exposure could only drive statistical analysis towards the null hypothesis and could never induce the highly significant relationships we report between BPA and adiponectin and ghrelin.

4.5. Limitations

This study was performed on a cohort of Caucasian elderly individuals and generalizing the findings to other ethnic- and age-groups should be done with caution.

This sample of elderly individuals is not generally very overweight or obese, which should be remembered in terms of generalizability.

4.6. Conclusion

The present study shows that BPA concentrations in serum are associated with levels of key hormones involved in hunger and satiety, but not with total or regional body fat measures by DXA or MRI. Thus, the present study adds further data suggesting an endocrine-disrupting action of BPA in humans, although possible health consequences of our findings are not obvious at present.

Disclosures

None.

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