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The effects of bisphenol A and bisphenol S on adipokine expression and glucose metabolism in human adipose tissue

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ABSTRACT

Purpose: The environmental endocrine disruptors, bisphenol A (BPA) and bisphenol S (BPS) are associated with the development of type 2 diabetes. We aim to study the effects of BPA or BPS exposure on adipokine expression in human adipose tissue and on adipocyte glucose uptake.

Methods: Human subcutaneous adipose tissue was treated for 24 or 72 h with environmentally-relevant and supraphysiological concentrations of BPA or BPS (1–10^4 nM). Following exposure, gene expression of proinflammatory cytokines, adipokines, and estrogen receptors was measured in adipose tissue. Glucose uptake and the insulin signalling pathway were analyzed in isolated adipocytes following adipose tissue culture with BPA for 24 h.

Results: Adipose tissue treated with BPA for 24 h had reduced expression of the proinflammatory genes (*IL6*, *IL1B, TNFA)* and adipokines (*ADIPOQ, FABP4*). BPA and BPS had no effect on the expression of other proinflammatory genes (*IL33*), adipokines (*LEP*), or receptors (*ESR1, ESR2*) after 72-h exposure. Adipose tissue treated with environmentally-relevant concentrations of BPA for 24 h had reduced insulin-stimulated glucose uptake, without altered gene and protein levels of key insulin signalling pathway markers.

Conclusions: We found that human adipose tissue treated with environmentally-relevant concentrations of BPA for 24 h, but not BPS, reduced expression of proinflammatory genes and adipokines. Furthermore, BPA reduced glucose uptake in adipocytes independently of insulin signalling. Such mechanisms can contribute to the development of insulin resistance associated with BPA exposure.

1. Introduction

Over the last few decades, the global prevalence of type 2 diabetes (T2D) has been steadily increasing. In addition to traditional risk factors (genetics, diet, sedentary), recent research has been focused on the role of environmental pollutants on the development of T2D (Jeon et al. 2015). Bisphenol A (BPA) is an endocrine-disrupting chemical (EDC) used in the production of polycarbonate products such as plastic bottles, food containers, and thermal receipts (Le et al. 2008). BPA is structurally similar to 17β-estradiol (E2) and has been shown to have hormone-mimicking properties (Delfosse et al. 2012).

In epidemiological studies BPA exposure has been linked to many adverse health outcomes in humans including the increased risk of T2D (Lang et al. 2008; Shankar and Teppala 2011; Wang et al. 2012) and obesity (Bhandari et al. 2013; Carwile and Michels 2011; Wang et al. 2012). In humans, BPA levels have been shown to range from low picomolar (Karrer et al. 2018) to low nanomolar concentrations (Welshons et al. 2006). In experimental studies, BPA exposure increases the differentiation of human preadipocytes into adipocytes and the expression of genes related to adipogenesis and lipid metabolism

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(Boucher et al. 2014; Boucher et al. 2016b; Chamorro-Garcia et al. 2012; Wang et al. 2013). Furthermore, BPA has been shown to interact with several nuclear receptors, such as estrogen receptors and glucocorticoid receptor (Acconcia et al. 2015; Prasanth et al. 2010), and to reduce the expression of adiponectin in human adipose tissue (Hugo et al. 2008). In human beta cells, BPA has been shown to decrease the activity of K_{ATP} channels and stimulate glucose-induced insulin secretion (Soriano et al. 2012), supporting the principle that BPA has adverse metabolic effects in humans. As a result of growing concerns over the adverse health effects of BPA, the chemical industry has replaced it with its analog, bisphenol S (BPS). BPS has been less studied than BPA, but recent publications suggest that BPS may also have endocrine-disrupting effects (Eladak et al. 2015) and affect human adipocyte differentiation and expression of adipokines in human adipose tissue (Boucher et al. 2016a; Boucher et al. 2016b; Peshdary et al. 2020). A wide range of concentrations have been used in these studies, with studies showing significant effects in the low nanomolar range (Chamorro-Garcia et al. 2012; Hugo et al. 2008; Soriano et al. 2012; Wang et al. 2013), whereas others only have shown effects in the micromolar range (Boucher et al. 2016a; Boucher et al. 2014; Peshdary et al. 2020).

Adipose tissue plays an important role in whole-body homeostasis, as it functions not only as a fat storage reservoir, but also acts as an endocrine organ. Adipose tissue dysfunction is central in the development of both obesity and T2D (Guilherme et al. 2008). As an endocrine organ, adipose tissue secretes adipokines, cytokines, and chemokines, which signal to organs such as the skeletal muscle, liver, and brain to regulate metabolism (Guilherme et al. 2008). Furthermore, low-grade chronic inflammation in adipose tissue is linked to metabolic disturbances and insulin resistance (Zatterale et al. 2019). Although there are several studies that have investigated the effects of BPA on adipocyte metabolism, the results are often conflicting, which may be due to factors such as differences in cell types, exposure time, and concentrations used (Chamorro-Garcia et al. 2012; De Filippis et al. 2018; Ohlstein et al. 2014). Furthermore, whole adipose tissue contains mature adipocytes, preadipocytes, fibroblasts, endothelial cells, and immune cells such as macrophages; therefore, investigating the effects of bisphenols on adipose tissue explants may provide better insight on the effects that occur *in vivo*.

It is evident that humans are consistently exposed to bisphenols, as they have been detected in nearly all urine samples from individuals tested (McGovern 2009). Mass production, chronic exposure, and the reported effects on endocrine disruption call for the need to continue research on the effects of bisphenols on human health. Based on previous publications we hypothesize that bisphenols can affect adipocyte glucose uptake and expression of adipokines and inflammatory markers at environmentally-relevant doses.

In this study, we investigated the effects of BPA and BPS exposure on human subcutaneous adipose tissue inflammation and glucose uptake. Exploring the effects of BPA and BPS on adipose tissue metabolism may provide a better understanding of the mechanisms that link bisphenols to the development of insulin resistance and T2D.

2. Material and methods

2.1. Human participants

Fasting blood samples were collected for biochemical analysis of study participants at the Department of Clinical Chemistry at the Uppsala University Hospital. After 10–12 h of fasting, human abdominal subcutaneous adipose tissue was obtained by needle biopsies from 16 healthy individuals (14 women and 2 men), age 21-72 yo, body mass index (BMI) 22.2-32.5 kg/m^2 after administration of local anesthetic (Xylocaine; AstraZeneca, Mölndal, Sweden). Anthropometric and fasting biochemical characteristics of subjects used in this study are described in Table 1. Subjects with T2D, endocrine disorders, cancer or other major illnesses, with systemic glucocorticoids, beta-blockers, and immune-modulating therapies, were excluded from the study. The Regional Ethics Review Boards in Uppsala approved the study (Dnr 2018/385). All participants gave their written informed consent.

Table 1
Anthropometric and fasting biochemical characteristics of subjects.

Variable	
Sex (female/male, n)	14/2
Age (years)	21–72
Body mass index (kg/m^2)	22.2–32.5 ± 5.7
Serum insulin levels (mU/L)	9.8 ± 7.3
Plasma glucose levels (mmol/L)	5.3 ± 0.3
Body fat (%)	34.2 ± 7.0
Plasma HDL-cholesterol (mmol/L)	1.44 ± 0.44
Plasma LDL-cholesterol (mmol/L)	2.86 ± 0.78
Plasma triglyceride (mmol/L)	0.96 ± 0.48
HbA1c (mmol/mol)	34.1 ± 3.9

Data are mean ± SD. LDL: low-density lipoprotein; HDL: high-density lipoprotein, HbA1c: glycosylated hemoglobin.

2.2. Tissue culture

Adipose tissue was cultured in a humidified incubator at 37 °C with 5% CO_2. Adipose tissue was incubated with biologically relevant (1 nM, 10 nM) and supra-physiological concentrations (10^4 nM) of BPA or BPS (Sigma-Aldrich, ON, Canada). Adipose tissue was incubated in low glucose DMEM (6 mM) (Gibco, Life Technologies, Paisley, UK), 10% charcoal-stripped fetal bovine serum (FBS) (Gibco, Life Technologies), and 1% penicillin-streptomycin (PEST, Gibco, Life Technologies), in the presence or absence of BPA (1 nM, 10 nM, 10^4 nM), BPS (1 nM, 10 nM, 10^4 nM), dexamethasone (Dex; 0.3 μM; positive control, Sigma, St. Louis, MO, USA), or dimethyl sulfoxide (DMSO, 0 nM; 0.1%; vehicle control) (Sigma) for 24 or 72 h. Dexamethasone has been shown to be associated with insulin resistance in subcutaneous adipose tissue (Pereira et al. 2014) and was thus used as a positive control. After treatment, adipose tissue was either snap-frozen in liquid nitrogen or directly used for experimentation.

2.3. Cell viability

Adipose tissue was incubated for 72 h in the presence or absence of BPA, BPS, or dexamethasone. Cell viability was measured using the Water Soluble Tetrazolium Salts (WST-1) Assay for Cell Proliferation (Roche, Manheim, Germany). This involves the cleavage and reduction of WST-1 to formazan which is largely dependent on the glycolytic production of NAD(P)H in viable cells (Ehrke et al. 2020). Treated adipose tissue was incubated with 10% WST-1 reagent according to the manufacturer's protocol. Absorbance was measured at 405 and 630 nm (reference wavelength). Cell viability was calculated using the optical density, which was normalized to control (vehicle).

2.4. Gene expression

Treated adipose tissue was washed once with ice-cold PBS (Medicago, Uppsala, Sweden) and snap-frozen in liquid nitrogen for gene expression analysis. RNA was isolated using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The concentration and purity of RNA were measured using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Rockford, IL, USA). RNA (400 ng) was then reverse-transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific). The data were calculated using the $2^{(-\Delta\Delta CT)}$ method. The results were normalized using the housekeeper gene 18S ribosomal RNA (18S rRNA). Gene expression of markers of inflammation (interleukin-6 (*IL6*), *IL1B*, *IL33*, tumor necrosis factor-alpha (*TNFA*)), fatty acid transport (fatty acid-binding protein 4 (*FABP4*)), glucose transport

(*GLUT4*, *GLUT1*), adipokines (adiponectin (*ADIPOQ*), leptin (*LEP*)), receptors (estrogen receptor (*ESR*) *1*, *ESR2*), and insulin signalling genes (*AKT, IRS1*) was performed with the QuantStudio 3 System (Thermo Fisher Scientific) using TaqMan assay probes listed in Table 2 (all from Thermo Fisher Scientific). All samples were run in duplicates.

2.5. Glucose uptake

Glucose uptake was performed as previously reported in Pereira et al. (2020). After exposure to BPA for 24 h, adipose tissue was digested using collagenase A (1 mg/mL) (obtained from *Clostridium histolyticum*, Roche), for 60 min shaking at 105 RPM and 37 °C in Medium 199 (Gibco, Life Technologies) supplemented with 6 mM glucose, 4% bovine serum albumin (BSA, Sigma), 150 nM adenosine (Sigma), pH = 7.4. Cells, filtered through 250 μm nylon mesh, were washed three times with glucose-free Krebs-Ringer bicarbonate medium (KRH) supplemented with 4% BSA, 150 nM adenosine, pH = 7.4. The supernatant was isolated, and cells were then subsequently diluted to 6–7% lipocrit with KRH. Adipocytes suspension shaking at 65RPM at 37 °C were stimulated with 25 or 1000 μU/mL (0.20 or 6.95 nM) of insulin (physiological and supraphysiological levels) for 15 minutes, followed by incubation with of Glucose-D-[U-^{14}C] (0.26 mCi/L, 0.86 μM, Perkin Elmer, Boston, MA, USA) for 45 min. Adipocytes were separated from the media thought centrifugation in 1 mL of silicon fluid (VWR Chemicals, Leuven, Belgium) and radioactivity was measured using Liquid Scintillation Analyser (Tri-Carb 4910 TR) (Perkin Elmer). Cell size was measured under a light microscope as a mean diameter (μm) of consecutively measured 100 adipocytes from the same individual. Cellular glucose uptake was measured according to this formula: Glucose uptake = (cell-associated radioactivity × volume) / (radioactivity of medium × cell number × time). Cell number was determined by adding heptane to lipocrit diluted in KRH and measuring mass after 8 days of evaporation.

2.6. Western blot

BPA-treated adipose tissue was washed three times with ice-cold PBS and snapped frozen in liquid nitrogen for protein expression analysis. Adipose tissue was homogenized in lysis buffer (25 mM Tris-HCl, 0.5 EGTA, 25 mM NaCl, 10 mM NaF, 1% NP-40, 100 nM okadaic acid, and 1 mM orthovanadate (all from Sigma), and 1 X protease inhibitor (Roche)). A clear cell lysate was obtained between the pellet and the fat cake layer using a blunt needle into the Eppendorf tube. Protein concentration was measured using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Anti-Akt (Cell Signaling, Beverly, MA, USA, #9272S), and phospho-Akt (Cell Signaling, #9271S) were the primary antibodies used, diluted 1:1000 in 1x PBS with 1% Tween-20 (Medicago). The secondary antibodies used were anti-mouse (Cell Signaling, #7076S) and anti-rabbit (Cell Signaling, #7074S) antibodies coupled to horseradish peroxidase, diluted 1:5000. Anti-glyceraldehyde-3-phosphate dehydrogenase (1:1000) was used as a loading control protein (Cell Signaling, #2118S). Proteins were visualized using ChemiDoc (XRS+, Biorad, Hercules, CA, USA). Protein expression was quantified by densitometry analysis using ImageJ program (National Institutes of Health, Bethesda, MD, USA).

Table 2
Taqman probes for genes used in this study.

Gene	Taqman probe
Adiponectin (*ADIPOQ*)	Hs00605917_m1
Fatty acid binding protein 4 (*FABP4*)	Hs01086177_m1
Interleukin 1 beta (*IL1B*)	Hs01555410_m1
Interleukin 6 (*IL6*)	Hs00985639_m1
Interleukin 33 (*IL33*)	Hs04931857_m1
GLUT4 (*SLC2A4*)	Hs00168966_m1
GLUT1 (*SLC2A1*)	Dm01821912_g1
Tumor necrosis factor alpha (*TNFA*)	Hs00174128_m1
Estrogen receptor 1 (*ESR1*)	Hs01046816_m1
Estrogen receptor 2 (*ESR2*)	Hs01100353_m1
Protein kinase B (*AKT*)	Hs00178289_m1
Insulin receptor substrate 1 (*IRS1*)	Hs00178563_m1

2.7. Statistical analysis

Data are presented as means ± SEM or ± SD. A comparison between two groups was done using a paired t-test and comparison between the mean of more than two groups was done using one-way ANOVA with repeated measures or mixed-effects model analysis as appropriate. The correction for the multiple comparisons was done by Dunnett's test. The analysis was performed using GraphPad Prism version 8.4.3 (La Jolla, CA, USA). Values with $p < 0.05$ were considered significant.

3. Results

3.1. The effects of BPA or BPS on human adipose tissue cell viability

First, we evaluated the effects of BPA, BPS, or dexamethasone exposure on adipose tissue cell viability. The reduction of the stable tetrazolium salt WST-1 to formazan, which is an indication of cell proliferation and viability, was measured in adipose tissue that was treated for 72 h with BPA, BPS or dexamethasone. We found that BPA, BPS, or dexamethasone had no significant effect on cell viability in adipose tissue explants (Fig. 1 A and B).

3.2. The effects of BPA and BPS exposure on adipokine and receptor gene expression in human adipose tissue

The effects of BPA and BPS exposure on expression of proinflammatory cytokines, adipokines, and receptor levels was measured in adipose tissue following 24 and 72-h treatments (Table 3). Following 24 h BPA treatment, there was a reduction in the expression of the proinflammatory cytokines *IL6* (10 nM and 10^4 nM), *IL1B* (10 nM), and *TNFA* (1 nM) ($p < 0.05$). The proinflammatory cytokine *IL33* was not affected by BPA or BPS treatment at 24 h (Table 3). Exposure to BPA for 24 h reduced gene expression of the adipokine *ADIPOQ* (10 nM) and *FABP4* (10 nM) ($p < 0.05$), with no effect on *LEP* expression (Table 3). *FABP4* was also reduced when exposed to 1 nM of BPS for 24 h ($p < 0.05$) (Table 3). Gene expression of the receptors *ESR1* and *ESR2* was unaffected by 24 h BPA and BPS exposure. Adipose tissue treated with BPA or BPS for 72 h had no effects on gene expression of proinflammatory cytokines, adipokines, and estrogen receptors (*ESR1* and *ESR2*) (Table 3).

3.3. The effects of BPA exposure on glucose uptake in human adipose tissue

Since 72 h bisphenol exposure had no apparent effect on adipose tissue gene expression, in addition to nearly no effect with 24-h BPS exposure, we focused on the effects of 24 h BPA exposure. To determine the effects of BPA on glucose uptake, we performed a radioactive ^{14}C-glucose uptake assay in human adipose tissue exposed to vehicle control (0.1% DMSO), BPA (10 nM or 10^4 nM), or dexamethasone, for 24 h. Following BPA incubation with adipose tissue, adipocytes were isolated and basal, and insulin-stimulated glucose uptake was measured. Maximal glucose uptake (1000 μU/mL insulin) was significantly reduced in adipocytes treated with 10 and 10^4 nM BPA, and dexamethasone (Fig. 2A). Control adipocytes treated with 25 and 1000 μU/mL had significantly increased glucose uptake (Fig. 2B), compared to basal. However, there was no insulin effect (25 and 1000 μU/mL) in adipose tissue treated with BPA (10 and 10^4 nM) or dexamethasone (Fig. 2B), compared to basal.

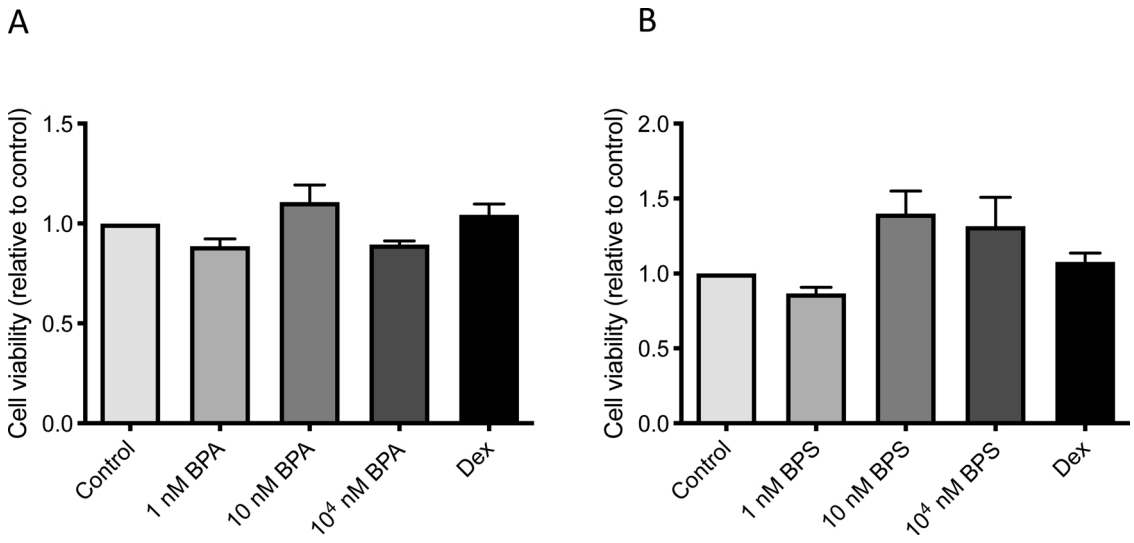

Fig. 1. The effects of BPA and BPS exposure on cell viability in human abdominal subcutaneous adipose tissue. Human subcutaneous adipose tissue was incubated with (A) BPA or (B) BPS (1 nM, 10 nM and 10^4 nM) *ex vivo* for 72 h and compared to the vehicle control (0.1% DMSO) and the positive control dexamethasone (Dex, 0.3 μM). Cell viability was measured by cleavage of the tetrazolium salt to formazan by cellular mitochondrial dehydrogenase (proportional to live cells). $n = 3$–4, each independent experiment was done in duplicate. Data represent mean ± SEM (one-way ANOVA).

3.4. The effects of BPA exposure on the insulin signalling pathway in human adipose tissue

Next, we determined whether the inhibitory effects of BPA on insulin-stimulated glucose uptake could be due to the effects of key proteins involved in the insulin signalling. The expression level of glucose transporters *GLUT4* and *GLUT1* was measured in adipose tissue treated with BPA for 24 h. BPA exposure did not result in a change in *GLUT4* or *GLUT1* mRNA levels (Fig. 3A and B). We then looked at whether BPA exposure had an effect on key regulators of the insulin signalling pathway in adipose tissue, by measuring expression levels of *AKT* and *IRS1*. We found no changes in gene expression of *AKT* or *IRS1* in adipose tissue exposed to BPA (Fig. 3C and D). Protein levels of GLUT4 were measured to determine if BPA altered protein levels; however, we found no significant effect (Fig. 3E). Next, we measured levels of phosphorylated and total protein levels of Akt. We found that 24 h BPA exposure did no effect on phosphorylation of Akt in adipose tissue treated with BPA (Fig. 3F).

4. Discussion

In this study, we investigated the effects of 24 and 72 h BPA and BPS on adipose tissue insulin sensitivity and inflammation. We show that exposure to environmentally relevant concentrations of BPA for 24 h results in reduced gene expression of proinflammatory cytokines and adipokines. Adipose tissue treated with BPS had no significant changes in the expression of proinflammatory genes, adipokines, or estrogen receptors. Furthermore, we found reduced glucose uptake and insulin response in adipose tissue treated with BPA for 24 h, with no changes in gene and protein levels of markers of the insulin signalling pathway.

Over the last few decades, there have been increased concerns regarding the effects of bisphenols on the development of metabolic diseases. Although there are studies that have investigated the direct effects of bisphenols on isolated adipocyte metabolism, to our knowledge, this is the first study exploring the direct effects of BPA or BPS exposure on human abdominal subcutaneous adipose tissue. Since adipose tissue contains mature adipocytes, preadipocytes, fibroblasts, endothelial cells, and immune cells such as macrophages, adipose tissue explants better represent molecular events that occur *in vivo*. Whole adipose tissue culture provides substantial benefits when investigating long-term gene expression of adipocytes in adipose tissue. One reason for this is that adipocyte-specific gene expression has been shown to be lost when isolated from adipose tissue (Carswell et al. 2012). Due to this, studies that have investigated the effects of bisphenols on human primary adipocytes may not precisely represent what occurs *in vivo* due to molecular changes that occur after isolation from adipose tissue. Widespread of bisphenols in consumer products results in nearly continuous exposure to humans. Therefore, we pre-incubated human adipose tissue for 24 and 72 h to mimic a longer exposure time. However, further studies are needed to address whether other concentrations and exposure times can induce different results.

Human pharmacokinetics studies of bisphenols have shown variable results. Some studies have shown that after single exposures BPA is rapidly metabolized, with the elimination of conjugates into urine largely occurring within 24 h (Vandenberg et al. 2007), while population-based studies suggest that BPA has a longer half-life (Stahlhut et al. 2009). Furthermore, the range of BPA concentrations found in humans has been reported from low picomolar (Karrer et al. 2018) up to 40 nM (Welshons et al. 2006). For instance, BPA has been found in amniotic fluid at approximately 36 nM (Ikezuki et al. 2002), in maternal serum at 10 nM (Yamada et al. 2002), and in breast milk at approximately 3 nM (Sun et al. 2004), which highlights that depending on different factors (tissue type, endpoint, sex, etc.) BPA can be found at levels that are higher than picomolar concentrations. As a lipophilic compound, BPA is also found in the adipose tissue (Artacho-Cordon et al. 2019; Fernandez et al. 2007; Geens et al. 2012) and the partition coefficient adipose:blood of BPA has been reported to be 5 (Yang et al. 2015), suggesting that adipose tissue is exposed to a higher concentration of bisphenols than urine and plasma. Therefore, 1 and 10 nM were used to ensure that we exposed adipose tissue to biologically relevant concentrations. The higher supra-physiological level of 10^4 nM was selected to ensure that we were able to achieve a maximal effect.

Adipose tissue insulin resistance is closely linked to increased inflammation (Gregor and Hotamisligil 2011). It has been shown that 3T3-L1 adipocytes cultured with BPA (1 nM and 3 nM) have increased expression of proinflammatory markers such as *Tnfa* and *Il6*. In contrast, other studies have shown that BPS (0.1 μM) reduces *IL6* expression in human adipocytes (De Filippis et al. 2018; Peshdary et al. 2020). The effects of BPA or BPS on the expression of inflammatory markers in whole adipose tissue have not been studied. Therefore, we wanted to determine whether human adipose tissue explants exposed to BPA or BPS would have similar alterations in inflammation. Interestingly, we

Table 3
The effects of 24 or 72 h BPA and BPS exposure gene expression in human subcutaneous adipose tissue.

	BPA			BPS			Dex
Gene	1 nM	10 nM	10^4 nM	1 nM	10 nM	10^4 nM	0.3 μM
24 h							
Proinflammatory cytokines							
IL6	0.72 ± 0.08	**0.56 ± 0.21***	**0.51 ± 0.11***	1.08 ± 0.15	1.41 ± 0.44	1.86 ± 0.76	**0.30 ± 0.09***
IL1B	0.74 ± 0.20	**0.58 ± 0.13***	0.55 ± 0.18	1.04 ± 0.27	1.06 ± 0.23	1.22 ± 0.13	**0.09 ± 0.02***
TNFA	**0.80 ± 0.04***	0.84 ± 0.14	0.68 ± 0.16	1.05 ± 0.14	1.12 ± 0.41	0.94 ± 0.17	**0.43 ± 0.10***
IL33	0.86 ± 0.04	0.82 ± 0.15	0.73 ± 0.13	0.90 ± 0.09	1.60 ± 0.47	1.13 ± 0.13	**0.07 ± 0.02***
Adipokines							
ADIPOQ	0.69 ± 0.10	**0.65 ± 0.12***	0.75 ± 0.15	0.81 ± 0.07	0.92 ± 0.16	1.11 ± 0.28	0.94 ± 0.15
FABP4	0.77 ± 0.09	**0.59 ± 0.11***	0.74 ± 0.20	**0.76 ± 0.05***	0.73 ± 0.09	1.06 ± 0.16	0.95 ± 0.15
LEP	0.84 ± 0.05	0.86 ± 0.14	0.82 ± 0.16	1.15 ± 0.11	1.32 ± 0.29	1.03 ± 0.10	**3.07 ± 0.73***
Receptors							
ESR1	0.91 ± 0.08	0.82 ± 0.13	0.79 ± 0.12	0.97 ± 0.08	1.10 ± 0.18	1.11 ± 0.17	0.98 ± 0.18
ESR2	0.94 ± 0.14	0.77 ± 0.12	0.77 ± 0.12	1.02 ± 0.19	1.02 ± 0.17	1.01 ± 0.17	**1.72 ± 0.29***
72 h							
Proinflammatory cytokines							
IL6	1.53 ± 0.15	1.66 ± 0.31	1.27 ± 0.20	5.25 ± 2.0	3.93 ± 0.96	3.74 ± 1.28	1.20 ± 0.65
IL1B	1.13 ± 0.39	0.91 ± 0.19	0.94 ± 0.28	1.73 ± 0.04	2.28 ± 0.73	2.93 ± 0.97	**0.64 ± 0.02***
TNF	0.78 ± 0.26	0.88 ± 0.30	0.95 ± 0.32	0.87 ± 0.20	1.89 ± 0.62	0.89 ± 0.20	**0.44 ± 0.11***
IL33	1.22 ± 0.16	1.27 ± 0.25	1.0 ± 0.17	1.87 ± 0.48	2.98 ± 0.88	2.68 ± 1.19	0.93 ± 0.51
Adipokines							
ADIPOQ	1.23 ± 0.18	1.87 ± 0.67	1.55 ± 0.22	1.47 ± 0.31	1.79 ± 0.46	4.45 ± 1.88	**2.36 ± 1.22***
FABP4	1.04 ± 0.21	2.27 ± 1.04	1.17 ± 0.29	1.79 ± 0.54	2.40 ± 0.77	5.62 ± 2.63	3.70 ± 2.80
LEP	1.02 ± 0.26	1.01 ± 0.17	1.04 ± 0.30	1.41 ± 0.52	1.38 ± 0.21	0.51 ± 0.13	2.15 ± 0.54
Receptors							
ESR1	0.93 ± 0.14	0.87 ± 0.14	0.89 ± 0.22	1.00 ± 0.14	1.07 ± 0.11	1.21 ± 0.11	0.82 ± 0.16
ESR2	0.93 ± 0.22	1.03 ± 0.21	0.95 ± 0.20	1.29 ± 0.24	1.11 ± 0.11	2.05 ± 0.48	2.30 ± 0.61

IL6 - Interleukin-6 (24 h: $n = 8$, 72 h: $n = 4$); *IL1B* - Interleukin-1β (24 h: $n = 7$, 72 h: $n = 5$); *TNFA* - Tumor Necrosis Factor α (24 h: $n = 6$, 72 h: $n = 5$); *IL33* - Interleukin 33 (24 h $n = 7$, 72 h: $n = 5$); *ADIPOQ* - Adiponectin (24 h: $n = 8$, 72 h: $n = 5$); *FABP4* - Fatty acid binding protein 4 (24 h: $n = 8$, 72 h: $n = 5$); *LEP* - Leptin ($n = 8$, 72 h: $n = 5$); *ESR1* - Estrogen Receptor 1 (24 h: $n = 8$, 72 h: $n = 8$); *ESR2* - Estrogen Receptor 2 (24 h: $n = 8$, 72 h: $n = 6$). Human subcutaneous adipose tissue incubated with BPA, BPS, dexamethasone (Dex, positive control), or DMSO (vehicle control), for 24 or 72 h. 18S was used as the housekeeping gene. Gene expression data are represented as a fold change to vehicle control. Relative expression was calculated as 2^(-ΔΔCt). Data represent mean ± SEM. $n = 3$–4, $^*p < 0.05$ (one-way ANOVA).

found reduced levels of the proinflammatory cytokines *IL6* (1 and 10 nM), *IL1B* (10 nM), and *TNFA* (1 nM) following 24 h BPA exposure. These results are consistent with our positive control, dexamethasone, which is known to induce insulin resistance in adipose tissue and also to reduce the expression of certain proinflammatory cytokines, such as *IL6* (Fried et al. 1998), which was confirmed in the present study. Dexamethasone is a glucocorticoid that exerts its effects by binding to the glucocorticoid receptor and has been shown to reduce glucose uptake in adipose tissue (Sarsenbayeva et al. 2019). It has been shown that BPA can bind the glucocorticoid receptor in a similar fashion and with similar binding energy to dexamethasone and cortisol (Prasanth et al. 2010). Therefore, it is possible that in adipose tissue BPA may be exerting its effects in a similar manner to dexamethasone. In insulin resistance, IL6 protein content is understood to be negatively associated with insulin-stimulated glucose disposal in adipose tissue (Nieto-Vazquez et al. 2008). Although adipocytes secrete IL6, it only accounts for approximately 10% of total tissue production; thus, other cells in the adipose tissue may contribute to increased IL6 production (Fried et al. 1998). Furthermore, IL1B mediates macrophage-induced impairments in the insulin signalling pathway, such as reduced phosphorylation of Akt expression in human adipocytes (Gao et al. 2014). Although BPA can increase inflammatory markers in adipocytes, it has also been shown to reduce proinflammatory cytokine production in macrophages (Pyo et al. 2007; Valentino et al. 2016). Therefore, it is possible that whole adipose tissue cultured with BPA may have a different inflammatory response than isolated adipocytes due to presence of the adipose tissue stroma.

Although BPS is considered a safe alternative to BPA, recent studies have indicated that BPS exposure may have adverse health outcomes. For example, Boucher et al. (2016) showed that preadipocytes treated with high concentrations of BPS (10 μM) increased lipid accumulation and gene expression of adipogenic markers (Boucher et al. 2016a). In our study, we found no changes in the expression of proinflammatory cytokines in adipose tissue exposed to BPS for 24 h. It is possible that at environmentally-relevant concentrations, BPS does not alter levels of inflammatory markers in adipose tissue. Also, we found that adipose tissue treated with BPA or BPS for 72 h did not have a similar reduction in proinflammatory cytokine expression. This may be the result of an adaptive mechanism of the adipose tissue to counteract the effects of BPA exposure.

In this study, we found that 24 h BPA exposure resulted in reduced levels of *FABP4* (10 nM) and *ADIPOQ* (10 nM). The release of *FABP4* from adipocytes and macrophages is positively related to obesity and insulin resistance (Nakamura et al. 2017). The reduction of *FABP4* in our study is consistent with a study that has shown that 3T3-L1 adipocytes exposed to BPA (0.1 nM-3 nM) during differentiation have reduced mRNA levels of *Fabp4* (De Filippis et al. 2018). The adipokine, adiponectin, is inversely related to adiposity, and increased plasma adiponectin levels are directly correlated with improved insulin sensitivity. Reduced adiponectin expression in our study corresponds to the study by Hugo et al. (2008) that showed pre-adipocytes isolated from human abdominal subcutaneous adipose tissue explants incubated for 6 h with 0.1, 1, and 10 nM of BPA to have reduced levels of adiponectin (Hugo et al. 2008). Furthermore, they showed that this did not occur through the estrogen receptor, as effects were not reversed by the use of an estrogen receptor antagonist (ICI 182,780) (Hugo et al. 2008). It was unclear, however, whether BPA or BPS is able to alter levels of estrogen receptors. It has previously been shown that BPA enhances ER expression in Jurkat cells, however, it was unknown whether this occurred in adipose tissue as well (Cipelli et al. 2014). Therefore, we measured levels of *ESR1* and *ESR2* to determine if BPA or BPS exposure can alter expression levels. We found no significant changes in the levels of these receptors. Although BPA has been linked to enhanced ER expression (Melzer et al. 2011), it is unlikely that this occurs in human adipocytes

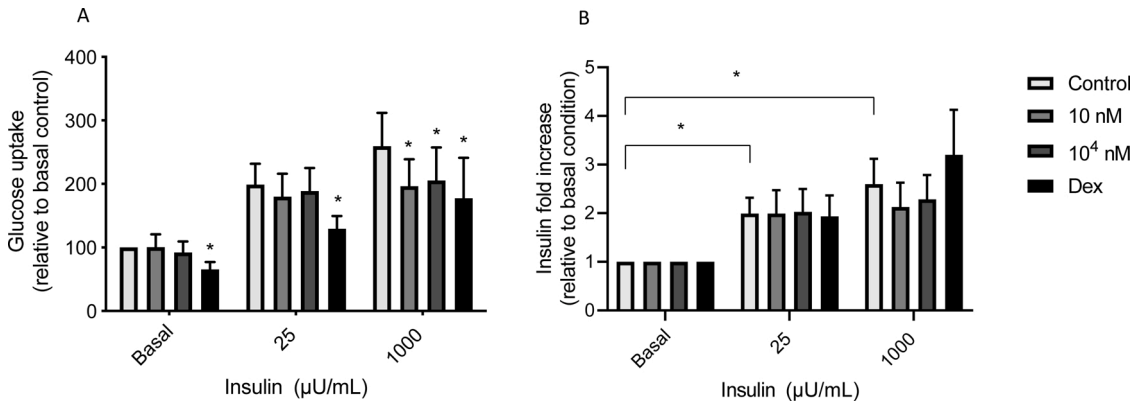

Fig. 2. The effects of 24 h BPA treatment on glucose uptake in adipocytes from human subcutaneous abdominal adipose tissue. (A) Uptake of radioactive glucose-D-[U-^{14}C] and (B) insulin-fold increase in isolated adipocytes from adipose tissue treated with vehicle (0.1% DMSO), BPA (10 nM, 10^4 nM), or dexamethasone (Dex, 0.3 μM, positive control). Adipocytes were exposed to (25 μU/mL, 1000 μU/mL) insulin for 1 h. For fold increase, basal conditions from each treatment were normalized to 1. $n = 7$, each independent experiment was done in duplicate or triplicate. *$p < 0.05$ compared to all other BPA concentrations in the same condition. (A-B) Data represent mean ± SEM. *$p < 0.05$ (one-way ANOVA).

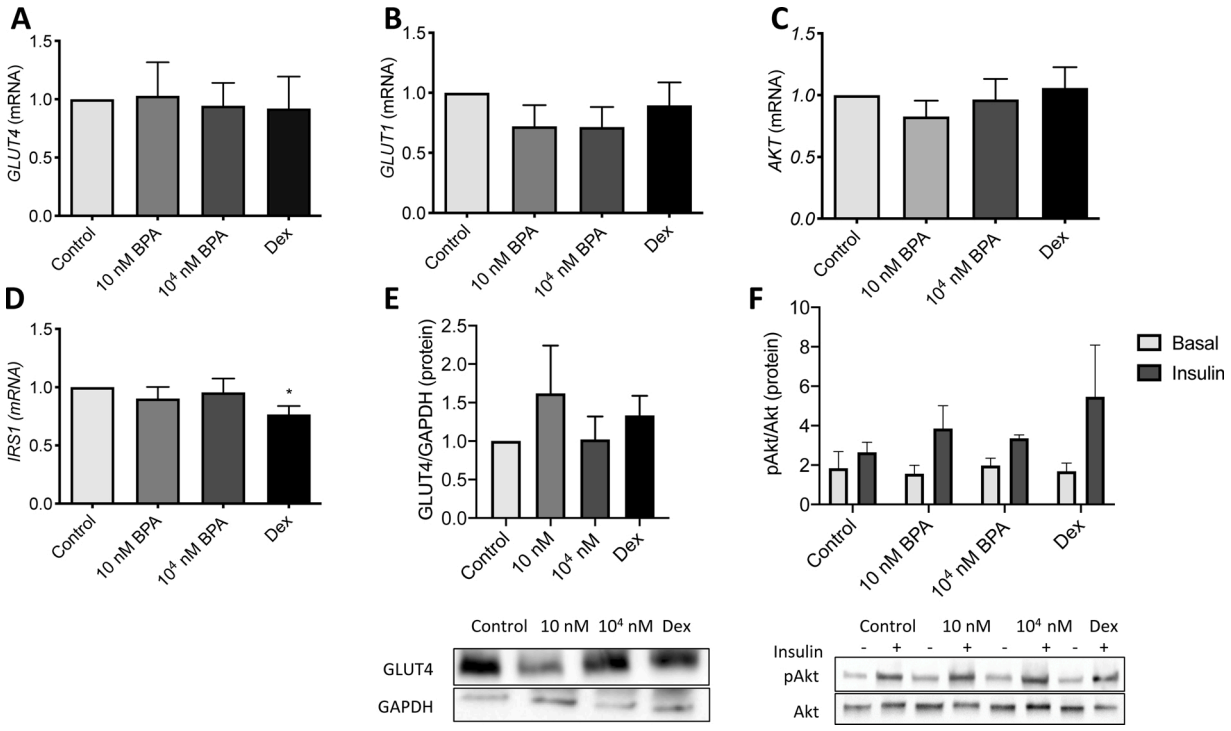

Fig. 3. The effects of 24 h BPA treatment on the insulin signalling pathway in human subcutaneous abdominal adipose tissue. Gene expression of (A) *GLUT4,* (B) *GLUT1,* (C) *AKT,* and (D) *IRS1* in adipose tissue treated with vehicle (0.1% DMSO), BPA (1–10^4 nM), or dexamethasone (Dex), measured by quantitative PCR (qPCR). $n = 3$, independent experiments. (E) Protein level of GLUT4. Top panel: quantification of GLUT4 by density analysis. Bottom panel: representative Western blot. (F) Ratio of phosphorylated and total Akt in adipose tissue treated with DMSO, BPA (10 nM, 10^4 nM) or dexamethasone (Dex). Adipose tissue was exposed to insulin (1000 μU/mL) for 15 min. Top panel: quantification of phospho-Akt/Akt by density analysis. Bottom panel: representative Western blot. $n = 3$ independent experiments. (A-F) Data represent mean ± SEM.

or adipose tissue.

Impaired glucose metabolism by the adipose tissue has been associated with the development of T2D (Pereira et al. 2016). Therefore, we incubated adipose tissue with BPA for 24 h to determine whether insulin sensitivity would be altered. We showed that adipose tissue exposed for 24 h with both environmentally-relevant (10 nM) and supra-physiological levels (10^4 nM) concentrations of BPA had a reduction in insulin-stimulated glucose uptake, with no effects on basal glucose uptake. This coincides with previous studies that have shown that adipocytes exposed to BPA have reduced insulin-stimulated glucose uptake (De Filippis et al. 2018). Interestingly, we found that 24 h BPA exposure did not alter total levels or phosphorylation of Akt, protein levels of GLUT4, or gene expression of *AKT*, *IRS1*, *GLUT1*, and *GLUT4*. Other diabetogenic drugs, such as the immunosuppressive agents' cyclosporin A and tacrolimus, can also inhibit glucose uptake, without altering activation/phosphorylation of the insulin signalling proteins IRS1/2, AS160, GLUT1, and GLUT4, but by altering the translocation of GLUT4 to the membrane (Pereira et al. 2014). Due to the difficulty of isolating sufficient adipocytes for isolation of the plasma membrane to measure GLUT4 (Pereira et al. 2014), future studies should determine an appropriate protocol to investigate the effects of BPA on GLUT4 transport machinery and overall translocation to the plasma membrane.

Furthermore, our results are consistent with a study investigating the effects of environmentally-relevant concentrations of BPA on 3T3-L1 cells treated chronically for 3 weeks (Ariemma et al. 2016). Specifically, it was shown that chronic BPA exposure of 3T3-L1 adipocytes resulted in reduced levels of glucose uptake, with no effect on gene expression of GLUT1 or GLUT4 (Ariemma et al. 2016). Interestingly, however, this study had increased levels of proinflammatory cytokines, such as *Il6* and *Ifnγ*, which differs from what we found in our study (Ariemma et al. 2016). As previously highlighted, it is possible that this difference can be due to differences in exposure duration, or the presence of the surrounding stroma and the communication between different cell types in adipose tissue.

Studies that have investigated the effects of BPA on adipocyte glucose metabolism often show conflicting and difficult to interpret results. In a study by Sakurai et al. (2014), 24 h BPA exposure of 3T3-L1 adipocytes leads to increased glucose uptake and GLUT4 expression at high concentrations (10^5 nM), with no effect at environmentally-relevant concentrations (1–10^4 nM) (Sakurai et al. 2004). Contrastingly, human adipocytes incubated with 1 and 100 nM of BPA for 8 h had no effect on glucose uptake, whereas 24 and 48 h had reduced glucose uptake, with the latter having the greatest reduction (Valentino et al. 2013). This emphasizes differences that can occur due to the experimental model, exposure time, and concentration of BPA or BPS. Furthermore, based on our study, it is apparent that there is a difference between exposing human adipocytes to bisphenols compared to human adipose tissue explants. This is likely due to communication between adipocytes and surrounding cells, such as macrophages. Hence, future studies should investigate the direct effects of BPA and BPS on the stromal vascular fraction cells and their cross-talk with adipocytes.

Previous studies have suggested that bisphenols follow a non-monotonic dose-response (Vandenberg 2014), but recent publications have challenged this notion (Badding et al. 2019). Our study protocol only included 3 concentrations, which does not allow for proper evaluation of non-monotonicity in dose-response. Such studies are needed to evaluate the biological effects of bisphenols in human adipose tissue metabolism.

Sex differences have been shown to influence fat storage, hormone secretion, and the brain's response to hormones (Shi and Clegg 2009). Therefore, since BPA and BPS can exert their effects through activation of hormone signalling pathways, there may be sex differences in adipose tissue exposure to these compounds. However, in our study, there were not enough subjects (14 females, 2 males) to make conclusions about the correlation between the sex of the subject and the effects of BPA. Future studies should determine whether there are sex differences when exposed to BPA and BPS. Another limitation of this study was the use of an *in vitro* system that does not take into account inter-organ communication which may also have an effect on adipose tissue metabolism.

5. Conclusions

Taken together, this study shows that 24 h BPA exposure at environmentally-relevant concentrations reduced gene expression of proinflammatory markers and adipokines in human subcutaneous adipose tissue and inhibited glucose uptake in adipocytes independently of insulin signalling. These mechanisms can contribute to the development of insulin resistance associated with BPA exposure. Furthermore, we demonstrate that BPS, a BPA analog, did not alter the expression of inflammatory markers in adipose tissue. Future studies are required to understand the mechanism by which glucose uptake is reduced in adipose tissue, and whether the effects of BPA on adipose tissue can affect inter-organ cross-talk.

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Declaration of Competing Interest

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

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