Bisphenol A and congeners proposed as substitutes promote adipogenesis of human ADSC at environmentally relevant doses

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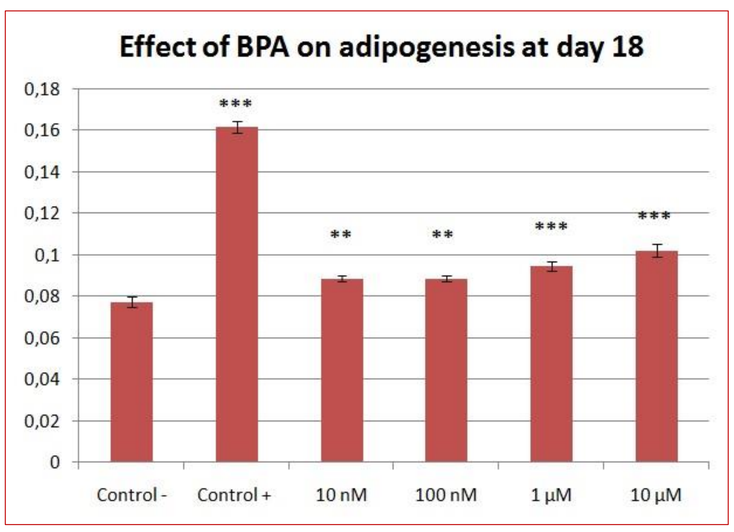
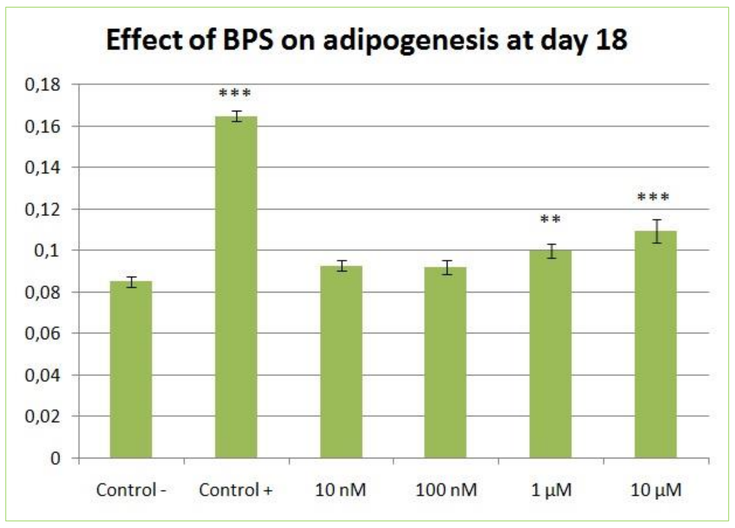
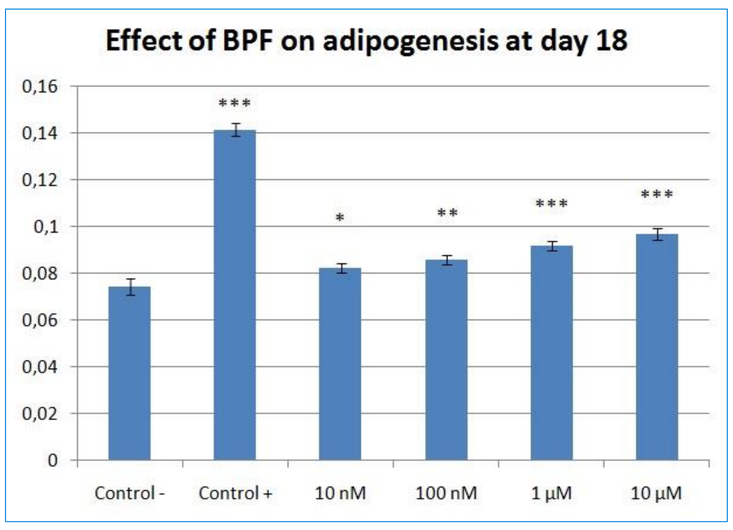
Background: Obesity epidemic is at the centre of worldwide public health concerns along with its associated co-morbidities. Apart from classical lifestyle factors, mounting evidence is signaling endocrine disrupting chemicals (EDCs) exposure as an additional risk factor for obesity and metabolic disorders. In this context, reliable *in vitro* screening systems of adipogenesis are needed. While several investigations have been conducted using murine cell lines, very few studies have explored this issue using human cell precursors.

Objective: To assess the impact of low levels of bisphenol A (BPA), as well as its proposed substitutes bisphenol S (BPS) and bisphenol F (BPF), on adipogenic differentiation and lipid metabolism of human adipose-derived stem cells (hADSC).

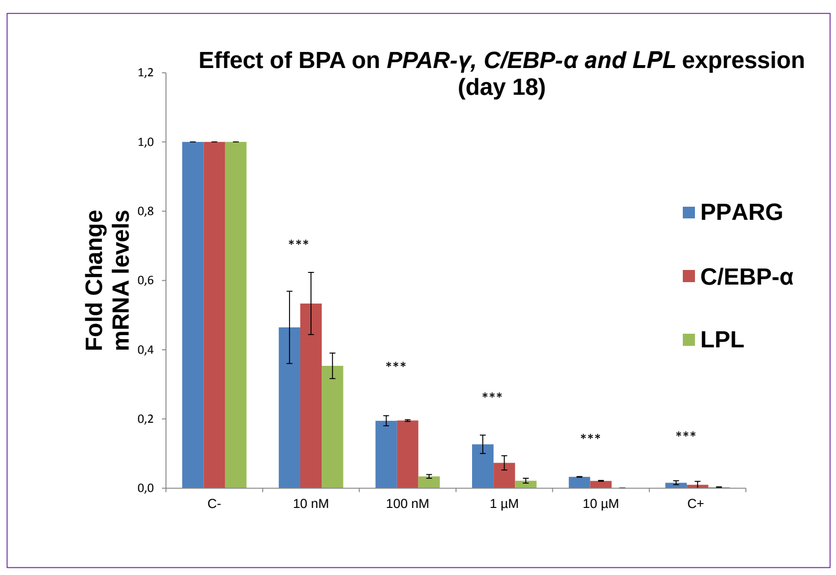
Methods: Serial doses of each compound (10nM- 100nM- 1µM- 10µM) were tested along the progressive maturation of hADSC for 18 days after culture in adipogenic differentiation media [dexamethasone (DEX), isobutil-metil-xantina (IBMX) and insulin (INS)]. The extent of adipogenic differentiation was assessed by staining intracellular lipid content with Oil Red O assay and quantified by optical density measurement of the retained dye at 520 nm. Gene expression of key adipogenic and lipid regulators, such as PPAR-γ, C/EBPα, LPL and FABP-4 was also assessed by qRT-PCR. Statistical analysis was performed using SPSS (22 version) by ANOVA adjusted by Bonferroni. Data are presented as mean±SD. *p<0.05, **p<0.01, and ***p<0.001 values were considered statistically significant.

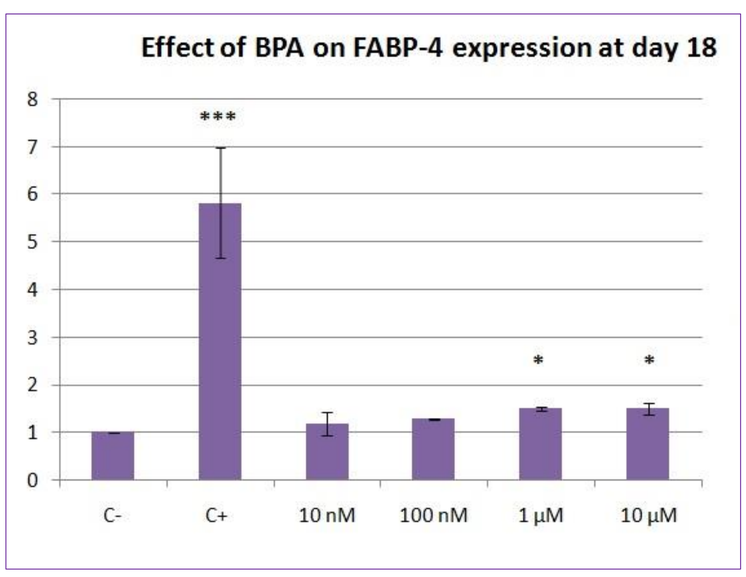






Negative control: DEX + IBMX + INS
Positive control: DEX + IBMX + INS + ROSIGLITAZONE





Discussion/Conclusion: PPAR-γ and C/EBP-α are mid-late genes crucial for the adipogenic differentiation process. Then, the expression of late genes as FABP4, among others, will complete the development of a mature and functional adipocyte phenotype. Our results support the obesogenic potential of BPA described by other authors and raise the concern about the use of BPF and BPS as candidate substitutes. Since BPA average urine levels are precisely 10 nM, the confirmation of these findings could be of great importance to public health.

Results: Oil Red assay showed that BPA and its congeners significantly enhanced adipogenesis in hADSC at low environmentally relevant concentrations, such us 10nM. BPA and BPF, subtly but significantly, promoted adipogenesis even at the lowest concentration tested (10nM) (BPA ≈ BPF > BPS). Our results showed a dose-dependent inverse relationship between the gene expression of ***PPAR-γ, C/EBP-α,*** and ***LPL*** of BPA-treated adipocytes (day 18/fully differentiated adipocytes). However, ***FABP4*** expression was significantly up-regulated (for 1µM and 10µM) at day 18 of differentiation.

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