Obesogenic endocrine disrupting chemicals? Testing this hypothesis with two compounds (26 D-LAB)

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Introduction

Although obesity is mainly considered to be caused by overeating and lack of physical activity in combination with a genetic predisposition, these conditions by alone can not account for the current disease trends. This led to hypothesis that other factors could be involved in the phenomenon of increasing obesity worldwide. Endocrine disrupting chemicals (**EDCs**) have been recently linked to metabolic syndrome and obesity and a subset of them apparently promotes adiposity. Until now, the majority of *in vitro* studies were done using murine cell lines grown in undefined media containing fetal bovine serum (**FBS**). This is far to reproduce *in vivo* conditions and therefore we tested two "standard" obesogenic EDCs on primary human adipose stem cells (**phASCs**) cultured in chemically defined xeno- and serum-free media.

Methods & Results

After a short period of expansion in defined conditions, phASCs were cultured in specially formulated xeno- & serum-free white adipose tissue (**SF-WAT**) induction medium with or without the addition of Bisphenol A (**BPA**) or Phthalate (**Pht**). After 10 days, the pro- or anti-adipogenesis effects of these two EDCs were monitored by analyzing the expression of typical cell surface antigens (FACS analysis), by quantitative RT-PCR, and by histochemical methods to quantify the amount of accumulated lipids.

Results

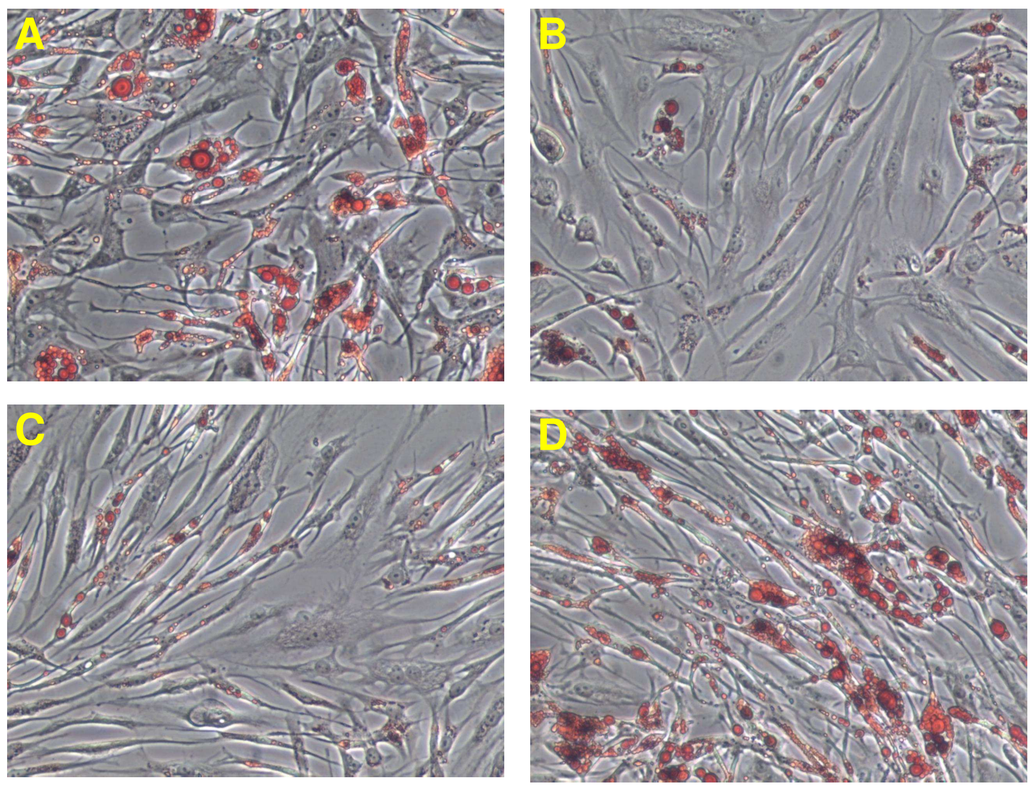

Figure 1

Representative phase contrast microphotograph of hASCs after 10 days of adipogenic induction and illustrating lipids accumulation. Cells were fixed with formaldheyde solution and stained with Oil-Red O. **A).** Xeno- & serum-free WAT induction medium (SF-WAT) only. **B).** SF-WAT medium supplemented with BPA **C).** SF-WAT medium supplemented with Pht. **D).** SF-WAT medium supplemented with β-Estradiol.

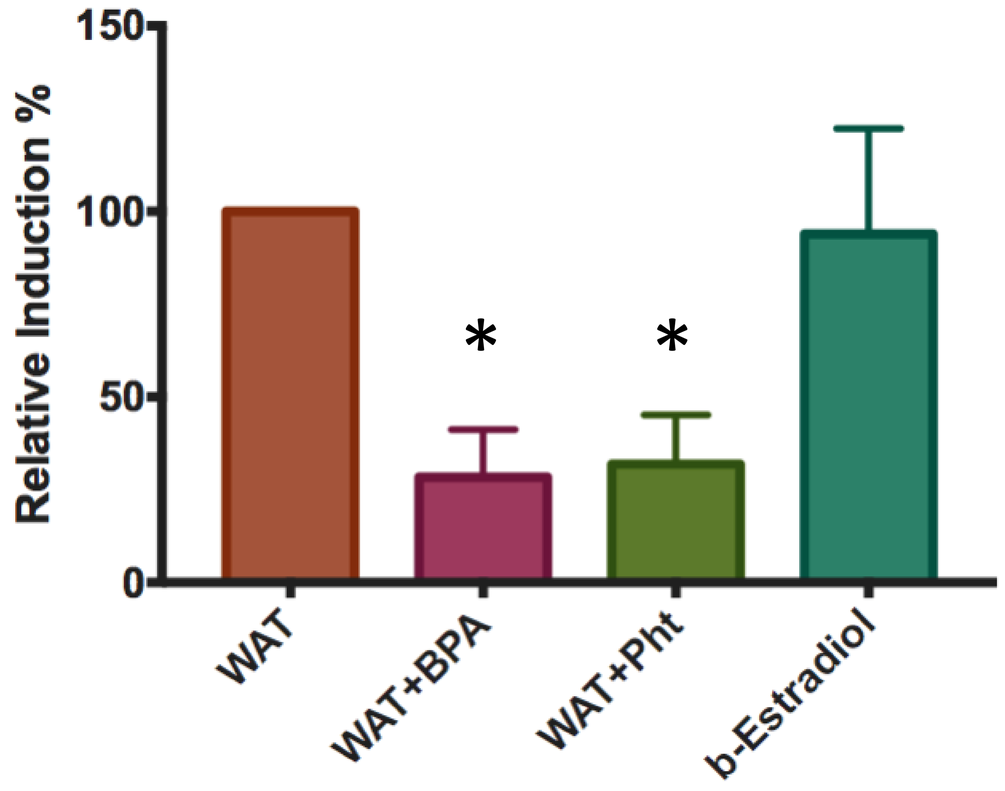

Figure 2

Evaluation of the adipogenic induction by Oil-Red-O quantification after extraction with isopropanol. Data are related to the SF-WAT induction medium without EDCs (100%). Each extracted Oil-Red-O sample is normalized by the number of cells present in the well (DAPI staining and nuclei counting). Graph show the relative induction average ± SD of different patients. There ic a significance reduction in the amount od extracted red dye in the two samples treated with the EDCs.

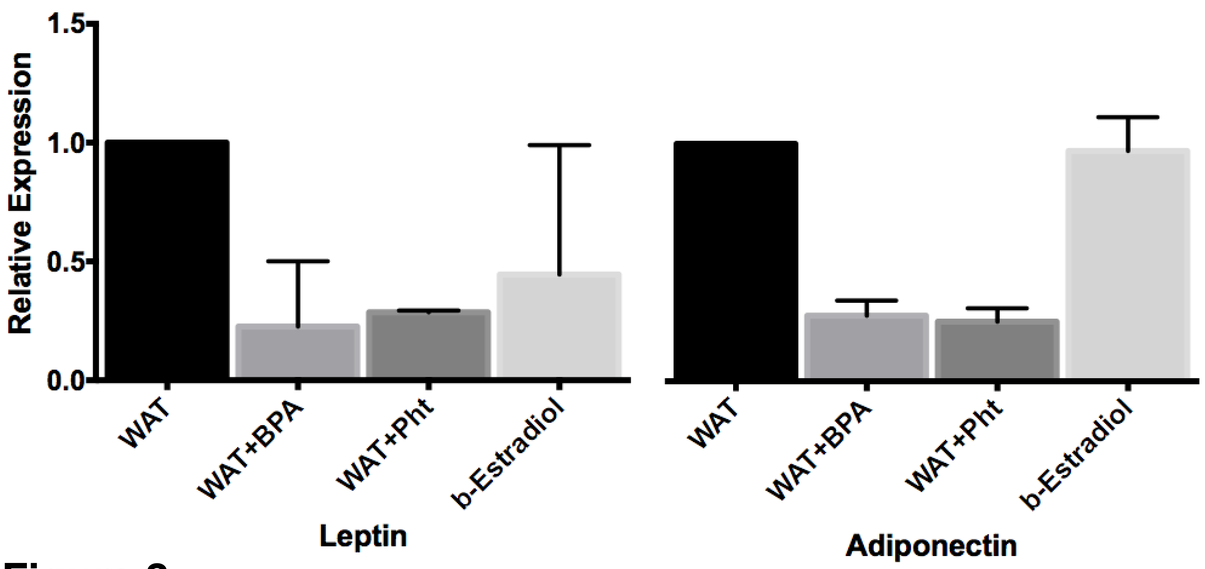

Figure 3

RT-qPCR analysis. In addition to the differences regarding typical pre-adipocytes signature genes (data not shown), there is a significant reduction also among two classical adipokines (Leptin & Adiponectin) between hASC cultured with only SF-WAT or with SF-WAT + BPA or + Pht .

Conclusion

Until now, most of the *in vitro* experiments have been done using murine tumor cell lines (e.g., 3T3-L1), in the presence of FBS and phenol red (itself an EDCs…). With these experimental conditions, EDCs acted normally as pro-adipogenic substances. On the other hand our results, obtained with phASCs cultured & induced in chemically defined xeno- & serum-free media, suggest that BPA & Pht reduce the accumulation of lipids. We conclude that the "pro-obesity" effect claimed for EDCs is much more difficult to explain than initially hypothesized and it will require numerous other experiments. It will be interesting to verify how the ASCs of the visceral adipose tissue will react in the presence of BPA and Pht. Subcutaneous and visceral adipose tissue derive from two different types of the lateral mesoderm. Perhaps this difference will also be reflected in their reactivity towards steroids hormones and EDCs.