



Comment on "In vitro effects of bisphenol A β -D-glucuronide (BPA-G) on adipogenesis in human and murine preadipocytes".

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Comment on “In Vitro Effects of Bisphenol A β -D-Glucuronide (BPA-G) on Adipogenesis in Human and Murine Preadipocytes”

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Boucher et al. reported that treatment of 3T3L1 preadipocytes with 10 μ M bisphenol A β -D-glucuronide (BPA-G) induced a significant increase in lipid accumulation, in mRNA expression of the adipogenic markers sterol regulatory element binding factor 1 (*SREBF1*) and lipoprotein lipase (*LPL*), and in protein levels of *LPL*, *aP2*, and *adipsin*. They concluded that their study was the first to show that BPA-G induced adipocyte differentiation and was not simply an inactive metabolite.

To justify the relevance of their 10- μ M effective BPA-G concentration, they claimed that this tested concentration was within the range found in human fluids. To support their statement, they cited Harthé et al. (2012), who reported an average BPA-G concentration of 4.64 μ g/L in human urine samples but wrongly converted this figure to a urine concentration of 11.5 μ M (their effective *in vitro* concentration) rather than to the correct figure of 11.5 nM, i.e., a value 1,000 times lower.

Apart from this error, it should be stressed that the plasma concentration and not the urine concentration is the driving concentration to explain any systemic effect. As the estimated ratio of total BPA (sum of BPA and its metabolites) concentration between serum and urine was less than 0.045 (Teeguarden et al. 2013), it can be concluded that the 10- μ M *in vitro* effective BPA-G concentration was several orders of magnitude higher than what would be expected to be measured in the general population.

Nevertheless, the fact that such high *in vitro* BPA-G displayed an estrogenic action merits some attention and was not totally unexpected. It should be recognized that BPA-G can be back-converted to BPA (aglycone) thanks to the ubiquitous presence of β -glucuronidases in many organs and bodily fluids (Sperker et al. 1997). Such a deconjugation process was reported to account for the estrogenic effect of soy isoflavone glucuronides on breast cancer cell lines (Islam et al. 2015). The likelihood that such a mechanism underlies the effects of BPA-G is supported by the *ex vivo* demonstration that BPA-G is readily converted back to its

parent compound in ovine gonads, BPA conjugation–deconjugation cycling favoring BPA-G hydrolysis (Corbel et al. 2015). Nishikawa et al. (2010) also observed deconjugation of BPA-G in fetal liver cells, suggesting that BPA-G can enter cells and that endogenous glucuronidases may convert BPA-G to its aglycone form, about 5% of BPA-G being reactivated after 2 hours. More generally, the lack of stability of BPA-G in the presence of rodent fetal tissue homogenates has been reported (Waechter et al. 2007). Thus, it can be hypothesized that BPA-G deconjugation by the preadipocytes could result in sufficiently high residual BPA concentrations to trigger the effects reported by Boucher et al. This issue could be easily explored by analyzing the extracellular medium and the intracellular BPA content of preadipocyte cells after 48 hours of exposure.

The authors declare they have no competing financial interests.

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Response to “Comment on ‘In Vitro Effects of Bisphenol A β -D-Glucuronide (BPA-G) on Adipogenesis in Human and Murine Preadipocytes’”

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Our paper was designed to investigate the possibility that the main metabolite of bisphenol A (BPA), BPA β -D-glucuronide (BPA-G), has biological activity distinct from estrogenic activity. Our results clearly show there are effects at the cellular level in human and mouse preadipocytes starting at a concentration of 0.01 μ M and peaking at 10 μ M (see Figures 1 and 3).

The concentrations used in this study are often used *in vitro* for evaluation of estrogenic activity (Matthews et al. 2001), and concentrations in the range of the ones where we saw significant effects in human preadipocytes (0.05 μ M; see Figure 3) were reported in human samples. The measurement 11.5 μ M was a typo and should be 11.5 nM. We apologize for the error, which has been corrected in the final article.

We agree that concentrations of plasma, not urine, are to be considered. However, the methodology to accurately assess BPA-G in serum or other bodily fluids is still developing (Kosarac et al. 2012), and to measure the intracellular concentrations is beyond our laboratory’s capabilities. Nevertheless, this paper was not designed to answer the question of whether urine concentrations are correlated to plasma or serum concentrations, but rather to assess the basic question of whether BPA-G has biological activity in our *in vitro* models. The answer was yes and at nanomolar concentration in human preadipocytes. However, it is extremely difficult to correlate *in vitro* concentrations to the *in vivo* situation.

We feel, though, that Gayraud et al. have seriously misinterpreted our article and its message. We did not find, nor did we claim, that BPA-G has estrogenic activity. As a matter of fact, we could not show direct estrogenic activity in ERE-luciferase assays when the cells were treated with BPA-G. That was the case in Cos-7 cells (see Figure 4) and 3T3L1 cells (data not shown) after 48 hours of BPA-G treatment. However when the same cells, in the same experiment, were treated with free BPA, we could readily detect estrogenic activity at concentrations as low as 10 nM. If the conjugation had been reversed intracellularly, one would have expected to see some estrogenic activity when the cells were treated with BPA-G; however, we detected no such activity in any of the cells.

In addition, even if the cells and the adipose tissue could deconjugate BPA-G into free BPA, the implication is the same—that BPA-G has biological activity.

The authors declare they have no actual or potential competing financial interests.

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