

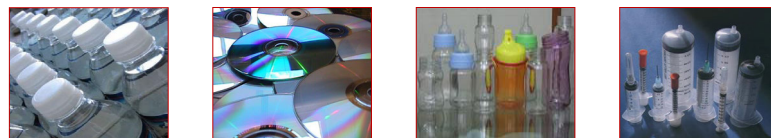
Background

Human exposure to synthetic chemicals has increased dramatically over the past half century, concurrently with the increase in obesity (Baillie-Hamilton, 2002).

Recent findings have shown the possible involvement of environmental obesogens, chemicals that can disrupt homeostatic energy balance and increase adiposity. One such a chemical is bisphenol-A (BPA). Studies have shown that mice exposed to BPA in utero or postnatally are fatter as adults.

BPA is a ubiquitous environmental contaminant with estrogenic properties. BPA is used in the manufacture of polycarbonate plastics and resins, which are commonly used in the packaging of canned foods and in beverage bottles. BPA may be ingested by humans, as it reportedly leaches from the lining of food and soda cans, polycarbonate bottles, and dental sealants.

Products containing BPA



We hypothesized that BPA exposure exerts effects on liver mitochondria. Considering the central role of the liver in fat and carbohydrate metabolism, it is possible that BPA alters energy homeostasis in a manner that promotes obesity

Aims

Determine the effects of BPA on metabolism and mitochondrial function by measuring the oxygen consumption rate (OCR), and ECAR in primary rat hepatocytes and Huh-7 cells using an XF24 analyzer (Seahorse Bioscience)

Determine the effects of BPA on rat hepatocyte and cell viability

Methods

Hepatocyte preparations. Rat primary hepatocytes were isolated as previously described. The viability of hepatocytes at isolation was 92±1.8%.

Measurement of Oxygen Consumption Rate (OCR). OCR in rat hepatocytes and Huh-7 cells was determined using an XF24 analyzer (Seahorse Bioscience, Billerica, MA). Hepatocytes were adhered to collagen-coated V7 plates (Seahorse Bioscience) for 24 hr, allowed to attach for 5 h, and then exposed to various concentrations of BPA (0, 1, 10, and 100 μ M) for 12 h before measuring OCR

Cellular mitochondrial function was measured as described previously using sequential injections of oligomycin (1 μ g/mL), carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP, 0.3 μ M), and antimycin A (10 μ M). The concentrations used were determined by titrating to yield their optimal effects (data not shown)

Results

Figure 1: Experimental design

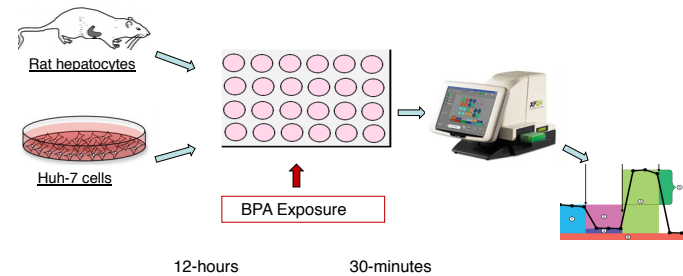


Figure 2: Oxygen consumption rate in rat hepatocytes exposed to several BPA concentrations (0, 1, 10 and 100 μ M) for 12-hours. Values represent mean OCR values \pm SE. * p <0.05 compared with corresponding controls. Results shown from 3 independent experiments

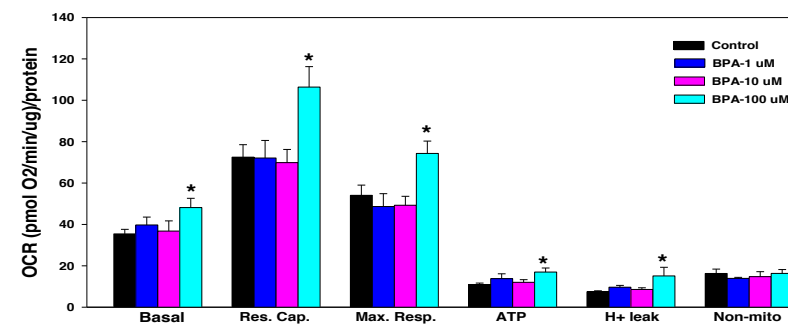


Figure 3: Oxygen consumption rate in rat hepatocytes exposed to several BPA concentrations (0, 1, 10 and 100 μ M) for 30 minutes. Values represent mean OCR values \pm SE. * p <0.05 compared with corresponding controls. Results shown from 2 independent experiments

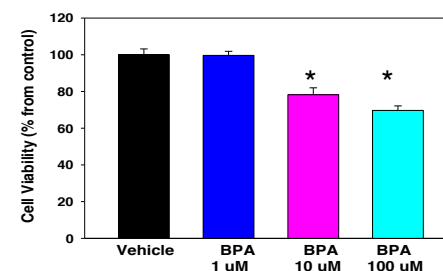
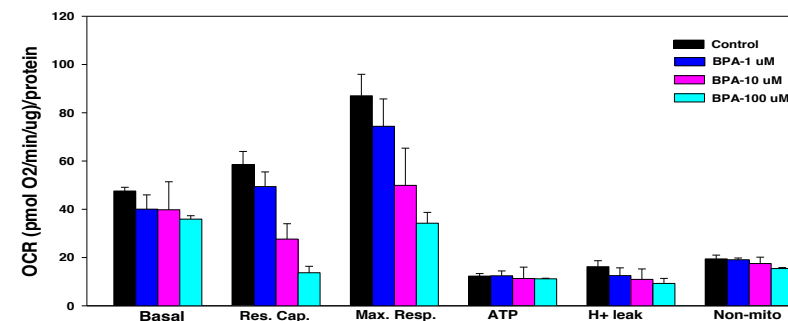


Figure 4: Cell viability determined with the MTT reagent in rat hepatocytes exposed to several BPA concentrations (0, 1, 10 and 100 μ M) for 12 hours. Values represent mean OCR values \pm SE. * p <0.05 compared with control

Figure 5: Extracellular acidification rate (ECAR) in rat hepatocytes exposed to several BPA concentrations (0, 1, 10 and 100 μ M) for 30-min. Values represent mean ECAR values \pm SE. Results shown from 2 independent experiments

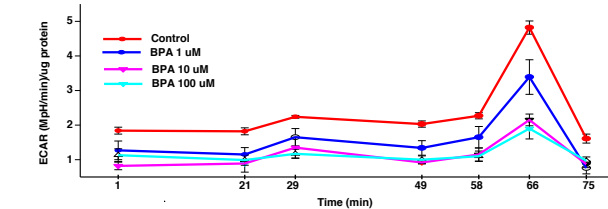


Figure 6: Oxygen consumption rate in Huh-7 cells exposed to several BPA concentrations (0, 1, 10 and 100 μ M) for 12 hours. Values represent mean OCR values \pm SE. Results shown from 2 independent experiments

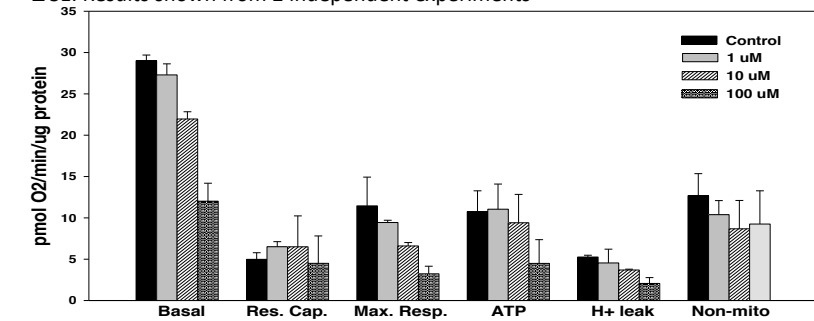
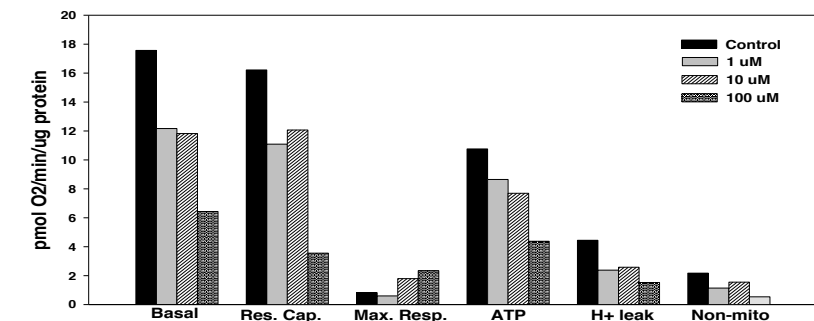


Figure 7: Oxygen consumption rate in Huh-7 cells exposed to several BPA concentrations (0, 1, 10 and 100 μ M) for 30 minutes. Values represent mean OCR values.



Conclusions

- OCR was significantly increased in rat hepatocytes following 12-hour exposure to only the highest BPA concentration (100 μ M). However, short-term exposure to BPA (30-min) induced a dose-dependent decrease in OCR, and a dose-dependent increase in ECAR.

-Possible mechanisms involved in these differential effects of BPA could include effects on mitochondrial biogenesis, and activation of mitochondrial estrogen receptors following the 12-hour exposure to BPA.

-Exposure to BPA disrupts hepatic mitochondrial bioenergetics and therefore, could potentially alter energy homeostasis