

**UNIVERSITI TEKNOLOGI MARA**

**THE IMPACT OF BISPHENOL A  
EXPOSURE IN UTERO AND  
POSTNATAL TRANS FAT DIET ON  
SMALL INTESTINE GLOBAL DNA  
METHYLATION AND GUT  
MICROBIOME OF SPRAGUE  
DAWLEY RATS**

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## ABSTRACT

A multitude of evidence has pointed out the repercussions of prenatal bisphenol A (BPA) exposure with a postnatal high fat diet on offspring's health. However, the information on how the interaction between these two variables affects the small intestinal cells and the gut microbiome is rather limited. Hence, we investigated the impact of postnatal trans fat diet (TFD) on the small intestinal morphology and global DNA methylation, as well as the gut microbiome of offspring exposed and non-exposed to prenatal BPA. Eighteen pregnant rats were administered with either unspiked control (CTL), vehicle control (P80) or BPA drinking water throughout pregnancy. Twelve weaned pups from each pregnancy group were given either normal diet (ND) or TFD from the postnatal week (PNW) 3 till PNW14; divided into CTLND, CTLTFD, P80ND, P80TFD, BPAND, and BPATFD groups. Body weight (BW), waist circumference (WC), food and water intake were measured weekly in offspring. At PNW14, small intestines were collected for global DNA methylation and histological analyses. Faecal samples were collected on day 50 and day 100 to differentiate the offspring's intestinal flora composition. Marked differences in BW and food intake were observed starting at PNW9, which persisted until PNW13 ( $p < 0.0001-0.05$  and  $p < 0.001-0.05$ , respectively). In contrast, the WC of offspring was only significantly different at PNW13 ( $p < 0.01$ ), whereas water intake among offspring was significantly different ( $p < 0.05$ ) from an earlier age (PNW5). Furthermore, substantial differences in the general parameters of the intestinal structures were exclusive to ileum crypt length alone, whereby BPA-ND ( $150.5 \pm 5.1 \mu\text{m}$ ;  $p < 0.001$ ) was significantly longer than CTLND ( $96.8 \pm 8.9 \mu\text{m}$ ). Moreover, BPAND ( $2.898 \pm 0.147\%$ ;  $p < 0.05$ ) and BPATFD ( $2.711 \pm 0.258\%$ ;  $p < 0.05$ ) demonstrated global small intestinal hypermethylation when compared to P80ND ( $0.991 \pm 0.409\%$ ). The alpha diversity indices of TFD offspring across all groups were markedly lower than their ND counterparts ( $p < 0.001-0.05$ ). The beta diversity, hierarchical cluster and network analyses of offspring's microbiome demonstrated that the microbiome species of the TFD group were distinct compared to the ND group. Consistently, TFD and ND offspring pairings exhibited a higher number of significantly different species ( $p < 0.0001-0.05$ ) compared to those of prenatal exposure and different life stages comparisons, as shown by multivariate parametric analysis DESeq2. Predictive functional profiling of the offspring's intestinal flora demonstrated altered expressions of genes involved in the metabolic pathways. Altogether, prenatal BPA exposure may significantly affect offspring's small intestinal function, whereas physiological parameters and gut flora are affected by the postnatal diet. The interaction between the perturbed function of the small intestine and the gut microbiome in the BPATFD group may hypothetically converge into obesity in middle or late adulthood, which requires extensive investigation.

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