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

SARAH BINTI ZULKIFLI

Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Medicine)

Faculty of Medicine

January 2023

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±5.1μm; $p<0.001$) was significantly longer than CTLND (96.8±8.9μm). Moreover, BPAND (2.898±0.147%; $p<0.05$) and BPATFD (2.711±0.258%; $p<0.05$) demonstrated global small intestinal hypermethylation when compared to P80ND (0.991±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.

ACKNOWLEDGEMENT

First and foremost, I would like to express my undivided gratefulness to Allah SWT for giving me the opportunity to embark on my PhD and for completing this long and challenging journey successfully. Alhamdulillah.

My heartfelt gratitude and appreciation go to my supervisors and role models, Associate Professor Dr. Noor Shafina Mohd Nor and Associate Professor Dr. Siti Hamimah Sheikh Abdul Kadir, for their unwavering support of my study and research, as well as their patience, encouragement, passion, and vast knowledge. Their guidance was invaluable during the research and writing of this thesis. Special thanks to my co-supervisor, Dr. Norashikin Mohd Ranai, for her guidance, advice and help.

Thank you to Associate Professor Dr. Noor Kaslina Mohd Kornain, Dr. Mardiana Abdul Aziz, Dr. Wan Nor I'zzah Wan Mohamad Zain, and Puan Juriah Togeman. Your expertise and assistance provided throughout my PhD journey are highly appreciated.

My appreciation goes to all IMMB staff, Puan Salina Othman and Encik Yusri Idorus, as well as to postgraduate students at IMMB, for their help and contributions to my research. Also, not to forget, I could not have completed this research and thesis without the continuous support of my friends in UiTM Sungai Buloh, and my siblings, Yumnaa and Rumaisa, who are always there for me.

Finally, this thesis is dedicated to my mother, [redacted] and the loving memory of my very dear late grandparents, Juriah and Abdul Halim, as well as my late father, Zulkifli Abd Khir, for their love and support throughout my life. This piece of victory is yours. Alhamdulilah.

TABLE OF CONTENTS