Effects of Perigestational Exposure to Chlorpyrifos and/or High Fat Diet on Sleep Apnea in Young Adult Rats

Hiba El Khayat El Sabbouri/ Wissam H. Joumaa
Laboratoire Rammal Hassan Rammal, équipe de recherche PhyToxE,
Faculté des Sciences, Université libanaise
Nabatieh, Lebanon
E-mails: hiba.sabboury@outlook.com; wjoumaa@ul.edu.lb

Walaa Darwiche
Hematim Laboratory, EA4666,
University of Picardy Jules Verne,
Amiens, France
E-mail: walaa.darwiche@u-picardie.fr

Jérôme Gay-Quéheillard/ Véronique Bach/ Marion Guibourdenche/ Narimane Djekkoun
PERITOX UMR-I-01
University of Picardy Jules Verne
Amiens, France.
E-mails:{jerome.gay; veronique.bach}@u-picardie.fr, marion.guibourdenche@outlook.fr
djekkoun.narimane@gmail.com

Wiam Ramadan
Lebanese Institute for Biomedical Research and Application (LIBRA),
International University of Beirut (BIU) and Lebanese International University (LIU)
Beirut, Lebanon
E-mail: wiam.ramadan@liu.edu.lb

***Abstract*-Chlorpyrifos (CPF) is an organophosphorus pesticide widely used in the world, which acts by inhibiting acetylcholinesterase (AChE). Exposure to this compound is harmful to the respiratory system during *in utero* and postnatal period. Such perturbations during prenatal and intrauterine life are associated with further diseases at adulthood through fetal programming. Some of these disturbances are linked to food composition and environmental pollutants. The aim of this study is to examine the effects of perigestational exposure to CPF and to a High-Fat Diet (HFD) on the occurrence of sleep apnea in young adult rats. Female rats were exposed for four months before and later during gestation and lactation periods to CPF (1mg/kg/day *vs* vehicle) with or without HFD. *In vivo* measurements of sleep apnea were performed by whole-body plethysmography for male pups at post-natal day (PND) 60. Then diaphragm, an essential respiratory muscle, were dissected and used for *in vitro* measurements of AChE activity assessment. The perigestational exposure to low dose of CPF and/or HFD induced an increase in the sleep apnea index in males at early adulthood, which was associated with a significant decrease in the AchE activity compared to controls. In conclusion, the chronic perigestational exposure to CPF combined with HFD feeding is associated with increased sleep apnea occurrence and reduced AchE activity at early adulthood in rats. Other studies are required to investigate the mechanisms underlying respiratory perturbations during development due to early life perturbations.**

Keywords- Chlorpyrifos; high-fat diet; sleep apnea; diaphragm.

I. INTRODUCTION

The perinatal period is characterized by high plasticity of the physiological systems, exposing the individual to higher vulnerability to his environmental factors. Then, any disruption in these physiological processes is described in the concept of Developmental Origin of Health and Diseases (DOHaD) **[**1**]**.

Maternal obesity constitutes an environmental risk favoring the occurrence of obesity or type 2 diabetes at adulthood [2][3].

Organophosphate (OP) insecticides are compounds commonly used for a variety of agricultural, industrial, and household applications [4] and are detected in food and drinking water [5]. They are potent AChE inhibitors resulting in the accumulation of acetylcholine at cholinergic synapses and consequent overstimulation of the central nervous system and neuromuscular junctions [6]. Recently, it has been shown that exposure to Carbofuran, an AChE inhibator, is positively associated with sleep apnea in US Farmers [7]. Central respiratory failure associated with apnea is considered to be the major cause of death following OP poisonings [8]-[10]. CPF, an OP pesticide, is suspected to affect the metabolic programming from the fetal period until adulthood [11]. Pre- and postnatal exposure to CPF has been shown to increase sleep apnea index during development [12]. However, continuous perigestational exposure for four months, before gestation till the end of lactation, without exposing the rats directly to CPF during early adulthood has not been examined.

It has been shown that sleep apnea is associated with moderate to severe levels of obesity [13]. Studies in obese populations have reported an association between metabolic dysfunctions and sleep apnea [14]. Furthermore, the increase in the incidence of apnea during sleep has been shown to be dependent on the metabolic disturbances such as insulin resistance induced by High-Fat Diet (HFD) feeding in rats despite the absence of obesity as reported by Ramadan et al. 2006 [15].

In this context, the present study aimed at determining the effects of long term perigestational exposure to CPF and/or HFD, 4 months before gestation and throughout gestational and lactational periods, on the occurrence of sleep apnea in young adult rats who were not exposed directly to CPF or HFD. Also, we measured the AChE

 ISBN: 978-1-61208-742-9

activity in the diaphragm, an essential respiratory skeletal muscle. The paper procedes as follow: Section II describes the exparimental design, data are analysed in Section III, and, finally, Section IV presents the discusion and draws the conclusions.

II. MATERIALS AND METHODS

This section describes the methodology and the experimental tools used.

A. Experimental Design

Female Wistar rats (age on arrival: 7 weeks) receiving a standard diet or HFD (60% kcal from fat) were force-fed with CPF (1 mg/kg/day) *vs* vehicle (rapeseed oil) for four consecutive months. Then, the females were mated with the male rats. During gestation, the females were subjected to the same treatment as before gestation until the end of the lactation period. At weaning; postnatal day (PND) 21, pups were separated from their mothers and four groups of male rats (n=7 per group) were identified according to their maternal exposure into: Control group (standard diet and vehicle), HFD group (HFD and vehicle), CPF group (standard diet and CPF), and HFD+CPF group (HFD and CPF). After weaning, the pups received only a standard diet without CPF until the PND60. Whole-body plethysmography was used to score apnea index during sleep for all male pups. Only male rats were selected for this study since female rats were used in another study.

B. Protocol for sleep apnea assessment

Each animal was familiarized for 60 min to the plethysmograph chamber during two consecutive days before the onset of measurements. On the second day, during the 60 min of measurement, behavioral observations were performed. We continuously noted whether the rat was awake (i.e., lying or standing with opened eyes) or was asleep (i.e., lying down without movements with closed eyes). Sleep apneas were scored using a previously described procedure, i.e., defined as two missed breaths [16]. Sleep apnea indexes were calculated as the number of apneas/h of behavioral sleep.

C. Acetylcholinesterase activity measurement

The Acetylcholinesterase activity in the diaphragm dissected from the midcostal region was measured according to the modified Ellman method [17]. The diaphragm samples were homogenized in lysis buffer (Abcam) and then centrifuged. 1 µl of protease inhibitor (Abcam) was added to the supernatant. The supernatant (diluted 1:2) was incubated with 100 µm of the butyryl cholinesterase inhibitor, tetra isopropyl pyrophosphoramide (Sigma-Aldrich) for 15 minutes before measuring AchE activity using the AChE colorimetric assay kit (ab138871; Abcam) according to the manufacturer's instructions. The AchE activity is expressed in µmol/min/mg protein.

D. Statistical Analysis

A two-way analysis of variance (ANOVA) was used to study the main effects of diet (standard diet/HFD) or exposure (Control/CPF) and the interaction between diet and CPF exposure. If a significant interaction was found, a non-parametric Mann-Whitney U test was then performed. Statistical significance was set to $p<0.05$ and indicative results ($p<0.1$) were shown when needed.

III. RESULTS

The main results obtained are presented in this section.

A. Sleep apnea index

A significant increase in sleep apnea index with either maternal CPF exposure ($p<0.05$) or HFD feeding ($p<0.05$) was observed. Indeed, there was a significant increase in the sleep apnea index by 96% in each of CPF group ($p<0.01$) and HFD group ($p<0.01$) and by 88% in CPF+HFD group ($p<0.05$) compared to the control group (Figure 1).

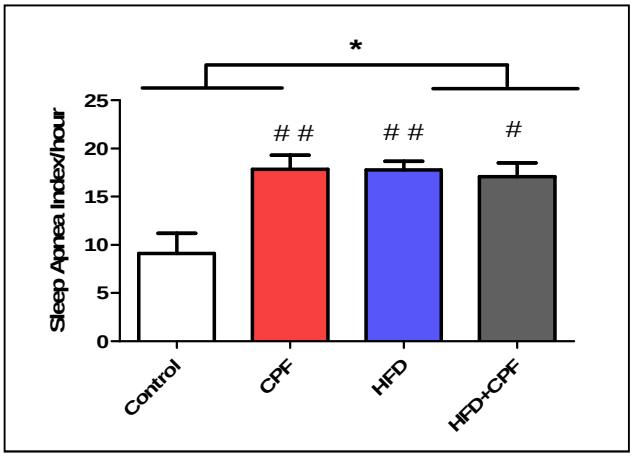

Figure 1. Effects of perigestational exposure to CPF and HFD on the sleep apnea index. Data are quoted as means ± SEM. Effect of diet *: $p<0.05$. CPF x diet interaction: #: $p<0.05$; # #: $p<0.01$ vs. control.

B. Acetylcholinesterase Activity

AchE activity levels were significantly reduced in CPF group ($p<0.05$) and HFD group ($p<0.05$) by 53% and by 29% respectively compared to controls. A significant decrease was also reported in CPF+HFD group by 40% ($p<0.05$ vs control) (Figure 2).

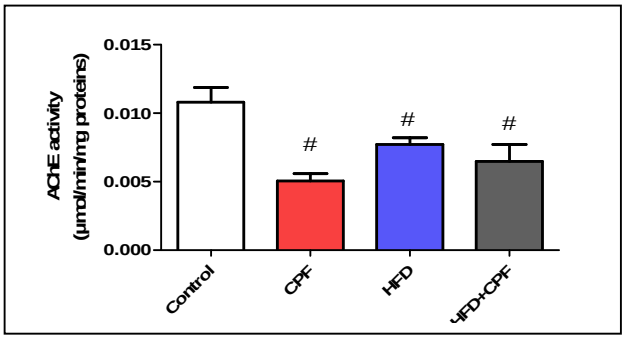

Figure 2. Effects of perigestational exposure to CPF and HFD on AchE activity. Data are presented as means ± SEM (n=7-10/group). CPF x diet interaction: #: $p<0.05$ vs. control.

 ISBN: 978-1-61208-742-9

IV. DISCUSSION AND CONCLUSION

This study is in line with the DOHaD concept. Since fetal development depends mainly on the maternal nutritional supply, then any disruption in the intrauterine milieu could predispose offspring to further diseases in later life. The present study was designed to evaluate, for the first time, the impacts of long term maternal exposure to two major alimentary risk factors; junk food and OP pesticide residues on the respiratory performance during early adulthood. The perigestational effects were studied through continuous exposure of female rats to CPF (1 mg/kg/day) and HFD (60% kcal from fat) four months before gestation till the end of lactation period at the PND21 and without exposing the pups after PND21 to neither CPF nor HFD.

The present study showed that perigestational exposure to CPF and/or HFD is associated with increased sleep apnea index. An increase of 67% in the sleep apnea index was reported in developing rats that are pre and postnatally exposed to CPF [12]. In our study, the increase in the sleep apnea was higher (around 100%) in CPF and/or HFD exposed rats. This incremental increase can approve the concept of DOHaD where maternal exposure to environmental toxicants can affect the life of the offspring even if they are not exposed to the same toxicants as shown in our study. The apnea episodes observed following OPs exposure can be attributed to either central effects caused by the AChE inhibition in the respiratory centers [18][19] or to peripheral effect resulting from airway obstruction [20]. On the other hand, HFD has been shown to increase apnea incidence in sleeping rats as a result of the induced metabolic disruptions [15].

The increase in the sleep apnea index could be linked to the reduced AchE activity in the diaphragm resulting in the overstimulation of the motor end plates. Obesity can affect the muscle force production by altering fiber type composition of the muscle and by disrupting calcium cycling mediated by the major calcium channels, ryanodine receptor and SERCA pump [21][22].

In conclusion, the maternal exposure of rats to CPF and/or HFD during the perigestational period alters the respiratory performance in their offspring at early adulthood despite they are not in direct contact to either CPF and/or HFD. Further studies are needed to determine the mechanisms underlying respiratory perturbations during development due to early life disturbances. The integration of this study can help in the prevention of chronic respiratory diseases in adults.

REFERENCES

[1] M. Kappil, R. O. Wright, and A. P. Sanders, "Developmental Origins of Common Disease: Epigenetic Contributions to Obesity," Annul Rev. Genomics Hum. Genet., 17, pp. 177–92, 2016.

[2] M. Z. Alfaradhi, et al., "Maternal Obesity in Pregnancy Developmentally Programs Adipose Tissue Inflammation in Young, Lean Male Mice Offspring," Endocrinology, 157(11), pp. 4246–56, 2016.

[3] S. Lecoutre, et al., "Depot- and sex-specific effects of maternal obesity in offspring's adipose tissue," J Endocrinol., 230(1), pp. 39–53, 2016.

[4] M. Maroni, C. Colosio, A. Ferioli, and A. Fait, "Biological Monitoring of Pesticide Exposure: a review. Introduction," Toxicology, 143(1), pp. 1–118, 2000.

[5] H. G. Bolles, et al., "U.S. market basket study to determine residues of the insecticide chlorpyrifos," J. Agric. Food Chem., 47(5), pp. 1817–22, 1999.

[6] B. Ballantyne, Clinical and Experimental Toxicology of Organophosphates and Carbamates [retrieved: July, 2019]. Available from: https://books.google.com/books/about/Clinical_and_Experimental_Toxicology_of.html?id=zgslBQAAQBAJ

[7] B. O. Baumert, et al., "Sleep apnea and pesticide exposure in a study of US farmers," Sleep Health, 4(1), pp. 20-26, 2018.

[8] Y. Lerman and H. Gutman, "The use of respiratory stimulants in organophosphates' intoxication," Med. Hypotheses, 26(4), pp. 267–9, 1998.

[9] M. Yamashita, M. Yamashita, J. Tanaka, and Y. Ando, "Human mortality in organophosphate poisonings," Vet. Hum. Toxicol., 39(2), pp. 84–5, 1997.

[10] P. M. Bartholomew, G. Gianutsos, and S. D. Cohen, "Differential cholinesterase inhibition and muscarinic receptor changes in CD-1 mice made tolerant to malathion," Toxicol. Appl. Pharmacol., 81(1), pp. 147–55, 1985.

[11] T. A. Slotkin TA, "Does early-life exposure to organophosphate insecticides lead to prediabetes and obesity?," Reprod. Toxicol., 31(3), pp. 297–301, 2011.

[12] W. Darwiche, et al., Impact of chronic exposure to the pesticide chlorpyrifos on respiratory parameters and sleep apnea in juvenile and adult rats," PLOS ONE, 13(1): e0191237, 2018.

[13] A. N. Vgontzas, E. O. Bixler, and G. P. Chrousos, "Sleep apnea is a manifestation of the metabolic syndrome," Sleep Med. Rev. 9(3), pp. 211–24, 2005.

[14] K. P. Strohl KP, et al., "Insulin levels, blood pressure and sleep apnea," Sleep, 17(7), pp. 614–8, 1994.

[15] W. Ramadan, et al., "Sleep apnea is induced by a high-fat diet and reversed and prevented by metformin in non-obese rats," Obes. Silver Spring Md., 15(6), pp. 1409–18, 2007.

[16] D. W. Carley and M. Radulovacki, "Mirtazapine, a mixed-profile serotonin agonist/antagonist, suppresses sleep apnea in the rat," Am. J. Respir. Crit. Care Med., 160(6), pp. 1824–9, 1999.

[17] G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Feather-Stone, "A new and rapid colorimetric determination of acetylcholinesterase activity," Biochem. Pharmacol., 7, pp. 88–95, 1961

[18] J. L. Carey, C. Dunn, and R. J. Gaspar, "Central respiratory failure during acute organophosphate poisoning," Respir. Physiol. Neurobiol., 1;189(2), pp. 403–10, 2013.

[19] R. J. Gaspari RJ and D. Paydarfar, "Dichlorvos-induced central apnea: effects of selective brainstem exposure in the rat," Neurotoxicology, 32(2), pp. 206–14, 2011.

[20] B. J. Proskocil, D. A. Bruun, C. M. Thompson, A. D. Fryer, and P. J. Lein, "Organophosphorus pesticides decrease M2 muscarinic receptor function in guinea pig airway nerves via indirect mechanisms," PloS One, 5(5), pp. 01-11, 2010.

[21] K. Funai, et al., "Skeletal Muscle Phospholipid Metabolism Regulates Insulin Sensitivity and Contractile Function," Diabetes, 65(2), pp. 358–70, 2016.

[22] F. Seebacher, J. Tallis, K. McShea, and R. S. James, "Obesity-induced decreases in muscle performance are not reversed by weight loss," Int. J. Obes., 41(8), pp. 1271–8, 2017.

 ISBN: 978-1-61208-742-9