



# **Assessment of Bisphenol A as a hormonal and lipid metabolism disruptor**

A thesis

Submitted for the degree of Ph. Degree of Science as a partial fulfillment for requirements of Ph. Degree of Science

BY

**Asmaa Mohamed Ahmed Elfiky**

*Under the supervision of*

**Prof. Dr. Magdy Mahmoud Mohamed**

Professor of Biochemistry  
Faculty of Science  
Ain shams University

**Prof. Dr. Fateheya Mohamed Metwally**

Professor of environmental and  
occupational medicine  
Division of Environmental Research  
National Research Center

**Prof .Dr. Mohamed Abel-Hady Ghazy**

Professor of Biochemistry  
Faculty of Science  
Ain Shams University

**Prof .Dr. Nevin Ezzeldin Sharaf**

Professor of environmental and  
occupational medicine  
Division of Environmental Research  
National Research Center

Biochemistry department

Faculty of science

Ain shams university

2017

## **ACKNOWLEDGEMENT**

I would like to seize this opportunity to offer my deep gratitude and appreciation to my dear Prof. **Dr. Magdy Mahmoud Mohamed**, professor of Biochemistry, Faculty of Science, Ain Shams University, for his endless and sincere help, instructive guidance, and valuable support throughout the course of this study.

I wish to offer my thanks to **Prof. Dr. Fateheya Mohamed Metwally**. Professor of environmental and occupational medicine, Division of Environmental Research, National Research Center, for her valuable effort, kind help and advice during this thesis.

My Sincere and profound thanks to **Prof. Dr. Mohamed Abel-Hady Ghazy**, Professor of Biochemistry, Faculty of Science, Ain Shams university, for his honest support.

Many thanks to **Prof. Dr. Nevin Ezzeldin Sharaf**, Professor of environmental and occupational medicine, Division of Environmental Research, National Research Center, for her helpful suggestion, supervision and great support.

I would like to acknowledge the **National Research Center** for its financial support through a project **Environmental Pollution and Obesogens: Role of Clinical Nutrition and Natural Products in treatment of Obesity and Diabetes Mellitus**.

Last but not least, my great and special thanks to all members of **my family** especially to **my father, my mother, my husband** and **my children** for their continuous attitude and help.

## **CONTENTS**

	<b>Page</b>
<b>Abstract</b>	<b>I</b>
<b>List of Abbreviations</b>	<b>III</b>
<b>List of Figures</b>	<b>VI</b>
<b>List of Tables</b>	<b>VIII</b>
<b>Introduction</b>	<b>1</b>
<b>Aim of work</b>	<b>3</b>
<b>1.Review of literature</b>	<b>4</b>
<b>1.1.1 Structure of BPA</b>	<b>4</b>
<b>1.1.2. Manufacture and industrial products of BPA</b>	<b>4</b>
<b>1.1.3. Source of exposure</b>	<b>6</b>
<b>1.2. Mechanisms of BPA Action</b>	<b>10</b>
<b>1.2.1 The Non-linear Dose Response Curves</b>	<b>14</b>
<b>1.3. BPA levels in human tissues and fluids</b>	<b>15</b>
<b>1.3.1. BPA levels in serum, &amp;tissues</b>	<b>15</b>
<b>1.3.2. BPA levels in Pregnancy-associated fluids</b>	<b>16</b>
<b>1.3.3. BPA levels in Breast milk</b>	<b>17</b>
<b>1.3.4. BPA levels in Urine</b>	<b>17</b>
<b>1.3.5. BPA levels in Semen &amp; follicular fluid</b>	<b>18</b>
<b>1.4. The relation between exposure to bisphenol A and chronic diseases</b>	<b>19</b>
<b>1.4.1. BPA and obesity</b>	<b>19</b>
<b>1.4.2. BPA and Type-2 Diabetes</b>	<b>21</b>

---

<b>1.4.3. BPA and Cardiovascular disease, hypertension, and cholesterol levels.</b>	<b>22</b>
<b>1.4.4. BPA and Liver enzymes</b>	<b>23</b>
<b>1.4.5. BPA and Thyroid function hormones</b>	<b>24</b>
<b>1.4.6. BPA and Immune signaling pathways</b>	<b>25</b>
<b>1. 4.7. BPA and Albuminuria</b>	<b>26</b>
<b>1.4.8. BPA and Oxidative stress and inflammation</b>	<b>27</b>
<b>1.5. BPA and adipocyte genes</b>	<b>30</b>
<b>1.6. BPA and Adiponectin</b>	<b>34</b>
<b>1.7. BPA and leptin</b>	<b>37</b>
<b>1.8. BPA and Insulin</b>	<b>38</b>
<b>2. SUBJECT AND METHODS</b>	<b>40</b>
<b>2.1. SUBJECT</b>	<b>40</b>
<b>2.1.1. Study design</b>	<b>40</b>
<b>2.2. METHODS</b>	<b>42</b>
<b>2.2.1. Questionnaire</b>	<b>42</b>
<b>2.2.2. Clinical examination</b>	<b>42</b>
<b>2.2.3. Investigated parameters</b>	<b>42</b>
<b>2.2.3.1. Blood sample collection</b>	<b>42</b>
<b>2.2.3.2. Calculation of Body Mass Index (BMI)</b>	<b>43</b>
<b>2.2.3.3. Biochemical analysis</b>	<b>44</b>
<b>2.2.3.4. Molecular studies</b>	<b>56</b>
<b>2.2.4. Statistical Analysis</b>	<b>58</b>
<b>3. Results</b>	<b>59</b>

---

---

<b>3.1.Biochemical studies</b>	<b>59</b>
<b>3.2. PPAR<math>\gamma</math> (Pro12Ala) genotyping</b>	<b>74</b>
<b>4. Discussion</b>	<b>76</b>
<b>5. Summary</b>	<b>92</b>
<b>6.recomendation</b>	<b>95</b>
<b>7. References</b>	<b>96</b>
<b>8. ARABIC SUMMARY</b>	

---

<b>9. Arabic abstract</b>	
---------------------------	--

---

## **Abstract**

Bisphenol A (BPA), is one of the highest volume chemicals produced and used to manufacture polymeric materials used in many products. Human can be exposed to it through its migration from polymers to food or water by heating. It is considered as an environmental obesogenic through promoting adipogenesis, lipid accumulation and endocrinal disrupting chemicals (EDCs) altering adipokine hormone release. The aim of this study was to assess the impact of BPA on lipid profile and metabolism. This work included 85 females aging from 16 to 58 years, after application of exclusion criteria. Among them 48 females with BMI  $\geq 25$  kg/m<sup>2</sup> group A (Gr-A) and 37 females with BMI < 25 kg/m<sup>2</sup> group B (Gr-B). All participants were subjected to detailed questionnaire and a clinical examination, sBPA, adiponectin, leptin hormones and lipid profile were assessed for all subjects. Results showed that a significantly high levels of leptin, cholesterol and LDL-c ( $p < 0.001$ ) were recorded with significantly low levels of adiponectin ( $p < 0.001$ ) & HDL-c ( $p < 0.05$ ) in (Gr-A) compared with those of (Gr-B). When the studied population was divided according to their BPA concentration, the adiponectin was significantly lower with high BPA concentration group. For most of these results non-monotonic dose–response relationships were observed. On the other hand, we observed no mutation at PPARG2 across the three percentiles of BPA. In conclusion, BPA generated a clear response in which the general obesity (BMI) and the central obesity (WC) showed significant increase at the low and high percentiles. Moreover, BPA had a disturbed action on lipid profile and suppressive effect on adiponectin release which support the claim that BPA is an endocrine disruptor increasing the risk of developing obesity associated disorders such

as glucose intolerance, hyperinsulinemia, hypertension and increasing the risk of diabetes and cardiovascular disease.

**Keywords:** BPA, obesogen, adipogenesis, non-monotonic dose–response relationships, adiponectin.

### **List of abbreviation**

---

<b>BF%</b>	<b>Body Fat percentage.</b>
<b>8-OHdG</b>	<b>8-Hydroxydeoxyguanosine</b>
<b>ADP</b>	<b>Adiponectin</b>
<b>AMPK</b>	<b>Adenosine Monophosphate-Activated Protein Kinase</b>
<b>APCs</b>	<b>Antigen-Presenting Cells</b>
<b>BADGE</b>	<b>Bisphenol A Diglycidyl Ether</b>
<b>BIA</b>	<b>Bioelectrical Impedance Analysis</b>
<b>BMI</b>	<b>Body Mass Index</b>
<b>BPA</b>	<b>Bisphenol A</b>
<b>CVD</b>	<b>Cardiovascular Disease</b>
<b>DBP</b>	<b>Diastolic Blood Pressure</b>
<b>DXA</b>	<b>Dual-energy X-ray absorptiometry</b>
<b>E2</b>	<b>17<math>\beta</math>-Estradiol</b>
<b>EDCs</b>	<b>Endocrine Disruptor chemicals</b>
<b>ELISA</b>	<b>Enzyme-Linked Immunosorbent Assay</b>
<b>ER</b>	<b>Estrogen Receptor</b>
<b>ERK</b>	<b>Extracellular signal-Regulated Kinases</b>
<b>ERR-<math>\gamma</math></b>	<b>Estrogen-Related Receptor Gamma</b>

---



---

<b>FBG</b>	<b>Fasting Blood Glucose</b>
<b>FBI</b>	<b>Fasting Blood Insulin</b>
<b>FFA</b>	<b>Free Fatty Acid</b>
<b>GLUT4</b>	<b>Glucose Transporter-4</b>
<b>HDL-c</b>	<b>High Density Lipoprotein cholesterol</b>
<b>HRP</b>	<b>Horseradish Peroxidase</b>
<b>HR</b>	<b>Heart Rate</b>
<b>IGF-1</b>	<b>Insulin-Like Growth Factor 1</b>
<b>INS-1</b>	<b>insulinoma</b>
<b>IR</b>	<b>Insulin resistance</b>
<b>IVF</b>	<b>In Vitro Fertilization</b>
<b>LDL-c</b>	<b>Low Density Lipoprotein cholesterol</b>
<b>LOAEL</b>	<b>Lowest Observed Adverse Effect Level</b>
<b>LPL</b>	<b>Lipoprotein Lipase</b>
<b>MAPKs</b>	<b>Mitogen-Activated Protein Kinases</b>
<b>MDA</b>	<b>Malondialdehyde</b>
<b>MS</b>	<b>Metabolic Syndrome</b>
<b>NHANES</b>	<b>National Health and Nutrition Examination Survey</b>
<b>NMDRCs</b>	<b>Non-Monotonic Dose–Response Curves</b>

---

---

<b>PCR</b>	<b>Polymerase Chain Reaction</b>
<b>PDI</b>	<b>Protein Disulfide Isomerase</b>
<b>PET</b>	<b>Polyethylene Terephthalate</b>
<b>PPAR<math>\gamma</math></b>	<b>Peroxisome Proliferator-Activated Receptor Gamma</b>
<b>Pro12Ala</b>	<b>Prolin 12 Alanine</b>
<b>PCOs</b>	<b>Polycystic Ovarian Syndrome</b>
<b>RFLP</b>	<b>PCR–Restriction Fragment Length Polymorphism</b>
<b>RXRs</b>	<b>Retinoid X Receptors</b>
<b>SBP</b>	<b>Systolic Blood Pressure</b>
<b>SP</b>	<b>Streptavidin- Peroxidase</b>
<b>SPSS</b>	<b>Statistical Package For Social Science</b>
<b>STAT</b>	<b>Signal Transducers And Activator Of Transcription</b>
<b>TDI</b>	<b>Tolerable daily intake</b>
<b>TMB</b>	<b>Tetramethylbenzidine</b>
<b>TNF-<math>\alpha</math></b>	<b>Tumor necrosis factor alpha</b>
<b>UGT2B1</b>	<b>UDP-glucuronosyltransferase 2B1</b>
<b>WB</b>	<b>Wash Buffer</b>
<b>WC</b>	<b>Waist Circumference</b>

---

## **LIST OF FIGURES**

---

	<b>Pages</b>
<b>Figure 1: Structure of BPA</b>	<b>4</b>
<b>Figure 2: Mechanism of BPA action</b>	<b>13</b>
<b>Figure 3: Cellular and molecular mechanisms of action of BPA in human chronic disease induction</b>	<b>29</b>
<b>Figure 4: Multiple roles PPAR<math>\gamma</math> in adipose tissue</b>	<b>33</b>
<b>Figure 5: The different factors that affect the obesity-related metabolic syndrome</b>	<b>36</b>
<b>Figure 6: Standard curve of BPA</b>	<b>47</b>
<b>Figure 7: Standard curve of insulin</b>	<b>49</b>
<b>Figure 8: Standard curve of adiponectin</b>	<b>52</b>
<b>Figure 9: Standard curve of leptin</b>	<b>55</b>
<b>Figure 10: Relationship between different concentrations of s. BPA on mean (<math>\pm</math> SE) waist circumference</b>	<b>66</b>
<b>Figure 11: Relationship between different concentrations of s. BPA on mean (<math>\pm</math> SE) BMI.</b>	<b>66</b>

---

<b>Figure 12:</b>	<b>Relationships between different concentrations of BPA on mean (<math>\pm</math> SE) lipid profile</b>	<b>68</b>
<b>Figure 13:</b>	<b>Relationship between different concentrations of BPA on mean (<math>\pm</math> SE) FBS</b>	<b>70</b>
<b>Figure 14:</b>	<b>Relationship between different concentrations of BPA on mean (<math>\pm</math> SE) insulin</b>	<b>70</b>
<b>Figure 15:</b>	<b>Relationship between different concentrations of BPA on mean (<math>\pm</math> SE) HOMA-IR</b>	<b>71</b>
<b>Figure 16:</b>	<b>Relationship between different concentrations of BPA on mean (<math>\pm</math> SE) adiponectin</b>	<b>73</b>
<b>Figure 17:</b>	<b>Relationship between different concentrations of BPA on mean (<math>\pm</math> SE) leptin</b>	<b>73</b>
<b>Figure 18:</b>	<b>Analysis of PCR–restriction fragment length polymorphism detection of the Pro12Ala PPAR<math>\gamma</math>2</b>	<b>75</b>

## **List of tables**

---

<b>Title</b>	<b>Pages</b>
<b>Table 1: Source of contamination with BPA and exposure pathways in different life stages</b>	<b>8</b>
<b>Table 2: In vivo studies of significant effects at and below the published LOAEL OF 0.05 mg/Kg/BW/ day</b>	<b>9</b>
<b>Table 3: primers used in PCR</b>	<b>57</b>
<b>Table 4: PCR reaction</b>	<b>57</b>
<b>Table 5: General characteristics of the study groups</b>	<b>59</b>
<b>Table 6: Mean levels of serum BisphenolA (BPA), Adipokine hormones in the studied groups</b>	<b>60</b>
<b>Table 7 Mean levels of fasting blood sugar, serum insulin and HOMA- IR in the studied groups</b>	<b>61</b>
<b>Table 8: Lipid Profile in the studied groups</b>	<b>62</b>

---

---

<b>Table 9: Distribution of Adipokines hormones, lipid profile and HOMA-IR according to BPA level in the study subjects</b>	<b>64</b>
<b>Table 10: general characteristics of the studied population according to BPA concentration</b>	<b>65</b>
<b>Table 11: Mean levels of serum lipid profiles in the study population according to BPA concentration</b>	<b>67</b>
<b>Table 12: Mean levels of fasting blood sugar, insulin and HOMA IR in the study population according to their BPA concentration</b>	<b>69</b>
<b>Table 13: Mean level of adipokine hormones in the study population according to BPA concentration</b>	<b>72</b>

---

## **Introduction**

Bisphenol A (BPA) is a monomer of polycarbonate plastics. It is amongst the highest volume of chemicals in commerce. Polycarbonates are found in numerous consumer products, including water and food bowls, baby bottles, linings of metal food and drink cans, medical tubing, epoxy resins, and dental fillings. Small quantities of BPA can be transferred from polymers to food or water, especially while heated (**Le *et al.*, 2008**).

BPA is an environmental endocrine-disrupting chemical (EDC) detected in ninety-five percent of human urine samples (**Calafat *et al.*, 2008**) as well as in serum, breast milk, and fat (**Rubin, 2011; Taylor *et al.*, 2011**). BPA was declared to change several metabolic functions at environmentally appropriate concentrations in the low nanomolar range (**Sakurai *et al.*, 2004; Masuno *et al.*, 2005; Alonso-Magdalena *et al.*, 2006**). Moreover, BPA often shows a lack of linear dose-dependent relationship, but U-shaped or inverted U-shaped curves instead. Consequently, extrapolation from an action or lack of the action of BPA at high doses to the assumed bioactivity at low doses is unwarranted.

Mechanistically, BPA binds to estrogen receptors (ER)  $\alpha$  and ER  $\beta$  and results in competition with estrogen (**Kurosawa *et al.*, 2002**) and disrupting the folding, assembly, and shedding of several cellular proteins by targeting protein disulfide isomerase (**Hiroi *et al.*, 2006**). Experimental data demonstrated that exposure to BPA alters normal lipid metabolism and adipogenesis (**Grün and Blumberg, 2009**), by binding to Peroxisome Proliferator-Activated Receptor Gamma (PPAR- $\gamma$ ) receptors, an important regulatory component of lipid metabolism and adipogenesis., BPA exposure has the possibility to promote weight gain (**Newbold *et al.*, 2007; Grün and Blumberg, 2009**) and displays its effects on metabolic function by inducing