A Diabetogenic Effect of Bisphenol A on Pancreatic β-cell Function

By
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April 1, 2013



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Department of Biology

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Senior Comprehensive Research Project

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Submitted in fulfillment of the senior thesis requirements for a Bachelor of Science Degree in the Department of Biology at Allegheny College and approved by the senior thesis committee.

_____	_____
Dr. James Palmer, Ph.D	Dr. Christine Donmoyer, Ph.D
_____	_____
Date	Date

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

_____
Pledge

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To my brother, Mac, I dedicate this body of work with the hope that someday, in our lifetime, we can celebrate a cure for the illness with which you have lived throughout your entire, innocent lifetime.

Lastly, I would like to express a mountain of gratitude to my mother. There was never a time in my life when she did not insist that I reach for the Moon and the stars. She fostered my love of all things challenging and gave me the confidence that there is nothing I cannot achieve. Her love, support, listening ear, and encouragement have helped me to succeed not only in academics, but in my personal growth, as well. Thank you, Mom, for always being unconditionally proud of my efforts.

Abstract[1]

Endocrine disrupters have been a major focal point in the investigation of diseases such as type 2 diabetes and obesity. Bisphenol A (BPA) is a chemical used in polycarbonate plastics that has been linked with the onset of these diseases by mimicking, or competing with the body's naturally produced estrogens. These chemicals are a very common component of objects that we come in touch with on a day-to-day basis. Little research had been conducted that focused on the effects of BPA, examining complications with the production of insulin in pancreatic cell lines. Here, I conducted an experiment using pancreatic islet cells that have been derived from C57BL/6 wild type mice exposed to a concentration of BPA (100 μg/kg/day) coupled with a high fat diet. Having an exposure coupled with a specific diet can give insight to how we, as Americans, could be affected by this everyday plasticide. Aside from the isolation of these cells, I isolated the pancreas from the 400-600 μg/kg/day BPA group and measured pancreata mass. These also have specific diets so as to observe the morphological effects at a much higher dose of BPA (400-600 μg/kg/day). Isolation of these islets would further provide evidence for increased insulin production from its origin, the pancreatic β-cell. Pancreata weights were not significantly different between treatment groups. BPA and a high fat diet did not significantly influence islet insulin levels. Between each test group, there were no significant differences in insulin concentration.

[1] *This research was made possible by financial support from the Department of Biology Senior Project Research Fund and the Robertson Research Fund.*

Introduction:

The immensity of the impact from endocrine disrupters has garnered interest of scientists around the globe. Their versatility within the body commands the attention of researchers and creates a need for further exploration into the widespread effects on health. These endocrine disrupting chemicals, or EDC's, have been shown to mimic estrogenic activity and alter the body's feedback regulation, yielding adverse effects on our oft-exposed population.

Bisphenol A: an endocrine disrupter

Bisphenol A is an endocrine-disrupting ingredient in the manufacture of polycarbonate plastics that, recently, has been linked with Type 2 diabetes and heart disease (Adachi et al. 2005). Early BPA research was associated with simple warnings, in the news and from parental groups, to use precaution when using certain plastics in the microwave, for example, because of the potential for long-term carcinogenic effects. In recent years, expanded research has broadened the awareness in the scientific community that BPA is more formidable than ever believed. Recently, for example, warnings were posted that BPA was a basic ingredient in epoxy resin, which is used to line canned goods and drinks (Freinkel 2011). These epoxy resins not only affect heart functions and yield carcinogenic effects, but they drastically affect the pancreas at its most fundamental state, the β-cell. It no longer warrants a simple warning, rather the comprehensive education of all health care providers, manufacturers, and legislators.

Normal β-cell function

Islets of Langerhans are clusters of cells in the pancreas that are central to normal pancreatic function. Most importantly, they include the pancreatic β-cell. A pancreatic β-cell's main function is to secrete insulin in response to concentrations of glucose in the plasma. Insulin

is a small protein that cannot fold properly itself, so it must travel to be manufactured as part of a larger protein to enable this proper folding. Before insulin is produced, there is an inactive protein that is translated from a messenger RNA called preproinsulin (BCBC 2013). Preproinsulin then travels through the membrane of the endoplasmic reticulum (ER) where it is then processed to proinsulin, which is further cleaved at the disulfide bonds to result in active insulin (BCBC 2013). Insulin is then stored in vesicles in the cytoplasm until release is initiated (BCBC 2013) (Figure 1).

Type 2 glucose transporters (GLUT2) regulate how glucose enters β-cells (BCBC 2013). After being phosphorylated by glucokinase, the derived glucose (glucose-6-phosphate) becomes trapped within the β-cells to be further oxidized into adenosine tri-phosphate or ATP, our most common source of energy (BCBC 2013). This oxidation of glucose increases the amount of ATP by such great extent that the voltaged-gated potassium channels in the β-cells close, causing a positive charge to fulfill the cell (BCBC 2013). Having a positive charge as such then causes depolarization of the cell, which results in an influx of calcium (Ca^{2+}) ions (BCBC 2013). Such an intense flow of the positive ions further triggers exocytosis of the insulin-saturated vesicles that diffuse into nearby capillaries (BCBC 2013).

Once the blood glucose begins entering the cells, a negative feedback loop is activated to stop secretion of insulin (Raven et al. 2002). In a normal adult, β-cells divide rather slowly, but in scenarios where the individual is in a state of pregnancy or insulin resistance, these cells have been observed to divide rapidly (Alonso-Magdalena et al. 2010). Along with this increased mitosis, when β-cells are within a human with obesity or diabetes, the mass of these cells is significantly higher than in lean individuals (Alonso-Magdalena et al. 2010). This increase in

size that has been observed may correspond to the amount of insulin that the cells are required to secrete to compensate for insulin resistance in obese or diabetic individuals.

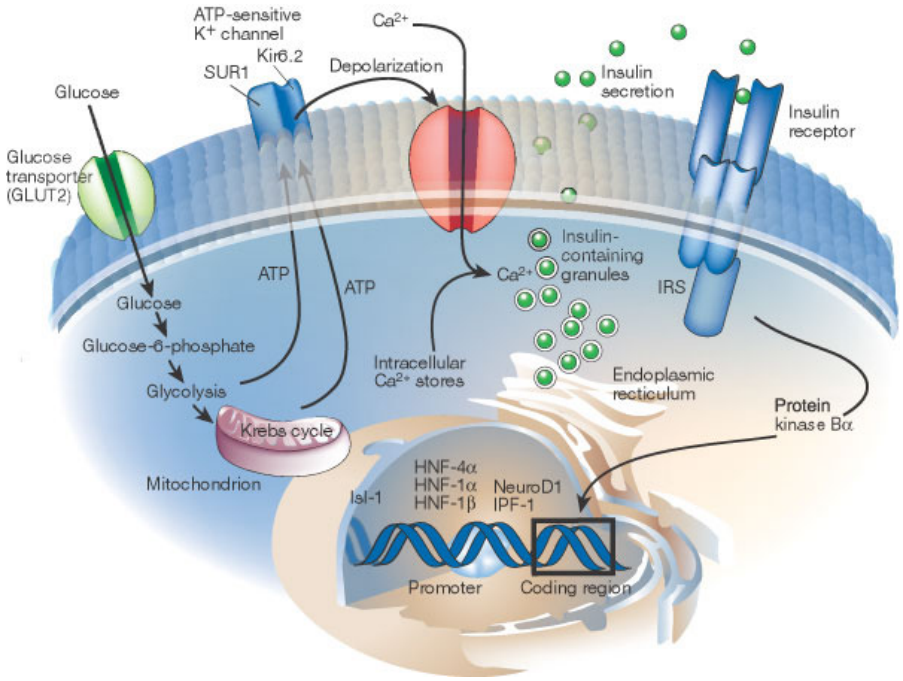

Figure 1. β-cell function -
http://www.nature.com/nature/journal/v414/n6865/fig_tab/414788a_F1.html#figure-title

β-cell function when exposed to BPA

Bisphenol A competes with the body's estrogens for endocrine receptor sites that, mechanistically, have yet to be defined, but the interactions involve rapid signal transductions initiated from plasma membrane receptors (Nagel et al. 1997). This competitive interaction has been observed to rapidly alter blood glucose levels in cells via insulin production. BPA and nonylphenol also affect insulin secretion via direct interaction with estradiol (E_2) endocrine receptors. The E_2 receptor-blocking chemical (ICI) to test this mechanism, and the observed results showed that these endocrine disrupters acted on insulin secretion by interacting with these

E_2 endocrine receptors themselves (Adachi et al. 2005). To study whether or not BPA regulates glucose uptake via endocrine receptors, adipose cells were incubated with a receptor-blocking chemical (ICI) and treated with BPA. Results showed that, over a period of time, the ICI could not inhibit the increased glucose uptake by BPA exposure (Sakurai et al. 2004). So, over a short period of time, ICI was able to block BPA interaction with the receptors, but over a longer period of time, the inhibitors could not completely block BPA's effect on the cell. BPA has also been shown to produce exhaustion of β-cells and also the increase of insulin resistance in cellular membranes by provoking the cells to produce an increased amount of insulin as a response (Nadal et al. 2009).

β-Cell mass expansion has been linked with this topic of increased pancreatic function (Prentki and Nolan 2006). In this study, an increased supply of glucose, food supply in general, and plasma insulin levels tested mass expansion of these cells. Another study hemodynamically infused glucose into the mouse via catheter. By doing so, this provided a new technique to measure the β-cell division *in vivo*. Over a 4-day period of infusion, results showed that β-cell division had significantly increased (Alonso et al. 2010). Cell mass, islet number, β-cell size, and β-cell death, however, were unaltered throughout the 4-day exposure. These findings suggested that the mild glycemia induced division of β-cells, but it did not have an effect on the physical attributes of these cells (Alonso et al. 2010). Cell lines were exposed to a media containing high amounts of glucose, and conducting an Ultra Sensitive Mouse Insulin ELISA kit, as was used in the experiment at Allegheny College. This key hallmark, of compensation by division, in type 2 Diabetes is not feasible (Gupta et al. 2007). The β-cells in a type 2 individual fails to increase the mass of these cells in order to maintain homeostatic levels

of insulin (Gupta et al. 2007). Although this study has not been repeated, there have been cases where, in rodents, insulin resistance has been an endpoint.

This research tested how the pancreatic β-cells respond to BPA exposure coupled with a high fat diet and how insulin secretion is affected. Based on the results of a study by Alonso-Magdalena and colleagues, I expected to see significant disruption of β-cell's insulin production, and functionality, due to the competitive nature of BPA with natural pancreatic hormones such as 17β-estradiol (Nagel et al. 2007). In this experiment, researchers investigated BPA and the way it mimicked 17β-estradiol *in vivo*, as well as compiling significant results and how the trend may show that this chemical may be involved in the onset of type 2 diabetes mellitus. The study used live Swiss albino OF1 male mice of 8-10 weeks of age. They determined that BPA rapidly produced hyperglycemia and hyperinsulinemia, increased β-cell insulin content, and triggered hyperinsulinemia and insulin resistance (Alonso-Magdalena et al. 2006). Another study, using similar procedure, was done at the University of Pittsburgh where mice received a high-glucose via catheter (Yokoe et al. 2008). By doing this, they were able to focus on the effects that were solely located in the islets. My experiment extended this work by determining the effects of BPA and insulin secretion of isolated islets of Langerhans. By using islet cells, the variable of how BPA would be metabolized, by varying tissues of the body, does not need to be taken into account. Some variables that had were accounted for are the prevention of bacteria that may develop in the cell lines, death of cells due to long exposure to histopaque, a toxic substance to the cells, or having small miscalculation throughout the very intensive steps. In C57BL/6 mice, the basal level of insulin content within the plasma is 60 ± 15 pmol/l; a deviation on the higher side of this would be expected (Park et al. 2005). Although my experiment did not collect the insulin from the plasma itself, I expected that, within the digested iselt cells, there would be a

higher level of insulin than within the plasma of these normal cells. The U.S. Environmental Protection Agency has concluded that BPA at 50 μg/ml/day is a safe level for humans (US EPA 2010). I hypothesized that pancreatic β-cells exposed to high levels of BPA would show the most drastic increase in insulin secretion. Furthermore, mice exposed to fatty diets will have increased insulin concentration when compared to groups that were on a normal diet.

Materials and Methods:

Test Subjects

Mice were obtained from Jackson laboratories after the weaning period of about three weeks. Eight male and eight female C57BL/6 mice were received and immediately divided into groups of two (n=2). Mice in both groups were maintained by cleaning cages, weighing weekly, and treated with BPA through food (daylong exposure). BPA was dissolved in ethanol for the vehicle. Groups that were on a normal diet were fed *Purina Rat Chow*, and the mice that were on a "fatty" diet were given *Breeder Chow Diet*. Dr. Laura Alonso and research group at the University of Pittsburgh maintained Group 3. Following this treatment group, another group of 18 mice (9 males, 9 females) were divided into these designated treatment groups. Another group of 8 mice, each randomly chosen with regards to gender were obtained from The University of Pittsburgh and were kept on a normal breeder chow diet. The groups are as follows:

Group 1 (6-week Treatment)

BPA (400-600μg/kg/day)

- **2 male/2 female** (~ 4.5 mg/mouse/day) + *Breeder Chow*
- **2 male/2 female** (~ 4.5 mg/mouse/day) + *Purina Rat Chow*

*****parenthesis indicate food intake/mouse/day

Untreated (Control)

- **2 male/2 female +** *Breeder Chow*
- **2 male/2 female** + *Purina Rat Chow*

Trial 2 Groups (4-week Treatment)

BPA (100µg/kg/day)

- **3 male/3 female** (~ 4.5 mg/mouse/day) + *Breeder Chow* 1x daily
- **2 male/2 female** (~ 4.5 mg/mouse/day) + *Purina Rat Chow* 1x daily

*****parenthesis indicate food intake/mouse/day

Untreated (Control)

- **2 male/2 female** + *Breeder Chow* 1x daily
- **2 male/2 female** + *Purina Rat Chow* 1x daily

Group Round 3 (Untreated)

- Untreated with BPA + ad libitum access to breeder chow and water at all times

*****These mice were not catheterized, but a detailed description of their maintenance is found in the online appendix (http://diabetes.diabetesjournals.org/content/56/7/1792/suppl/DC1).

Pancreata Mass Measurement

Once the mice in Group 1 only had been euthanized by CO_2 asphyxiation in, they were then dissected and their pancreata were removed. This step did not need to be sterile due to the measurements that were being taken. Each pancreas was removed by making a V-incision on the ventral side of the mouse, and the obstructing organs were pulled to one side of the mouse cavity, to obtain the majority of the pancreas. After each pancreas was removed, I took note of any morphological abnormalities that were obvious, then I weighed each.

Collagenase Preparation

Collagenase was obtained from Sigma Aldrich (C7657-500mg). Collagenase comes as a powder and had been stored at -20°C. Hanks Balanced Salts Solution (HBSS) was obtained from Sigma Aldrich (H2387-10x1L) and stored at 4°C. 1X Hanks was mixed with collagenase

(1.7mg/ml). The collagenase and HBSS were mixed to get the desired goal of 3ml per pancreas. This collagenase solution was mixed immediately prior to injections in order to keep a stable activity for each digestion of the pancreas.

Pancreas Isolation

Cells were taken from C57BL/6 mice through process of tissue removal and collagenase digestion. Once mice were obtained, they were euthanized by CO_2 asphyxiation. By doing this in this manner there was no laceration of organs or blood contamination from decapitation or injection. Once euthanized, mice were placed under a dissecting microscope that had been properly cleaned in order to reduce contamination. Dissecting instruments were flame-sterilized and dipped in 100% EtOH for expediting the cooling process. Once the mouse was placed under the dissecting microscope, it was sprayed with 70% EtOH over the abdominal area to keep the fur from contaminating the collagenase mixture. Using sterile dissecting scissors, the abdomen was opened with a V-incision from the pubic region to both front legs (Zmuda et al. 2011). Once the abdomen was opened, the liver was repositioned by pressing it up against the diaphragm (Zmuda et al. 2011). When the mouse was opened, the tail was directed away from me and a hemostat clamp was placed on the small intestine (where the bile duct drains), just far enough onto the intestine so that an amount of collagenase was able to pass into the pancreas. By using a 28 gauge syringe, a ductal injection of 3ml collagenase mixture was used to inflate the pancreas. This allowed for a clean removal of the pancreas without exogenous tissue. Each excised pancreas was placed into a polypropylene 50ml conical tube and put on ice. The pancreata are then incubated for 17 minutes at 37°C in 50ml conicals. These were kept on ice (0°C) for approximately 15 minutes, while the other pancreata were being excised. They were separate from each other in conical tubes for a better islet yield. After this step, I inactivated the

collagenase with 15ml of HBSS with 10% Fetal Bovine Serum (FBS) and had vortexed each tube 5-10 seconds to disrupt the tissue (Yokoe et al. 2008).

Islet Isolation

After each of the injections, the pancreata were incubated, washed three times with Hanks and FBS, and then contents of each tube were poured through a 530micron filter in order to remove any tissue that may have adhered to the pancreas. Once this filtration step had been done, the remaining tissue was centrifuged in a Ficoll/histopaque gradient. These cells were suspended in a density gradient allowing for easy removal of islets from debris in the gradient. Islets sat on top of the Histopaque, which was at 1.077g/mL density. Removed islets were then washed three times with 1X HBSS to further clean the islets of ficoll, and then approximately three sedimentation steps would follow the clean, washing away further waste from the islets. I then re-suspended the islets in 10mL islet medium and transferred to a petri plate where islets were picked.

Enzyme-linked immunosorbent assay (ELISA)

Manufacturer's Instructions were followed for Ultra Sensitive Mouse Inuslin ELISA Kit (Cat# 90080) that were obtained from Crystal Chem Incorporated – Downers Grove, IL (Crystal Chem Inc., 2010) (Appendix I). A Wide Range Assay (0.1-12.8 ng/mL) was used to see the insulin concentrations of my experiment and another experiment on the same 96-well plate. All reagents were prepared for use before the ELISA began. A stock solution of 12.8 ng/mL was prepared and used for the serial dilutions of the following polypropylene microtubes: 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 ng/mL, respectively. 95µL of sample diluent was dispensed into each well followed by the 5µL samples of both standards and samples. These standards were then run in duplicates (not including duplicates for 12.8 ng/mL and 6.4 ng/mL for first ELISA) in both

ELISA Assays used. Since the antibody is already in existence on the plastic, each serum dilution is introduced to the wells and incubated for 2 hours at 4°C. After this, a thorough wash (5 washes) was completed using 300 µL wash buffer per well, and 100 µL enzyme conjugate was dispensed into each well, followed by another incubation period 30 minutes at room temperature. Once incubating was done, the plate is washed (7 washes) with 300 µL wash buffer per well, and 100 µL enzyme substrate was then added to the wells. During a 40 minute time period at room temperature, reaction was specifically noted to avoid exposure to light. Enzyme reaction stop solution is added after this incubation and the sample was complete. Within 30 minutes of the previous step, the A_{450} and A_{620} values were measured using a plate reader. These values were run through ANOVA StatView program to observe trends and results. A more simplified version and pictorial layout are seen in Appendix Figure 4.

Results

Pancreatic Weight

Pancreatic weight to body weight ratios show significant differences (.0065mg to .0047mg and .007mg to .006mg) due to treatment, diet, and the interaction between treatment and sex (Figure 2., Table 1). Each treatment group had two mice that had been treated under the same conditions throughout the 6-week exposure period. Over the 6-week exposure, there was a significant difference (Table 1) between the diets used in the experiment. Due to the increased weight of the mice in the "high fat" groups (Figure 2.), a significantly smaller pancreata weight/body weight ratio was observed. There are trends that show how, as the diet and treatments are analyzed as one, the ratio decreases as the groups get to more "exposed" conditions (fatty diet + BPA, healthy diet + BPA, etc) (Appendix I). The insignificant differences between sex, treatment+diet, diet+sex, and the combination of these, show how the multifaceted

approach cannot be categorized as significantly different (Figure 2., Table 1).

Table 1. ANOVA results pancreatic weight/body weight ratio for 6-week treatment groups. Significant values (P=.0401, P=.0209, and P=.0113) showed significant differences in groups.

ANOVA Table for P.Wt/BdWt

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	1	1.563E-6	1.563E-6	5.993	.0401	5.993	.573
Diet	1	2.146E-6	2.146E-6	8.232	.0209	8.232	.715
Sex	1	4.623E-8	4.623E-8	.177	.6848	.177	.066
Treatment * Diet	1	1.124E-6	1.124E-6	4.310	.0716	4.310	.439
Treatment * Sex	1	2.789E-6	2.789E-6	10.697	.0113	10.697	.826
Diet * Sex	1	5.402E-7	5.402E-7	2.072	.1880	2.072	.237
Treatment * Diet * Sex	1	5.760E-8	5.760E-8	.221	.6509	.221	.070
Residual	8	2.086E-6	2.607E-7				

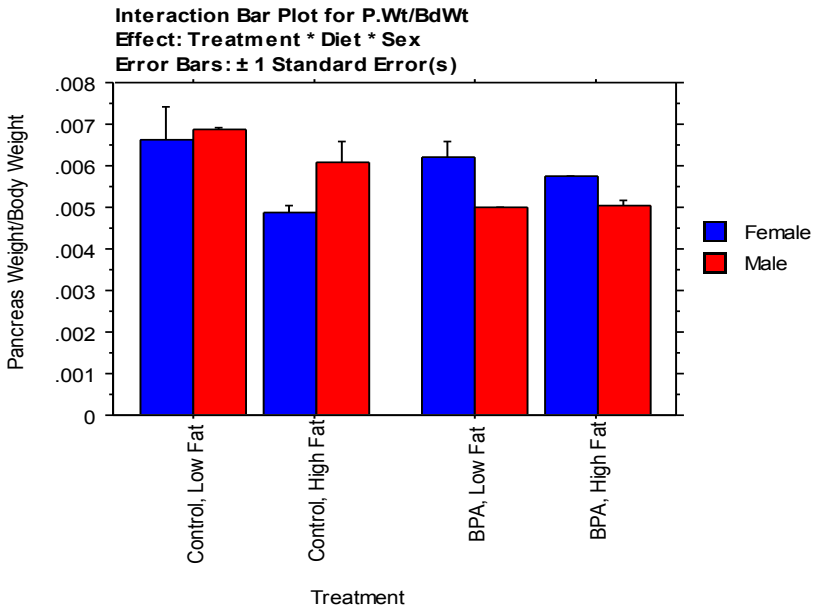

Figure 2. The pancreatic weight/body weight ratio of 6-week exposure and treatment groups.

In Figure 3, trends are observed between the male and female pancreata when exposed to BPA and without such exposure. Although these data were not observed through statistical

analysis, there were expected trends between both males and females, and fatty diet and healthy diets. With males, these results show increased pancreata weight when compared between the healthy and fat groups; however, in females, there is quite the opposite effect (Figure 3.). More investigation must be completed for interpretation.

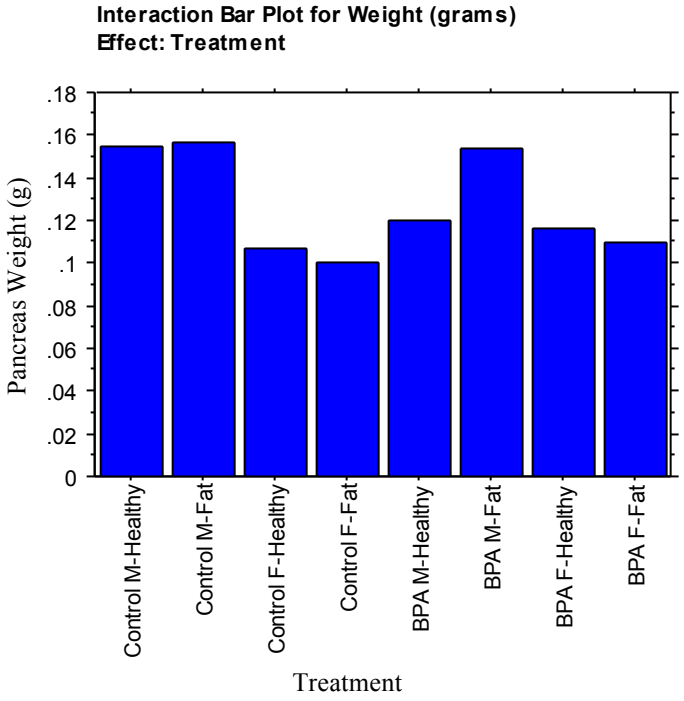

Figure 3. The pancreata weight in mice over a 4-week period that were exposed to chemical through food consumption and BPA Fat was coupled with *Breeder Chow* diet.

Insulin Concentration

The insulin concentration between the treatment groups was not affected by treatment groups of BPA Fat and BPA Healthy when compared to the control groups (Figure 4., Table 2). Samples came from the 4-week exposure of 100 µg/kg/day (n=2) and were all placed under uniform living conditions.

Table 2. ANOVA table of treatment group, dilution, and insulin content (ng/mL) of each treatment group. No significant differences were observed between groups.

ANOVA Table for Insulin (ng/mL)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Treatment	2	.001	4.745E-4	.105	.9010	.210	.063
Dilution	1	.001	.001	.178	.6807	.178	.067
Treatment * Dilution	2	.001	3.857E-4	.086	.9186	.171	.060
Residual	12	.054	.005				

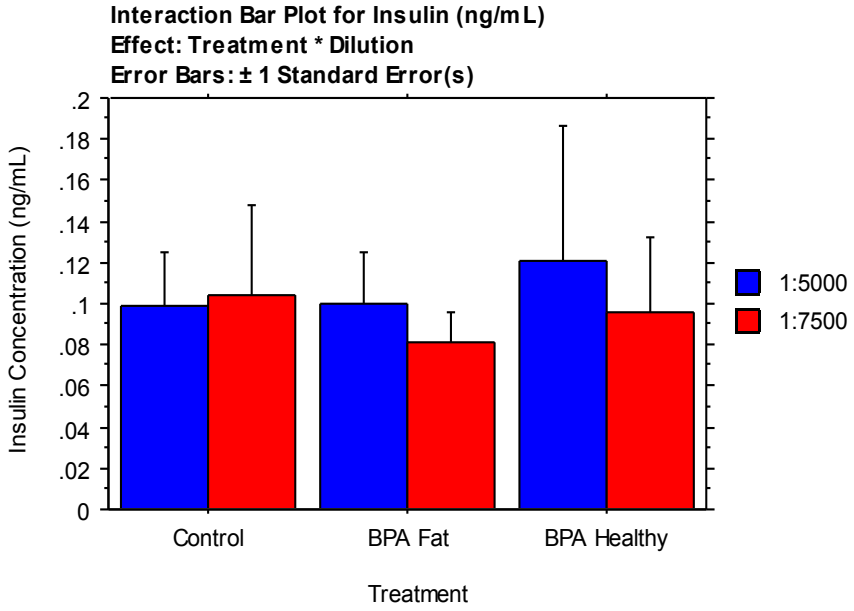

Figure 4. 1:500 dilution and 1:7500 dilution for each treatment group, measuring total insulin concentration.

Since the effect of two different serum dilutions (1 serum : 5000 H_2O and 1:7500) was not significant, dilutions were treated as replicates and pooled for analysis (Appendix II). There were, in fact, no significant relationships between insulin concentration and treatments. These trends were unreliable with relationship to the treatments, but the difference between BPA healthy and control seem to have an expected difference in insulin concentration (Appendix II).

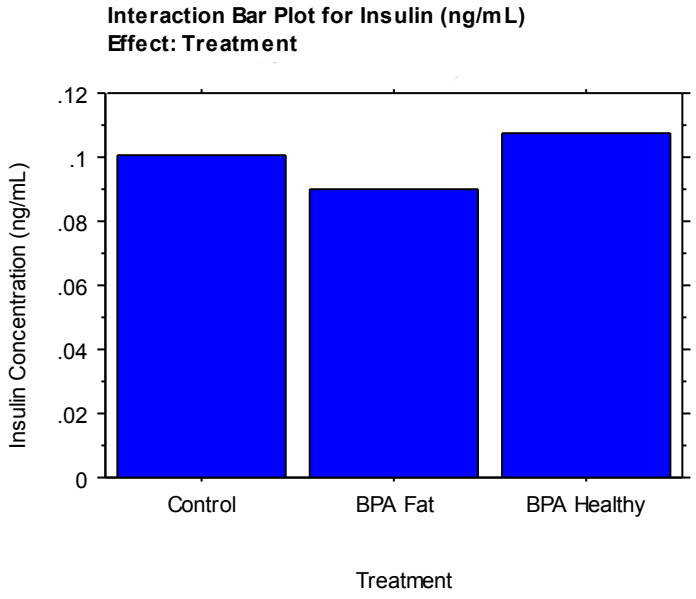

Figure 5. Pooled dilutions of 1:5000 and 1:7500 to further analyze relationships between insulin concentration, dilutions, and sex on a broader data set.

**Data from Pitt mice was drastically different than treatments and isolation done by me. By removing the outlying data, I was more readily able to analyze my personal data to reach the endpoints that were seen at Allegheny College.*

Discussion

In order for these worldwide complications of diabetes and other diseases to be understood, health care administrators, pharmaceutical companies, and other diabetic research foundations must focus on the origin on the problem. To solve this rising problem, these

administrators must allocate additional funding into developing a thorough understanding of the basic mechanisms of diabetes and how β-cells interact with xenoestrogens. While this research was done on a much less rigorous scale, I was able to quantify differences between treatments and the mass of pancreas through precise dissections. This pathway of research should be continued in the future to further observe morphological differences between mice that have been exposed to endocrine disruptors and mice that have not. Although not all data are conclusive of the main experiment, there is significant momentum within this type of cellular pancreatic research that gives a promising explanation to the way the β-cells function.

Pancreas Weight to Body Weight Ratio

This work, using whole islets of Langerhans from mice being treated for a 6-week and 4-week time period, shows how concentrations and exposure time of BPA can alter pancreatic function and morphology. BPA played a significant role in the size of pancreas due to its capability to promote an increase in β-cell mass (Alonso-Magdalena et al. 2010). Significant differences were seen due to the interaction of sexes and their pancreatic weight/body weight ratios (Figure 2., Table 1). Pancreatic weight to body weight ratio was significantly smaller in subjects treated with BPA (both male and female) than control groups (both male and female) (Figure 2., Table 1). These data support the hypothesis that, when the mice are treated with BPA and high fat diets; the β-cell mass increases, thus increasing overall pancreas weight. β-cell mass must increase to supplement the increased metabolic demand of the mouse that has been exposed to BPA and/or a high fat diet (Merino et al. 1997). A high glucose diet indicates that a much higher level of glucose is entering the body; as a result, the β-cells should naturally grow to compensate for the insulin needed to maintain homeostasis (Weir and Bonner-Weir 2007). Within these results, the male mice had a more consistent trend than in the female mice. This

type of consistency was also seen in the 4-week exposure between the female mice and male mice and their pancreata weights (Figure 3., Table 2), respectively. Differences between male and female subjects may be explained by the decreased insulin sensitivity that is seen during developmental (luteal) phases of female mice (Bonaventura et al. 2013). Weighing the pancreata separately from the body weight showed a trend between the male pancreata and the diets to which the groups were exposed. In the more saturated mice (BPA + High Fat and Control + Fat), the pancreata were considerably more massive than the mice put on a healthy diet (Figure 3.). An earlier explanation of how female mice have a different estrogenic response would be a good explanation as to why these differences are not consistent with female data. Methodological and surgical difficulties with these procedures may explain some variability between data. During the 6-week dissections, I had not been extremely familiar with the surgical process of cleanly removing the entire pancreas from the mice; however, I performed the task to the best of my capabilities with the dissecting tools at hand. The tissue around the pancreata might be another explanation, as my inexperience might have led to a measure of attached adipose tissue to pancreata that I removed. By doing so, this would increase the pancreata mass that was recorded and analyzed.

Treatment and Insulin Concentration

Insulin concentrations were not affected by treatment groups for either dilution (1:5000 and 1:7500), in the 4-week exposure (Figure 4.). These trends were not observed in other research studies that may provide evidence for error in this procedure. In the islets of Langerhans, BPA was shown to increase insulin release in a dose dependent manner when administered to both human and mouse islets (Soriano et al. 2012). Also, when the cells were exposed to BPA, they became much more responsive to glucose, which would then trigger a much higher

secretion of insulin (Soriano et al. 2012). Although my data does not support these trends, the data that was generated at Allegheny could be repeated for more prominent trends. I obtained results that contrast research at The University of Pittsburgh, my experimental data here does not support what was found in a professional laboratory. By using the same protocol, the lab found a much higher insulin concentration in the control, even compared to a BPA + Fatty diet at Allegheny College. This discrepancy is most likely due to the amount of islets that were harvested in the lab at Allegheny. Pitt's lab isolated a standard 50 IE of sample, while my protocol was only able to yield around 20 IE. With this procedure, it takes many hours of experience and expertise to properly complete ductal injections and islet isolation. Another obstacle was the timing of shipments to perform the protocol. If this procedure were repeated, it would be much more beneficial to have all materials and proper dissecting tools assembled prior to the experiment. The ductal injections were done with a syringe that was two gauges too large for the injections; this was crucial because the duct left a very small opening to make the collagenase injection into the pancreas, and by having a syringe that was too large might have lacerated a portion of either the pancreas or the duct. Another crucial portion of the procedure is to have the solutions mixed before initiating steps. This leaves little to no delay in between procedural steps, and time-sensitivity may be an important factor, so it is advised to mix solutions beforehand. During sedimentation steps, the 530 micron filter had not arrived, so improvisation allowed for a makeshift filter to be made from larger pieces of mesh. Using these larger pieces of mesh could have allowed for islets to pass through the gaps in between, potentially leaving behind few or no islets. This is extremely important to have, because, once all other steps are complete, these islets are the only source of insulin left in the solution.

In conclusion, over a 6-week exposure period, BPA significantly increased the pancreas weight when compared to body weight, but BPA did not affect the insulin concentration within islets of Langerhans over the 4-week exposure. BPA is in fact an endocrine disruptor that can lead to type 2 diabetes and obesity through disruption of β-cell functionality. More studies like this must be tried to conclude how BPA works, mechanistically, to cause these worldwide complications.

Future Experiments

Studies leading up to present day research, on pancreatic function and BPA exposure, have been mainly focused on how an organism metabolizes the exogenous estrogen and the pancreatic output thereafter. Additional studies to supplement the findings of BPA exposure and pancreatic output could include things such as, observing the mass of β-cell in an organism after exposure or to study how receptor genes are down regulated and up regulated on the cell's surface because at 1nM BPA, Ca^{2+} oscillations increase in β-cells (Ropero et al. 2008).

Measuring insulin gene transcription and the exposure to BPA could also be a contingency "plan B" for my experiment. β-cells are activated once a glucose molecule has been detected and binds to the receptor on the pancreatic cells. Insulin secretion begins once this interaction occurs as the ATP-regulated potassium channels close, causing an opening of the voltage-dependent calcium channels (Melloul et al., 2002). A study that could be conducted could be how BPA causes these receptor channels to close and how insulin secretion is altered otherwise. Measurements would be conducted as they were before using a sensitive ELISA kit (Figure 6.)

Further exploration of how this endocrine disruptor may be affecting insulin secretion could follow a protocol, like this, that tests the F1 generations. By testing F1 generations, sex

specificity and developmental changes would be observed. Kendig et al. treated mice with concentrations of BPA within their diet, like this project, except they waited for reproduction to test the offsprings' changes due to BPA exposure. In this study, the F1 females showed little to no response to the BPA diet while males showed increased glucose tolerance, adiposity, weight gain, and improved metabolic function (Kendig et al. 2013). This type of study may explain the insulin differences that were observed between males and females. Taking this experiment one step further might give better understanding as to how males and females metabolize this endocrine disruptor differently.

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APPENDIX



Figure 6. – Ultra Sensitive Mouse Insulin ELISA Kit –
http://www.crystalchem.com/media/document/1316114602Ultra_Mouse_specs-07.pdf

Pancreas Weight/Body Weight Ratio

Treatment	Diet	Dose	Sex	Panc Wt/Body Wt
Control	Low Fat	High	Male	.00691
Control	Low Fat	High	Male	.00683

Control	Low Fat	High	Female	.00585
Control	Low Fat	High	Female	.00741
Control	High Fat	High	Male	.00561
Control	High Fat	High	Male	.00658
Control	High Fat	High	Female	.00504
Control	High Fat	High	Female	.00472
BPA	Low Fat	High	Male	.00499
BPA	Low Fat	High	Male	.00501
BPA	Low Fat	High	Female	.00579
BPA	Low Fat	High	Female	.00659
BPA	High Fat	High	Male	.00515
BPA	High Fat	High	Male	.00494
BPA	High Fat	High	Female	.00577
BPA	High Fat	High	Female	.00571

Dilution Pool

Treatment	Rep	Dilution	Adj. Absorbance	Insulin (ng/mL)
Control	1	1:5000	.052	.099
Control	1	1:7500	.053	.102
BPA Fat	1	1:5000	.052	.099
BPA Fat	1	1:7500	.047	.081
BPA Healthy	1	1:5000	.058	.120
BPA Healthy	1	1:7500	.051	.095

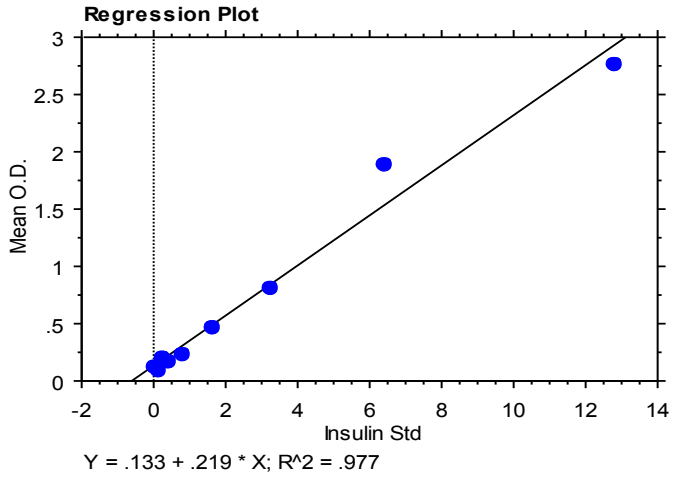
Insulin Concentrations (Rep1-3)

Treatment	Rep	Dilution	Adj. Absorbance	Insulin (ng/mL)
Control	1	1:5000	.065	.145
Control	2	1:5000	.052	.099
Control	3	1:5000	.039	.053
Control	1	1:7500	.078	.191
Control	2	1:7500	.043	.067
Control	3	1:7500	.039	.053
BPA Fat	1	1:5000	.066	.148
BPA Fat	2	1:5000	.043	.067
BPA Fat	3	1:5000	.048	.085
BPA Fat	1	1:7500	.055	.110
BPA Fat	2	1:7500	.044	.071
BPA Fat	3	1:7500	.042	.064
BPA Healthy	1	1:5000	.095	.251
BPA Healthy	2	1:5000	.039	.053
BPA Healthy	3	1:5000	.041	.060
BPA Healthy	1	1:7500	.072	.170

BPA Healthy	2	1:7500	.042	.064
BPA Healthy	3	1:7500	.039	.053

Regression Summary
Mean O.D. vs. Insulin Std

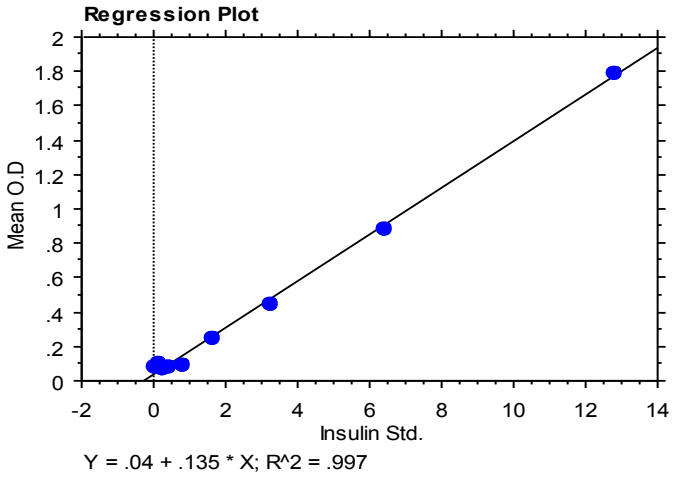
Count	9
Num. Missing	3
|R|	.988
R Squared	.977
Adjusted R Squared	.973
RMS Residual	.155



First standard regression

Regression Summary
Mean O.D vs. Insulin Std.

Count	9
Num. Missing	0
|R|	.998
R Squared	.997
Adjusted R Squared	.996
RMS Residual	.036



Second standard regression

Data that I have recorded from exposed mice and weight of pancreas:

○ Mouse	Weight	Pancreas Weight
BPA M1 Fat	29.93 g	.1540 g
BPA M1 Healthy	24.03 g	.1200 g
Control M1 Fat	27.98 g	.1570 g
Control M1 Healthy	22.42 g	.1550 g
BPA F1 Fat	19.06 g	.1100 g
BPA F1 Healthy	20.05 g	.1160 g
Control F1 Fat	19.85 g	.1000 g
Control F1 Healthy	18.29 g	.1070 g

BPA M2 Fat	25.95 g	.1281 g
BPA M2 Healthy	24.85 g	.1245 g
Control M2 Fat	24.53 g	.1157 g
Control M2 Healthy	22.04 g	.1506 g
BPA F2 Fat	20.22 g	.1154 g
BPA F2 Healthy	19.00 g	.1252 g
Control F2 Fat	18.87 g	.1242 g
Control F2 Healthy	20.67 g	.1531 g