I. Project Title

Effects of oral ingestion of Bisphenol A on cardiovascular disease and type 2 diabetes risk factors in healthy adults

II. Abstract

Bisphenol A (BPA) is a mass-produced endocrine disrupting compound used in a variety of consumer products such as food and beverage containers, dentistry materials, and baby bottles.[1] While BPA is quickly metabolized after exposure, the relationship between BPA and diseases such as type 2 diabetes and cardiovascular disease (CVD) may be stronger than previously understood, as continuous exposure may result in sustained blood concentrations of the compound. Environmental exposure to BPA has been positively associated with increased risk for developing type 2 diabetes and CVD in several epidemiological studies and cross-sectional analyses of 2003-2008 National Health and Nutrition Examination Survey (NHANES).[2-7] The findings of these studies are severely limited however, as they provide only correlational data and do not show cause and effect. Thus, experimental studies are needed evaluating the direct effects of BPA on disease markers. The goal of this study is to determine the effects of oral ingestion of BPA on CVD and diabetes risk markers. Ten healthy adults (20 to 50 years old) will be randomized to oral ingestion of BPA or placebo on separate days, in a double-blinded, crossover fashion. Thirty minutes after ingestion of BPA or placebo, blood concentrations of lipid profile (total cholesterol, LDL, HDL, triglycerides), insulin, glucose, and BPA will be assessed for up to 3 hours. The results of this study will provide much-needed experimental data as to whether oral ingestion of BPA poses any health risk, and will inform public health recommendations for food packaging.

III. Introduction

The prevalence of both CVD and diabetes is increasing at an alarming rate, with CVD being the leading and diabetes the 7th leading cause of death in the United States.[8,9] Clearly, genetics, diet, and physical activity play major roles in the development of CVD and type 2 diabetes. However, emerging data suggests a novel hypothesis that synthetic non-persistent endocrine disruptors used in a variety of common consumer goods, including the industry-produced chemical BPA play a pivotal role in development of CVD and type 2 diabetes. BPA is one of the most largely produced endocrine disrupting compounds and can be found in foods, drinks, toys, and beauty products. In a 2003-2004 analysis of exposure to BPA among the U.S. adult population, over 93% of individuals were shown to have detectable urinary BPA concentrations.[1] Several other cross-sectional analyses of the NHANES and Nurses' Health Study II have shown positive associations between urinary BPA concentrations and CVD and type-2 diabetes.[5,7,10] In vitro animal studies have shown that BPA causes disruption of endocrine pathways resulting in dysregulation of glucose metabolism and decreased insulin sensitivity, which may explain the positive correlation between BPA exposure and type 2 diabetes.[11,12] Additionally, BPA exposure is positively associated with cardiovascular disease, an effect that may be secondary to insulin and glucose dysregulation, or may be directly caused by upregulation of cholesterol biosynthesis.[13] This will be the first study to experimentally test the effect of oral ingestion of BPA on CVD and diabetes risk markers, and will address whether increasing rates of these diseases may be caused BPA exposure.

IV. Objectives

1. To determine the effects of oral ingestion of BPA on CVD risk markers. We hypothesize that oral ingestion of BPA, compared to placebo, will increase total cholesterol, low-density lipoprotein, triglycerides, and decrease high-density lipoprotein.
2. To determine the effects of oral ingestion of BPA on type 2 diabetes risk markers. We hypothesize that oral ingestion of BPA, compared to placebo, will increase insulin and glucose concentrations.

V. Methodology

Subjects

Ten healthy, adults (18.5–35 kg/m^2 BMI, 20-50 years old) will be recruited from Cal Poly San Luis Obispo campus and nearby area. Exclusion criteria include postmenopausal women, smoking, diagnosed disease, consuming an extreme diet (e.g. Atkins, Paleo, etc.), taking prescription medications (e.g. metformin, thyroid medication, anxiety medication), and pregnant women or women trying to become pregnant, assessed by a health history questionnaire.

Screening and Informed Consent

During recruitment, those who are interested in participating in this study will be given a brief overview of the study. The study protocol will be explained in full detail, and participants will be given the opportunity to read the informed consent provided and ask questions. This study will be submitted for approval to the Human Subjects Committee at Cal Poly San Luis Obispo, and all subjects will give verbal and written informed consent.

Experimental Protocol

At baseline (prior to randomization), weight, height, body composition (assessed by BodPod), and a health history questionnaire will be assessed. After an overnight fast, subjects will arrive in the Human Performance laboratory in the Kinesiology department and a fasting blood sample will be collected. Subjects will then be randomized to oral ingestion of BPA or placebo (calcium supplement), in a double-blinded, crossover fashion. Oral BPA ingestion in capsule form will be administered at a dose of 100 μg/kg body weight (e.g. 70 kg person will consume 7 mg of BPA), which is a typical daily dose of BPA in cross-sectional studies. The pharmacokinetics of this dose of BPA has previously been reported with a blood concentration max occurring at approximately 1 hour after ingestion, and with 94% of BPA present in urine at 24 hours.[14] Another blood sample will be collected 30 minutes after ingestion of BPA or placebo. Then, an oral glucose tolerance (OGTT) test will be performed in which subjects will consume 75g of carbohydrate and additional blood samples will be collected every 30 minutes for 2.5 hours. Also, an appetite questionnaire using a visual analog scale and gastric distress questionnaire will be assessed in concert with each blood draw. Within one week, subjects will return for the additional condition. Twenty-four hours prior to each trial, subjects will refrain from exercise, caffeine, and alcohol. **NOTE**: The risks for this study are minimal, as the single dose of BPA administered to subjects is a daily typical dose assessed on cross-sectional studies. However, after both conditions we will offer subjects a "fresh food" diet, which has previously been shown to reduce BPA concentrations by 66%.[15]

Biochemical Collection and Analysis

A certified phlebotomist in the Kinesiology Department will insert catheters into a forearm vein, and venous blood samples will be collected (by the phlebotomist and/or student researchers) in sterile syringes and transferred to tubes containing a serum separator for analysis of lipid profile (total cholesterol, LDL, HDL, triglycerides), sodium fluoride and potassium oxalate (for analysis of glucose concentrations) and EDTA (for analysis of insulin concentrations). Samples will be immediately centrifuged at 3000g for 15 minutes, and plasma will be aliquoted into polystyrene tubes and stored at -80°C until analyzed. After completion of experimental trials, plasma lipid profile and glucose concentrations will be assessed by the oxidase method using an autoanalyzer (Analox Instruments, Lunenburg, MA), and insulin and BPA concentrations by an ELISA kit (Millipore Research, St Charles, MO or R&D Solutions, Detroit, MI).

Statistical Analysis

Repeated measures ANOVA, adjusting for baseline measurements sex, weight, BMI, physical activity levels, age, and demographics will be used to analyze both CVD and type 2 diabetes outcome measures. An alpha <0.05 will be considered significant, and a Tukey's HSD post hoc will be used if differences occur.

VI. Timeline

This project will be submitted for IRB approval, and upon approval, recruitment will commence. All subjects will be recruited, assessed, randomized, and participate in experimental trials on an individual basis, thus the timeline of this study will accommodate availability of subjects.

2016-2017 Academic Year	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
Submit Project for IRB Approval	■	■										
Study Recruitment		■	■	■	■							
Baseline Assessment/ randomization			■	■	■	■	■					
Intervention Trials			■	■	■	■	■					
Biochemical Analysis								■	■			
Statistical Analysis								■	■			
Data Cleaning									■	■		
Presentation and Manuscript Writing											■	■

Table 1. Project Timeline

VII. Final Products and Dissemination

The purpose of this project is to serve as Alyssa Bird's and Dana Williams's Honors Senior Project. The project will be uploaded to Cal Poly Digital Commons as a scientific research paper. Furthermore, it will be presented at the CSM research fair in Spring 2017, and preliminary data will be presented at the Southwest ACSM conference in Fall 2017. The results of this study will also be submitted to a peer-reviewed journal in 2017.

VIII. Budget Justification

We are requesting a total of $4,250 for this study. Additional costs, if any, will be provided by either the faculty mentor (Dr. Todd Hagobian), the Kinesiology Department, or STRIDE.

Non-computer Supplies and Materials: A total of $300 will be budgeted for purchasing of Bisphenol A and calcium placebo supplements for 10 trials. A total of $200 will be budgeted for blood draw supplies. $500 will be budgeted for lipid profile and glucose testing reagents. Four insulin kits (Millipore Corp.) capable of analyzing 39 samples each will be purchased for a total of $2,250.

Contracted Services: A total of $750 will be provided for subject remuneration ($75 each) and $250 will be provided for a fresh food diet upon completion of the study.

References

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Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

CAL POLY

PROPOSAL BUDGET

Student Applicant(s):	Alyssa Bird Dana Williams	
Faculty Advisor:	Todd Hagobian	
Project Title:	Effects of oral ingestion of Bisphenol A on cardiovascular disease and type 2 diabetes risk factors in healthy adults	**Requested Endowment Funding**
Travel	*subtotal*	**$0**
Travel: In-state		**$0**
Travel: Out-of-state		**$0**
Travel: International		**$0**
Operating Expenses	*subtotal*	**$3,250**
Non-computer Supplies & Materials		**$3,250**
Computer Supplies & Materials		**$0**
Software/Software Licenses		**$0**
Printing/Duplication		**$0**
Postage/Shipping		**$0**
Registration		**$0**
Membership Dues & Subscriptions		**$0**
Multimedia Services		**$0**
Advertising		**$0**
Journal Publication Costs		**$0**
		0
Contractual Services	*subtotal*	**$1,000**
Contracted Services		**$1,000**
Equipment Rental/Lease Agreements		**$0**
Service/Maintenance Agreements		**$0**
	TOTAL	**$4,250**

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April 21, 2016

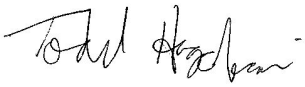
To Warren J. Banker Endowment Committee,

This letter is in strong support of Alyssa Bird's and Dana Williams's pursuit of the Warren J. Baker Endowment award. I have known Alyssa and Dana for over one year in an academic and mentorship setting at Cal Poly State University, and overall, both students are in the top 20 of all students I have been associated with. Both Alyssa and Dana are in their 3rd year at Cal Poly, and are exceptional academic students, and are extremely interested in clinical research studies emphasizing human physiology. The proposed study is a natural extension of their hard work and dedication. Moreover, experimentally testing whether bisphenol A poses any public health risk is highly novel and innovative, and if shown to be efficacious, will inform US public policy on food packaging and move the clinical research field substantially forward. In regards to the current application, both Alyssa and Dana were involved with all aspects of the study thus far, including conception of the idea and research question to writing the proposal application. The proposed project will expand their research skills and knowledge, and I have no doubt that they will meet the objectives of the study.

I will have formal, weekly meeting with both Alyssa and Dana, and more frequently as needed, especially during recruitment and data collection periods when I anticipate daily interactions. They will have access to equipment and supplies in the Human Performance Laboratory on the 2nd floor of the Kinesiology tower and to the STRIDE research center. This includes, but not limited to, using clinical exam rooms for recruitment of subjects, assessments and data collection, blood collection and processing supplies, -80 freezer, biochemical analyzers, BodPod for body composition assessment, body weight scales, stadiometer, and copier supplies. In addition, Alyssa and Dana will attend our weekly laboratory meeting, and present updates to me and research staff in regards to study progress, which is standard procedure for all studies in our laboratory. Additionally, attending this meeting will allow them to learn from our experienced clinical research staff, aid in problem solving skills for their particular project, learn how to statistically analyze data, and attend out bi-weekly journal club. This proposed project will be their Senior Honors Project, and I am requiring (and will oversee) that they submit a scientifically written research paper based on these results to Digital Commons in spring 2017, and present results at the College of Science and Math Study Research Conference also in spring 2017. Additionally, they will present preliminary findings at the Southwest regional American College of Sports Medicine in fall 2016 in Costa Mesa, CA. Finally, this study will be submitted to a refereed journal for publication, and both students will be heavily involved in that entire process.

In summary, the proposed experimental research study by Alyssa and Dana is highly novel, and will inform US public policy and food packaging. They will have all the necessary equipment and supplies from the Kinesiology Department, STRIDE research center, and my funded laboratory to complete the study. Most importantly, this project will enhance their skills, knowledge, and engagement, consistent with Cal Poly "Learn by Doing" approach. It is without hesitation that I highly recommend Alyssa Bird and Dana Williams for the Warren J. Baker Endowment Award.

Sincerely,



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