Title: Randomized Trial Examining Oral Consumption of Bisphenol A on Type 2 Diabetes Risk Markers

NCT03771066

PI: Todd Hagobian, PhD
Professor
California Polytechnic State University
Department of Kinesiology and Public Health

Funding: American Diabetes Association

Date: January 1, 2019

Title: Randomized Trial Examining Oral Consumption of Bisphenol A on Type 2 Diabetes Risk Markers
PI: Todd Hagobian, PhD
Funding: American Diabetes Association

Background and Overview

The prevalence of diabetes is well established, affecting >29 million Americans, with 90-95% of these individuals diagnosed with type 2 diabetes.[1,2] Although, diet, physical activity, obesity, and genetics play important roles in the etiology of type 2 diabetes, those established factors explain 30-60% of variance[3]; thus much still remains unknown. Emerging data suggests that synthetic non-persistent endocrine disruptors used in a variety of common consumer goods, including the mass industry-produced chemical bisphenol A (BPA), may play a pivotal role in type 2 diabetes rates.[4-13] In support of this hypothesis, National Health and Nutrition Examination Survey (NHANES), Nurses' Health Study II (NHSII), and other cross-sectional data have shown associations between urinary BPA concentrations and type-2 diabetes[14,15], pre-diabetes[16], insulin resistance[17], and hemoglobin A1c.[18] As a preliminary test, we conducted one of the only known studies at Cal Poly (IRB approved) in humans and found that consumption of a single, oral BPA dose of 50 μg/kg body weight immediately decreased glucose responses to an oral glucose tolerance test over 3 hours. These novel data were consistent with the one animal study in mice that showed that consumption of 10 μg/kg BW of BPA immediately (after 1 day) and significantly reduced glycemia. Interestingly, in longer mice studies, after 4 days of administration of 100 μg/kg BW of BPA, glycemia drastically increased and the mice became hyperinsulinemic.[4] The primary purpose of this 2-group, randomized, double-blinded, experimental study is to determine whether oral consumption of BPA at a dose consistent with the US EPA reference safe dose,[19] while controlling for energy intake and energy expenditure, has an independent effect on muscle insulin sensitivity and hepatic glucose suppression. Forty, normal-weight, sedentary adults will be randomly assigned to a 4-day energy balance diet plus oral BPA consumption at 50 μg/kg body weight (Diet+BPA) or 4-day energy balance diet plus oral placebo consumption (Diet+No BPA). The proposed experimental study will be the first to isolate the direct effects of BPA consumption on muscle insulin sensitivity and hepatic glucose suppression in humans, and will explore fecal microbiome species diversity and communities. The nature of this study is short-term (4 day exposure), and our and other previous preliminary studies showed that markers returned to normal after short-term BPA exposure.[20] Findings from this study will inform public health recommendations for food packaging, provide a framework for other studies in this area, and provide the first, much needed experimental evidence using gold standard measures as to whether BPA consumption over several days poses any risk of type 2 diabetes.

Specific Aims

1. To determine the effects of Diet+BPA, compared to Diet+No BPA on muscle insulin sensitivity (assessed by euglycemic hyperinsulinemic clamp technique and glucose stable isotope infusion) and insulin-stimulated hepatic glucose suppression.

2. To determine the effects of Diet+BPA, compared to Diet+No BPA, on fasting hormones (C-peptide, Pro-insulin), inflammatory markers (adiponectin), and sex hormones (17-beta-estradiol), linked to the progression to type 2 diabetes.

Exploratory Aim will examine fecal microbiome species diversity and communities in participants randomized to Diet+BPA compared to Diet+No BPA.

APPROACH

Preliminary Studies. The investigative team on this proposal has several prior collaboration and complementary expertise in pathophysiology of type 2 diabetes (Drs. Todd Hagobian, Suzanne Phelan, Michael La Frano, Steven Malin, Ryan Hubbard), experimental feeding and assessment studies (Drs. Hagobian, La Frano, Malin), energy intake and expenditure controlled studies (Drs. Hagobian, Malin, Hubbard), assessment of insulin sensitivity and hepatic glucose suppression (Drs. Hagobian, La Frano, Malin), assessment of endocrine disruptors (Drs. Hagobian, Phelan), and statistical methods of randomized controlled designs (Drs. Hagobian, Phelan, Schaffner, Malin).

Oral BPA Consumption in Adults. The PI (Hagobian) and Co-I's (Phelan and Schaffner) recently completed the first human study to our knowledge examining the effect of a single, oral dose of BPA on diabetes risk markers. [21] After an overnight fast, eleven, healthy college students (8W, 3M; 40% Hispanic, 21.0 ± 0.8 yrs.; 24.2 ± 3.9 kg/m^2) were randomized in a balanced, double-blinded, cross-over fashion to oral consumption of Placebo (PL), BPA at 4 μg/kg BW (BPA 4), and BPA at 50 μg/kg BW (BPA 50). Blood BPA, glucose, insulin, and C-Peptide were assessed at baseline, minutes 15, 30, 45, 60, and then every 30 minutes for the next 2 hours in response to a 75g oral glucose tolerance test using a linear mixed model (See Figure). BPA concentrations in the BPA 50 condition compared to both PL and BPA 4 was higher at minutes 30 through 180 (P<0.05), and BPA 4 was significantly higher than PL at the same time points (P<0.05). **Participants reported no gastrointestinal distress or changes in subjective appetite rating with the varying doses of BPA.** We also investigated effects on diabetes biomarkers. While group x time interactions did not reach significance, significant (or near) main effects for condition were observed for lower glucose (Tukey-adjusted P=0.036; Figure 1A), lower insulin (Tukey adjusted P=0.05; Figure 1B) and C -Peptide concentrations (Tukey adjusted P = 0.003; Figure 1C) in the BPA 50 vs BPA PL or BPA 4 conditions. These data are consistent with animal data[4] suggesting that oral BPA consumption had an immediate impact to <u>*lower*</u> blood glucose, and may potentially alter insulin sensitivity and/or hepatic glucose suppression, both of which will be assessed in the current proposal.

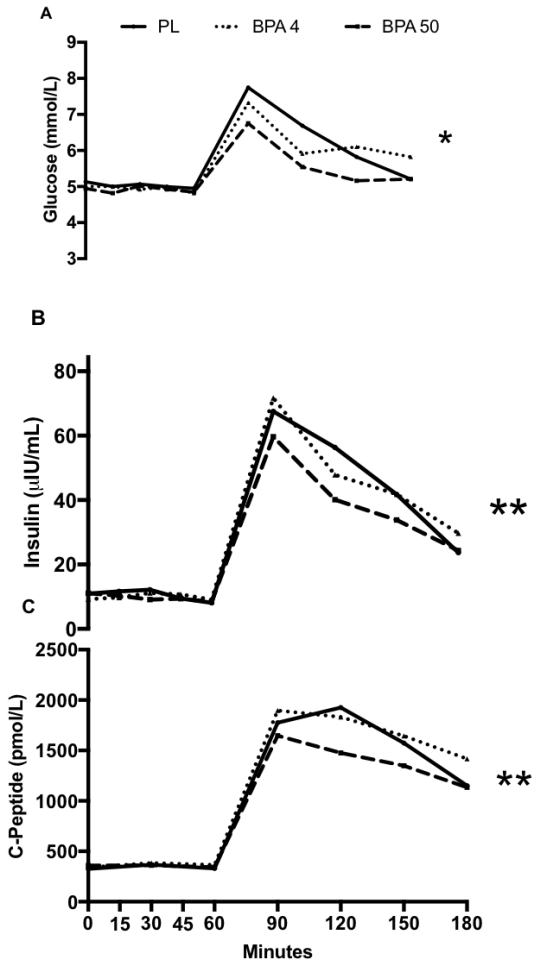


Figure 1. Plasma geometric mean glucose concentrations (A), insulin concentrations (B), and C-Peptide concentrations (C).
* BPA 50 significantly different than PL (P<0.05)
**BPA 50 significantly different than BPA 4 (P<0.05)
PL, Placebo; BPA 4, bisphenol A 4μg/kg BW; BPA 50, bisphenol A 50μg/kg BW

Intervention studies to reduce BPA exposure*.* The PI (Hagobian), and Co-I's (Phelan and Schaffner) have conducted two, novel 3-week intervention studies to reduce BPA exposure in women with normal-weight and obesity, respectively. Results indicate that from study entry to 3-weeks in women with normal-weight, the intervention significantly decreased geometric mean creatinine-adjusted urinary BPA by -0.71 ng/m (-50%) whereas women in the control significantly increased urinary BPA by 0.32 ng/mL (+66%; P=0.04).[22] We then adapted the 3-week intervention to women with obesity (Hagobian et al, *BMC Women's Health*, In review February 2018). From study entry to 3-weeks, a significant treatment x time (P<0.02) effect on creatinine-corrected urinary BPS concentrations was observed such that BPS decreased by 1.27 μg/g creatinine in the intervention group and decreased slightly by 0.09 μg/g creatinine in the control group. There were no significant changes in BPA concentrations (P>0.05).

METHODS

Overview This experimental study is a 2-group randomized, clinical trial comparing a 4-day energy balance diet plus oral BPA consumption at 50 μg/kg body weight (Diet+BPA) vs. 4-day energy balance diet plus oral placebo consumption (Diet+No BPA). Forty participants will be randomized to Diet+BPA and Diet+No BPA and will reside in a supervised environment at Cal Poly's sleep research facilities in the College of Liberal Arts under the supervision of Dr. Kelly Bennion for 6 days (2-day baseline run-in, followed by 4-day treatment). Main outcome measures (muscle insulin sensitivity and hepatic glucose suppression) will be assessed at baseline and treatment periods.

Experimental Protocol

Participants Forty, 18 to 50 years old, non-dieting, sedentary (≤ 3 hour/week of aerobic exercise), normal-weight (BMI = 18.5 to 24.9 kg/m^2) participants will be recruited. All participants will be healthy, weight-stable for the previous 6 months, free of any metabolic or chronic disease, non-smoking, and sedentary, assessed by

Health and Fitness History and Physical Activity Readiness (PAR-Q) questionnaires. We chose normal-weight participants to minimize the potential confound of high BPA exposure and insulin resistance as it relates to obesity.[8,23-25] Both men and women will be included in the study, as previous reports have shown that total BPA exposure may not differ by sex.[26] A fasting hemoglobin A1C blood draw will be conducted by a certified phlebotomist and assessed at Central Coast Pathology, as done in our previous studies. *Exclusion criteria include:* Hemoglobin A1C based on the American Diabetes Association guidelines of 5.7 to 6.4% (Prediabetes) or >6.4% (Diabetes), any metabolic or chronic disease, including impaired glucose tolerance or type 1 or type 2 diabetes, colitis or any inflammatory bowel condition, neurologic or psychiatric conditions, smoking, unsafe dieting practices, special diets (e.g. vegetarian, low-carbohydrate, Paleolithic, etc.) assessed by a Health History questionnaire, and pregnant women or women trying to become pregnant. All women will be given a pregnancy test (First Response, Princeton, NJ) that detects urine human chorionic gonadotropin prior to their participation. All testing in women will occur in the early follicular phase (1 to 4 days after start of menstruation) of the menstrual cycle. San Luis Obispo county has approximately 30-40% Hispanic adults, 55-65% non-Hispanic white, 5% non-Hispanic African-American, and 1% non-Hispanic Asian. All race/ethnicities will be eligible for this study with a target enrollment of 20% Hispanic and 80% non-Hispanic. We have previously successfully recruited this racial/ethnic target.[27] Because all participants will undergo a 2-day baseline run-in with a diet low in BPA, which has been shown to reduce BPA by 66%[28], background BPA exposure is not an exclusion.

Recruitment Our previous BPA consumption, and other feeding and exercise trials showed that recruitment of normal-weight, sedentary participants are extremely feasible with limited participants lost to follow-up (>98% retention).[29-33] In the current study, we anticipate similarly high retention. We will employ comparable retention procedures, including financial incentives ($500 total) for completing all visits. The breakdown of participant payment is: Baseline testing = $100, Treatment days = $400. During the treatment phase for 4 days participants will have a safety blood draw (see below for more detail) to assess liver, kidney and immune function at the Cal Poly Health Center. Cal Poly students will be charged $7.50 per draw (total $30). However, treatment days compensation will be increased to $430 to cover these costs if the participant is a Cal Poly student.

BPA Dose and Duration The dose of BPA selected in the proposed study is consistent with the US EPA reference dose of 50 μg/kg body weight. The National Toxicology Program reported that that typical BPA exposures range from 0.43 to 1.5 μg/kg body weight.[34] Based on occupation work, BPA exposure can be as high as 100 μg/kg body weight however.[34] The BPA dose selected is well below the FDA **no-observed-adverse-effect level** of 5 mg/kg BW per day.[35] The pharmacokinetics of 50-100 μg/kg BW of BPA has a blood concentration max occurring at ~1 hour after consumption, 92% of BPA is present in urine at 24 hours, and urinary half-life of 4-5.5 hours, with no reported side effects.[36-38] Our preliminary oral BPA consumption protocol at 4 μg/kg BW and 50 μg/kg BW had no gastrointestinal distress or subjective appetite changes. Thus, we anticipate no long-term negative effects of BPA consumption at 50 μg/kg BW as this is the reference dose in humans per the US EPA. Nevertheless, we will have medical supervision by Ryan Hubbard, MD (Board certified internist at the Cal Poly Health Center), throughout the entire study and will assess four hepatic function tests, one at baseline and three during treatment on days 4-6. Also, after completion of the study, all subjects will be offered a 2-day "fresh food" diet that has been shown to reduce BPA exposure by 66%[28] or will be provided with a $50 gift card to a natural food market (participant will choose). In our previous BPA consumption study, we successfully used a similar protocol to limit BPA exposure after completion of the study.

Diet, Activity and Sleep Monitoring and Control

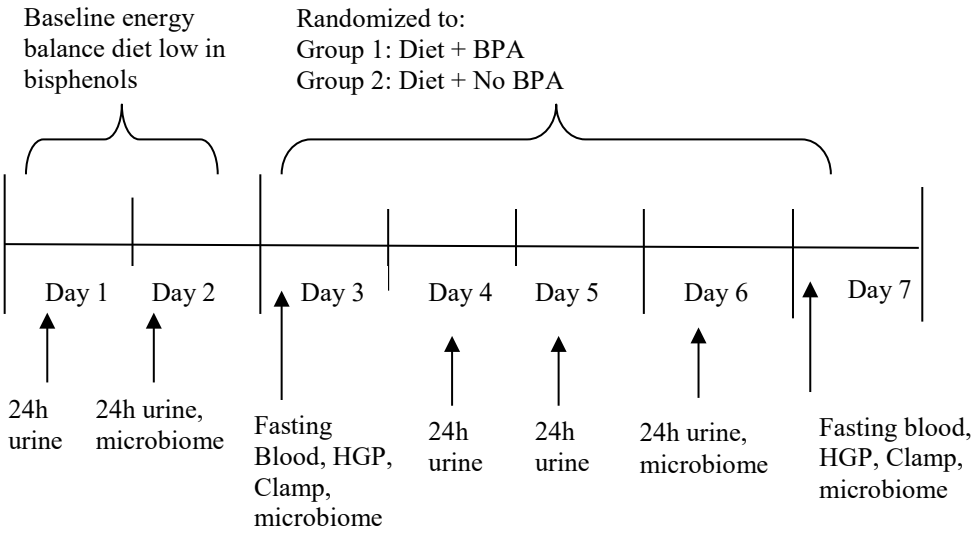
Participants will reside in our laboratory facilities in the College of Liberal Arts sleep facility for a total of 6 days (2-day baseline run-in followed by 4-day treatment) during which energy intake, energy expenditure, and sleep will be closely monitored and controlled.[39] Maintaining and controlling energy balance is necessary to isolate whether BPA has an effect on muscle insulin sensitivity and hepatic glucose suppression independent of changes in weight. Participants will be provided all food, will wear an Actigraph GT3X accelerometer on their non-dominant wrist for the entire 6 days, and ActiPal on their dominant leg (to assess sitting and standing), and perform no physical activity beyond daily living.[40] Participants will be allowed to leave our facilities and attend school or work but will be required to wear the Actigraph and ActiPal to ensure their typical low activity levels. Participants will consume all foods at our facilities and must return each night by dinnertime to sleep in our facilities. Participants in both conditions will be provided an energy balance diet low in bisphenols for all 6 days

(BPA <0.20 ng/g fresh weight; e.g. organic, whole foods, etc.). In our laboratory we have pretested foods from local grocery stores in San Luis Obispo, CA that are low in BPA (<0.20 ng/g fresh weight) using a commercially available kit (Detroit R&D, Inc., Detroit MI), to be used during the feeding portion of the proposed study. The composition of the diet will be approximately 55% carbohydrate, 30% fat, and 15% protein, consisting of all natural, organic foods, and all food will be prepared and stored in BPA-free containers, glass containers, etc. The 2-day baseline "fresh food" diet low in BPA is sufficient to reduce background BPA exposure.[28] Dr. La Frano (Co-I and Registered Dietician), will aid the Dr. Hagobian in estimating calorie needs and in preparing all meals and macronutrient composition for both groups. During the treatment phase, both groups will have similar diets with the only difference in BPA or placebo consumption. Oral BPA and placebo consumption will be administered on a vanilla wafer cookie, similar to our preliminary study and other previous pharmacokinetic studies.[36,38] A single dosing solution (10 mg/ml) for BPA will be prepared by dissolving d6-BPA (C/D/N Isotopes, Pointe-Claire, Quebec) in absolute 95% ethanol (Acros Organics, Janssen Pharmaceuticalaan, Belgium). For placebo, d6-BPA will not be included in the ethanol solution. A research assistant not involved in any other aspect of this study will make the dosing solutions. Approximately 1 ml aliquots will be passed twice through a sterile micro filter to aid in removal of bacteria and placed onto a vanilla wafer cookie (20 grams), allowing the ethanol to dry six hours before daily consumption. The vanilla wafer cookies with BPA and placebo look, weigh, and taste identical.

Sequence of Events

Participants will complete a demographic questionnaire assessing age, race, ethnicity, and weight history, as previously used in our studies.[30,41] Participants will also complete a health and fitness history questionnaire. Both of these questionnaires ask personal questions including the use of illegal substances as well as confidential mental and physical health information. This information will be collected using RedCap, an encrypted data storage program. Any paper copies of participant's answers will be stored in a locked filing cabinet and destroyed after 7 years. Only trained study staff who have undergone significant training on confidentiality and privacy, HIPAA regulations and patient safety will collect these completed questionnaires and have access to the results for research purposes only. No research volunteers including students will administer theses questionnaires or have access to the questionnaires. Height will be measured by stadiometer (Ellard Instrumentation LTD., Monroe, WA) and weight by balance scale (Continental Scale Corporation, Bridgeview, IL), and BMI (kg/m^2) will be calculated. Resting metabolic rate (RMR) will be measured in the morning after an overnight fast using a ventilated hood and indirect calorimetry to estimate energy requirements consistent with best practice methods[42]. After a 60-minute period of relaxation, subjects will sit comfortably in a reclining chair for up to 60 minutes while expired air is collected using the metabolic measurement system (Parvomedics TrueMax 2400, Sandy, UT). Kien and Ugrasbul[43] reported that energy requirements estimated from RMR and the appropriate activity factor were strongly correlated (r = 0.73) with energy requirements measured during 28-days of controlled feeding. Also, participants will complete a 3-day (2 weekdays, 1 weekend day) dietary recall using the automated NCI ASA-24 (https://epi.grants.cancer.gov/asa24/). Thus, total energy requirements will be estimated using RMR and the appropriate activity factor and 24 h dietary recalls, as others and we previously described[29,32,41,43]. Body composition will be measured as a preliminary test using a Lunar iDXA scan (General Electric Healthcare Company, USA), the new gold standard for assessing body composition. It will require the

Figure 2. Overview of experimental study design. Participants will reside in our laboratory facilities during which energy intake, energy expenditure and sleep will be monitored and controlled.



participant to lay flat on their back without moving for approximately 10 minutes. Cell mutation can occur with excessive exposure to radiation, however the Lunar iDXA has technology that enables extremely precise and safe measurements. It uses a unique "K-edge filter" that absorbs X-rays to protect the patient against unnecessary exposure, resulting in a low radiation exposure of approximately 0.0003 mSv per scan. The exposure from one scan is equivalent to 17.6% of the average person's daily exposure to radiation. For comparison, a normal chest X-Ray causes .010 mSv of radiation exposure per scan which is 588% of an individuals average daily exposure. There are no complications expected with the DXA procedure.

After preliminary tests, a 2-day run-in baseline energy balance diet low in BPA will occur (see Figure 2 above) in which 24-hour urine, fasting blood, muscle insulin sensitivity, hepatic glucose suppression and fecal microbiome will be assessed. Participants will then be randomized (blocked by sex and ethnicity) to either Diet+BPA or Diet+No BPA, in a double-blinded fashion. Similar to our previous intervention studies, the study statistician (Dr. Schaffner) will computer generate the randomization scheme but will not have contact with any participant. Repeated assessment of 24-hour urine collection of BPA for 3 consecutive days, and fasting blood, muscle insulin sensitivity, hepatic glucose suppression, and gut microbiome will occur at the end of the treatment period. Consumption of the final BPA or placebo will occur 1 hour prior to the muscle insulin sensitivity measurement and glucose stable isotope infusion.

Participant Comfort: The goal of the experimental protocol is to try and control for as many confounds as possible including dietary intake, activity, and sleep that could influence outcome measures. Thus, participants will sleep in the Cal Poly sleep research facilities in the College of Liberal Arts under the supervision of Dr. Kelly Bennion. A Research Assistant or Postdoctoral Research Fellow will sleep in an adjacent room. Participants will be able to leave during the day, attend class or work, do laundry and other activities, but must consume food and sleep in the Cal Poly sleep research facilities. Building 52 is close to the recreation center, and participants will be allowed to walk over and shower, if they are a student. Non-students will be allowed to go back to your home and shower, etc. in the morning after activities are completed. Restrooms are available down the hall from the sleep research facility.

Outcome Measures

Urinary BPA and Creatinine Concentrations At baseline and treatment periods, 6-hour intervals of urine will be collected for 24-hours[38], on 5 days to minimize day-to-day variability of BPA[44]. After measuring urine volume with a graduated cylinder, twenty-five milliliters will be aliquoted into 5 separate BPA-free polypropylene tubes at each 6-hour interval and stored at -80°C. All urine samples will be analyzed by Washington State Department of Health (Director Blaine Rhodes) using established CDC protocol[45,46] and online solid-phase extraction coupled to high-performance liquid chromatography (HPLC) isotope dilution tandem mass spectrometry with peak focusing as described previously[47]; urinary creatinine concentrations will be assessed by a colorimetric assay. The laboratory will be blinded to the identity of the samples and treatment allocation.

Fasting Hormones, Inflammatory Markers, and Muscle Insulin Sensitivity At baseline and treatment periods, fasting hormones, endocrine factors, and inflammatory markers linked to the pathogenesis of type 2 diabetes, including insulin, glucose, C-peptide, Pro-Insulin, adiponectin, 17-beta-estradiol, free fatty acids (FFA) and BPA will be collected by trained staff (phlebotomist and research assistants). ***Muscle Insulin Sensitivity*** Then, a priming bolus of 200 mg [6,6-^{2}H] glucose will be given, followed by a 90-min infusion of [6,6-^{2}H] glucose at a rate of 2.5 mg/min delivered by a peristaltic infusion pump (Harvard Apparatus Pump 22; Harvard Apparatus, Holliston, MA). ***Muscle Insulin Sensitivity (euglycemic hyperinsulinemic clamp technique***) will then be assessed as others and we previously described.[25,48,49] Prior to insulin infusion, exhaled air will be collected for 20 min, using a ventilated hood and indirect calorimetry to determine basal substrate oxidation. Two infusions will be started using a peristaltic infusion pump: 1) primed (250 mU/m^{-2}) constant infusion (40 mU/m^{-2}•min^{-1}) of insulin diluted in saline containing 3%(vol/vol^{-1}) of the participants own blood; and 2) a variable infusion of a 20% glucose saline solution with 2% spiked [6,6-^{2}H] glucose, adjusted to maintain plasma glucose at 90 mg/dl, and continued for 120 minutes. Sterile techniques are used at all times, including the mixture of the participants own serum into insulin. A small amount (<3ml) of patients' blood will be collected in a sterile syringe and then inserted with a 18g needle into the insulin infusion bags through the port, using sterile techniques. Including the participants own serum to insulin prevents insulin absorption to glassware and plastic surfaces, as noted in the original Matsuda and DeFranzo paper.[50] Dr. Hubbard will be on call during all clamps. Blood glucose analysis will occur every 5 minutes using the glucose oxidation method (GL5, Analox Instruments). Rates of glucose appearance (Ra) and glucose rates of disposal (Rd) will be

calculated using non-steady-state Steele equations[51]: glucose Ra (mg/min) =F − V[(C1+C2)/2] [IE2−IE1)/(t2−t1)] ÷ [IE2+IE1)/2] and glucose Rd (mg/min) = Ra−V[C2−C1)/(t2−t1)]; where F is the isotope infusion rate, IE1 and IE2 are enrichments of plasma glucose with isotope label at times t1 and t2, C1 and C2 are plasma glucose concentrations, and V is the estimated volume of distribution for glucose (180 ml/kg). Rates of glucose disposal (Rd), which reflect mostly skeletal muscle glucose uptake, will be averaged during the last 30 min of the clamp and used to characterize *muscle insulin sensitivity*. Basal rates of endogenous glucose appearance (Ra), which is comprised primarily of hepatic glucose production (HGP), will be averaged from t = −30 to t = 0 min. HGP during the clamp will be calculated as the difference between Ra clamp and the exogenous glucose infusion rate. The suppression of HGP will be defined as [1 − (HGPclamp/HGPfast) × 100%] and used to estimate hepatic insulin sensitivity.[49] Additionally, we will account for ambient insulin to infer effect on HGP. Insulin-stimulated suppression of free fatty acids (FFA) will be calculated as [1 − (FFAclamp/FFAfast) × 100%]. Glucose kinetics and clamp-derived carbohydrate oxidation will be determined by indirect calorimetry during the last 20 min of the clamp using standard equations.[51] Nonoxidative glucose disposal (NOGD) will be calculated (NOGD = Rd − total carbohydrate oxidation). Metabolic flexibility will be calculated as RQclamp − RQfast. Blood samples will be centrifuged at 4°C for 15 min at 3000g and then stored at −80°C until subsequent analysis. Glucose isotopic enrichment will be measured by liquid chromatography–mass spectrometry, as we described previously.[49] Plasma insulin, C-peptide, adiponectin, and 17-beta-estradiol will be measured using an Enzyme Linked-Immuno-Sorbent assay (Millipore, Billerica, MA or Invitrogen Corporation Camarillo, CA). Plasma FFAs will be analyzed by a colorimetric assay (Wako Chemicals, Richmond, VA), and plasma BPA using HPLC (described above). All samples (except BPA) will be analyzed under the direction of the PI, and repeated for CV >7-10%.

Fecal Microbiome will be assessed by 16S rRNA gene sequencing for classification and relative quantitation of bacterial taxa. Fecal samples will be collected <1 hour of defecation and DNA will be extracted from well-homogenized aliquots using industry-standard fecal DNA extraction kits. PCR will be used to amplify the V3-V4 variable regions of the 16 rRNA genes with 25 cycles of amplification to minimize PCR biases. PCR products will be sequenced in a paired-end protocol using the MiniSeq DNA sequencing platform. Sequence data will be run through a standard microbiome analysis pipeline. Bacterial diversity will be assessed at multiple taxonomic levels from Operational Taxonomic Units through Phylum, using observed counts as well as Simpson and Shannon diversity indices.

STATISTICAL CONSIDERATIONS.

Statistical Analysis The ultimate goal in our analyses will be to compare participants randomized to Diet+BPA vs. Diet+No BPA. We conservatively expect 10% of participants will be lost to follow up. All data will include assessment for model validity (e.g. distribution, homoscedasticity) and will be appropriately transformed to meet model conditions as needed. Outliers will be included in the analysis and, when substantive, their impact on parameter estimates will be noted. Statistical test will be conducted at the $P<0.05$ level. ***Hypothesis 1*****:** We hypothesize that participants randomized to Diet+BPA will have decreased reduced muscle insulin sensitivity and blunted insulin stimulated hepatic glucose suppression, compared with Diet+No BPA. Under the condition of sphericity, a linear mixed effect model will be used to examine differences in muscle insulin sensitivity and hepatic glucose suppression between groups adjusting for age, sex, education, income, physical activity levels, ethnicity/race, baseline BMI, baseline weight, baseline dietary intake and macronutrient intake. In the event the data do not meet the sphericity condition, multivariate analysis of variance (MANOVA) models with profile analyses will be used with the same covariates. ***Hypothesis 2***: Multiple linear regression or repeated measures MANOVA will be used to examine associations between BPA and insulin sensitivity and hepatic glucose suppression adjusting for the same covariates. ***Hypotheses 3:*** Linear mixed effect model will be used to examine differences in hormones related to the pathogenesis of type 2 diabetes (i.e. C-peptide, Pro-insulin, adiponectin, 17-beta-estradiol). ***Exploratory Aim***: Changes in microbiome community structure will be evaluated for each individual by comparing Bray-Curtis similarity measures between samples collected before and after treatment using a linear mixed model.

Timeline The proposed study is currently in review with the American Diabetes Association and National Institutes of Health. We anticipate enrolling 1-2 participants per month with a total of 40 subjects being enrolled through year 3.

PROTECTION OF HUMAN SUBJECTS and DATA SAFETY MONITORING

Proposed level of risk: The risks for physical, psychological, social or legal harm are considered low for all testing procedures and treatment doses. The dose of BPA chosen was based on the US EPA report that that

BPA at 50 μg/kg BW is the reference dose.[19] The Reference Dose is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used." (https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system)

Potential risks to subjects: Minor physical risks are related to forearm/hand catheterization, such as bruising, fainting, or nausea. Moreover, there are some risks associated with the hyperinsulinemic euglycemic clamp technique including fainting, nausea, and tingling sensation in fingers and toes, sweating, and uncomfortableness. The direct effect of the dose of BPA on insulin sensitivity, hepatic glucose production and fecal microbiome is not known, and we hypothesize that it will reduce insulin sensitivity and blunt insulin-stimulated glucose production. Participants may experience nausea, upset stomach, and/or diarrhea with consumption of BPA. However, in our preliminary study no participants reported any side effect with consumption of BPA at the same dose. Also, the repeated dose of BPA on liver and kidney enzymes is not known. Risks of receiving the placebo are considered minimal. Participants may feel uncomfortable answering questionnaire data and may omit any question. There is a minor risk of low dose radiation exposure from the body composition Lunar iDXA scan, but exposure is only 0.0003 mSv, 17.6% of the average daily exposure. There is a small possibility of cell mutation caused by excessive radiation exposure however a DXA scan is a low dose of exposure. There are no complications expected with the DXA procedure. There may be a loss of work time or family time by residing in our facilities for 6 consecutive days. Although every effort will be made to keep all personal health information confidential, there is an unforeseen risk of a breach in this confidentiality. It is possible that the data stored could be hacked. We will follow National Institutes of Health guidelines (2 unique de-identifiers for each participant, encryption of all data, data stored and backup on Cal Poly servers monitored by COSM IT department) to minimize this risk.

Adequacy of Protection Against Risks. Risks will be minimized by continuous safety monitoring, highly qualified staff, careful screening prior to enrollment, and by our medical board-certified internist consultant (Dr. Ryan Hubbard). Dr. Hubbard will be on call during the study. We are using standard laboratory techniques that we have used extensively in our prior studies; laboratory staff is experienced in all these testing procedures using the CDCs universal precautions standards. All laboratory staff is trained in CPR.

Blood draws and hyperinsulinemic euglycemic clamp technique. The PI has studied diabetes and appetite regulation for over 18 years and has extensive experience with blood draws and assessment of insulin action. The total amount of blood taken for the entire study is relatively small, only about 70 ml. There are some minor risks involved with catheters and blood draws. To minimize these risks, we will follow the CDC universal precautions in preventing transmission of agents in health care settings. Catheters will only be inserted by trained individuals (e.g. phlebotomist), using sterile technique at all times. There may be some pain associated with the needle stick at the moment of insertion, followed by minor discomfort of having the needle withdrawn and the catheter advanced slightly into the vein. There may potentially be a local infection, slight bleeding, or bruising, and fainting in some participants. To minimize this, subjects will be sitting for all blood draws and during the clamp. Moreover, Dr. Ryan Hubbard (medical consultant) will be "on call" during all clamps. The study staff and Dr. Hubbard will be available for emergencies and will refer and help participants obtain appropriate medical care, if needed.

Bisphenol A Consumption. As mentioned above, the BPA dose selected is well below the **no-observed-adverse-effect level** of BPA of 5 mg/kg body weight per day, according to the U.S. FDA[35] and is reference dose as reported by the US EPA.[19] Our preliminary study examined the effects of varying doses of BPA consumption, including 50 μg/kg body weight, on markers of type 2 diabetes. We observed no gastrointestinal distress side effects, or changes in subjective appetite ratings with this or a smaller dose. Pharmacokinetics studies of similar dosages (50-100 μg/kg body weight) of BPA have also reported no side effects, no adverse effects, or no unintended participant harms.[36-38] Because this proposed study is relatively short (4 treatment days), we anticipate no long-term negative effects of BPA given that the dose is within a typical exposure range seen in US adults. We will provide all participants with a 2-day "fresh food" diet after they have completed the study (or drop out during the study). A similar "fresh food" diet has been shown to reduce urinary BPA concentrations by 66%.[28] To minimize risks, medical supervision will occur throughout the study and we will now expand daily blood tests to include both liver, kidney, and immune functioning. In the morning during all treatment days a Comprehensive Chemistry Panel (liver and kidney functioning) and Complete Blood Count test will be completed and evaluated by Dr. Hubbard. The Comprehensive Chemistry

Panel includes, Glucose, BUN, Creatinine, BUN/Creatine, Sodium, Potassium, Chloride, Total Carbon Dioxide, Calcium, Total Protein, Albumin, Globulin, Albumin / Globulin Ratio, Alkaline Phosphatase, AST, ALT, Total Bilirubin, and glomerular filtration rate (GFR) Estimated. The Complete Blood Count (CBC) includes white blood cell, red blood cell, hemoglobin, hematocrit, mean corpuscular volume, platelet count. These tests will be done in "real time" and reviewed by Dr. Hubbard each day. Should any abnormally occur, Dr. Hubbard will inform the PI and DSMB, and Dr. Hubbard has the authority to cease participant treatment at any time and inform the PI and DSMB. If needed, Dr. Hubbard will contact a participant's physicians to discuss treatment/assessment continuation for participant. **We will thoroughly discuss with all participants that the BPA dose is above typical exposure ranges in US adults (i.e. up to 1.5 μg/kg BW per day).** The nature of this study is short-term (4 day exposure), and our and other previous preliminary studies showed that markers returned to normal after short-term BPA exposure.[20]

***Confidentiality, Data Storage, and Security of Data*:** All data will be de-identified, with two unique participant identifiers appearing on collection documents. Data collected will be placed in a locked file cabinet within 24 hours of its acquisition. An encrypted, password-protected file will be maintained that associates the subject name with identification number. Access to electronic data will be encrypted, password protected and restricted to the investigators. Paper data will be stored in a locked file cabinet. Data will be removed for the purpose of coding, data entry, or auditing only. Personal and sensitive health information will be collected directly into RedCap, an encrypted, password protected data collection tool and no paper copies with participant's personally identifying information will be kept. Only study staff will have access to this information and it will be restricted to all volunteers.

Data Safety Monitoring Plan. The data safety-monitoring plan for this trial focuses on close monitoring by the PI/Co-Is, IRB, and consultants. The Safety Officer Report template will be finalized with input from the entire investigative team at the time of award. The investigative team will review quarterly recruitment, retention, and safety information on all participants, including any adverse events and unintended harms. Daily/weekly internal investigator meetings will also occur to review recruitment, attendance, retention, and safety data on an ongoing basis. Annual DSMB meetings, via conference call, will occur with the entire research team. *Adverse Events/ Reporting of Events:* The investigators are responsible for reporting all adverse events, serious adverse events, and unintended harms within pre-specified times to the DSMP and IRB. Adverse events will include any event that causes or increases risk of harm to the subject or others. Serious adverse event will include any event that results in death, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability or incapacity, or a congenital anomaly. A fatality will be reported immediately. Adverse events reported at an assessment visit or informally at any time will be evaluated by the investigators to determine if they are unanticipated problems involving risk to subjects and others or not. The subject's situation will also be assessed with regard to study and/or intervention continuation. The Postdoctoral Research Fellow, reported to the PI and investigative team, the DSMP, and the IRB, will record any serious adverse event. American Diabetes Association and/or National Institutes of Health will be informed of serious adverse events and actions taken.

Importance of Knowledge to be Gained. The potential for minimal risks to human subjects is considered reasonable in relation to the importance of the knowledge that is expected to result from this study. We believe this project is significant because it deals with endocrine disruptors and risk markers related to type 2 diabetes. If found effective, the proposed study could inform food packaging and labels containing BPA (or BPA-free). Moreover, if found to be efficacious, results from the current study will inform larger BPA dosing studies and large clinical trials to reduce BPA exposure. This study will provide the first comprehensive picture of how oral BPA consumption impacts insulin sensitivity and hepatic glucose production.

References

1. Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States. Atlanta, GA: US Department of Health and Human Services; 2014.
2. Wadden TA, Vanltallie TB, Blackburn GL. Responsible and irresponsible use of very-low-calorie diets in the treatment of obesity. JAMA 1990;263:83-5.
3. Poulsen P, Kyvik KO, Vaag A, Beck-Nielsen H. Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance--a population-based twin study. Diabetologia 1999;42:139-45.

4. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. Environ Health Perspect 2006;114:106-12.
5. Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect 2001;109:675-80.
6. Somm E, Schwitzgebel VM, Toulotte A, et al. Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ Health Perspect 2009;117:1549-55.
7. Teppala S, Madhavan S, Shankar A. Bisphenol A and Metabolic Syndrome: Results from NHANES. Int J Endocrinol 2012;2012:598180.
8. Shankar A, Teppala S, Sabanayagam C. Urinary bisphenol a levels and measures of obesity: results from the national health and nutrition examination survey 2003-2008. ISRN Endocrinol 2012;2012:965243.
9. Shankar A, Teppala S, Sabanayagam C. Bisphenol A and peripheral arterial disease: results from the NHANES. Environ Health Perspect 2012;120:1297-300.
10. Eladak S, Grisin T, Moison D, et al. A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. Fertil Steril 2015;103:11-21.
11. Helies-Toussaint C, Peyre L, Costanzo C, Chagnon MC, Rahmani R. Is bisphenol S a safe substitute for bisphenol A in terms of metabolic function? An in vitro study. Toxicol Appl Pharmacol 2014;280:224-35.
12. Peyre L, Rouimi P, de Sousa G, et al. Comparative study of bisphenol A and its analogue bisphenol S on human hepatic cells: a focus on their potential involvement in nonalcoholic fatty liver disease. Food Chem Toxicol 2014;70:9-18.
13. Valvi D, Casas M, Mendez MA, et al. Prenatal bisphenol a urine concentrations and early rapid growth and overweight risk in the offspring. Epidemiology 2013;24:791-9.
14. Silver MK, O'Neill MS, Sowers MR, Park SK. Urinary bisphenol A and type-2 diabetes in U.S. adults: data from NHANES 2003-2008. PLoS One 2011;6:e26868.
15. Ahmadkhaniha R, Mansouri M, Yunesian M, et al. Association of urinary bisphenol a concentration with type-2 diabetes mellitus. J Environ Health Sci Eng 2014;12:64.
16. Sabanayagam C, Teppala S, Shankar A. Relationship between urinary bisphenol A levels and prediabetes among subjects free of diabetes. Acta Diabetol 2013;50:625-31.
17. Beydoun HA, Khanal S, Zonderman AB, Beydoun MA. Sex differences in the association of urinary bisphenol-A concentration with selected indices of glucose homeostasis among U.S. adults. Ann Epidemiol 2014;24:90-7.
18. Sun Q, Cornelis MC, Townsend MK, et al. Association of urinary concentrations of bisphenol A and phthalate metabolites with risk of type 2 diabetes: a prospective investigation in the Nurses' Health Study (NHS) and NHSII cohorts. Environ Health Perspect 2014;122:616-23.
19. EPA. Chemical Assessment Summary, Bisphenol A; CASRN 80-05-7. In: Agency USEP, ed.1988.
20. Stahlhut RW, Peterson J, Taylor JA, Nadal A, Dyer JA, vom Saal FS. Experimental BPA Exposure and Glucose-Stimulated Insulin Response in Adult Men and Women. Journal of the endocrine society 2018.
21. Hagobian TA, Bird A, Stanelle S, Williams D, Schaffner A, Phelan S. Pilot Study on the Effect of Orally Administered Bisphenol A on Glucose and Insulin Response in Nonobese Adults. J Endocr Soc 2019;3:643-54.
22. Hagobian T, Smouse A, Streeter M, Wurst C, Schaffner A, Phelan S. Randomized Intervention Trial to Decrease Bisphenol A Urine Concentrations in Women: Pilot Study. J Womens Health (Larchmt) 2017;26:128-32.
23. Hou X, Liu J, Song J, et al. Relationship of Hemoglobin A1c with beta Cell Function and Insulin Resistance in Newly Diagnosed and Drug Naive Type 2 Diabetes Patients. J Diabetes Res 2016;2016:8797316.

24. Derakhshan A, Tohidi M, Arshi B, Khalili D, Azizi F, Hadaegh F. Relationship of hyperinsulinaemia, insulin resistance and beta-cell dysfunction with incident diabetes and pre-diabetes: the Tehran Lipid and Glucose Study. Diabet Med 2015;32:24-32.
25. Abdul-Ghani MA, Matsuda M, DeFronzo RA. Strong association between insulin resistance in liver and skeletal muscle in non-diabetic subjects. Diabet Med 2008;25:1289-94.
26. Kim YH, Kim CS, Park S, Han SY, Pyo MY, Yang M. Gender differences in the levels of bisphenol A metabolites in urine. Biochem Biophys Res Commun 2003;312:441-8.
27. Evero E, Hackett, L, Clark, RD, Phelan, S, Hagobian, T. Aerobic exercise reduces neuronal responses in food reward brain regions. Journal of Applied Physiology 2012;In press.
28. Rudel RA, Gray JM, Engel CL, et al. Food packaging and bisphenol A and bis(2-ethyhexyl) phthalate exposure: findings from a dietary intervention. Environ Health Perspect 2011;119:914-20.
29. Hagobian TA, Braun B. Interactions between energy surplus and short-term exercise on glucose and insulin responses in healthy people with induced, mild insulin insensitivity. Metabolism 2006;55:402-8.
30. Hagobian TA, Jacobs KA, Subudhi AW, et al. Cytokine responses at high altitude: effects of exercise and antioxidants at 4300 m. Med Sci Sports Exerc 2006;38:276-85.
31. Hagobian TA, Sharoff CG, Braun B. Effects of short-term exercise and energy surplus on hormones related to regulation of energy balance. Metabolism 2008;57:393-8.
32. Hagobian TA, Sharoff CG, Stephens BR, et al. Effects of exercise on energy-regulating hormones and appetite in men and women. Am J Physiol Regul Integr Comp Physiol 2009;296:R233-42.
33. Hagobian TA, Yamashiro M, Hinkel-Lipsker J, Streder K, Evero N, Hackney T. Effects of acute exercise on appetite hormones and ad libitum energy intake in men and women. Appl Physiol Nutr Metab 2013;38:66-72.
34. NTP. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental effects of Bisphenol A. NTP CERHR Monograph Series 2008;v:vii–ix:1-64.
35. Aungst J, Anderson S. Final report for the review of literature and data on BPA by FDA Bisphenol A Joint Emerging Science Working Group. Department of Health and Human Services.2014.
36. Thayer KA, Doerge DR, Hunt D, et al. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ Int 2015;83:107-15.
37. Volkel W, Bittner N, Dekant W. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. Drug Metab Dispos 2005;33:1748-57.
38. Volkel W, Colnot T, Csanady GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chem Res Toxicol 2002;15:1281-7.
39. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients) %@ 978-0-309-08525-0. Washington, DC: The National Academies Press; 2005.
40. Kozey-Keadle S, Libertine A, Lyden K, Staudenmayer J, Freedson PS. Validation of wearable monitors for assessing sedentary behavior. Med Sci Sports Exerc 2011;43:1561-7.
41. Hagobian TA, D'Amico A, Vranna C, Brannen A, Phelan S. Prospective Changes in Energy Intake, Physical Activity, and Resting Energy Expenditure During Pregnancy. California Journal of Public Health 2015;13:66-71.
42. Compher C, Frankenfield D, Keim N, Roth-Yousey L, Evidence Analysis Working G. Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. J Am Diet Assoc 2006;106:881-903.
43. Kien CL, Ugrasbul F. Prediction of daily energy expenditure during a feeding trial using measurements of resting energy expenditure, fat-free mass, or Harris-Benedict equations. The American journal of clinical nutrition 2004;80:876-80.
44. Ye X, Wong LY, Bishop AM, Calafat AM. Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. Environ Health Perspect 2011;119:983-8.

45. Hagobian T, Smouse A, Streeter M, Wurst C, Schaffner A, Phelan S. Randomized Intervention Trial to Decrease Bisphenol A Urine Concentrations in Women: Pilot Study. J Womens Health (Larchmt) 2016.
46. Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ Health Perspect 2005;113:391-5.
47. Ye X, Kuklenyik Z, Needham LL, Calafat AM. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. Anal Chem 2005;77:5407-13.
48. Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. Diabetes Care 2007;30:89-94.
49. Sharoff CG, Hagobian TA, Malin SK, et al. Combining short-term metformin treatment and one bout of exercise does not increase insulin action in insulin-resistant individuals. Am J Physiol Endocrinol Metab 2010;298:E815-23.
50. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. The American journal of physiology 1979;237:E214-23.
51. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol Respir Environ Exerc Physiol 1983;55:628-34.

California Polytechnic State University

Informed Consent to Participate in a Research Project on a Randomized Trial Examining Oral Consumption of Bisphenol A on Type 2 Diabetes Risk Markers

INVESTIGATORS: A research project focused on the effects of Bisphenol A (BPA) on type 2 diabetes risk markers is being conducted by Todd Hagobian, Ph.D. (thagobia@calpoly.edu), Suzanne Phelan, Ph.D. (sphelan@calpoly.edu), Hannah Brunner-Gaydos, B.S. (hbrunner@calpoly.edu), Andrew Schaffner, Ph.D. (aschaffn@calpoly.edu), and Michael La Frano, Ph.D. (mlafrano@calpoly.edu) in the Departments of Kinesiology and Public Health, Department of Statistics, and Food Science and Nutrition at Cal Poly, San Luis Obispo.

NOTE: You are being asked to participate in a research study. Your participation is voluntary. If you are a student, your decision whether or not to participate will not have any effect on your academic status or grades. Please feel free to ask questions at any time if there are anything you do not understand. Please be aware that you are not required to participate in this research, you may omit any questions you prefer not to answer, and you may discontinue your participation at any time without penalty or loss of benefits.

PURPOSE: BPA is a chemical used to make plastics, cans, and other food storage containers. It is estimated that up to 93% of the US population has detectable urine levels of BPA. BPA, which may act like estrogen in the body, is related to an increased risk of diabetes. The purpose of this study is to determine whether 4 days of consumption of BPA alters type 2 diabetes risk markers. A total of forty, healthy men and women will be recruited for this study. The study will have preliminary tests, a 2-day baseline period, and 4-day treatment period. You will sleep in our facilities at Cal Poly, and all food for 6 days will be provided to you. You will be randomized to either BPA at an amount of 50 µg/kg body weight (approximately 3.5 mg for 155 lb. person) or placebo (no BPA). The BPA or placebo will be provided on a vanilla wafer cookie to you. You and the researchers will not know whether you consumed BPA or placebo. The 50 µg/kg body weight amount of BPA is a lot higher than what is typical is US adults and higher than the recommended of BPA exposure by the European Food Safety Authority. The BPA dose of 50 µg/kg body weight is the referent dose (safe dose throughout a lifetime) approved by the US Environmental Protection Agency (EPA). You will be closely monitored by the research team and board-certified internist, Ryan Hubbard, M.D.

MEASURES:

Preliminary Tests (Approximately 2.5 hours)

Your height and weight will be measured, and you will be given questionnaires on your demographics and health history. You will also have your body composition (muscle, fat and bone density) measured using a complete body DXA scan. You will lay on your back for about 10 minutes while the DXA machine scans your body. All women will be given a urine pregnancy test, and pregnant women or women wanting to become pregnant are not eligible to participate in the study. You will then have a fasting blood draw taken by a trained phlebotomist and we will assess hemoglobin A1C, which measures your glucose levels over 2-3 months. If your hemoglobin A1C is $\geq$5.7% you cannot participate in study and will be referred to your physician. You will then complete a resting metabolic rate test to determine how many calories you burn in a day. You will sit quietly in a reclining chair for 60 minutes with a ventilated hood placed on your head (it looks like a space helmet), and we will collect your expired air. You will then wear accelerometers on your wrist and thigh for the next 6 days to determine your activity levels.

2-day Baseline and 4-day Treatment

Diet, Activity, and Sleep*:* You will sleep and reside in Cal Poly's sleep facilities in the College of Liberal Arts for a total of 6 days (2-day baseline followed by 4-day treatment) in which diet, activity, and sleep

will be monitored. You will be provided all food during the entire 6 days, consisting of all natural, organic foods, and all food will be prepared and stored in BPA-free containers, glass containers, etc. The composition of the diet will be approximately 55% carbohydrate, 30% fat, and 15% protein. During the 4-day treatment phase, you will be randomized (you do not get to choose) to either consumption of BPA at 50 μg/kg body weight (approximately 3.5 mg for 155 lb. person) or placebo (no BPA). Your diet will be similar to what you normally eat and will have enough calories to maintain your weight. You will also wear an accelerometer on your wrist and another accelerometer on your thigh for the entire 6 days to measure activity and sleep. You can leave our facilities during the day to attend school, work, go home, etc., but will have to come back at night to sleep.

Urinary and Fecal Collection You will urinate in a collection container to assess urine BPA levels. Urine collection will happen for 24 hours at baseline (2 days) and treatment (3 days) periods for a total of 5 days. You will also provide a single, small amount of fecal waste at baseline (2 days) and treatment (2 days) periods.

Fasting Blood Draw (approximately 30 minutes): You will have a fasting blood draw administered by a phlebotomist prior to and during each treatment day to assess hormones and/or liver, and kidney enzymes.

Insulin Sensitivity Test (approximately 3 hours): This test helps determine the level of insulin response by the body. This test involves intravenous (IV) infusion of insulin and glucose (sugar) by vein, and providing multiple blood samples for up to 3 hours. We will use sterile techniques at all times during the infusion. To make the blood sampling easier, we will place a small tube (catheter) in the vein we are going to take blood samples from, and leave that tube in place until the end of the test. The small tubes (catheters) will be placed in different forearms; one for administration of insulin and glucose, and one for blood draw. You will receive a constant infusion of insulin mixed with your own blood, which will raise your blood level of insulin to about the level it would normally reach after a large meal such as a big bowl of pasta. You will then receive an infusion of glucose to maintain normal levels. Your blood sugar will then be checked every five minutes through one of the small tubes (you will not need a finger stick) to help maintain normal levels. We will change the amount of glucose infused to maintain normal levels. After completing the test, the insulin infusion will be turned off, and you will be given lunch. During the next 30 to 60 minutes the sugar (glucose) solution will gradually be tapered and stopped. You will then be allowed to leave.

Risks and Discomforts:
The risks are low in this study. Trained individuals will conduct all laboratory procedures with your well-being as their first priority. All procedures will be explained and demonstrated until you are comfortable with the proposed study.

Staying at Cal Poly: You will live, sleep, and eat at Cal Poly's Sleep Facilities in the College of Liberal Arts for the entire 7 days. We want you to be comfortable during your stay. There are restrooms in our facilities and showers in the recreation center nearby. You will be allowed to leave during the day to go to school, work, or your house, etc. A researcher will sleep in an adjacent room to make sure that you are fine during the night.

Accelerometers: There is a very minimal risk that a device or devices you are wearing become uncomfortable or cause you discomfort such as skin irritation. You are free to remove any device if you feel that causes you a problem.

DXA Scan: There is minimal risk of radiation exposure with use of the body composition measurement machine. The DXA Scan has a special filter that limits radiation to less than 18% of your daily exposure from just being outside. In comparison, one X-ray is 588% of the average person's daily exposure.

Blood draw: A catheter (a small flexible tube) will be inserted into a forearm vein by a trained professional for blood sampling. While you are resting comfortably, a blood sample will be collected from the catheter. The total amount of blood taken from you during the entire study is relatively small, only about 70 tablespoons, which is spread out over 6 days. However, there are some minor risks involved with catheters. To minimize these risks, catheters will only be inserted by trained individuals, using sterile technique at all times following the Centers for Disease Control and Prevention Universal Precautions for Preventing Transmission of Blood borne Infections. There may be some pain associated with the needle stick at the moment of insertion, followed by minor discomfort of having the needle withdrawn and the catheter advanced slightly into the vein. There can be local infection if the site is not kept clean following the procedure. Some slight bleeding may also occur following the withdrawal of the catheter. There is the possibility of bruising of the skin in the area around the catheter that poses no health risk and should subside within a few days. Fainting also occurs in some persons following the drawing of blood. To minimize this, you will be sitting for all blood draws. **NOTE**: Since the blood samples are essential to this study, if you are at all uncomfortable with providing blood samples you should not participate.

Insulin Sensitivity: There is a very small chance of having a low blood sugar reaction (called hypoglycemia) while getting the insulin and glucose IV, but the glucose is being given to prevent this and your blood sugar is being tested frequently to help adjust the glucose to keep it normal. A low blood sugar reaction can have many symptoms, most commonly feelings like shakiness, sweating, clammy skin, weakness, hunger, nausea, confusion, fast heartbeat, or mood change; you could feel any combination of these types of symptoms. Treatment for a low blood sugar reaction would involve giving you more sugar directly into your vein, at which point your symptoms would stop. You may also experience some burning or stinging sensation as a result of the glucose infusion.

BPA consumption: The BPA dose of 50 μg/kg body weight is higher than the typical exposure in adults (up to 1.5 μg/kg body weight) and higher than the European Food Safety Authority recommendation of 4 μg/kg body weight. Because BPA may act like estrogen in the body, it could affect diabetes and other risk markers. The 50 μg/kg body weight amount of BPA you may consume is the referent dose (safe dose throughout a lifetime) approved by the US Environmental Protection Agency (EPA). It is also well below the no-observed-adverse-effect level of BPA of 5 mg/kg body weight per day, according to the U.S. Food and Drug Administration (FDA). However, we will have medical supervision during the study and you will give a daily blood draw that we will test liver, kidney, and immune functioning.

BENEFITS: There are no direct benefits to you in participating in this study. These data are collected purely for the purposes of research and do not have a clinical or diagnostic value. These data may further our understanding whether BPA impacts the risk for type 2 diabetes. After completion of the study, you will be offered the opportunity to see the findings of the study.

CONFIDENTIALITY: All records and assessment data from this study will be treated as confidential and de-identified. Your name and the fact that you are in the study will be kept confidential. Information stored on our computer will be password protected. Participant ID will identify information on worksheets and questionnaires and decoded using a list kept in a locked cabinet. Only research staff and the principal investigator will have access to the locked cabinet. Study staff who have extensive training in confidentiality and privacy will be the only ones collecting completed questionnaires with sensitive health data and personal information, and no research volunteers including students will have access to this information. All questionnaires and data collection material completed in this study will be shredded within seven years after the study's completion. It is possible that there may be a breach of confidentiality (e.g. computer hackers, etc.), but we will follow National Institutes of Health protocols (2

unique de-identifiers for each participant, encryption of all data, data stored and backup on Cal Poly servers monitored by computing support). All volunteers will undergo extensive training in good clinical practice, biosafety training, bloodborne pathogen training and training on confidentiality and privacy before they are allowed to help in any way with the study.

COSTS/PAYMENTS: There are no costs to you for your participation in this study. You will be paid a maximum of $500 for your participation using the following guidelines:
Completion of Preliminary and Baseline Periods = $100
Completion of Treatment Period = 400

If you are a Cal Poly Student, you will be charged $30 for participating in the safety blood draws at the Cal Poly Health Center. However, your compensation for the completion of the treatment period will be $430.

After completion of the study, you will be offered a two-day fresh food, organic diet which has been shown to reduce BPA levels by 66%. If you refuse this diet, you will be offered a $50 gift card to a health food store.

QUESTIONS: If you have questions regarding this study, concerns about BPA exposure, or would like to be informed of the results when the study is completed, please feel free to contact Hannah Brunner-Gaydos (hbrunner@calpoly.edu; 805-756-5573) or Dr. Todd Hagobian (thagobia@calpoly.edu; 805-756-7511). If you have concerns regarding the manner in which the study is conducted, you may contact Dr. Michael Black, Chair of the Cal Poly Institutional Review Board, at (805) 756-2894, mblack@calpoly.edu, or Ms. Debbie Hart, Compliance Officer, at (805) 756-1508, dahart@calpoly.edu.

If you agree to voluntarily participate in this research project as described, please indicate your agreement by signing below. Please keep one copy of this form for your reference, and thank you for your participation in this research.

_____ _____
Signature of Volunteer Date

_____ _____
Signature of Researcher Date

Initials_____