

Video Article

A Method for Manipulating Blood Glucose and Measuring Resulting Changes in Cognitive Accessibility of Target Stimuli

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Abstract

Much research in social psychology has investigated the impact of bodily energy need on cognition and decision-making. As such, blood glucose, the body's primary energy source, has been of special interest to researchers for years. Fluctuations in blood glucose have been linked to a variety of changes in cognitive and behavioral processes, such as self-control, political attitudes, and eating behavior. To help meet growing interest in the links between bodily energy need and these processes, this manuscript offers a simple methodology to experimentally manipulate blood glucose using a fasting procedure followed by administration of a sugar-sweetened, unsweetened, or artificially-sweetened beverage. This is followed by presentation of a method for measuring resulting changes in implicit cognition using a lexical decision-task. In this task, participants are asked to identify whether strings of letters are words or non-words and response latencies are recorded. Sample results from a recent publication are presented as an example of the applications for the experimental manipulation of blood glucose and the lexical decision task measures.

Video Link

The video component of this article can be found at <https://www.jove.com/video/54211/>

Introduction

Researchers in cognitive psychology and neuroscience have long studied the impact of blood glucose fluctuations on the brain, cognition, and behavior¹. For example, researchers have found that fluctuations in blood glucose predict differences in memory (verbal, digit span, working, and episodic)¹⁻⁵, attention^{6,7}, puzzle solving⁸, and performance on tasks of varying levels of cognitive demand^{2,9,10}. This research finds that increasing blood glucose enhances attention and memory, and that these effects are strongest when working on cognitively demanding tasks, and in those of older age^{1,3}.

In recent decades, research on the impact of blood glucose fluctuations on human psychology has also become a topic of interest in the field of social psychology¹¹⁻¹³. Fluctuations in blood glucose have been linked to changes in mood¹⁴, motivation¹⁵, self-control¹¹⁻¹³, attitudes^{16,17}, prejudice¹⁸, and to changes in decision-making in consumer¹⁹, eating^{20,21}, and financial domains²². Because these results transcend a wide variety of dependent variables, the message about the role of blood glucose in decision-making is clear: blood glucose is the critical form of energy to the brain, and depletions in blood glucose lead to important changes in cognition and self regulation^{11,13,15}.

Given the important role that blood glucose plays in various psychological and behavioral outcomes, the current manuscript offers a simple method for manipulating blood glucose that researchers can use for testing a range of cognitions and behaviors. Additionally, the current manuscript provides a method for assessing the cognitive accessibility of target words or concepts that may change in response to fluctuations in blood glucose using a lexical decision task. The efficiency of the present methods make them ideal for experimental research, as they effectively manipulate blood glucose and measure cognitive changes using relatively inexpensive, commercially-available products. The affordability of the present blood glucose manipulation makes it easier to run the large numbers of participants needed to obtain the adequate power needed to detect statistically significant differences in behavior caused by any manipulated changes in blood glucose²³. Additionally, the lexical decision task is useful in that it can reveal implicit changes in cognition not easily accessed through more explicit measures such as self-report surveys²⁴.

The described blood glucose manipulation task involves an 8 hour fasting period followed by the directed consumption of a caloric or a calorie-free drink²². After fasting for eight hours, participants are randomly assigned to consume either a sugar sweetened beverage or water. This method also allows for the testing of any unique effects produced by non-caloric sweeteners (e.g. aspartame, sucralose), which taste sweet like traditional caloric sweeteners, but do not contain the sugars needed to raise blood glucose levels^{20,22,25}. If interested in the effects of non-calorically sweetened beverages, this task includes an optional, 3rd between-subjects condition in which participants consume a "zero-calorie" drink. The current method has been applied successfully in multiple social psychological paradigms^{12,18,22}, and offers a shorter way to test for the effects of changes in blood glucose upon self-regulation than previously used measures like glucose tolerance tests²⁶.

The authors also provide a procedure for assessing the cognitive accessibility of target words, or concepts, using a lexical decision task^{27,28}. In this task, letter strings and words are presented via computer in rapid-fire format, and typically fall into a few distinct conceptual categories (in the described research, the categories were: high fat food words, low fat food words, non-words). Participants are asked to quickly identify (typically via keystroke) whether a string of letters (e.g. fhens; pizza) is a word or a non-word. The reaction speed in which participants correctly identify letter strings in each conceptual category measures the relative ease with which the participant is able to mentally process words in that concept category. Quicker reactions mean that a concept is more mentally accessible, and thus, "on someone's mind"^{24,29,30}. As a consequence, lexical decision tasks make useful dependent variables, and can also be used as effective manipulation checks to measure if a recently primed concept (see reference³⁰ for more information on priming) is indeed subsequently more cognitively accessible than it would be in the absence of a prime. Although the lexical decision task is a task that has been used in social psychology for decades²⁹, the current manuscript presents some minor variations to the procedure, a link to an editable program template²⁷, and an empirically validated list of words from the authors' previous work that measures the cognitive accessibility of healthy versus non healthy foods²⁰. It is the authors' hope that the further adaptation of these two procedures will help lead to innovations in the fields of social psychological research, in eating behavior and energy regulation research, and will facilitate further comparative research that combines the perspectives and methodology of multiple disciplines.

Protocol

Ethics Statement: Procedures and incentives involving human subjects have been approved by the Institutional Review Board (IRB) at Texas Christian University.

1. Participant Inclusion Factors and Recruitment

1. Exclude people who have sugar sensitivities or any other health condition, like diabetes or other conditions that impair glucoregulation, and that would preclude them from being able to safely consume a sugar sweetened or artificially sweetened beverage. Exclude participants with a body mass index (BMI) over 30, as obesity is associated with energy dysregulation³¹.
NOTE: If any of the following behavioral dependent variables in the study involve food or beverage consumption, ensure that participants do not suffer from food allergies towards the food or beverages planned for use.
2. Schedule participants several days in advance of the session.
NOTE: Participant recruitment and session scheduling do not have to be done in person. These procedures can be performed by email contact or phone call.
 1. When scheduling a participant to do this study, ask the participant to abstain from consuming all food and non-water beverages for at least 8 hours before the scheduled session time³².
NOTE: Alternately, many clinicians and researchers instead require an overnight fast for their standard fasting period for measuring blood glucose tolerance¹.
 2. Schedule all participant sessions for a consistent time of day to help control for natural fluctuations in blood glucose that may occur as a result of diurnal variation rather than fasting.
NOTE: Morning (between 8 and 11 am) is an ideal time to schedule sessions, as it increases compliance with the fasting procedure and minimizes participant discomfort (e.g. light headedness) from prolonged fasting.
 3. Schedule single participant sessions or group participant sessions, depending on how sensitive of a topic the dependent variable of choice reflects.
NOTE: For example, if measuring eating behavior as the primary dependent variable, it may be more prudent to let a participant consume food privately rather than publicly because group presence and size are known to impact food consumption^{33,34}. When using a lexical decision task or a survey-based dependent measure, it may be more pragmatic to run participants in small groups in semi-private, partitioned laboratory space.
 4. Send participants a reminder email 24 hr in advance of a scheduled appointment to remind participants of the scheduled session time and the fasting requirement.
NOTE: Ensure that participants have a way to contact the researcher and cancel or reschedule without penalty, if needed. Please see Appendix A for a sample reminder email.

2. Survey and Lexical Decision Task Programming

1. Set up a survey that includes any questionnaire-based dependent variables, moderating variables, and mediating variables needed to test the hypothesis.
 1. Begin the survey with a consent form and end with a debriefing statement.
 2. Create the survey using survey-building software³⁵. Divide the full survey into enough separate survey portions to accommodate a break for participants to complete the lexical decision task.
NOTE: The current research survey was programmed into two halves using a survey building software. To do this, two separate surveys were built and then later linked together by a batch file. The first half contains steps 2.1.3.1-2.1.3.6. The second survey contains step 2.1.4. Participants completed the lexical decision task during the gap between the two survey halves.
 3. Create the first survey half so that it contains the consent document and the items described in steps 2.1.3.1-2.1.3.6.
NOTE: If collecting blood glucose data, some research institutions may require that researchers also include a HIPAA (Health Insurance Portability and Accountability Act) form for participants to sign to authorize the recording of any blood glucose readings.
 1. Create a page near the beginning of the survey half that assesses how long the participant has fasted, and how hungry the participant is feeling.
NOTE: Please see Appendix B for exact questions and scale-points used in the authors' research.
 2. Insert a "stop" instruction that tells participants to stop and contact the experimenter to start the next portion of the survey.
NOTE: See Appendix C for sample set of stop instructions.

3. Program the beverage consumption task. To do this, first program a set of instructions for consuming the beverage. Follow the instructions by including a 2 min neutral filler observational task that participants can observe during the two minute beverage consumption period. Program into this task a 30 sec warning indicating that participants have 30 sec left to finish the drink.
NOTE: In the current research, authors programmed a block in their survey that automatically advanced pictures taken by the Hubble Telescope for 1 min and 30 seconds (see Appendix D for exact coding and how to implement it). This was followed by a 30 sec warning indicating to participants that there were 30 sec left to finish their drink.
 4. Create a page in the survey to be completed after the two minute drinking procedure that prompts the participant to rate the beverage for (a) pleasantness, (b) liking of the beverage and (c) desirability as a future product for purchase. (See Appendix B for exact questions and scale-points).
 5. Create a 10 min neutral filler task that participants will complete after consuming and evaluating the beverage.
NOTE: The purpose of this 10 min neutral filler task is to allow enough time to pass for blood glucose changes to occur. Thus, the specific content that comprises the task is up to the experimenter to choose. In the current research, participants were asked to name as many brand names of office products, household cleaning products, and school supplies that they could think of in 3 min intervals (with additional 1 minute programmed to include instructions), in keeping with the consumer product testing ruse. The same JavaScript coding used for the blood glucose manipulation task was used to program open answer response blocks to appear for exactly 3 min (**Appendix C**).
NOTE: If concerned about participant fatigue due to the filler task, another slideshow presenting pictures of a neutral subject (which does not require word generation) could be substituted for a more active task (such as the brand name generation task mentioned above).
NOTE: Alternately, if interested in measuring cognitive function, *per se*, the task can be modified to be more cognitively demanding if desired; given that research using various cognitive paradigms has revealed that blood glucose fluctuations may facilitate certain types of cognition under conditions of high load^{1,10,36}.
 6. If measuring post-manipulation blood glucose, program stop instructions for a second, post-manipulation reading immediately after the end of the neutral filler task to allow the implementation of the blood glucose assessment protocol.
4. Create a second survey half that contains all the questions that will go after the lexical decision task, as well as a debriefing statement where participants are informed of any deception (e.g. in the current line of research: the consumer product testing ruse).
NOTE: In the current line of research, this portion asked participants to report on various sociodemographic factors, such as: age, height, weight, and socioeconomic status. Optionally, including a measure of mood in this portion will allow for the ability to control for differences in cognitive accessibility that may result from changes in mood¹⁴.
2. Program the lexical decision task as a computer task using behavioral task-building software³⁷ that is able to record reaction times down to the millisecond.
NOTE: The coding details used to program this task will vary depending on the software that is used to execute the lexical decision task. Please see Appendix E for a sample coding template for the authors' task-building software of choice, and sample lists of words and non-words to program into task²⁰.
 1. Program the letter string words/non-words in the lexical decision task to appear in random order and not repeat by manipulating the portions of the coding labeled "block" and "trial" in the behavioral task building software.
NOTE: Randomizing will help to control for any potential priming effects upon participants' behaviors from seeing a specific order of word/non-word letter strings. See the sample template in Appendix E for the exact code for that can be used to randomize letter string order.
NOTE: In the current research, the authors programmed each letter string to show on the computer screen for 250 ms. The task was programmed so that participants indicate whether each letter string was a word or non-word by pressing the "z" or "m" keys on the keyboard, respectively. This task was also programmed so that there was a practice trial where participants categorized 15 neutral valence words and 15 non-word letter strings to gain familiarity with the task. The stimulus presentation time, response key, and task length can all be easily manipulated by altering the template code included in Appendix E.
 3. Integrate the surveys and lexical decision task.
NOTE: One way to integrate computerized surveys with computerized behavior tasks is to program a batch file in a notepad application, saving it as a .bat file. This method helps to reduce procedural awkwardness by eliminating the need for an experimenter to open each survey portion and the lexical decision task manually. Please see Appendix F for sample batch file code used in the authors' current research.
 1. If running the survey on a computer, ensure that upon opening the batch file an internet browser (or survey software program) opens and directs to the hyperlink containing the first survey half.
 2. Program the batch file to automatically open the lexical decision task upon the close of first survey.
 3. Program the batch file to automatically open an internet browser that directs to the last survey half upon completion of the lexical decision task.
 NOTE: The sample batch file in Appendix F provides the code to execute steps 2.3.1-2.3.3.

3. Day of Study Setup

1. Before participants arrive to the laboratory, assign each scheduled participant a unique ID number (e.g. "101, 102"...) using a participant log. Write each participant ID number being used in a session on an individual sticky note.
NOTE: Give a sticky note to the participant when the participant enters the experiment room and enter the number into the computerized survey. Later use the participant's number to link the data from all survey parts and tasks.
2. Randomly assign each scheduled participant into a drink manipulation condition prior to their arrival in the lab. Pour each participant's chilled drink into an opaque disposable cup labeled with that participant's participant ID number. Pour all drinks before the session while the room is empty of any participants, so that participants are blind to the type of drink that was assigned.

NOTE: Ensure that the experimenter (or research assistant) is blind to the hypothesis of the experiment. To make the procedure fully double-blind, add a second research assistant to the procedure. The research assistant will pour the drinks, number the cups, and then hand the drinks to the experimenter, who then distributes them to participants.

1. Record the participants' drink condition next to the corresponding participant ID number in the participant log.
 1. To test the unique effects produced by non-caloric sweeteners relative to naturally sweetened drinks, ask each participant to drink either a "sugar sweetened" lime soda beverage, "zero-calorie sweetened" lime soda beverage, or a "non-sweetened" lime flavored sparkling water beverage.
 2. To test the contrasting effects of high energy need versus low energy need, ask each participant to drink either a "sugar sweetened" lime soda beverage or "plain spring water".

NOTE: Participants should be given 12 oz of the assigned drink (approximately one can).

NOTE: In the authors' prior research, "seltzer water"^{20,38} has also been used instead of spring water to control for differences in palatability that might occur from having carbonated drinks presented versus plain water.

NOTE: If consistency of drink constituency poses a concern to the readers' research, non-carbonated drinks can be used³. To do so, in the experimental condition mix 25 grams of pure glucose into water. In the non-caloric sweetener condition mix an equivalent amount of non-caloric sweetener (such as aspartame or saccharin) into water^{1,2}. Sugar free squash pulp can be mixed to drinks in these conditions for improved palatability.

3. Prepare the computerized surveys for administration. On the computer, open the study folder, double-click on the batch file icon and select run to open the survey.

NOTE: If unable to run the survey on a computer and the survey is in paper format, write the participant ID on top of all forms.

4. If measuring blood glucose using a glucometer, gather blood testing strips (1 per reading), lancets (1 per reading), a glucometer, and a participant log with a spot built in to record readings.
 1. Have Band-Aids, hand sanitizer, medical-grade exam gloves, and sterilizing wipes present and ready for clean-up after the reading.

4. Protocol for Running Participants Through the Study

1. Bring participants into the lab. Give brief introduction to the study purpose. Distribute a consent form (digital, or on paper) for participants to sign before starting the experiment. Once consent is given, hand the participant the sticky note containing the appropriate, assigned participant ID number.

NOTE: It is ok to use some deception (e.g. a cover story) when initially explaining the study purpose to participants if telling them the true purpose will affect participants' survey and task responses. If deception is used, it is required to inform participants of the true study purpose upon debriefing.
2. If taking pre-manipulation blood glucose readings, guide participant to a sterile spot away from the survey. Wear exam gloves to minimize any chance of exposure to blood while taking blood glucose readings. Clean the participant's finger with a sterile wipe prior to reading blood glucose.
 1. Using lancet (or lancet tipped blood testing strip), gently prick the side of participant's index finger. Squeeze a small amount of blood onto blood testing strip and insert into glucometer.
 2. Write down the corresponding blood glucose reading and time the blood glucose reading is taken in the participant log. Wipe down the area with a sterilizing wipe.
 3. Offer the participant hand sanitizer and a tissue to clean their finger. Provide a small, adhesive bandage if the participant desires to cover their finger.
3. Direct all participants to take a seat at the computer terminal where their drink and survey are located.
 1. Instruct each participant to place the sticky note with the participant ID number on the desk next to the computer where it is visible to both the researcher and the participant.
 1. Instruct the participant to type in the participant ID number on the sticky note when prompted at the beginning of the survey to ensure later ability to link survey responses to the participant's drink condition.
4. Instruct participants to complete a consumer evaluation task, and as a consequence, consume a brand blind beverage.
 1. Hand the participant the cup containing the participant's assigned experimental drink. Double check that the cup matches the assigned condition and participant number recorded on the participant log.
 2. Tell participants that the computer will prompt them to consume the entire beverage in two minutes and that this will be followed by a survey.
 3. Ask the participant to take the cup and begin to drink the beverage. At this time, click the next arrow on the survey page to begin the timed beverage consumption task.

NOTE: The 2 minute timing can be performed manually by the researcher using a stopwatch, or can be programmed into a computer survey using JavaScript programming language (see Appendix C), such that upon clicking a "begin button" the survey will not progress until two minutes have passed and it has instructed the participant to finish the beverage and inform the researcher to come collect the cup.

 1. After the two minute period has elapsed, participants will be instructed by the computer to begin a survey on their impressions of the drink by clicking the "begin" button.

NOTE: Participants will first be asked about their impressions of the drink to buttress the cover story. This brief survey is immediately followed by a 10 min neutral filler task to allow time for changes for blood glucose to occur¹⁶.
 4. Collect all used beverage cups from the participants' workspaces but do not throw away.
 5. Note in the experimental log next to a participant's ID number if a participant did not finish the entire beverage.

5. If recording blood glucose, immediately take another blood glucose reading at conclusion of the 10 min filler task before proceeding to the lexical decision task. Repeat steps 4.2, 4.2.1, 4.2.2, and 4.2.3.
NOTE: It is important to record both the time of first and second blood glucose readings, because there is always some small amount of variation in the time between readings across participants, due to differences in the time it takes to finish portions of the survey. Recording the time of readings allows the researcher the option of controlling for time between readings by entering it as a covariate in later statistical analyses.
6. Upon completion of the 10 min filler task and blood glucose reading, submit and close the survey.
NOTE: Allow the batch file to automatically open the behavioral task by ensuring that the internet browser running the survey is completely closed once the survey is submitted (e.g. do not simply minimize the window), and then verify that the batch file has successfully opened the behavioral task software by checking to see if a window prompting the participant to enter the participant ID number has appeared.

5. Protocol for Running the Participant Through the Lexical Decision Task

1. Double click and open the software to run the task. Enter the participant's ID number into the participant ID prompt given by the behavioral task software and initiate the programmed lexical decision task by clicking the "run" button on the prompt window.
2. Briefly describe the lexical decision task to the participant by verbal instruction or by including an instructions page at the beginning of the computerized task.
3. Allow the participant time to complete the lexical decision task on the computer and instruct the participant to notify the researcher when the task is finished.
NOTE: It typically takes participants approximately 3-4 min to complete the lexical decision task.
4. After finishing the task, close the lexical decision task out fully by hitting spacebar, and ensure that the batch file opens the final survey portion that contains the sociodemographic and debriefing questions outlined in step 2.1.4.
5. At the conclusion of the entire study, debrief the participant. Give the participant the agreed upon compensation for participation and release them from the session.
6. If the participant did not finish the drink during the beverage consumption task, use a measuring cup at the end of the experimental session to measure and then record how much of the beverage is left, in fluid ounces, in the participant log.
7. Flag the data for any participant who did not finish the assigned drink, and for participants who presented a fasting blood glucose level of higher than 100 mg/dL (5.6 mmol/L).
NOTE: Discarding participants' data for those that pass this blood glucose threshold is recommended because a fasting blood glucose level higher than 100 mg/dl is considered to be a marker of impaired glucose processing/prediabetes³⁹. Heightened blood glucose at the first reading may also be an indicator that a participant did not comply with the pre-session fasting instructions and ate something prior to session.

6. Scoring the Results and Preparing the Data for Analysis

1. Using the participant's ID number, match the participant's hand recorded log data (e.g. blood glucose readings, drink condition, amount of drink left if any, and any noted study session problems) with the participant's survey data and the computerized lexical decision task data.
2. Import all data (log, survey, and lexical decision task) into a single dataset in any statistical analysis software format of choice⁴⁰. Discard any flagged session data.
 1. Accomplish this either by entering data manually into a computerized spreadsheet and importing it into the statistical analysis software, or by entering it directly into a file format that is supported by the statistical software.
3. Code and trim reaction time data from the lexical decision task, as outliers are known to distort reaction time data^{24,29}.
 1. Trim reaction time data by either deleting any individual responses that fall outside the a priori determined cut-off latency, or by recoding all responses in a single word category into a new trimmed variable that does not include responses outside of the a priori cut-off window.
NOTE: The authors suggest adopting the following a priori standards to trimming reaction time data: eliminating single item responses that are shorter than 100 msec and longer than 1,000 msec, and eliminating incorrect responses³⁰.
4. Using the trimmed response latencies, create all dependent variables needed to answer the experimental hypothesis^{24,29}.
 1. Create mean reaction time composites for all correct responses in each word category that was included in the lexical decision task (e.g. high fat words, low fat words, non-words). Create sum composites of total number of correct responses for each word category^{24,29,30}.

Representative Results

Participants

The above methods were implemented in a study run by Hill and colleagues²⁰ at a midsize, private university in the southern United States. The undergraduate population at the university provided the subject sample and participants received partial course credit as compensation for study participation. Using the exact methods outlined in the current manuscript protocol, the authors ran participants through a blood-glucose manipulation procedure followed by a lexical decision task (see **Table 1** for information about drinks used). At the conclusion of data collection, data from participants who did not meet the fasting preparation requirements [e.g. those who ate or drank anything besides water in less than eight hours prior to the experimental session ($n = 18$)] were excluded, leaving a total of 116 undergraduates in the final sample for analysis (75 women, 41 men; 36 in the Sprite condition (12 men), 40 in the Sprite Zero condition (14 men), and 40 in the mineral water condition (15 men)), aged 18 to 25 years ($M = 19.81$, $SD = 3.27$).

Condition	Energy/100g	Drink Ingredients
Sugar sweetened	167 kJ/40 kcal	Carbonated water, sugar, other sweetener (steviol glycosides), citric acid, malic acid, acidity regulator (sodium gluconate), lemon-lime flavorings (natural).
Non-caloric sweetener	0 kJ/0 kcal	Carbonated water, citric acid, lemon-lime flavorings (natural), non-caloric sweeteners (aspartame, acesulfame-K), preservative (E211), acidity regulator (E331).
Water	0 kJ/0 kcal	Carbonated water, natural flavors

Table 1: Characteristics of the Drinks Used Across All Studies. Table modified from original publication by Hill and colleagues²⁰.

Data Analysis

First, participants' lexical decision time data were cleaned and trimmed using the procedure outlined in protocol section 6 of this manuscript. The authors next created mean score composites for each word category (high fat, low fat, and non-word), using only the data from correct responses. The data for incorrect responses were not considered because faster reaction times for incorrect categorizations might simply be a result of random pecking on the keyboard, rather than reflecting differences in cognitive accessibility of a word, per se. Next, a preliminary 2 (Sex) x 3 (Drink) x 3 (Word Category) mixed model ANOVA was run to examine whether drink condition or participant sex influenced word categorization accuracy. Results demonstrated no differences in accuracy by drink condition across any word category ($p = 0.25$) and participant sex did not interact with any of the manipulated variables ($ps \geq 0.28$). Thus, sex and accuracy variables were excluded from further analyses.

To test the hypothesis that non-calorically sweetened beverages (NCS) would increase the cognitive accessibility of high calorie, but not low calorie or non-word letter strings, a 3 x 3 mixed-model ANOVA was run, with drink type entered as the between-subjects factor and response word category (high calorie words, low calorie words, and non-words) entered as the within-subjects factor. Therefore, the authors expected that participants assigned to drink a non-calorically sweetened drink would have quicker reaction times when categorizing high calorie food words, but not when categorizing low calorie or non-food words. Results revealed that there was a significant interaction between drink type and response word category, $F(4, 224) = 2.98$, $p = 0.02$, $\eta^2 = 0.05$ (see **Figure 1**). Next, to probe this interaction, the authors ran three separate ANCOVA models examining the effect of drink condition on an individual word category (e.g. high calorie words) and controlling for response times to each alternate word category while controlling for response times to each alternate word category. Bonferroni's correction ($\alpha = .017$) was applied to preserve alpha level and reduce any inflated likelihood of committing a Type 1 error due to running multiple, separate ANCOVA models. As predicted, unpacking the interaction revealed a significant main effect of drink type on participants' reaction times to high calorie food words, $F(2, 111) = 6.03$, $p = 0.003$, $\eta^2 = 0.10$. There were no effects of drink type on participants' reaction times to low calorie food words, $F(2, 111) = 1.93$, $p = 0.15$, or non-words, $F(2, 111) = 0.90$, $p = 0.41$. Supplemental analyses run using a Sidak correction, in which the formula for calculating the new alpha cut-off is more conservative than a Bonferroni correction⁴¹ revealed the same cut-off value for alpha significance as the Bonferroni test, $\alpha = 0.017$, and thus re-confirmed the significance of our main effect.

Lastly, Helmert orthogonal planned contrasts ($\alpha = 0.025$) were conducted to probe any differences in reaction time between participants who consumed a NCS beverage and those who consumed sugar-sweetened or unsweetened drinks. The authors first compared participants in the NCS conditions' reaction times to those of participants in both the sweetened and unsweetened drink conditions. Results revealed that participants who drank the NCS beverage responded more quickly to high calorie words than participants who drank sugar sweetened or unsweetened beverages ($p = 0.001$, $CI: -84.89, -20.11$). A final contrast was run to investigate if there were any differences in reaction times to high calorie words within the two control conditions (sugar sweetened vs. unsweetened beverages). Results revealed no differences in mean reaction time to high calorie words between participants who drank sugar sweetened or unsweetened beverages ($p = 0.28$).

The results of this research found that participants who consumed a beverage sweetened with NCS, compared to those who consumed a sugar-sweetened or unsweetened beverage, had shorter response latencies to the names of high-calorie food items than did those who had consumed a sugar-sweetened or unsweetened beverage. No such differences were found for the names of low-calorie food items or non-words, suggesting that drinking non-caloric sweeteners may increase cognitive accessibility (and hence, may reflect preoccupation) with high-calorie foods. These results suggest that consuming NCSs may influence implicit desires for calorie dense foods in ways that may encourage increased calorie consumption over time.

	High Calorie				Low Calorie				Non Word			
	<i>M</i>	<i>SD</i>	<i>Min</i>	<i>Max</i>	<i>M</i>	<i>SD</i>	<i>Min</i>	<i>Max</i>	<i>M</i>	<i>SD</i>	<i>Min</i>	<i>Max</i>
<u>Words Correct</u>												
Sweetened	6.97	0.17	6	7	6.83	0.38	6	7	13.42	1.02	10	14
Non Calorically Sweetened	6.98	0.16	6	7	6.88	0.34	6	7	13.33	1.23	8	14
Unsweetened	6.85	0.36	6	7	6.75	0.44	6	7	13.33	1.42	7	14

Table 2: Descriptive Statistics for Number of Letter Strings Categorized Correctly by Word Category and Drink Condition (Sugar Sweetened, Non-calorically Sweetened, and Unsweetened).

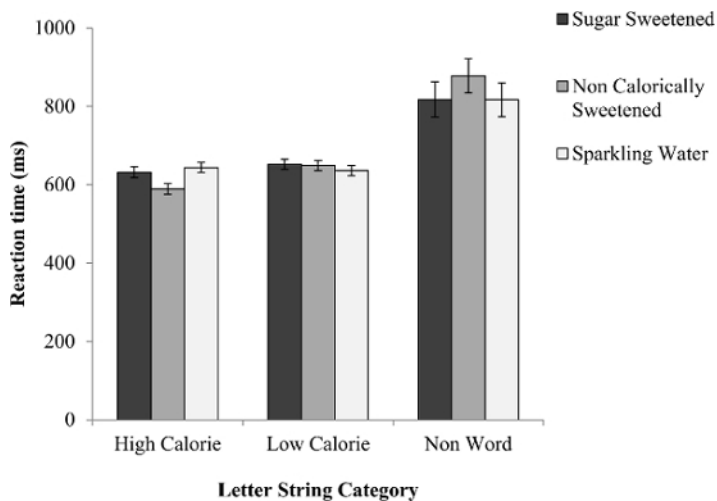


Figure 1: Reaction Time Results. Mean reaction time (in msec) of letter strings categorized correctly during a lexical decision task are plotted by word category and drink condition. Longer reaction time latencies indicate lesser cognitive accessibility of a word category. Error bars reflect the standard error of the adjusted means. Only the comparisons of drink group within the high calorie condition are significant. [Please click here to view a larger version of this figure.](#)

Discussion

This manuscript outlines a simple, inexpensive procedure for manipulating blood glucose, as well as a procedure for measuring resulting changes in the cognitive accessibility of target words and concepts. The above outlined methods can be applied to a wide range of research areas including social psychology, cognitive psychology, and nutrition sciences. Given that this method may be used by people in multiple research areas (some that do not typically use survey data), this section will present some tips on critical steps and trouble-shooting to help researchers that wish to implement this procedure in their studies.

One of the most critical steps to this procedure is having an error-free and rigorous survey/lexical decision task setup. Whether working with a survey building software, or implementing the survey manually using paper/pencil measures and a stopwatch to time participants completing beverage consumption, the clarity and accuracy of all procedures is paramount for both data collection and interpretation, and for maintaining control of the conditions across sessions. It is strongly recommended to proofread and pre-test all survey and lexical decision task measures for clarity and accuracy before data collection. It is best to engage multiple people (both familiar and blind to the hypothesis) to take the survey and offer feedback. Many survey building and experimental task building software companies also offer online message forums and troubleshooting help pages to help guide setting up and editing these survey measures and the lexical decision task. Accordingly, it is also important to have well-trained experimenters and research assistants helping with data collection and data entry. Write a session script with detailed instructions on session setup, scripted dialogue on how to communicate tasks to participants, and clearly outlined procedures for completing the experimental log. This will ensure that procedures are consistent across sessions, and across multiple experimenters that may be running the study. Additionally, another step to ensure that all participant data is accurate and linked properly by participant ID is to have double data entry procedures where two hypothesis blind research assistants separately enter and link the same data (see protocol section 6.1-6.2.1).

The methods presented in the current manuscript are significant for several reasons. First, the method for manipulating blood glucose is relatively simple and inexpensive to implement. Other methods of manipulating blood glucose such as a glucose tolerance test can cost a minimum of \$26 to buy per person⁴². The current methods require, at minimum, that the researchers purchase of boxes of commercially available soft drinks (which can often be purchased in bulk at a discount). When the authors of this paper calculated the cost per participant for drinks (1 assigned drink) and blood testing supplies (2 lancets and 2 blood testing strips), plus factored in the cost of buying a blood glucometer to run the study, the calculated price per participant ranged from \$1.34 to \$3.34 (US dollars), depending upon where the supplies were purchased. Further, these methods have a proven track record of being able to change blood glucose enough to impact both cognitive and behavioral dependent measures^{16,20,22,43}. Further, these methods have allowed researchers to test the effects of non-caloric artificial sweeteners, whose novel combination of sweet taste with no glucose may trigger unique changes in self-regulation^{20,22}. Thus, using a simple blood glucose manipulation such as the one described can allow for research examining the complex interplay between one's physiological state and their changes in cognition and decision-making^{4,6,8,22}. Additionally, the described lexical decision task provides a useful tool for measuring implicit changes in cognition that might be otherwise hard to test²⁷. Together, these methods may be useful for nutrition researchers who wish to incorporate more self-report methods into their research, and for psychologists who wish to add more behavioral and physiological methods into their research.

Given that the incidence of overweight and obesity in the U.S. and the rest of the world has steadily increased for more than 30 years⁴⁴⁻⁴⁶, research on the psychological impact of blood glucose fluctuations on the processes that guide energy regulation represents an important domain of research for psychologists and nutritionists alike^{20,21,43}. Although the changes in energy regulating decisions caused by blood glucose fluctuations at any given time may be small (such as picking a less healthy snack at the grocery store²⁰), these small decisions can create surpluses in energy budget that add up to significant weight gain over a long period of time⁴⁷. Therefore, examining the impacts of momentary blood glucose fluctuations on food related cognitions and health decision-making is a critical next step to build from the current self-regulation research. Thus, the current methodology offers an opportunity to both manipulate such fluctuations and measure changes in the subtle psychological underpinnings that may contribute to overweight and obesity.

The current methods have important limitations. One limitation of the methods is that the waiting period provided for blood glucose changes is at the shorter end of the range of acceptable time needed to allow for blood glucose change^{11,12,16}. These methods were designed to balance the need to allow for energy change while also helping to minimize participant fatigue and discomfort. Although previously published research from the authors' lab and other social psychologists showed consistent results using periods of 10 to 12 min^{11,12,18}, others have found that it may be advantageous to wait 20 min or more after the drink manipulation before measuring the dependent variable^{4,8}. For example, one could increase the delay by extending the time of the neutral filler task to allow for more time for change in blood glucose. The current methods are also limited in that they have not yet been tested or demonstrated effective in individuals with metabolic disorders (such as diabetes) or other health conditions associated with energy dysregulation (such as obesity). Thus, results cannot be generalized to obese individuals, and therefore must be treated with caution. Although investigating the underpinnings of calorie-regulation related cognition in healthy weight individuals represents a first step in investigating these processes, a critical next step will be to examine these effects in individuals who are obese (or who have dysregulated glucoregulation) and thus, may react to fluctuations in blood glucose differently than healthy weight individuals. Future research could benefit from trying to adapt these methods to safely investigate the effects of blood glucose change (and non-change) in such populations. This may mean making changes to the type of drink provided, time for blood glucose change allowed, or having proper medical equipment on hand to help re-balance blood glucose to safe levels if needed at conclusion of the research. Last, given that cognitive research has found that the facilitation effects of blood glucose are greatest in older adults¹⁻³, it may be important to include age-diverse populations when running the current protocol. As the authors of this paper have not studied such populations yet (and have primarily relied on undergraduate populations to provide study samples), this paradigm cannot be seen as representing or generalizing to the effects of blood glucose fluctuations in older adult performance.

This manuscript provides a series of methods for manipulating changes in blood glucose and sample methods for a way to measure corresponding changes in cognition. It is the authors' hope that these methods will help to increase interest in research examining the effects of energy fluctuation in cognition, decision-making, and behavior. The given methods provide a simple, and experimentally validated option to implementing such research. As such, they may be an important contribution to an ever growing area of research that is of interest to people across many research disciplines.

Disclosures

The authors have nothing to disclose.

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References

1. Smith, M. A., Riby, L. M., van Eekelen, J. A. M., & Foster, J. K. Glucose enhancement of human memory: A comprehensive research review of the glucose memory facilitation effect. *Neurosci Biobehav Rev.* **35**, 770-783 (2011).
2. Meikle, A., Riby, L. M., & Stollery, B. The impact of glucose ingestion and gluco-regulatory control on cognitive performance: A comparison of younger and middle aged adults. *Hum Psychopharm Clin.* **19**, 523-535 (2004).
3. Riby, L. M. The impact of age and task domain on cognitive performance: A meta-analytic review of the glucose facilitation effect. *Brain Impair.* **5**, 145-165 (2004).
4. Martin, P. Y., & Benton, D. The influence of a glucose drink on a demanding working memory task. *Physiol. Behav.* **67**, 69-74 (1999).
5. Adan, A., & Serra-Grabulosa, J. M. Effects of caffeine and glucose, alone and combined, on cognitive performance. *Hum Psychopharm Clin.* **25**, 310-317 (2010).
6. Benton, D., Owens, D. S., & Parker, P. Y. Blood glucose influences memory and attention in young adults. *Neuropsychologia.* **32**, 595-607 (1994).
7. Serra-Grabulosa, J. M., Adan, A., Falcón, C., & Bargalló, N. Glucose and caffeine effects on sustained attention: An exploratory fMRI study. *Hum Psychopharm Clin.* **25**, 543-552 (2010).
8. Donohoe, R. T., & Benton, D. Cognitive functioning is susceptible to the level of blood glucose. *Psychopharmacology.* **145**, 378-385 (1999).
9. Scholey, A. B., Harper, S., & Kennedy, D. O. Cognitive demand and blood glucose. *Physiol Behav.* **73**, 585-592 (2001).
10. Meikle, A., Riby, L. M., & Stollery, B. Memory processing and the glucose facilitation effect: The effects of stimulus difficulty and memory load. *Nutr Neurosci.* **8**, 227-232 (2005).
11. Gailliot, M. T., & Baumeister, R. F. The physiology of willpower: Linking blood glucose to self-control. *Pers. Soc. Psychol. Rev.* **11**, 303-327 (2007).
12. Gailliot, M. T. *et al.* Self-control relies on glucose as a limited energy source: Willpower is more than a metaphor. *J. Pers. Soc. Psychol.* **92**, 325-336 (2007).
13. Baumeister, R. F., Vohs, K. D., & Tice, D. M. The strength model of self-control. *Curr. Dir. Psychol.* **16**, 351-355 (2007).
14. Benton, D. Carbohydrate ingestion, blood glucose and mood. *Neurosci. Biobehav. Rev.* **26**, 293-308 (2002).
15. Baumeister, R. F., & Vohs, K. D. Self-regulation, ego depletion, and motivation. *Soc. Personal. Psychol. Compass.* **1**, 115-128 (2007).
16. Aaroe, L., & Petersen, M. B. Hunger games: Fluctuations in blood glucose levels influence support for social welfare. *Psychol. Sci.* **24**, 2550-2556 (2013).
17. DeWall, C. N., Pond, R. S., Jr., & Bushman, B. J. Sweet revenge: Diabetic symptoms predict less forgiveness. *Pers. Individ. Dif.* **49**, 823-826 (2010).

18. Gailliot, M. T., Michelle Peruche, B., Plant, E. A., & Baumeister, R. F. Stereotypes and prejudice in the blood: Sucrose drinks reduce prejudice and stereotyping. *J. Exp. Soc. Psychol.* **45**, 288-290 (2009).
19. Baumeister, R. F., Sparks, E. A., Stillman, T. F., & Vohs, K. D. Free will in consumer behavior: Self-control, ego depletion, and choice. *J. Consum. Psychol.* **18**, 4-13 (2008).
20. Hill, S. E., Prokosch, M. L., Morin, A., & Rodeheffer, C. D. The effect of non-caloric sweeteners on cognition, choice, and post-consumption satisfaction. *Appetite.* **83**, 82-88 (2014).
21. Campfield, L. A., & Smith, F. J. Blood glucose dynamics and control of meal initiation: A pattern detection and recognition theory. *Physiol. Rev.* **83**, 25-58 (2003).
22. Wang, X. T., & Dvorak, R. D. Sweet future: Fluctuating blood glucose levels affect future discounting. *Psychol. Sci.* **21**, 183-188 (2010).
23. Cohen, J. A power primer. *Psychol. Bull.* **112**, 155-159 (1992).
24. Fazio, R. H. in *Research methods in personality and social psychology*. eds C. Hendrick & M. S. Clark 74-97 Sage Publications, (1990).
25. Swithers, S. E. Artificial sweeteners are not the answer to childhood obesity. *Appetite.* **93**, 85-90 (2015).
26. Donohoe, R. T., & Benton, D. Glucose tolerance predicts performance on tests of memory and cognition. *Physiol Behav.* **71**, 395-401 (2000).
27. Lepore, L., & Brown, R. The role of awareness: Divergent automatic stereotype activation and implicit judgment correction. *Soc. Cognition.* **20**, 321-351 (2002).
28. Meyer, D. E., & Schvaneveldt, R. W. Facilitation in recognizing pairs of words: Evidence of a dependence between retrieval operations. *J. Exp. Psychol.* **90**, 227-234 (1971).
29. Fazio, R. H., Sanbonmatsu, D. M., Powell, M. C., & Kardes, F. R. On the automatic activation of attitudes. *J. Pers. Soc. Psychol.* **50**, 229-238 (1986).
30. Bargh, J. A., & Chartrand, T. L. in *Handbook of research methods in social and personality psychology*. (eds Harry T. Reis, Charles M. Judd, Harry T. Reis, & Charles M. Judd) 253-285 Cambridge University Press, (2000).
31. Zigman, J. M., & Elmquist, J. K. Minireview: From anorexia to obesity-The yin and yang of body weight control. *Endocrinology.* **144**, 3749-3756 (2003).
32. Wisse, B. in *Medline Plus medical encyclopedia*. (eds I. Oglivie & D. Zieve) National Institute of Health- U. S. National Library of Medicine, online, (2014).
33. Patel, K. A., & Schlundt, D. G. Impact of moods and social context on eating behavior. *Appetite.* **36**, 111-118 (2001).
34. Redd, M., & de Castro, J. M. Social facilitation of eating: effects of social instruction on food intake. *Physiol. Behav.* **52**, 749-754 (1992).
35. *Qualtrics Research Suite*. Provo, UT, USA, (2015).
36. Van Dillen, L., Papies, E., & Hofmann, W. Turning a blind eye to temptation: how cognitive load can facilitate self-regulation. *J Pers. Soc. Psychol.* **104**, 427-443 (2013).
37. *Inquisit 4.0.8.0*. [Computer software] Millisecond Software LLC, Seattle, WA, (2014).
38. Hill, S. E., Prokosch, M. L., DelPriore, D. J., Griskevicius, V., & Kramer, A. Low childhood socioeconomic status promotes eating in the absence of energy need. *Psychol. Sci.* (2016).
39. American Diabetic Association. Standards of medical care in diabetes-2014. *Diabetes Care.* **37**, S14-S80 (2014).
40. *IBM SPSS Statistics for Windows, Version 22.0*. IBM Corp, Armonk, NY, (2013).
41. Abdi, H. in *Encyclopedia of Measurement and Statistics*. (ed N.J. Salkind) Sage, Thousand Oaks, CA, (2007).
42. Zhang, P. *et al.* Costs of Screening for Pre-diabetes Among U.S. Adults: A comparison of different screening strategies. *Diabetes Care* **26**, 2536-2542 (2003).
43. Hill, S. E., Prokosch, M. L., DelPriore, D. J., Griskevicius, V., & Kramer, A. Low childhood socioeconomic status promotes eating in the absence of energy need. *Psychol. Sci.* (2016).
44. Flegal, K. M. Epidemiologic aspects of overweight and obesity in the United States. *Physiol Behav.* **86**, 599-602 (2005).
45. Flegal, K. M., Carroll, M. D., Kit, B. K., & Ogden, C. L. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *J.A.M.A.* **307**, 491-497 (2012).
46. WHO. Fact Sheet No 311: Obesity and Overweight. *World Health Organization.* (2014).
47. Hill, J. O., Wyatt, H. R., Reed, G. W., & Peters, J. C. Obesity and the environment: Where do we go from here? *Science.* **299**, 853-855 (2003).