Emotional Arousal, Blood Glucose Levels, and Memory Modulation: Three Laboratory Exercises in Cognitive Neuroscience

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The relationships between emotional arousal and cognition in humans represent an important field in cognitive neuroscience. Studies examining the characteristics of emotion-induced memory enhancement and the mechanisms through which these effects occur are becoming increasingly common. This article describes three affordable laboratory exercises of relevance to the growing interest in this field. Specifically, Experiment one reviews a protocol for examining memory, hypermnesia, reminiscence, and primacy/recency effects for emotional and neutral words. Experiments two and three provide opportunities to examine the relationships between blood glucose level and memory for either a list of pictures or the spatial location of pictures. Each laboratory exercise contains a certain amount of flexibility and is malleable to the specific needs of the instructor. For example, the use of blood glucose monitoring may be of value to a variety of different exercises examining stress and/or emotional arousal and the stimuli used in each of the protocols may be varied, creating opportunities for a number of different novel exercises. A series of questions have been provided at the end of each exercise in order to help stimulate in-class discussion. The potential application of this line of research in cognitive neuroscience is conveyed through a list of references where glucose has been used to attenuate cognitive deficits in Alzheimer’s disease, age-related cognitive decline, and other neuropsychological conditions.

Key words: glucose; emotional arousal; memory; memory modulation; blood sugar; neuroscience education; humans; teaching methods

The impact of acute emotional arousal on subsequent memory performance has been a topic of interest to cognitive neuroscientists for decades (Flint, 2002). The enhancing effects of arousal may be favorable, as in the flashbulb memory and Polyanna effects, or they may be detrimental, as in the case of post-traumatic stress disorder and learned helplessness. In this article I focus on the favorable characteristics of arousal, provide a foundation for the role of glucose in memory modulation of acute emotional arousal, and review three cost-effective laboratory exercises associated with the impact of emotional arousal on memory. Two of these exercises specifically examine the role that blood glucose may play in the memory enhancing effects associated with arousal and review some simple techniques that may be utilized in other laboratory exercises.

Acute emotional arousal, often a result of proximal environmental stimuli and frequently involving the to-be-remembered stimuli, activates a collection of responses known as the stress cascade. This stress cascade involves a series of parallel neuroendocrine responses (reviewed by Kalman & Grahn, 2004). To summarize, the hypothalamic-anterior pituitary-adrenocortical axis (HPA axis) is associated with the endogenous release of adrenocorticotropic hormone (ACTH) and glucocorticoids, whereas the sympatho-adrenomedullary axis (SAM axis) releases epinephrine and norepinephrine. All of these substances have been shown to have memory modulating effects (Gold & van Buskirk, 1975; Haroutunian & Riccio, 1977, 1979; Richardson et al., 1984; Ferry et al., 1999; McGaugh & Roozendaal, 2002).

Our understanding of the body’s responsiveness under emotional stress and the need for immediate energy in the form of glucose allows us to modify and extend the stress cascade. Glucocorticoid release produces increases in blood glucose by directly affecting glucose output from the liver, decreasing glucose absorption from peripheral tissue, and by releasing glucogenic amino acid (Figure 1). Similarly, epinephrine increases blood glucose levels directly via the liver.

Figure 1. Extension of the Traditional Stress Cascade. The stress cascade, including the HPA-axis and SAM-axis, has been extended to reveal the pathways through which glucocorticoids and epinephrine elevate plasma glucose levels.
examine memory acquisition (Pavone et al., 1998), although one could not rule out a memory consolidation effect. Glucose administered following the completion of training allows researches to examine memory consolidation (Flint & Riccio, 1999), and glucose administered shortly prior to testing allows for the examination of memory retrieval (Flint & Riccio, 1997). Much of the research with human participants has involved administering glucose shortly prior to training in paradigms where testing is conducted right after training has been completed. In a protocol such as this, one is unable to determine whether an effect of glucose results from influences on memory acquisition, memory consolidation, or memory retrieval. In much of the non-human animal literature, glucose is administered immediately after the training episode is completed, and testing is delayed for at least 24 hours allowing the animal’s system to return to baseline. In a paradigm such as this, any effect of glucose must result from alterations in processes associated with memory consolidation, as the glucose was not present during training or testing. Research with human participants has begun to use this consolidation paradigm more frequently since it is difficult to argue that glucose influenced the participant in some other way, giving the false appearance of memory facilitation (e.g., enhanced attention to task stimuli).

In the context of memory modulation resulting from acute emotional arousal, it is important to demonstrate that increases in blood glucose are associated with emotionally arousing stimuli. Research has shown, for example, that a strong footshock will produce a natural elevation in blood glucose that is comparable to the level in an animal that received a mild footshock plus an injection of 100 mg/kg of glucose (Hall & Gold, 1986). Importantly, animals in both conditions commonly display strong memory retention that is greater than animals that receive a mild footshock alone. This may actually represent an endogenous memory modulating mechanism where the body is capable of increasing the salience of memory in situations of high emotionality and/or stress.

Based on these results and similar studies in humans, one might hypothesize that memory for emotionally arousing material combined with systemic administration of a memory enhancing dose of glucose would actually impair performance in comparison to each treatment alone. We have, in fact, recently demonstrated this to be true (Mohanty & Flint, 2001) and argue that this result is similar to what one would find when systemically administering a large dose of glucose (i.e., glucose increase due to emotional arousal plus glucose increase from systemic administration equals higher than optimal glucose on inverted-U dose response curve).

**EXPERIMENT 1**

**HYPERMNESIA AND REMINISCENCE FOR EMOTIONALLY AROUSING WORDS**

The purpose of this exercise is to provide a demonstration of some basic characteristics of memory, specifically, enhanced memory for positive and negative emotionally arousing stimuli, hypermnesia, and
reminiscence. Hypernesia is described as an improvement in performance with repeated testing in the absence of additional study sessions, whereas reminiscence is the recall of previously forgotten or unrecalled items. Instructors may also utilize these data from this exercise to demonstrate other characteristics of memory including primacy and recency effects.

Participants The participants for all three of these exercises are typically students who volunteer to participate in the laboratory activity. If students outside the class are used it is recommended that the instructor consult the local Institutional Review Board (IRB) for research with human participants to determine whether or not IRB approval should be obtained.

For this exercise, a minimum of 20 participants works best. There are no specific requirements or restrictions, although it is recommended that participants refrain from eating or drinking for two hours and smokers refrain from smoking for at least 30 minutes prior to the exercise.

| Neutral Words | Negative Words | Positive Words |
|---------------|----------------|--------------|
| Bench 655     | Bomb 46        | Cash 503     |
| Banner 649    | Demon 106      | Orgasm 920   |
| Cord 698      | Rape 344       | Thrill 438   |
| Jelly 238     | Slap 396       | Sexy 530     |
| Statue 995    | Tumor 459      | Kiss 248     |
| Errand 150    | Ambulance 15   | Ecstasy 75   |
| Habit 775     | Cancer 60      | Glory 189    |
| Pamphlet 925  | Tornado 444    | Treasure 449 |
| Hay 784       | Crash 89       | Fame 157     |
| Prairie 325   | Killer 244     | Joke 826     |
| Hairpin 776   | Pervert 312    | Flirt 754    |
| Cow 554       | Slave 398      | Graduate 192 |

Table 1. Neutral, Negative, and Positive Words. Words, word number, and mean ratings for valence, arousal, and word frequency from the Affective Norms for English Words (Bradley & Lang, 1999) used in Experiment 1.

Methods Inform the participants that they will be viewing a slideshow that will present a total of 36 common words and that each word will appear on the screen for five seconds followed by a three second blank screen. At the end of the slideshow there will be a word recall test, so participants should pay close attention to the words when they are presented. In addition, participants will be rating the pleasantness, to ensure somewhat equal processing of each word, by circling the number on the Likert scale that best represents their gut feeling regarding the word. This rating should be done during the three second blank screen after each individual word appears.

Immediately following the slideshow presentation, collect the pleasantness rating forms and distribute one word recall form face down. Instruct the participants to write their name or some unique identifier on the back of the page and tell them that they will have two mintues to write down as many words as they can recall when told to turn the paper over. Do not tell them that there will be more recall tests. Once the first recall test is completed, collect the forms and distribute another blank recall sheet face down. Instruct the participants to write their name/identifier and the number two on the sheet and tell the participants that you want them to try to improve upon their previous recall performance. Give them another two minutes to recall as many words from the wordlist as possible. Once the second recall test is complete, repeat the procedure again, informing the participants that this is the last recall test, to write their name/identifier on the back along with test three, and that the participants should do their very best to recall more words from the wordlist than they did on the previous two trials.

Results & Discussion Once the final recall test is completed, ask each individual to score his/her own data. Distribute to each participant a list of the neutral, positive arousing, and negative arousing words and have them tabulate the following: total number of words recalled, number of neutral words recalled, number of positive arousing words recalled, and the number of negative arousing words recalled for each of the three tests. In addition, have the students calculate the number of each type of word recalled on test two that was not recalled on
test one and the number of words recalled on test three that were not recalled on test two. Finally, if interested in using this exercise to demonstrate primacy and recency effects, participants should be given three lists of words corresponding to each third of the list and asked to tabulate the number of words they recalled each time in each third of the list.

A variety of options exist for examining these data, although they may be unnecessary given that the purpose is to demonstrate simply some basic characteristics of memory in cognitive neuroscience. I recommend that students share their data and create a line graph of the class mean recall performance for neutral, negative arousing, and positive arousing words across the three trials. Figure 3 displays the combined results of a few similar exercises. The increase in performance across the three trials and the clear enhanced performance for emotionally arousing words should suffice to stimulate discussion. Some additional questions to stimulate discussion of these include: Why does performance improve across trials when no additional exposure to the test stimuli occurred? Why is performance greater for emotionally arousing stimuli? Did the class perform equally well with negative and positive arousing stimuli and if no, why not? Why is reminiscence necessary for hypermnesia to occur? Did any students find that they forgot some previously recalled items on tests two and/or three and if so, how does this impact reminiscence if hypermnesia is still demonstrated? How might word imagery have influenced recall performance? Did the emotional valence of the wordlist impact the traditional J-shaped primacy/recency curve? Finally, students may find it interesting to speculate about the arousing nature of the words and whether or not negative words were equally arousing as positive words.

EXPERIMENT 2
EMOTIONAL AROUSAL-INDUCED MEMORY ENHANCEMENT AND BLOOD GLUCOSE ELEVATION

The purpose of this exercise is to examine the relationship between blood glucose level and memory for negative emotionally arousing and neutral pictures. The exercise is based, in part, on studies by Blake et al. (2001) and Mohanty & Flint (2001).

Participants For this exercise it is best to have a minimum of ten participants per group which usually means a class size of 20 or more. Participants must fast (no food or drink except water) for a minimum of two hours prior to the lab exercise, and each student will need to provide a small blood sample. Thus, participants with blood sugar disorders (diabetes, hyper-/hypo-glycemia) or individuals with phenylketonuria, blood clotting disorders, or blood-based contagious infections must refrain from participating. Smokers are also encouraged to refrain from smoking for 30 minutes prior to the lab exercise.

Materials Instructors will need a copy of the International Affective Picture System (IAPS; Lang et al., 1999, see author notes for url) which may be obtained from Peter Lang at the Center for Research in Psychophysiology at the University of Florida. This database of pictures comes with normative values for level of arousal (high/low), valence (positive/negative), and dominance (high/low). A set of 25 highly arousing negative pictures and 25 neutral non-arousing pictures should be selected and placed into a computerized slideshow presentation system such as PowerPoint. If the exercise is conducted in a group, instructors may wish to use a room equipped with a projection system and projector screen. Table 1 displays the picture description and picture number for a pre-selected set of negative arousing and neutral pictures along with the mean valence and arousal ratings for each set (based on a nine point Likert scale). A generic pleasantness rating sheet should be developed with 25 Likert scales ranging from one (very unpleasant) to five (very pleasant). At least one, depending on class size, standard diabetic testing kit including glucometer, lancets, and enzyme strips will be needed. The One Touch Basic Glucometer from LifeScan has proven to be an inexpensive and durable glucometer for this purpose. These are available at most local drug stores and pharmacies. Instructors will also need gauze pads, rubbing alcohol, and band-aids. Finally, instructors will need a stopwatch and should prepare a single page of difficult multiplication and division problems to be distributed as a distracting task to prevent rehearsal.

![Graph showing recall test results](image)

**Figure 3.** Neutral, Negative Arousing, and Positive Arousing Word Recall on Trials 1, 2, and 3. The increasing performance on each subsequent recall test is evidence of hypermnesia and reminiscence. Negative and positive arousing words were clearly recalled at a higher rate than non-arousing words (Need & Flint, 2000; Need et al., 2001; Shatynski & Flint, 2000).

Procedure Review the participant requirements outlined in the participants section above and explain to the students that some of the pictures may include disturbing negative pictures and if they do not wish to participate in the exercise, they do not have to. Divide the participants into two groups and dismiss one group from the classroom for 30 minutes. Distribute glucometers, enzyme strips, lancets, gauze pads, rubbing alcohol, and band-aids to the participants. Explain that during this exercise participants...
will be required to prick their finger twice to obtain two drops of blood, view and rate the pleasantness of 25 pictures, and complete a picture recall test.

Ask participants to select and clean a finger-tip on their non-dominant hand with gauze and rubbing alcohol. Once the fingertip has dried, prepare a lancet and enzyme strip, prick the fingertip, and place a drop of blood onto the enzyme strip in the glucometer to establish a baseline blood glucose level. Fasting blood glucose levels may be expected to range between 60 and 120 mg/dl, although results from a recent study in our lab indicated that blood glucose level ranged from 63 to 111 mg/dl with a mean of 83.92 (SD=8.35) mg/dl after eight hours of fasting (Flink & Zak, unpublished). Participants may wish to put pressure on the finger-prick with a gauze pad for a moment and apply a band-aid. Have each student record the blood glucose value on their datasheet.

| Neutral Pictures | Negative Pictures | Positive Pictures |
|------------------|-------------------|------------------|
| **Description**  | **IAPS #**        | **Description**  | **IAPS #**        |
| Fingerprint      | 2206              | Erotic           | 4290              |
| Neutral Face     | 2210              | Female           | 4680              |
| Boy              | 2280              | Erotic Male      | 4520              |
| Elderly          | 2516              | Erotic           | 4520              |
| Woman Shadow     | 2680              | Erotic Male      | 4520              |
| Rocks            | 5130              | Erotic           | 4520              |
| Mushrooms        | 5534              | Erotic           | 4520              |
| Plant            | 5740              | Erotic Male      | 4520              |
| Bicyclist        | 5757              | Erotic           | 4520              |
| Outlet           | 6150              | Erotic           | 4520              |
| Rolling Pin      | 7000              | Erotic           | 4520              |
| Bowl             | 7006              | Erotic           | 4520              |
| Woman            | 2271              | Erotic           | 4520              |
| Girl             | 2381              | Erotic           | 4520              |
| Shoes            | 7031              | Erotic           | 4520              |
| Hair Dryer       | 7050              | Erotic           | 4520              |
| Car              | 7096              | Erotic           | 4520              |
| Trash Can        | 7060              | Erotic           | 4520              |
| Lamp             | 7175              | Erotic           | 4520              |
| File Cabinets    | 7224              | Erotic           | 4520              |
| Building         | 7491              | Erotic           | 4520              |
| Car Crash        | 7920              | Erotic           | 4520              |
| Runner           | 8010              | Erotic           | 4520              |
| Rain             | 9210              | Erotic           | 4520              |

Table 2. Neutral, Negative, and Positive Pictures. Picture description, picture number, and mean valence and arousal ratings for 25 neutral, 25 negative emotionally arousing pictures, and 25 positive emotionally arousing pictures selected from the IAPS database (Lang et al., 1999) for Experiment 2.

Distribute a pleasantness rating sheet with 25 five point Likert scales to the participants. Instruct them that they will be viewing a picture slideshow where each picture will appear for five seconds, followed by a three second blank screen before the next picture appears. Ask participants to study each picture while it is on the screen and to circle the number on the Likert scale that best represents their gut feeling of pleasantness when the blank screen appears.

Once the slideshow is completed (either negative arousing or neutral pictures), distribute the math worksheet and tell the participants to complete as many problems as possible in the next two minutes. After two minutes have passed, ask participants to turn over their math sheets and write down brief descriptions of as many of the pictures as they can recall. After five minutes, instruct the participants to stop writing, and to conduct another blood glucose test in the same manner as previously described.

This procedure should be repeated with the other group of participants using the remaining picture slideshow. Instructors should be careful to keep the datasheets from the two groups separate for data analysis.

**Results & Discussion** Once data collection is complete, have all students return to class and go through score his/her answers. Create summary tables where students can indicate their group (neutral or negative arousing pictures), their blood glucose levels (pre and post slideshow), and the number of pictures correctly recalled. Once each student has a copy of the class data, instruct them to create a line graph for blood glucose levels for the two groups and to create a bar graph for recall performance. Results should indicate elevated blood glucose levels post-viewing for the group that saw negative emotionally arousing pictures as well as a higher rate of recall on the performance test. Engage the class in discussion of these results. Some specific questions instructors may wish to pose include: Why did blood glucose levels increase in one group and not the other and what mechanisms are likely responsible for this result? Can you trace the neurobiological pathways responsible for emotion-induced increases in plasma glucose? How might glucose have affected the brain to improve recall? What other neuroendocrine mechanisms might have contributed to an improvement in performance? Is this a memory effect, or could the results be confounded by attention? Do you think the same results would have occurred if positive emotionally arousing pictures had been used instead of negative emotionally arousing ones?

**EXPERIMENT 3**

### GLUCOSE-INDUCED ENHANCEMENT OF SPATIAL MEMORY

The purpose of this exercise is to demonstrate the memory-enhancing effects of glucose on acquisition of a spatial memory task. This exercise is originally based on a study by Mohanty & Flint (2001) where they demonstrated that 100 mg/kg of glucose administered prior to training enhanced learning (Figure 4).

**Participants** Participant requirements for this exercise are the same as those for Experiment 2.
Materials For this exercise instructors will need glucose (free samples are available from Arthur Daniels Midland Corn Processing), a non-nutritive artificial sweetener (e.g., saccharine solution Sweet-10 available at many grocery stores), and unsweetened lemon-flavored drink mix (e.g., Kool Aid). Two scales will be needed, one to weigh the participants and one to weigh 100 mg/kg of glucose to be mixed with eight oz of the lemon-flavored beverage. If using Sweet-10 as a control, two ml should be mixed with eight oz of the lemon-flavored beverage. Two 2-gal pitchers, weighing boats, stir sticks, paper towels, and large plastic cups will also be needed. Two blood samples will also be obtained during this exercise so glucometers, enzyme strips, and lancets will been needed as in Experiment 2. Finally, a stopwatch and a set of 16 neutral non-arousing pictures from the IAPS database (Lang et al., 1999, see author notes for url) will be needed. Once selected, the pictures should be printed on white paper, numbered on the back, and laminated. If the participants have not seen the IAPS pictures from Experiment 2, then instructors may simply select 16 of the neutral pictures in Table 2 for use in this exercise.

Procedure Data for this exercise is collected from one student at a time and thus can be time consuming. In order to facilitate the data collection process, instructors may opt to create a second set of 16 neutral pictures and run the experiment twice, once with half the class acting as the researcher and then switching roles. The restrictions for participating in this exercise should be reviewed as described in Experiment 2 with the exception that no negative arousing pictures will be viewed. Participating students should be weighed and randomly assigned to either the saccharin or glucose group. Those in the glucose group should have the appropriate lemon-flavored beverage mixed to ensure a 100 mg/kg dose of glucose. Care should be taken to keep the participants blind to their condition, so the instructor should code the cups to ensure that conditions can be revealed following the exercise. Ask all participants to take a baseline blood glucose measure per the instructions provided in Experiment 2 and then consume the lemon-flavored beverage.

The pictures should be arranged on a large flat surface (e.g., tabletop) in a 4 x 4 grid where the picture labeled number 1 is in the upper left corner and the picture labeled 16 is in the lower right. The participant should be facing away from the table when the pictures are arranged. Once the pictures have been arranged, the participant should be instructed to turn around and study the pictures paying particular attention to their position. Give the participant 20-sec and instruct them to turn back around and begin counting backward from 200 by 3 out loud (as a distraction to prevent rehearsal). While the participant is counting, shuffle the pictures well and place them into a randomly ordered pile. Tell the participant to turn around, hand him/her the pile of pictures with instructions to place them into a 4 x 4 grid as accurately as possible based on their original location. Once the participant has completed this task, ask him/her to turn back around, flip over all the pictures, and record the number of correct responses. Return the pictures to their correct location on the 4 x 4 grid and repeat this process two more times. After completing the last trial, ask the participant to repeat the blood glucose test.

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Results & Discussion Analysis should begin with revealing the group assignment for each participant. Summary tables should be created including group assignment, baseline and post-consumption blood glucose levels, and recall performance on each spatial memory trial. Students should create a line graph, as in Figure 5, demonstrating an increase in plasma glucose levels for those participants that received 100 mg/kg of glucose in their beverage. A line graph for recall performance across the three trials in each group should also be drawn, followed by a class discussion of these results. If the glucose-induced facilitation of performance is difficult to see in a line graph, instruct the students to create a bar graph of these data collapsed across trials (as in Figure 4). Spatial memory has also been shown to be sensitive to gender differences. While the study by Mohanty & Flint (2001) lacked sufficient numbers to address potential gender differences, instructors with a large enough sample may find that an additional analysis of gender differences a useful exercise.
Instructors interested in expanding this exercise further may wish to add a verbal recall task that might allow them to do cross-task comparisons. For this it is recommend that the California Verbal Learning Test be utilized, as prior research has shown that it is sensitive to the memory enhancing effects of glucose in unimpaired adult participants (Sunram-Lea et al., 2002). Some focus questions for this lab include: Was performance in the glucose group greater than that in the saccharin group on each trial, or did the glucose group display facilitat performance on the 2nd and 3rd trials? What is spatial memory and what sorts of strategies were utilized to complete this task? If endogenous glucose release is one mechanism through which memories are enhanced under conditions of stress and/or high emotional arousal, how might the enhancement of spatial memory translate into real-world situations? What areas of the brain and neurotransmitter systems are particularly important for spatial memory? Given that acetylcholine in the hippocampus is important for spatial memory, how might glucose facilitate these processes (hint: glucose facilitates acetylcholine neurotransmission, Messier et al., 1990; Ragozzino et al., 1996)? (See also Results & Discussion for Experiment 2 for additional questions of relevance).

These exercises are based largely on primary empirical research which has been referenced with each activity.

The role of glucose in memory is also of interest because of its potential involvement in age-related cognitive decline, Alzheimer’s disease, and other neuropsychological conditions. While the laboratory exercises presented here are not directly applicable to these conditions, instructors may wish to assign some additional reading before or after an exercise in order to stimulate discussion or provide sources for a written report of the laboratory exercise. Table 3 provides a list of a number of references where glucose has been effectively used to attenuate cognitive deficits for a variety of neuropsychological conditions in humans.

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| Reference                | Sample         |
|--------------------------|----------------|
| Craft et al. (1992)      | Alzheimer’s Disease |
| Craft et al. (1993)      | Alzheimer’s Disease |
| Gonder-Frederick et al. (1987) | Elderly |
| Hall et al. (1989)       | Elderly         |
| Manning et al. (1990)    | Elderly         |
| Manning et al. (1992)    | Alzheimer’s Disease |
| Manning et al. (1993)    | Down’s Syndrome |
| Messier & Gagnon (1996)  | Alzheimer’s/Diabetes |
| Messier et al. (1997)    | Elderly         |
| Newcomer et al. (1999)   | Schizophrenia   |
| Pettersen & Skelton (2000)| Head Injury    |

Table 3. List of References for Further Reading on Glucose-Induced Memory Modulation in Humans. These references may provide additional context within which students can evaluate these laboratory exercises.

GENERAL DISCUSSION
This article reviews some of the literature regarding enhanced memory for emotionally arousing stimuli, provides a foundation from which to argue that increases in plasma glucose levels may be one of many endogenous mechanisms contributing to this effect, and describes three experiments associated with these effects in cognitive neuroscience. The materials necessary for these human laboratory exercises are quite affordable and the protocols are easily adapted to suit the needs of individual instructors. In particular, the techniques for monitoring blood glucose levels used in Experiments 2 and 3 are relatively simple, and may be easily applied to other experimental protocols where stress/emotional-arousal are of interest. For example, in non-human animals, a simple tail cut can be used to obtain a drop of blood for examination of blood glucose levels or assessment of plasma glucose levels could be added to another exercise such as the one described by Kalman & Grahn (2004).
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