

Sex-differences in Cognitive Function and Blood Pressure: The Effects of a Meal.

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Abstract

Sex-specific responses to acute hyperinsulinemia have the ability to influence cognitive function. We sought to examine the effects of a meal on cognitive function and blood pressure, as well as sex-differences in cognitive function and blood pressure in response to a meal. There were a total of 13 subjects, six male and seven female that performed memory and reaction time tests to assess cognitive function. In a successive two-week study, both tests were performed by each subject in randomized order, one week under fed conditions and the other week under fasted conditions. Blood pressures were taken at periodic intervals throughout the study. In the fasted state, females had significantly lower average MABP than males ($84.8 \pm 4.6 \text{ mmHg}$ vs $88.5 \pm 1.9 \text{ mmHg}$; $P < .01$); in the fed state females also had a significantly lower average MABP versus males ($83.8 \pm 4.8 \text{ mmHg}$ vs $89.0 \pm 2.5 \text{ mmHg}$; $P < .01$). There was no significant difference between fasted male and female groups for reaction time tests ($19.6 \pm 1.6 \text{ s}$ vs $17.7 \pm 1.5 \text{ s}$; $P > .05$). There was no significant difference between fed male and female groups for reaction time tests as well ($20.4 \pm 2.2 \text{ s}$ vs $18.1 \pm 1.8 \text{ s}$; $P > .05$). We conclude there were no interactions between a meal and sex on blood pressure and cognitive function. Since this is not consistent with previous research, further studies would be needed to resolve confounding variables.

Introduction:

Cognitive challenges increase neuronal activity and metabolic demand leading to an increase in cerebral perfusion (Bookheimer et al., 1995). Inadequate cerebral perfusion is a characteristic of Alzheimer's disease and stroke, which are leading causes of cognitive impairment (Roher et al., 2012, Go et al., 2013, Association, 2012). The risk of developing Alzheimer's disease or stroke is highly sex-specific (Appelros et al., 2009). Specifically, Alzheimer's disease strikes women more than men, possibly due to a post-menopausal decline in estrogen (Association, 2012). Women who undergo hormone replacement therapy are protected from the development and advancement of Alzheimer's disease (Paganini-Hill A, 1996).

Despite these sex-biases, the sex-specific mechanisms of cerebral vascular control remain largely unexplored.

Insulin resistance, characterized by chronic hyperinsulinemia, increases Alzheimer's disease and stroke risk nearly three-fold and exacerbates the sex-bias seen in cerebral vascular dysfunction (Calleja et al., 2011, Hyvarinen et al., 2009, Vermeer et al., 2006, Kuusisto et al., 1997). Therefore, insulin resistance is emerging as a fundamental characteristic of cognitive dysfunction (Steinberg et al., 1996, Cosentino et al., 2001, Erdös et al., 2002, Kuusisto et al., 1997). In contrast to chronic hyperinsulinemia, acute hyperinsulinemia has positive effects on the vasculature. Acute insulin exposure induces vasodilation of peripheral vasculature and increases blood flow to the arm, leg, and heart (Chen and Messina, 1996, Steinberg et al., 1994, Anderson et al., 1991, Laine et al., 2000). The acute effects of hyperinsulinemia may also increase cerebral blood flow and improve cognitive function as insulin evokes vasodilation of rat cerebral arteries "*in vitro*" (Katakam et al., 2009).

Sex-specific responses to acute hyperinsulinemia have the ability to influence cognitive function. Women are protected from the adverse effects of chronic hyperinsulinemia and ensuing cerebral vascular dysfunction. Estrogen promotes vasodilation and improves blood flow through cyclooxygenase (COX) mediated prostacyclin formation (Sobrino et al., 2010, Turner and Kinsella, 2010). Conversely, testosterone promotes vasoconstriction and decreases blood flow through COX mediated thromboxane production (Gonzales et al., 2005). This is consistent with data from isolated cerebral arteries of male rats indicating insulin mediated vasodilation is

restrained by COX derived products; insulin-mediated vasodilation in the cerebral arteries of female rats was not studied (Katakam et al., 2009).

Considered collectively, cognitive function is associated with increased cerebral perfusion. Acutely, insulin has positive effects on the cerebral vasculature as it vasodilates isolated cerebral arteries of male rats. However, there appears to be sex-specific cerebrovascular responses to acute insulin exposure due to sex-hormones that can influence cognitive function.

With this in mind we would like to address whether or not there are sex-differences in the cognitive response to acute hyperinsulinemia elicited by a meal, in humans.

Stroop and memory recall tests will be utilized to examine the sex-specific differences in the cognitive response to a meal. The Stroop Effect is the human tendency to read words automatically before being able to comprehend the colors of the words. If a word such as green is written in blue, (green) a person would name the word more readily than the color of the word. The task of creating a response to these two conflicting signals has been associated with the anterior cingulate region of the brain known for having an effect on a wide range of cognitive functions (De Young). The cognitive mechanism at work during the Stroop Test is called selective attention, where the reader must inhibit the faster and stronger word recognition process in order to create a color response as the final response. When subjected to continual conflicting stimuli, reaction time increases with attentional fatigue. Thus, the Stroop Test can be used as an effective test of reaction time.

Memory recall tests are another form of testing cognitive function. The first process of memory formation is called Sensory Information Storage (SIS). This involves the holding of sensory stimuli for just about a second after being received by the sensory organs, including things that are seen and heard. From here, sensory information is moved into short-term memory (STM). The information is held here longer than in SIS, but still for a very short period of time (a few seconds or minutes). In this time, the information is processed to decide if it is important enough to store in the long-term memory (LTM). Since the information in STM has never left the conscious mind to be stored in the LTM, its retrieval is direct and relatively easy. A process called rehearsal, in which the information in the STM is consciously repeated over and over, allows information to stay in the STM for longer periods of time. However, while rehearsing information to retain it in STM, new information can't be added. Although STM is convenient for its direct recall abilities, it has a very limited capacity. If a person hears a list of 20 numbers, they will retain about 5 or 6 of them (CSI, 2013).

These cognitive tests will investigate whether or not there is a difference in cognitive function between males and females after ingesting a carbohydrate-rich meal. In this study, participants will be instructed to refrain from eating before coming to lab. Once the participants arrive, they will either be given nothing to eat (serving as the control group) or they will be instructed to eat a 'plain' bagel. After waiting 30 minutes, the participants will complete a reaction time test (Stroop test) and a word memory task to test their cognitive function capabilities. Additionally, participants' blood pressure will be monitored throughout the study to determine if there is a relationship between blood flow and cognitive function.

Under fasting conditions we predict that there will be no differences in cognitive function between sexes. Due to the vascular effects of sex-hormones we believe blood pressure in fasted males will be greater than females. Based upon the evidence that increased cerebral blood flow is associated with cognitive function, the effects of glucose on cognitive performance, and the acute positive effects of insulin on vascular blood flow, we hypothesized that ingesting a carbohydrate-rich meal will improve cognitive function regardless of gender. Additionally,

given the vasodilatory effects of estrogen and insulin, we hypothesized that ingesting a carbohydrate-rich meal will improve cognitive function in both sexes, but more so in females. Furthermore, we predict that a meal will only increase blood pressure in males.

Methods:

Participants were six male and seven female UW-Madison students enrolled in physiology 435. Ages ranged from 20-31 years old. No participants had a history of diabetes. Subjects were in the normal BMI range (18.5-24.9) (Centers of Disease Control and Prevention). Subjects gave informed consent prior to voluntary participation. Each participant was tested as part of a within subjects design, meaning each person served as their own control.

To test the effects of a meal on reaction time and the ability to form memories, we conducted Stroop Tests and memory recall tests on the participants. Each subject participated in two study visits one week apart, once having fasted before the experiment and once after being given a small meal before the experiment. Whether the meal was given during the first or second visit was randomized. In addition, blood pressure was measured prior to, during and after the cognitive function tests.

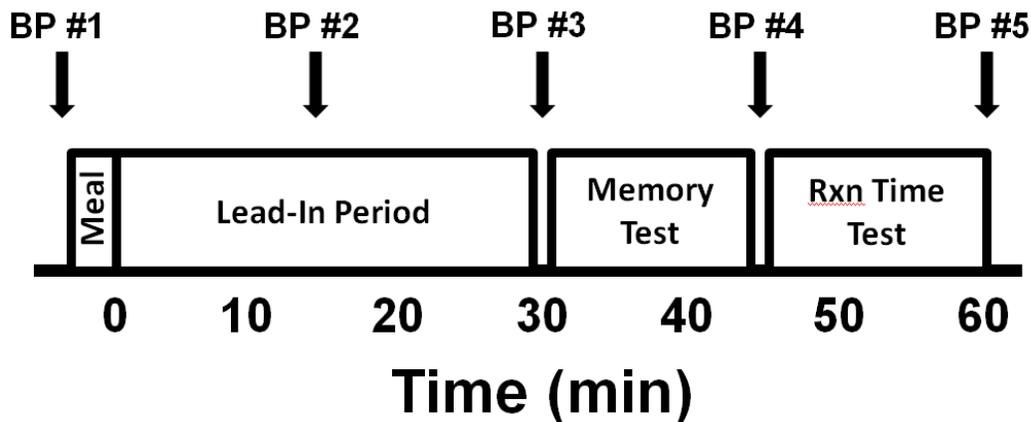


Figure 1. Study timeline. Order of memory and rxn time test randomized.

Meal administration:

Subjects in the “fed” group were given a bagel consisting of 52 grams of carbohydrates as their meal, and given as much time as they needed to finish it. Once the bagel was finished, the 30-minute “lead-in period” began (see Figure 1). Blood pressure was measured once at 15 minutes post-meal consumption, and once at 30 minutes post-meal consumption. Cognitive tests began after the 30 minute blood pressure measurement. The tests were delayed to allow for an adequate insulin response from the meal. Test order was randomized to decrease the possibility that one test may improve the subject’s performance on the other test. Fasted trials were the same as fed trials except for the consumption of a meal.

Reaction time:

The colors of the 24 words for each trial were pre-recorded on a sheet of paper. Subjects then named the colors of 24 words from a table as fast as possible. If a color was incorrect, the

experimenter said “incorrect” and didn’t allow the subject to proceed until the color was stated correctly. Once all of the colors were stated correctly, the time to complete the test was recorded. For the second trial, we utilized the same procedure as the first with a new set of colored words.

Subjects took a computerized Stroop Test online week one:



Subjects performed this Stroop Test week two:

BROWN	RED	GRAY	PURPLE	PINK
GRAY	BLACK	YELLOW	RED	GREEN
BLUE	PINK	PURPLE	ORANGE	BROWN
YELLOW	ORANGE	BLUE	GREEN	BLACK

Memory test:

Subjects were given a list of 25 words and one minute to study them. Participants were instructed to memorize as many words as they could. Next, they were given a one minute break to perform math problems as a distraction in order to prevent rehearsal of the list. Then, subjects were given as much time as they needed to recall and write down as many of the words as they could. The first time that a subject participated in the study, the subject used word list #1. The second time, word list #2 was used.

Word List #1

Nine	Swap	Cell	Ring	Lust
Plugs	Lamp	Apple	Table	Sway
Army	Bank	Fire	Clap	Worm

Clock	Horse	Color	Baby	Sword
Desk	Hold	Find	Bird	Rock

Word List #2

Orange	Paper	Pond	Tonight	Bucket
Dinner	Climb	Left	Queen	Garden
Train	Kitchen	Circus	Think	Teacher
Holiday	Ladder	Grass	Stone	Uncle
Dress	Balloon	Watch	Dance	Castle

Distraction Task

28 156 98 33 14 14 48 3 16 33 90
+37 +364 -62 x 40 x 26 x 7 +168 +12 x 6 ÷ 3 ÷ 12

Blood Pressure:

Blood Pressure was measured non-invasively using a manual sphygmometer. Measurements were taken when the participants were relaxed and in a seated position. The same investigator took all measurements to eliminate variability between measurements. A baseline blood pressure reading was taken for each participant, as well as measurements at timepoints of 15, 30, 45, and 60 minutes post-carbohydrate consumption. All blood pressures were converted to the Mean Arterial Blood Pressure (MAP), which is calculated as $\frac{1}{3}$ systolic pressure + $\frac{2}{3}$ diastolic pressure. This accounts for the fact that the heart spends $\frac{2}{3}$ of its time in a diastolic state, and simplifies the comparison of blood pressures across trials and subjects.

Statistical Analysis:

For reaction and memory tests, an Analysis of Variance (ANOVA) F-test was performed to compare differences of means between groups (e.g. Female/Fasted, Female/Fed, Male/Fasted, Male/Fed). $P < .05$ was considered statistically significant.

The average mean arterial blood pressure (MABP) was calculated for each condition, at each time point. An ANOVA was performed to compare the differences of means between each group. Additionally, MABP was compared at each time point (e.g. 0, 15, 30, 45, 60 minutes) between groups.

Results

In the fasted state, females had significantly lower average MABP than males (84.8 ± 4.6 mmHg vs 88.5 ± 1.9 mmHg; $P < .01$); in the fed state females also had a significantly lower average MABP versus males (83.8 ± 4.8 mmHg vs 89.0 ± 2.5 mmHg; $P < .01$) (Figure 1, Figure 2). Throughout the time course of the experiment there were no significant changes in blood pressure in either sex. Additionally, there were no significant differences in MABP within sexes between fasted and fed conditions (Figure 3, Figure 4).

There were no significant differences between fasted male and female groups for reaction time tests (19.6 ± 1.6 s vs 17.7 ± 1.5 s; $P > .05$). There were no significant differences between fed male and female groups for reaction time tests as well (20.4 ± 2.2 s vs 18.1 ± 1.8 s; $P > .05$) (Figure 5). Neither males nor females had change in reaction time when fed versus fasted. There were no significant differences between fasted male and female groups for memory tests (9.3 ± 0.8 words vs 11.2 ± 1.1 words; $P > .05$). There were no significant differences between fed male and female groups for reaction time tests as well (8.4 ± 0.8 words vs 10.3 ± 1.8 words; $P > .05$).

Discussion

Previous research has shown estrogen promotes vasodilatation and increases blood flow through its actions on prostacyclin formation, whereas, testosterone has the ability to vasoconstrict and decrease blood flow through its effect on thromboxine (Sobrinho et al., 2010, Turner and Kinsella, 2010, Gonzales et al., 2005). Additionally, animal and epidemiological studies suggest that insulin may interact with sex hormones to regulate blood flow. The regulation of blood flow is important when considering cognitive function as elevated brain activity requires adequate brain perfusion. Specifically, it has been shown that there are sex differences in cerebral perfusion in disease states (Appelros et al., 2009, Association, 2012). Inadequacy of cerebral perfusion due to the interaction of insulin and sex hormones may explain some of the sex differences in disease states such as stroke and Alzheimer's disease.

For our study, we chose to examine sex differences in cognitive function after a meal based upon the previous research showing that insulin and sex hormones effect cerebral blood flow. Based upon our data, females had significantly lower MABP. This finding is consistent with prior research that has shown estrogen to have beneficial effects on the vasculature, such as promoting vasodilation.

We did not find any effect of a meal on cognitive function or blood pressure. Insulin is known to promote vasodilation, and our meal should have been adequate to significantly elevate circulating insulin levels. However, despite our presumed rise in insulin levels, no differences in blood pressure or cognitive function were observed. Without measuring insulin or cerebral blood flow, our data supports the idea that insulin does not interact with sex hormones to have an effect on the vasculature, such as increase in blood flow.

A possible reason for contradictory findings with past research may have been due to the lack of standardization. We did not control for the phases of the female menstrual cycle, measure plasma hormone levels in relation to insulin, or measure cerebral blood flow. Additionally, other factors such as circulating catecholamines, endothelin-1, angiotension-II, dehydration, exercise, BMI, medications, and genetic predispositions were not accounted for, which could have had an effect on our results. Future studies could correct for these confounding variables by increasing sample size, measuring insulin directly, and using transcranial Doppler or magnetic resonance imaging to quantify cerebral blood flow. One final limitation to our study was the environment in which the cognitive tests were performed. Surrounding noise in the laboratory room may have interfered with subjects' concentration, and potentially underestimated their cognitive scores.

Additional studies on cognitive performance would be administered in a quiet, distraction-free environment with a larger sample size. Furthermore, to increase the external validity of our study, hormone levels would need to be tested with consideration for the female menstrual cycle. These future studies have the potential to increase understanding of sex differences in cognitive function and disease.

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Figures

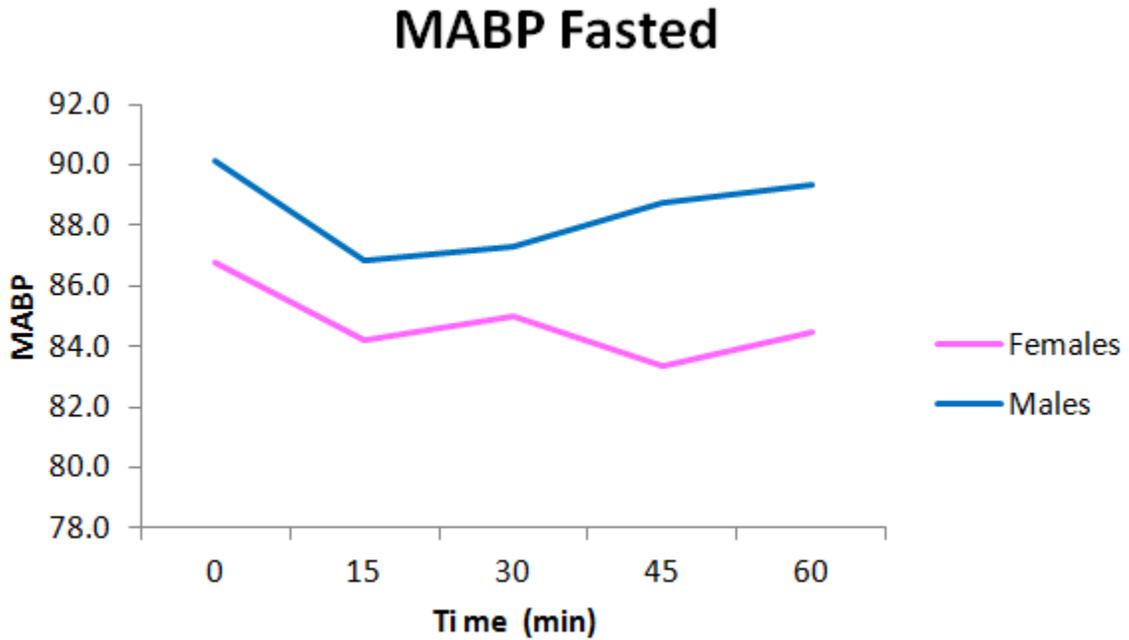


Figure 1. Mean Arterial Blood Pressure for the fasted condition

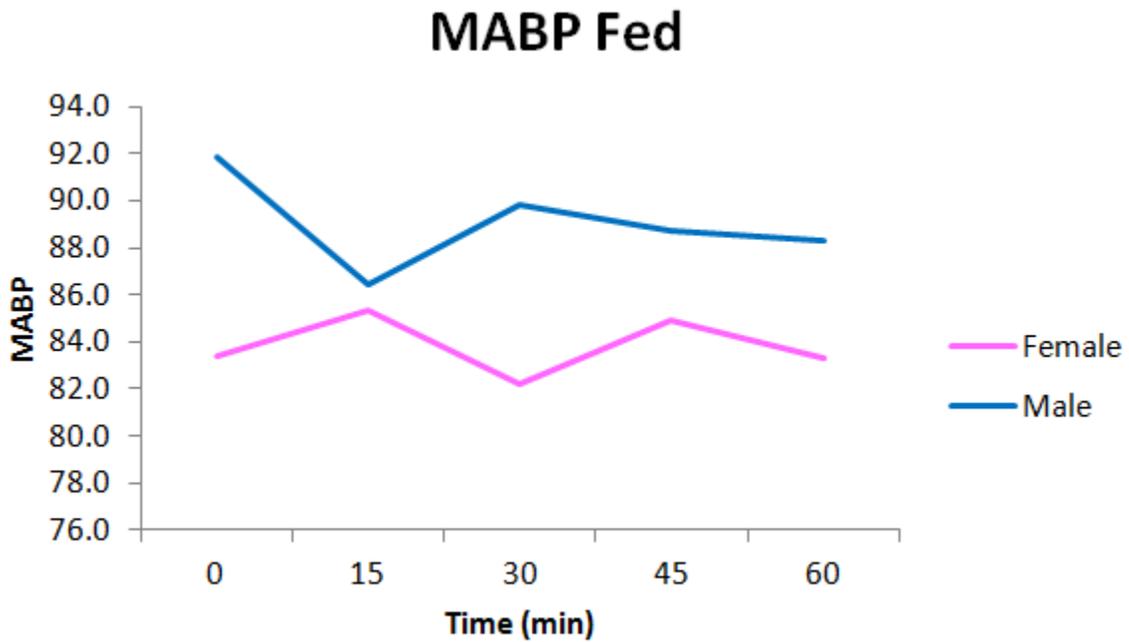


Figure 2. Mean Arterial Blood Pressure for the fed condition.

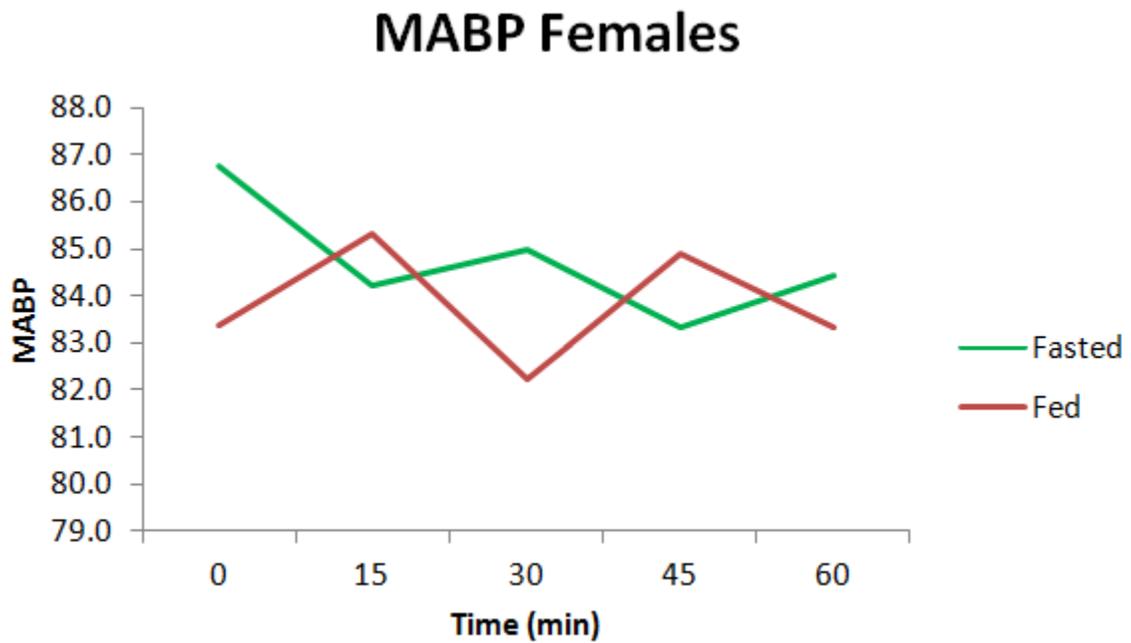


Figure 3. Mean Arterial Blood Pressure for females in both fasted and fed conditions.

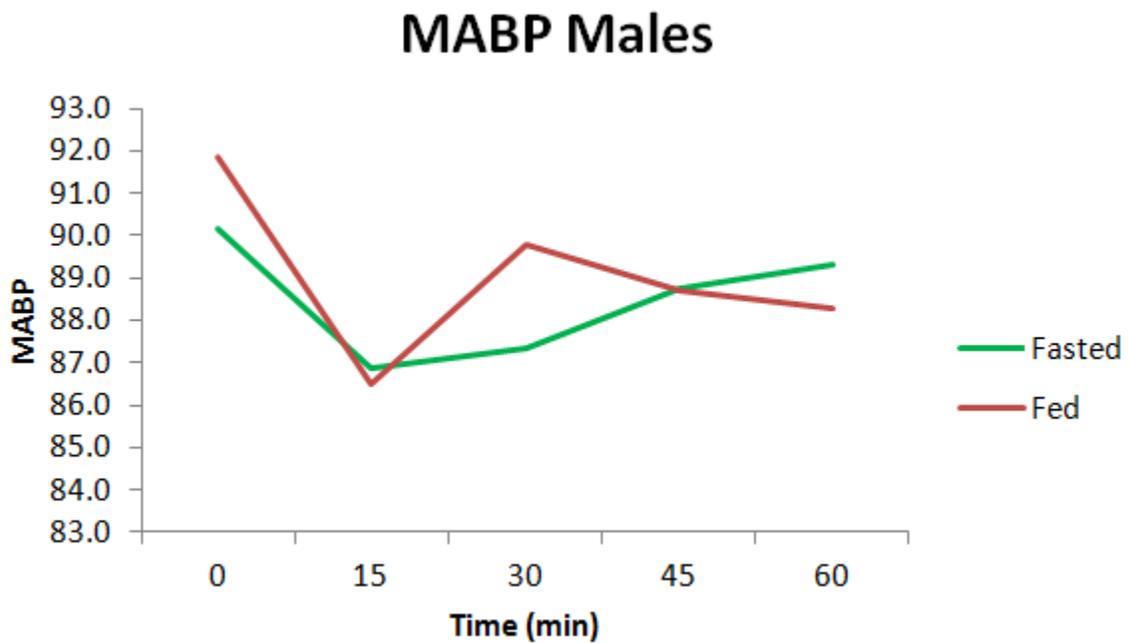


Figure 4. Mean Arterial Blood Pressure for males in both fasted and fed conditions.

Stroop Test

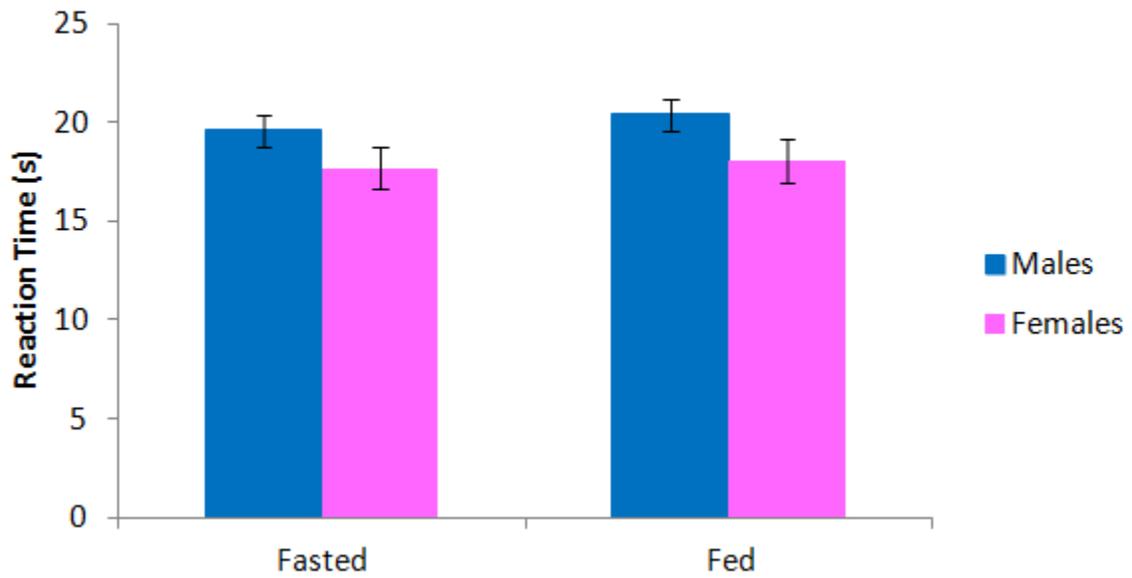


Figure 5. Stroop Test results comparing males and females during fasted and fed conditions.

Memory Test

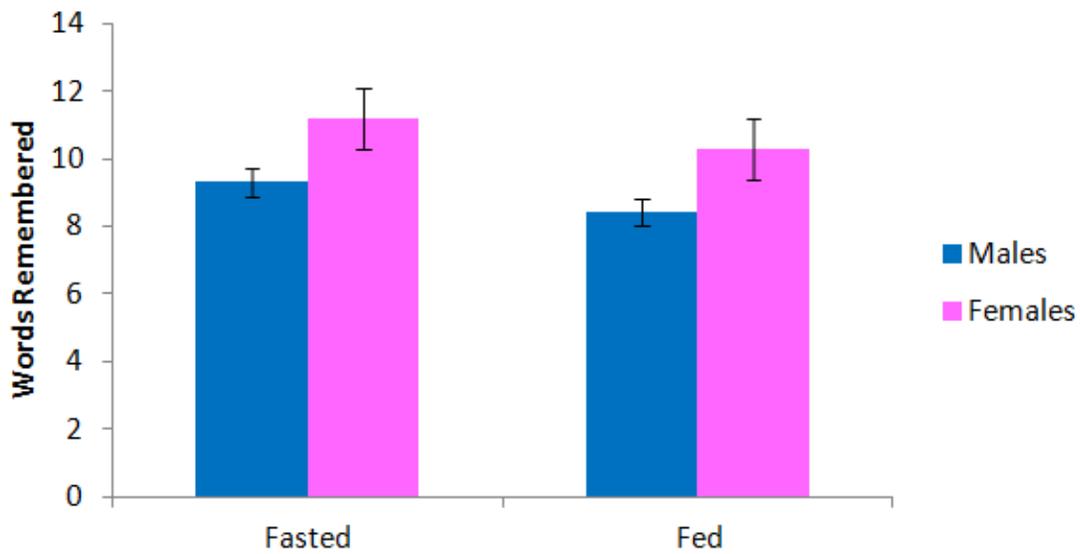


Figure 6. Memory test results comparing males and females during fasted and fed conditions.