Olga Bulgurova

Independent Study in Biology, Bio 0499, Spring 2011

Missouri Southern State University

TITLE

Testosterone Effects on Mood, Energy Level, and Libido of College-Age Females

ABSTRACT

Circulating free testosterone was measured by means of ELISA salivary assays from three specific points in the menstrual cycle (Days 1, 13, and 24) in 15 college-age females. All participants answered the same ten-question survey on the same three days they collected saliva samples; questions were asked to evaluate mood, energy level, and sex drive. The aim of the study was to find out if there is a general trend in how women feel throughout their menstrual cycle and if varying levels of testosterone show a correlation with survey responses. Mean testosterone concentration was lowest on Day 1 ($p<0.05$). Day 1 also generated most negative responses to being in a good mood and open to sexual activity, feeling energetic, and having a good outlook on the future ($p<0.05$). Day 13 generated the most positive responses to the same questions ($p<0.05$). While it was shown that women tend to feel worse around the onset of their menses and better around the time of ovulation, the data was not sufficient to determine whether changing testosterone level correlated with energy, libido, and mood.

PURPOSE:

The purpose of this study was to determine if a change in testosterone levels during the menstrual cycle affected mood, energy level, and sex drive in healthy young women. Salivary testosterone levels were measured at three specific points in the menstrual cycle (days 1, 13, and 24) in 15 females using ELISA assays and compare the results to the self-reported mood, sex drive, and energy levels of the women. A strong connection may confirm that testosterone is responsible for creating a sense of well-being in women and increasing their libido at specific times in the menstrual cycle, and that when its levels are low, mood and libido are also diminished.

The goal of this study was to provide women with solid statistical evidence that the monthly ailments associated with the menstrual cycle many of them experience have a physiological basis, as well as to demonstrate the importance of having normal levels of testosterone for their sexual and mental health.

HYPOTHESIS:

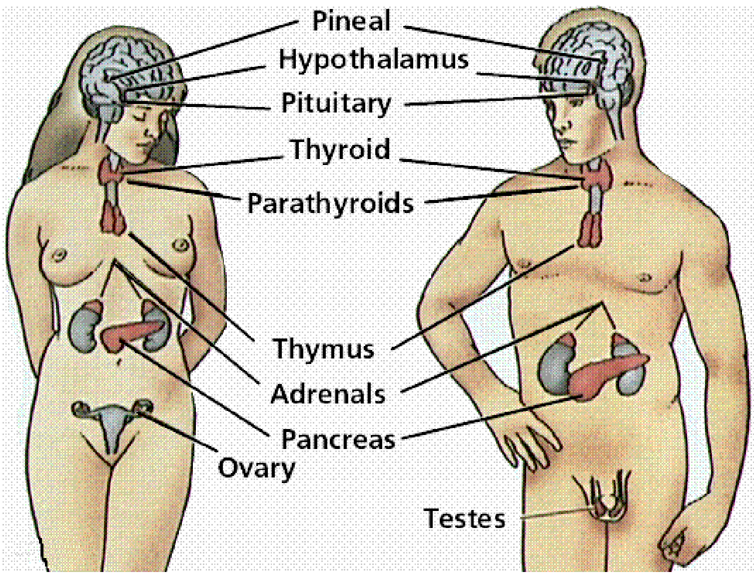
Upon review of relevant literature, I hypothesized that testosterone levels would be higher during ovulation than during other stages of the menstrual cycle. My second hypothesis was that higher levels of testosterone would correspond to better mood, higher energy, and increased sexual libido, and that mood, energy, and libido would also be highest around ovulation. Based on background research, I hypothesized that when bioavailable testosterone levels are highest (around ovulation), the participants will feel most content and optimistic, have the most energy, and be the most open to sexual activity, with the opposite being true when testosterone is at its lowest (during menstruation).

BACKGROUND

a) Testosterone

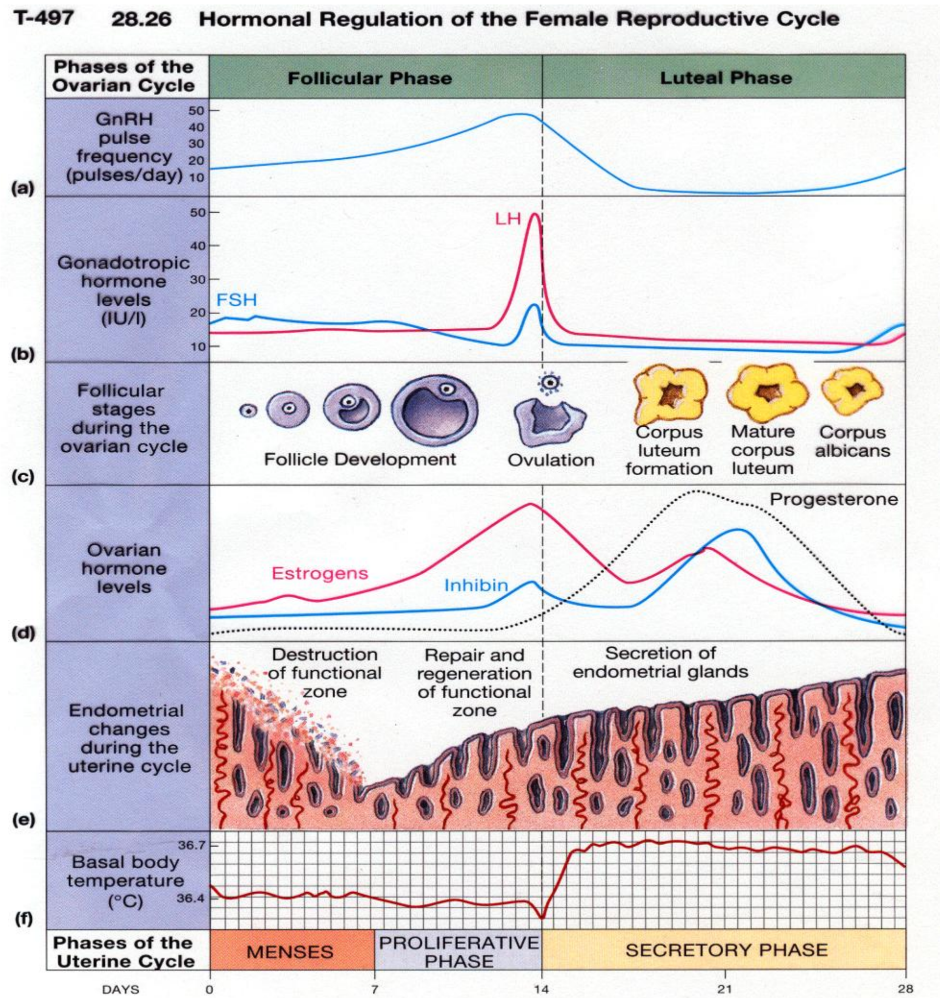
Hormones are naturally-produced chemical messengers that travel within the body to allow different body systems to communicate and maintain homeostasis, a set of optimal internal conditions. Chemically, hormones are capable of altering cellular metabolism wherever receptors for them are present; hormones bind to their specific receptor and initiate metabolic chain reactions. Hormones travel through the blood stream, usually target multiple tissues, and can have a variety of effects, depending on what cells receive the signals and which metabolic pathway is initiated. Only a small amount of a hormone is needed, and its actions can be local or wide-spread. For example, adrenaline release leads to global effects including immediate dilation of air passages, a spike in heart rate, increased blood flow to muscles, and a burst of energy and alertness associated with the "fight-or-flight" response. Gastrin, secreted by G cells in the stomach, stimulates gastric acid secretion from adjacent parietal cells. Hormones are interdependent because they moderate each others' activity through feedback mechanisms, can have amplifying or negative effects, and a few of them can even be interconverted. The importance of proper levels of hormones is evident from the considerable and wide-spread consequences that result from deficiency or excess. Hormones are secreted by the endocrine system. Although the primary endocrine structures are the pituitary and thyroid glands, pancreas, and the hypothalamus, nearly all organs and some tissues produce hormones, in locations such as particular reproductive organs, the thymus, kidney, liver, heart, and adipose tissue (Figure 1).

Figure 1. Endocrine organs (http://www2.estrellamountain.edu/faculty/farabee/biobk/biobookendocr.html)



A class of hormones known as “sex hormones” regulates reproductive development and function in both sexes. These have a variety of roles outside of the reproductive system, but in females, controlling the menstrual cycle is one of their main purposes. The average menstrual cycle lasts 28 days (but in extreme cases can range from 15 to 45) and is divided into three distinct phases: the follicular phase, during which menstruation occurs and the follicle develops, ovulation, when a non-fertilized egg is released from the ovary; and the luteal phase, when the uterine lining thickens and secretes mucus in preparation for possible implantation of an embryo and pregnancy and the corpus luteum or the shell of the ovum which remains in the ovary produces hormones to maintain the endometrium (Figure 2). The egg is only viable 24 hours after ovulation, but a zygote (fertilized egg) has two weeks to implant. If the egg is not fertilized or implantation is unsuccessful, corpus luteum degenerates and is reabsorbed from the ovary. The endometrial lining is shed, and the cycle starts over. It is widely believed that every woman's luteal phase lasts 14 days and that difference in the lengths of follicular phase accounts for the total discrepancy in the total lengths of the cycle (Bullivant). Cycle length can also vary from month to month, and variation of 8-20 days is considered moderately irregular, while variation of over 21 days is classified as "very irregular". Many studies have demonstrated how sex hormone levels fluctuate throughout the menstrual cycle. Estradiol level (main form of estrogen) rises throughout the follicular phase, while both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels peak during ovulation. Then during luteal phase estradiol rises again more steadily, and progesterone level increases also (Bancroft Mood). The anterior pituitary produces both LH and FSH. Estradiol is made mainly by the ovaries, corpus luteum, and the placenta. Cholesterol and testosterone are intermediate molecules in the biosynthesis of estradiol.

Figure 2. Human female menstrual cycle and major regulating hormones (Vander's Human Physiology – McGraw-Hill)



Although testosterone is commonly and falsely perceived to be associated with male physiology, this androgen is one of the main sex hormones produced by women. Its effects in women are completely different than in men, but the scope of its actions in both sexes and even how it affects gender interaction remain to be fully understood. Testosterone secretion is stimulated by the gonadotropin releasing hormone (GnRH), which acts on the FSH and LH, which in turn stimulate production of testosterone. Many positive effects have been historically (but not all always scientifically) attributed to testosterone in women: higher bone density, higher muscle mass percentage and lower fat percentage, higher energy levels, better mood and more positive outlook, better sexual health, and even higher red blood cell production. In a female, 50% of testosterone is made by the ovaries and adrenal glands and is released into the blood stream directly. The remaining testosterone is produced by body fat and skin (Bangcroft Mood). Some of the testosterone is converted to estrogen and some to dihydrotestosterone (DHT) in other tissues. DHT is one form of testosterone, and very little of it is present in women, because most of it is immediately converted to estradiol by enzyme aromatase. In a healthy female with a regular cycle, testosterone levels peak around ovulation and again late in luteal phase in some women (Oka). Testosterone production reaches its highest levels between the ages of 20 and 30 and then declines gradually, as does production of estrogen. When menopause occurs, typically between the ages of 45 and 55 (Bolour), testosterone concentration is about 50% of what it was at its highest, although in some women it rises after menopause, with the largest portion of the hormones originating in adipose tissue.

It has been shown that many women experience cyclical patterns of mood and behavioral changes. Studies have demonstrated that women tend to dress more impressively, feel more attractive and less lonely, and have greater interest in attending social gatherings around the time of ovulation. The mood improvement and increase in energy level around the time of ovulation may be due to improved receptivity and openness to sexual intercourse (Pawlowski). Testosterone has also been demonstrated to influence women's libido, and this was even demonstrated in other animal species. Scientists have hypothesized that the rise in testosterone which occurs around the time of ovulation is responsible for an increase in sexual desire in women as well as higher energy levels and better mood. Evolutionally-speaking, this would make sense as an adaptation: a surge in testosterone would increase the likelihood of sexual activity when the woman is most likely to conceive, which would carry out the natural goal of reproducing and passing on her genetic material to offspring. Also based on this line of reasoning, it makes sense for the body to reduce testosterone production once a woman has reached the age of menopause and lost the ability to have children. Because several other hormones levels peak around ovulation time, this correlation does not necessarily mean it is the changes in testosterone levels that are affecting sex drive. Finding one causal agent has been proven difficult, and it is more likely to be a combination of chemical messengers and other factors (Bangcroft Oral).

Because there are few studies attempting to correlate fluctuating testosterone levels with mood in women of reproductive age, I first looked at studies of menopausal and post-menopausal women. Clinical studies done to attempt to correlate low testosterone levels and diminished sex drive, unexplained fatigue and low energy, and higher body fat percentage in post-menopausal women or women who have undergone oophorectomy (ovary removal) have been inconclusive. In the majority of these women testosterone is decreased, and many of them suffer from depressive symptoms (Center). However, there are many other reasons which could explain bad emotional disposition at that stage such as poor sleep and health, hot flashes, stressful life events, employment status, aging, and others. Therapy combining testosterone and estrogen has been effective for treating diminished libido in a moderately high percentage of patients and has been commonly used for over 40 years even despite the lack of FDA-approved androgen supplement (Martin-Du, Bolour). While some research finds this treatment to be a "miraculous" cure, not everyone agrees with this solution. A literature review published in *Journal Fertility and Sterility* concluded that androgen levels are not correlated with sexual libido, while factors such as pain and stress are. Any improvements associated with testosterone therapy demonstrated by some studies could be due to a placebo effect and certainly do not outweigh the increased risk for breast cancer and negative cardiovascular side effects associated with chronic androgenic hormonal replacements (Schover).

In younger women, hormone surges are also blamed for many ailments, most commonly, premenstrual syndrome (PMS), which is infamous for causing mood swings, food cravings, bloating, aches, cramps, acne, etc. However, scientific evidence has not as yet confirmed this wide-spread belief. Experts believe the connection exists, but because every individual's chemical composition is different, it has been difficult to isolate and study the effects of different hormones. These symptoms typically appear a week before menstruation as progesterone and estrogen levels peak and testosterone levels plateau. Still, testosterone deficiency as determined through lab tests clearly reveals the importance of this hormone. Women with testosterone deficiency present with symptoms such as loss of sexual desire, unexplained fatigue, mood changes, sleep disturbances, reduced motivation, and body shape changes (Basson). For younger women, oral contraceptives are the main cause of low testosterone.

Research attempting to correlate testosterone levels with sex drive, emotionality, and energy in younger women directly has produced fairly consistent findings. One study showed the sense of general well-being to be greatest in the late follicular phase and “subjective sexuality [to be] highest in mid-follicular and late-luteal stages.” Sexual activity was maximal in mid-follicular (Bangcroft Mood). Another study found that physical discomfort and poor sleep peaked during menstruation, and attraction to the opposite sex increased around the time of ovulation (Guillermo). Another study referred to three days prior to ovulation and two days after as the “sexual” phase and concluded that sexual desire and activity were, indeed, increased then (Bullivant). In yet another study, half of the participants reported premenstrual complaints and an increase in sexual interest in premenstrual phase, while the other half reported feeling mostly energetic throughout and increased libido around ovulation (Van Goozen). However, the significant hormonal differences between the groups were in the estradiol-progesterone ratios and estradiol levels, not testosterone. A study of women diagnosed with anorexia nervosa found that “strong inverse associations were observed between both total and free testosterone and anxiety and depression severity, independent of weight” as the levels varied throughout the month (Miller). One of the possible reasons for lack of consensus among related studies is thediffering methods of approximating ovulation and of evaluating mood, libido, and energy level. While mood, amount of energy compared to normal, and sexual behavior may not be too difficult to assess, sexual desire is extremely subjective and is affected by a multitude of factors, such as partner satisfaction, stress, health, self-image, lifestyle, personal preference, and others. In addition, sexual desire will not necessarily correlate to sexual activity.

b) Why salivary tests

Testosterone is a steroid hormone, which means it is hydrophobic, and blood is approximately 83% water. In order to circulate in the blood testosterone must bind to carrier proteins, which are mainly hydrophilic with hydrophobic parts to bind testosterone. Typically, about 60% of the total hormone in the blood is inactivated and bound to sex hormone-binding globulin or SHGB (Dabbs). In this state testosterone can no longer enter body cells and trigger intracellular receptors, so it is permanently inactivated. About 38-39% is bound to protein albumin, which has low affinity for testosterone and thus releases it easily when the albumin enters target tissue. The remaining 1-2% represents the testosterone that can travel freely in the blood stream and can still be used (American). Albumin-bound testosterone and free testosterone add up to "bioavailable" testosterone, which is what needs to be measured to determine testosterone production and activity, and this can also be referred to as "active" testosterone.

Free testosterone easily diffuses into saliva from blood, but its concentration levels in saliva are very low. Testosterone bound to carrier proteins (SHGB or albumin) is not present in the saliva, but knowing that the fraction that is present in the blood is only 1-2% of the testosterone that is bioavailable helps laboratory technicians diagnose hormone deficiency or excess. Besides being more accurate, when performed properly, and assuming the assay is sensitive enough to measure low levels, saliva assay testing is inexpensive and convenient. Salivary testosterone is stable, and samples can be frozen and thawed multiple times and stored for a week at 4°C and much longer at lower temperature (Khan-Dawood). One does not have to rely on a professional to draw blood, which is very helpful especially when multiple samples are needed for increased confidence in the measurement.

Today researchers prefer a salivary test over the blood-based assay method of measuring testosterone levels in the body. When testosterone is measured in serum using radioactive or immune-based assays, the hormone that is attached to albumin and SHGB is included in the total level (Vertkin). The result of a blood test can misrepresent the actual level of bioavailable testosterone. It is biochemically possible to separate albumin-bound testosterone, SHGB-bound testosterone, and free testosterone in the collected blood samples, but it requires more laboratory work. However, researchers were able to approximate accuracy of the salivary tests using these assays. Also, multiple samples must be taken to account for the high fluctuation in the release rate of free testosterone throughout the day when performing serum or salivary analysis (Campbell).

Samples should be collected before a meal, because food (in particular, meat) can contain high amounts of testosterone. If there is any visible blood contamination, sample must be discarded, because it will provide an elevated value of testosterone. It is suggested in the instruction manual to take five samples within two hours, but the participants were instructed to take two samples during the day to account for variation in steroid secretion over several hours. Rinsing with water five minutes prior to sample collection was recommended.

METHODS

a) Materials

For the study 2 IBL International salivary 96-well ELISA assay kits (product number IB79303), pipette tips, deionized water, and disposable microtubes were needed. Some other materials were required, such as a timer, precision pipettes, test tube racks, a freezer, and a plate reader spectrophotometer.

b) Participant selection and instruction

Participants for the study were found using personal connections with the women's track and field team, MSSU Honors Program, and making announcements in my classes. Body chemistry can vary greatly from person to person, which is why it was so important to narrow down the group of study subjects as much as possible in attempt to reduce factors which may contribute to variance and make the study subjects more comparable. Many factors can influence the percentage of bioavailable testosterone, such as age, weight, stress, levels of other hormones in women throughout the menstrual cycle, diabetes, alcohol, smoking, pregnancy, reduced liver function, and high cholesterol. Factors that lower SHGB will result in more free testosterone and thus, possible masculinizing effects in women. Factors that increase SHGB (such as increased estrogen in some contraceptives) will result in more testosterone being bound to it and less being bioavailable and causing symptoms associated with testosterone deficiency. I limited the study to women of age 18-25 who have regular menstrual cycles, are not on birth control, hormone therapy, or antidepressants, and are in the normal BMI (body mass index, or height-to-weight ratio) range for their age. Oral contraceptives change levels of hormones. Because there are many different combinations of hormones given as birth control, it would be a huge undertaking to try to determine how each affects testosterone and thus my dependent factors, but there is a general tendency for them to lower testosterone. Birth control, hormone therapy, and antidepressants would artificially alter body chemistry and would, therefore, interfere with the study. The normal BMI parameter was intended to eliminate significant differences in body composition, such as extremes in muscle mass or fat percentage, and to provide a measure of good health.

As Plan B is an emergency hormonal contraceptive, it would alter a woman's levels of hormones including testosterone, and I had to inform my participants before beginning of the study that they would have to drop out if they took it at any point during the period of sample collection. I also informed them that if they were to become pregnant, they would no longer be eligible and to let me know; however, I told them they did not have to provide a reason for leaving the study.

Participants were also instructed to take mental note of their energy, mood, and sex drive on a daily basis so that they could more easily respond to surveys, as most of the questions asked how they felt "compared to normal".

A detailed confidentiality agreement was signed by study subjects to assure the participants of the privacy of their responses and laboratory results (See Appendix 1).

To avoid experimenter bias, the double-blind method was used with Dr. Roettger as the mediator. She assigned each participant a number and labeled the vials appropriately. When responding to surveys each participant identified herself with her number.

c) Surveys

Surveys used in the study are attached as Appendices 2 and 3. The purpose of the initial survey was to ensure participants fit the minimal criteria of the study and were not on medication that could affect the outcome, had regular menstrual cycles, were in the correct age group and in the normal BMI range. Also, they provided information about the typical levels of physical and sexual activity, overall attitude towards life, and whether or not they notice any regularly-occuring mood swings. The subsequent surveys were handed out to the participants on the same day as the sample tubes, and they were instructed to return them soon after their completion. These surveys were worded in the format “I feel more energetic today compared to normal” followed by the same question “compared to the last few days” to differentiate if a change may have occurred within the last few days or suddenly. Eight questions were asked designed to evaluate mood, energy, and libido, and a scale was provided ranging from 1 to 6, 1 being Completely Agree to 6 being Completely Disagree, on which they circled their responses.

d) Sample, storage, and assay procedure

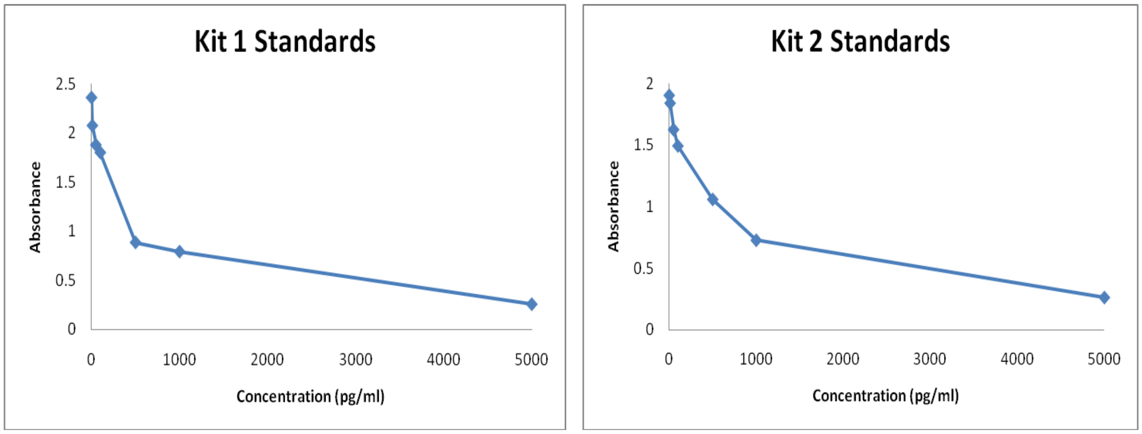
Participants were provided with six 2-ml capped microtubes for the samples. On days of collection I instructed them to obtain the samples before a meal and recommended rinsing with water about five minutes before. They were to give two samples during the day. After collection they were to store them in the freezer until they were able to return them to Dr. Roettger. If any blood was visible, they were to retake the sample and ask for a replacement tube.

Refer to the instruction manual for samples and kit storage and assay procedure protocols (Appendix 4). Samples and assay kits were kept in 2°C freezer. Wash solution was prepared immediately before running the first kit and was used four days later again for Kit 2. After dilution it was stored at room temperature for two weeks. Reagents, samples, and controls were warmed up to room temperature prior to the start of the procedure.

The assay is based on the competition principle. The wells of the microplate are coated with anti-testosterone antibodies. When samples are added, the testosterone molecules bind to them. Next I added a known amount of testosterone conjugated to horseradish peroxidase (EC). This is an enzyme that is attached to testosterone and thus competes for the binding sites on the antibodies with the testosterone from the samples. The more testosterone is present in the sample, the more of it will take up those binding sites and less EC will bind. After an incubation period the wells are rinsed, thus removing unbound testosterone and EC. Colorless substrate for the horseradish peroxidase is added and when it reacts with the enzyme, a color is produced; thus, the more enzyme is present, the more substrate is cleaved, and the greater the color intensity. Next, the absorbance of the solutions in the wells is read using a spectrophotometeric plate reader (model Tecan A-5082), an instrument that determines the transparency of a liquid. The higher the absorbance and the more intensely-colored the final solution is, the more EC, and thus, less testosterone is present in the sample. Actual concentration is calculated by comparison to known standards, whose absorbance is also read. Standards are provided in the kit, and using their absorbance values a standard curve is plotted and used to determine unknown concentrations. It is important to note that each sample and standard was done in replicate and the read values were averaged. No dilution of samples was necessary.

Because two kits were used on two different days, two standard curves had to be created (Figure 3). The kits provided typical absorbance values for the standards but advised the user to run the standards due to variation in equipment. When I compared the determined values from each kit against one another and provided absorbance values, one standard was too high in absorbance. The standards provided were 0 pg/ml, 10 pg/ml, 50 pg/ml, 100 pg/ml, 500 pg/ml, 1000 pg/ml, and 5000 pg/ml. The absorbance should have increased at approximately similar intervals in both sets, and in the case of 100 pg/ml standard from Kit 1, the absorbance was almost equal to that determined for the 50 pg/ml standard, and was thus disregarded in calculating the standard curve.

Figure 3. Standards used to determine testosterone concentration



Although plotting concentration vs. absorbance of the standards resulted in exponential regressions, calculations were done using linear regressions, because they allowed us to have a simple y=mx+b formula into which we were able to plug in absorbance values as "y". The linear regression curves were obtained by taking natural log of concentration of the standards. Calculated x-values were then converted to concentration using x=e^y formula.

e) Statistics:

ANOVA tests were done on testosterone concentration and survey responses to individual questions to see if statistically significant difference among the three test days 1, 13, and 24 existed. For the testosterone concentration, a standard parametric repeated measures ANOVA was done, with Tukey and Gaussian assumption post-tests. A repeated measures test was chosen because same subjects provided data for the different test days. The parametric option was selected because we assumed Gaussain distribution of values, which is also known as the "bell-shaped curve". Most are somewhere in the middle with some high and some low. A p-value less than 0.05 was considered significant.

Survey responses were marked on a six-point Likert scale with possible responses ranging from “Completely agree” to “Completely disagree”. This allowed statistical analyses to be performed. To analyze survey response data, I used an ordinary ANOVA test followed by the Dunn post-test, because the normality post-test failed when a parametric ANOVA was used.

Then I had to determine if there was a statistically significant correlation between testosterone concentration and responses. However, because there were two different types of data involved, one that used a rank scale and another that was continuous, in addition to the samples not being independent, much higher level statistics analysis was required which we were unable to perform. Thus, we had to observe the trends displayed by the data and estimate to conclude whether or not there appeared to be a correlation.

RESULTS

The average values for testosterone concentration were 51.12 pg/ml $\pm$ 34.1 (SD) for Day 1, 66.19 pg/ml $\pm$ 51.4 for Day 13, and 73.83 pg/ml $\pm$ 47.7 for Day 24 (Figure 4). Standard parametric repeated measures ANOVA found that there was a statistically significant difference between testosterone levels ($p=0.0417$). Tukey’s post-hoc showed the statistically significant difference to occur between Day 1 and Day 24 ($p<0.05$). The data passed the Gaussian assumption test, but the trend was not consistent for all participants (Figure 5). Testosterone concentration was the highest on Day 1 in 2 of the

15 participants, on Day 13 for 6 of the 15 participants, and on Day 24 for 7 of the 15 participants. It was lowest on Day 1 for 8 of the 15 participants, on Day 13 for 4 of the 15 participants, and Day 24 for 3 of the 15 participants. 11 out of the 15 participants had increased in testosterone concentration from Day 1 to Day 13, while 9 participants showed increased in testosterone concentration from Day 13 to Day 24.

Figure 4. Trend in testosterone concentration. Error bars represent standard deviation. The asterisk marks that Day 1 was statistically different from Day 24 (p-value<0.05).

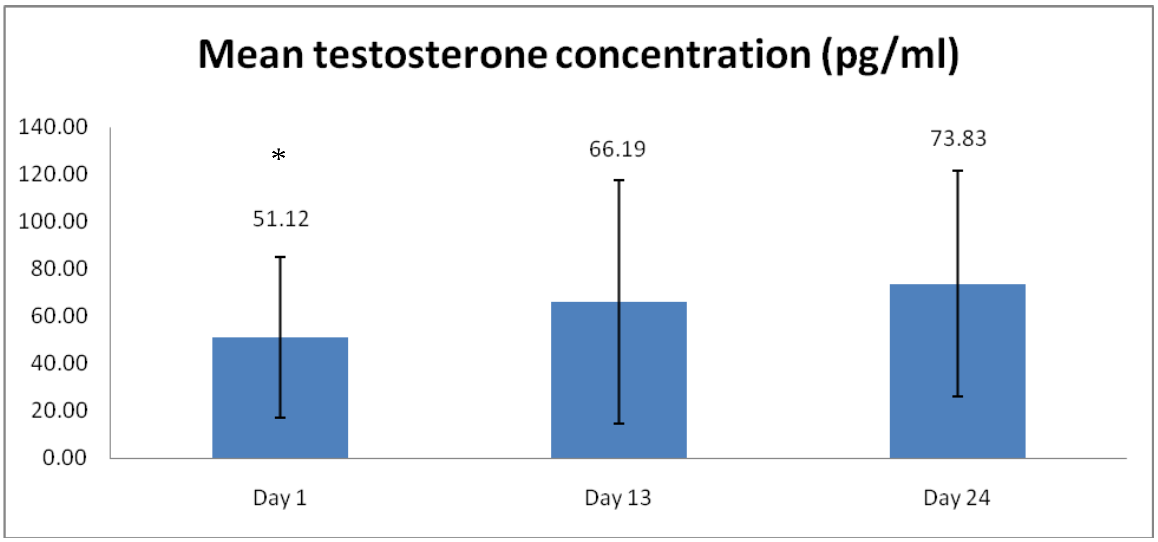
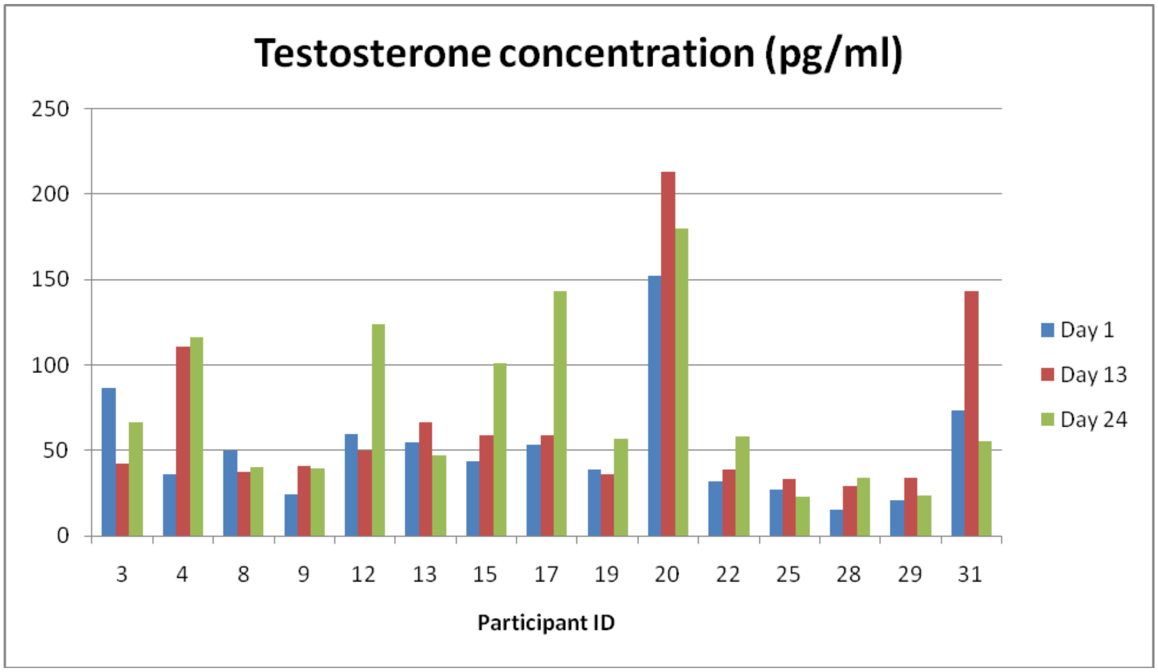
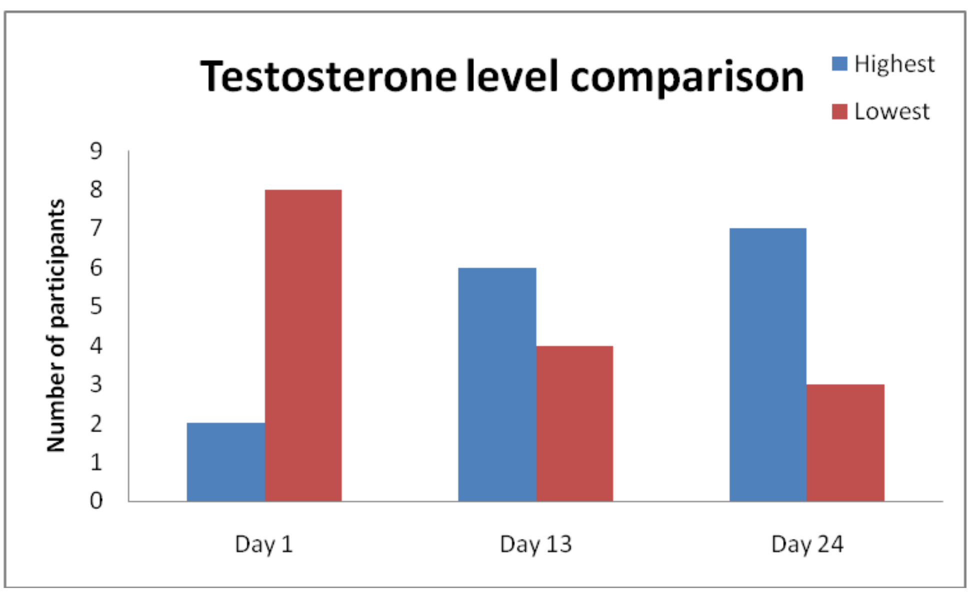


Figure 5. Variation in testosterone concentration for each individual



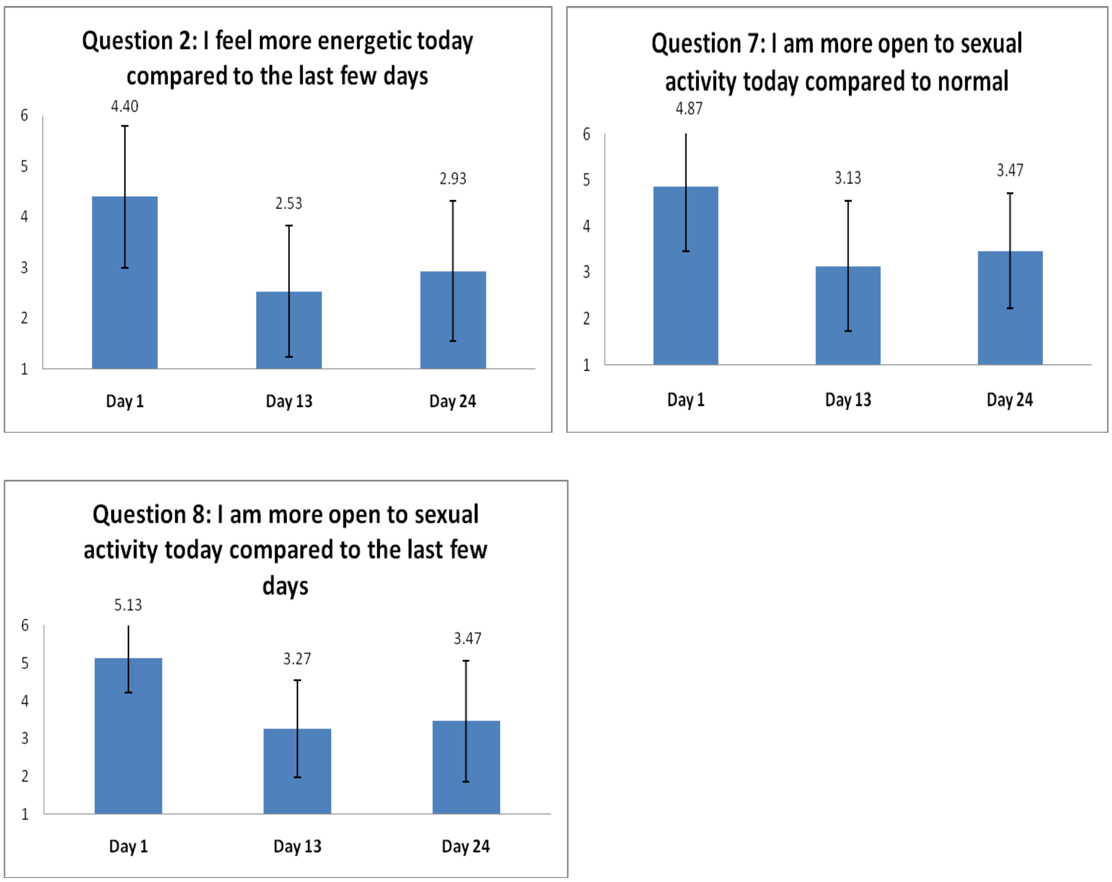
Two of the participants had their highest testosterone levels on Day 1, 6 showed the highest levels on Day 13, and 7 on Day 24 (Figure 6). Conversely, 8 had lowest testosterone concentration on Day 1, 4 on Day 13, and 3 on Day 24.

Figure 6. Variation in high and low testosterone levels throughout the month



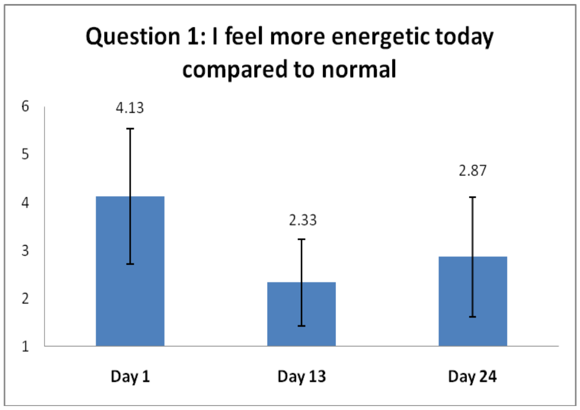
For Questions 2, 7, and 8 Day 1 was significantly different from both Day 13 and Day 24 ($p<0.05$) (Figure 7), meaning on Day 1 study subjects were most likely to disagree to being more energetic compared to the last few days or open to sexual activity compared to the previous few days or normal.

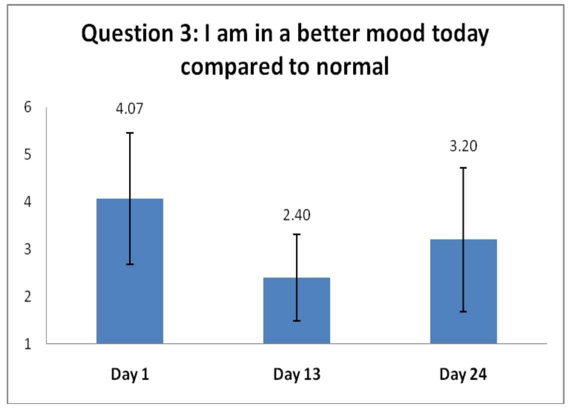
Figure 7. Responses to questions 2, 7, and 8.

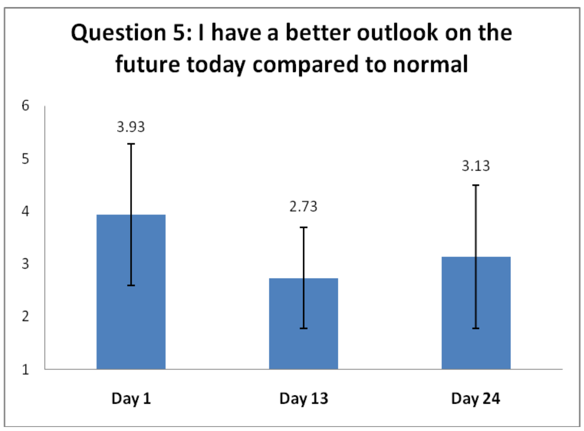


Questions 1, 3, and 5 showed statistically significant difference ($p<0.05$) between Days 1 and 24 (Figure 8), meaning on Day 1 participants were most likely to disagree to being more energetic, in a better mood, and having a better outlook on the future compared to normal.

Figure 8. Responses to Questions 1, 3, and 5.







Responses to Questions 4 and 6 did not differ statistically with p-values of 0.0906 and 0.1107, respectively (Figure 9).

Figure 9. Mean responses to Questions 4 and 6, 1 being "Completely agree" and 6 being "Completely disagree".

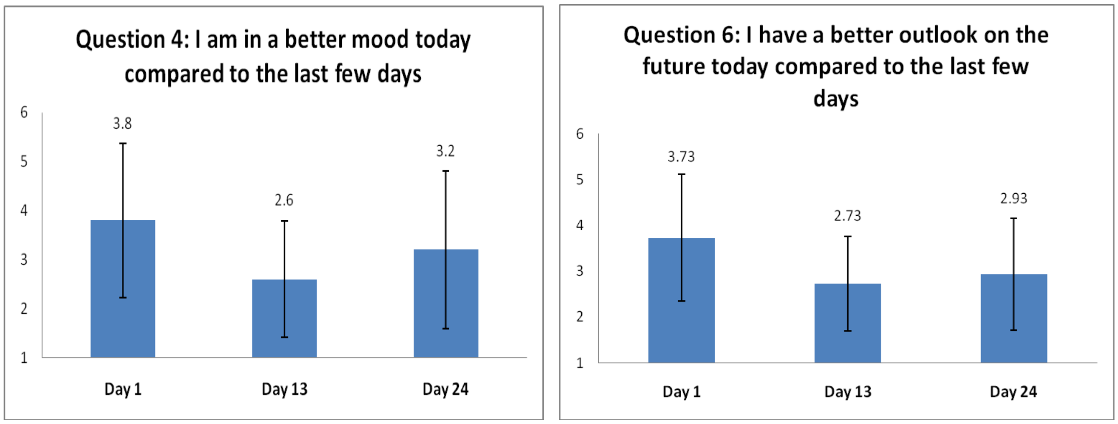
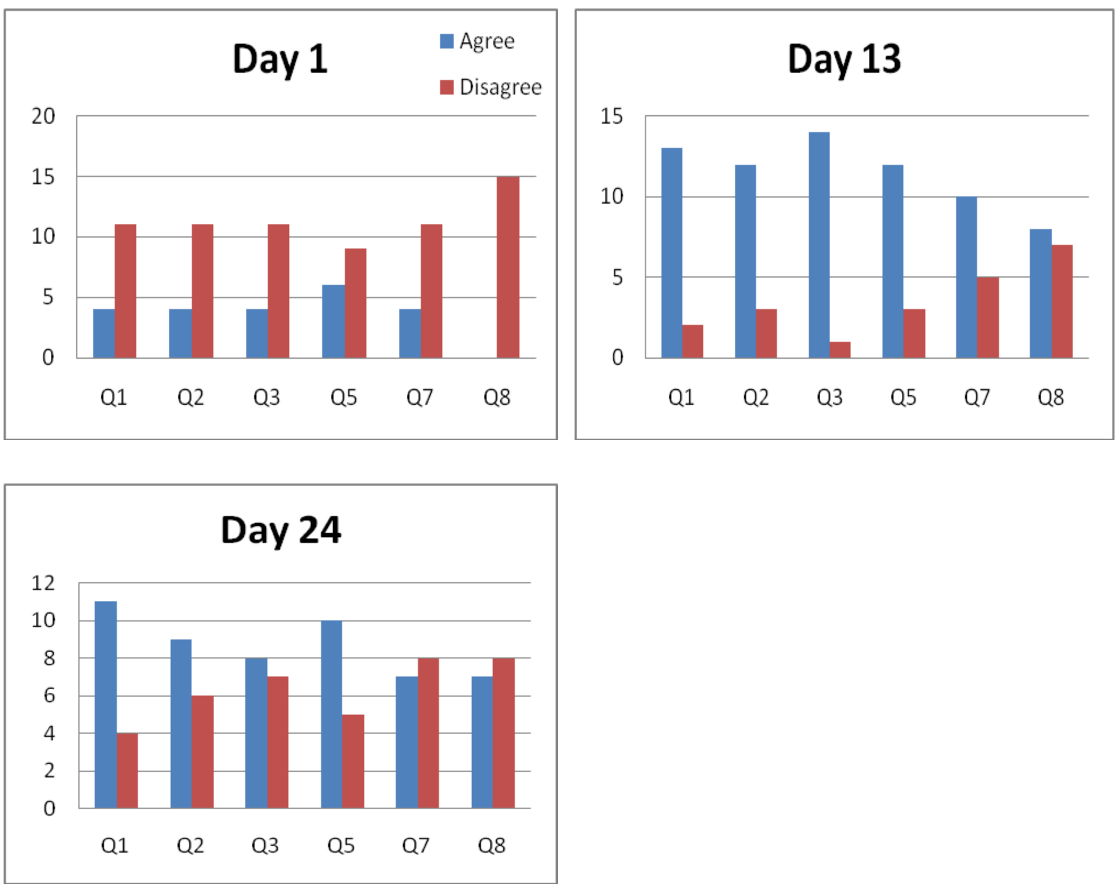


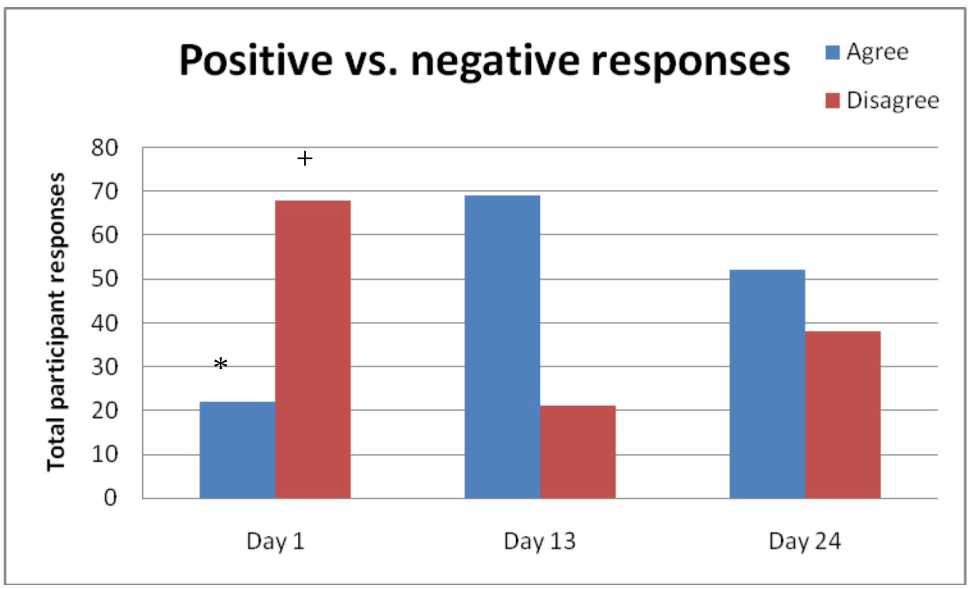
Figure 10 provides a tally of "Agree" and "Disagree" responses without considering the 1-6 scale or the extent of agreement or disagreement and disregards the statistically insignificant Questions 4 and 6.

Figure 10. Positive and negative responses to Questions 1,2,3,5,7, and 8



Noticing the tendency to respond negatively (Disagree) on Day 1 and positively (Agree) on Day 13, I found the total numbers of positive and negative responses on each day (Figure 11). Day 1 Agree was statistically different from Day 13 and Day 24 Agree columns ($p<0.001$). Day 1 Disagree was statistically different from Day 13 and Day 24 Disagree ($p<0.001$).

Figure 11. Total positive and negative responses on each day. The asterisk marks that Day 1 positive responses were significantly different from Day 13 and Day 24. The plus marks that Day 1 negative responses were significantly different from Day 13 and Day 24.



DISCUSSION

a) Results

I rejected my hypothesis that highest testosterone concentration would be found around the time of ovulation. Testosterone level appeared to increase from the beginning of menses through the menstrual cycle, but this was not a statistically significant trend, as it mostly varied from person to person. This variation around the mean was due to the low sample number and definitely caused the standard deviation to be high, thus affecting the statistics. I am not sure why my results were not consistent with previous research and testosterone did not peak at ovulation and then decrease. The only conclusion I can make with statistical certainty is that testosterone concentration was definitely lowest on Day 1 compared with the other two test days.

Day 1 generated negative responses across the board: participants tended to report low energy, bad mood, and low sexual desire. Because we did not find significant difference between Days 13 and 24, we were unable to accept the hypothesis that the best mood, highest libido, and highest energy occur around ovulation. The data, however, did show this trend. The general trend seemed to suggest that responses were lower on the scale on Day 13 for all questions. I was able to say with statistical certainty that mood, libido, and energy were lowest on Day 1, or the onset of menses.

I also could not accept my hypothesis that there would be a direct relationship between testosterone concentration and my variables: mood, energy, and libido. I was unable to perform statistical analysis and had to resort to observing patterns in responses and comparing them to patterns in testosterone levels. Testosterone concentration was lowest on Day 1, and most negative responses also tended to be generated on that day, including worst mood, outlook, lowest energy reported, and least openness to sexual activity. However, because we did not find significant difference between Days 13 and 24 for testosterone concentration or the surveyed variables, we certainly cannot conclude even based on trends that any relationship exists. Looking at the background information, however, and the trend in estrogen secretion, it appears to be consistently in an inverse relationship with our survey data. Estrogen is lowest during menses, spikes at ovulation, and decreases slightly right after, only to have a second, much flatter peak during the luteal stage. It is possible that estrogen has a more obvious, if not causative, relationship with mood, energy, and libido.

b) Limitations and critique

Energy and mood can be affected by the amount of sleep one is getting, diet, amount of exercise, illness, and other events, not all of which were documented by the surveys resulting in significant limitations. Something like a death in the family would have been an out of the ordinary occurrence and would have affected mood, energy, and sex drive. Daily journals of sexual activity and exercise as well as moods and possibly significant events (stressful and happy) would have been a more effective method to keep track of the women's desires and energy expenditures, but also more time-consuming and possibly harder to objectify. It is likely that outside events may have affected responses. For example, if one of the study subjects competed in a sporting event the day before sample collection or has been sick, that would have been a likely cause for fatigue. To question "I would describe myself as an energetic person" participants responded 2.35 on average on a scale from 1 being Completely Agree to 6 being Completely Disagree and 11 placed themselves in the "I get 5 or more hours of physical activity each week" category, although the effects of exercise on energy are debatable. Approximately half of my participants are collegiate athletes and participate in high-intensity training and competition, and obviously that might induce fatigue. Most agreed to "I am content with my life" at 1.94. Also, wording of the questions in the subsequent surveys was somewhat problematic. When a subject disagrees to feeling better compared to normal, does that mean she feels worse or about the same as normal? It makes it hard to interpret the responses.

Many factors affect libido for women: self-image and confidence, stability of the relationship with sexual partner, happiness, amount of energy, moral views, and even positive or negative life outlook. It has also been shown that women tend to initiate sex rarely, but get aroused during foreplay, so they are not necessarily opposed to sex, but do not get excited about it when initially considering, which would affect the responses. There have been studies showing that female-initiated sexual activity is more correlated to the menstrual cycle than male-initiated sexual activity. This would also imply that perhaps men are not able "sense" when a woman is most fertile as proposed by some scientists. I would have liked to only have sexually-active (perhaps married) women to avoid any preconceived notions about sexual activity due to moral values and background. This even complicated finding appropriate wording for the survey questions. For example, it may be difficult for a woman to agree with the statement "I am more open to sexual activity today compared to normal" if she has never had any sexual experiences or shown any interest. The initial survey showed that on a scale of 1 to 6, 1 being "Completely Agree" and 6 being "Completely Disagree", average response to “I regularly in sexual activity” was 4.53. A tactful question about previous experience with sex could also have been helpful in the initial survey. In fact, whether or not a participant enjoys sex is important, because while it may be viewed as a positive aspect of someone’s life, for a rape victim it could be quite traumatic and her responses would be much affected by that experience. Partnership status and proximity to partner should also have been surveyed, although studies of partnered and unpartnered women showed increases in sexual activity and desire in both groups in sexual phase (Bullivant). Journals also would have been helpful to allow us to differentiate between when sexual activity initiated by the woman herself as opposed to her partner; however, previous studies attempting to find patterns by looking at incidence of sexual activity have failed. There are simply too many circumstantial factors involved.

Using Days 1, 13, and 24 was our best attempt at finding the times at which the fluctuations in testosterone levels should be most evident. However, due to individual differences estimating onset of ovulation presented great room for error, because each woman’s cycle length varies. We assumed ovulation would occur around 14 days after onset of menses, but length of follicular phase can vary from 3 to 19 days and total length of cycle can range from 24 to 35 days. This is probably the biggest reason there was not a consistent trend among the women. Other methods of approximating ovulation that were used in the studies I reviewed are detecting a surge in luteinizing hormone in the blood, an increase in basal body temperature, or a change in the composition of cervical mucus, which would have been unrealistic to do at this level of research (Bullivant). In addition, it would have been interesting to see if the average testosterone concentration was, indeed, higher in women who are collegiate athletes vs. the non-athletes.

Testosterone secretion varies throughout the day with the time period between 4 a.m. and 12 p.m. being the highest and can even be affected by changes in season (Vierhapper). While all the samples were taken in the fall, I had no control over what time of the day samples were collected, as the participants were simply told to take them at their convenience on the respective days. Collecting more samples in one menstrual cycle or over the course of a year would have provided us more data and likely more accurate results. Monitoring cycle length would also have been helpful prior to sample collection.

Participant compliance played a major role in the study. Although subjects were expected to follow instructions closely, human error and inconsistency are likely to have occurred. With more resources, I would have been able to provide compensation to the research subjects to give them incentive to follow directions precisely and turn in samples in a timely manner. I was approached by several of the participants as late as six weeks after the informational meeting to ask how many times saliva has to be collected or whether they have time to start over because they forgot to take a sample. Several had to opt out of the study, and thus I ended up with fewer reliable samples than I hoped for (15, as opposed to 30 sets of tubes handed out). Unexpected circumstances also arose, for example, one participant informed me that she got her period two weeks after beginning of sample collection and should be removed from the study.

This project was a big and slightly too ambitious undertaking for undergraduate research. In retrospect, I should have selected one of the factors that may be affected by testosterone and devoted more time to it, looking at its different aspects and considering all methods of evaluation before selecting one or more. I also should have been more realistic and done a study that did not involve human participation, at least to the extent that mine did, not to mention it was hard to find participants that fit all my criteria. Increasing sample number would account for many factors that vary among the individuals that could affect responses, and would provide more accurate and statistically significant results.

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Appendix 1. Confidentiality agreement

Privacy Statement

The data and survey responses will be kept strictly confidential. If you are interested, you can receive them after the conclusion of the study.

You will be asked about the level of your sexual interest on the surveys. If you do not feel comfortable with this, you may leave.

Consent

I am not on birth control or anti-depressants, pregnant, or planning to become pregnant during the duration of the study.

I understand that I will be asked to provide multiple saliva samples which I will collect on my own throughout the study and fill out the surveys on corresponding days. I will follow the instructions to the best of my ability and respond to the surveys with honesty.

Date

____

Printed name

____

Signature

____

Appendix 2. Initial survey

INITIAL SURVEY

Participant ID number:

Date of birth:

Height:

Weight:

BMI:

What medications, vitamins, and supplements do you take?

Age at first menstruation:

Do you experience mood changes that seem to be cyclical (monthly)?

Date of first day of your last menstruation:

Approximately, how long does your period last and how long between each?

Please circle your response:

I regularly engage in sexual activity or masturbate.

Completely agree 1 2 3 4 5 6 Completely disagree

I would like to have sex more often.

Completely agree 1 2 3 4 5 6 Completely disagree

I would describe myself as an energetic person.

Completely agree 1 2 3 4 5 6 Completely disagree

I am content with my life.

Completely agree 1 2 3 4 5 6 Completely disagree

On average, how much physical activity do you get during the week?

0-2 hours 3-4 hours 5-8 hours More than 9 hours

Appendix 3. Sample day survey

Participant ID number:

Day of menstrual cycle:

I feel more energetic today compared to normal.

Completely agree 1 2 3 4 5 6 Completely disagree

I feel more energetic today compared to the last few days.

Completely agree 1 2 3 4 5 6 Completely disagree

I am in a better mood today compared to normal.

Completely agree 1 2 3 4 5 6 Completely disagree

I am in a better mood today compared to the last few days.

Completely agree 1 2 3 4 5 6 Completely disagree

I have a better outlook on the future today compared to normal.

Completely agree 1 2 3 4 5 6 Completely disagree

I have a better outlook on the future today compared to the last few days.

Completely agree 1 2 3 4 5 6 Completely disagree

I am more open to sexual activity today compared to normal.

Completely agree 1 2 3 4 5 6 Completely disagree

I am more open to sexual activity today compared to the last few days.

Completely agree 1 2 3 4 5 6 Completely disagree