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HL Bio P4
Ms. Soule

**IRP: The Effect of Chlorinated Water on Hair Strength**

## EXPLORATION and PERSONAL ENGAGEMENT

### Introduction:

Personal engagement:

Swimmers may experience dry and brittle hair as a result of swimming in chlorinated pools. Even with the use of conditioners and other hair care products there may be obvious damage to hair. As a member of the swim team, I am personally interested in the nature and extent of the damage that chlorine causes to hair.

This led to questions about the effect of exposure to chlorine over time for swimmers and non-swimmers, as well as the rate of damage.

Research question:

*What is the relationship between the amount of time in which hair from a swimmer or non-swimmer is soaked in chlorinated water and the strength of hair as measured by the amount of force that can be applied before the hair breaks?*

Background Theory:

Chlorine is a popular chemical to add to pool water, as it works as a disinfectant. It has three main purposes: it sanitizes the water by killing bacteria and inactivating viruses, it oxidizes the water as it controls the organic debris from bodily perspiration and oils, and chlorine deters algae (1). Unfortunately, the over-exposure to chlorine has negative side effects as well, as chlorine causes dryness in the skin and the hair.

When chlorine is added to water, hypochlorous acid and hydrochloric acids form:

$$Cl_2 + H_2O \rightarrow HOCl + HCl$$

Hydrochloric acid is a strong acid and dissociates completely. Hypochlorous acid is non-polar so it can enter cells. It is a weak acid, and, depending on pH, it partially dissociates to hypochlorite ions:

$$HCl \rightarrow H^+ + Cl^- (aq)$$
$$HOCl + H_2O \leftrightarrow H_3O^+ + OCl^- (aq)$$

The result of these chemical reactions is to attack the chemical bonds in microorganisms such as bacteria, making pools less likely to cause infection in humans (3). As a side effect, exposure to chlorine affects both the outer lipid layer of hair and also the core protein structure. The lipid layer is removed by interaction with chlorine causing a dull appearance and allowing greater interaction between chlorine and protein (4). Research done on the keratin fibers in wool suggests that chlorine can then break disulfide bridges found in keratin and also break peptide linkages, meaning that less force is necessary to stretch hair and that the hair has less tensile strength (5).

It was hypothesized that the longer the hair was soaked in the chlorinated water, the weaker the hair would become, as the chlorine and other chemicals would penetrate the lipid layer and have greater time and exposure to disrupt the protein structure of the human hairs. It was also hypothesized that the hair of a non-swimmer would be stronger but also have greater initial damage from exposure to chlorinated water. This is because the swimmer's hair would already have long-term damage but be less likely to have a dramatic change when exposed to the familiar situation of chlorinated water.

**Method:**

Variables:

The *independent variables* in this experiment were

- the amount of time the hair was exposed to the chlorine
  - The time on classroom clock was noted when the hair samples entered the water and then again when the hair was removed.
- the source of the hair (swimmer and non-swimmer) and
- whether the water was chlorinated (experimental group) or not (control group)
  - Water was taken from the school pool and from the tap

The *dependent variable* in this experiment was

- the maximum force (in Newtons) that the hair strand could withhold before breaking
  - An electronic spring scale force meter was used and the data collected by Logger Pro

Theoretically, the strength of the hair is affected by age of the person, the outside temperature which affects the strength of the bonds, area of the hair on the scalp, and length of the hair strand tested. (3)

Some *controlled variables* in this experiment were

- Genetic factors:
  - The volunteers were teenaged sisters who had similar color hair. As siblings, they share many alleles. Similar hair color suggests alleles relevant to hair may also be shared.
- Environmental factors:
  - The hair was not dyed or permed and used the same hair products.
  - temperature of the room and the water temperature of the experiment were controlled by keeping all samples in the same location
  - the hair was taken from the same place on the scalp, behind the left ear, as hair at the base of the scalp is often weaker than hair on the top.
  - The length and age of the hair sample measured was kept a constant, by cutting from 1 cm from the scalp and trimming to a length of 10 cm thus creating an equal length of hair for each trial.
  - The same sample of pool water with a chlorine level of 2.05 ppm was used in all the trials to minimize the differences in chlorine levels
  - The pool water was filled to the brim and sealed in Tupperware to prevent the evaporation of water or chlorine which could change the concentration over time
  - The same force meter was used to avoid problems with calibration.
  - Dampness of hair, which can affect hair strength, was controlled by removing hairs one at a time for each trial
  - Angle of pull on the hair was controlled by taping the ends of each hair together, creating a loop and pulling directly down

Procedure:

The experiment was explained to the subject and the parents and **approval** was received. Sixty strands of **hair** were cut using **scissors** from the two subjects (one swimmer and one non-swimmer) at 1.0 cm from the scalp on the left side behind the ear. Scissors were used to trim the hair to a length of 10.0 cm.

About 800 mL of 2.05 ppm **chlorinated pool water** was collected from the school pool in a **1 L beaker**.

**Four small Tupperware** containers were labeled: swimmer (chlorine), swimmer (tap), non-swimmer (chlorine), and non-swimmer (tap). 30 hairs from the swimmer were placed in each swimmer container and 10 hairs from the non-swimmer were placed in each non-swimmer contained. All Tupperwares were then filled to the brim with about 115 mL pool water and sealed.

After two days, all the containers were opened and a **tweezers** was used to remove one hair at a time, for a total of 10 hairs from each volunteer. The hair was placed on a **paper towel** to absorb any extra water and the ends were **taped** together to create a loop. The loop of hair was placed over the hook of the **electronic spring scale force meter** and "collect data" was selected. The taped end of the hair loop was pulled directly downward slowly and steadily until the hair broke. The highest recorded value of force was then recorded.

After the data were collected, the containers were resealed. After four and six days the process was repeated.

Data were analyzed in terms of means and standard deviations. Normality tests and t-tests were used to check for significant differences between samples. Once it was shown that control trials did not change in strength during soaking, percent change in strength of hair was calculated using the mean of all control trials for that volunteer.

Safety and Ethical Concerns:

- Informed consent of volunteers and parents was received
- Scissors were used with care with the points of blades turned away from the scalp
- The glass beaker was handled with care to prevent breaking.
- Chlorine can be a dangerous chemical but at the low concentrations of pool water no special precautions or disposal techniques were required. Hands were washed after working with chlorinated water.
- The force meter was firmly affixed to a ring stand which was clamped to the edge of the table to prevent it from moving or toppling over
- All extra materials were kept out of the way to prevent accidents.
- Used hair, water, and tape were non-hazardous and were disposed of properly with solids in the trash cans and liquids down the sink.

**ANALYSIS:**

Data Collection:

Quantitative Data:

Table 1 (Controlled Group): the maximum amount of force (in Newtons) that a strand of hair can withstand after being soaked in tap water for 2, 4, and 6 days

| | Swimmer Hair | | | Non-Swimmer Hair | | |
|---|---|---|---|---|---|---|
| | **2 Days** (±0.001 N) | **4 Days** (±0.001 N) | **6 Days** (±0.001 N) | **2 Days** (±0.001 N) | **4 Days** (±0.001 N) | **6 Days** (±0.001 N) |
| **Trial 1** | 0.432 | 0.427 | 0.437 | 0.632 | 0.697 | 0.649 |
| **Trial 2** | 0.478 | 0.431 | 0.442 | 0.533 | 0.787 | 0.651 |
| **Trial 3** | 0.456 | 0.484 | 0.476 | 0.736 | 0.732 | 0.687 |
| **Trial 4** | 0.436 | 0.502 | 0.493 | 0.694 | 0.745 | 0.670 |
| **Trial 5** | 0.397 | 0.447 | 0.503 | 0.638 | 0.683 | 0.668 |
| **Trial 6** | 0.501 | 0.483 | 0.480 | 0.701 | 0.694 | 0.664 |
| **Trial 7** | 0.476 | 0.437 | 0.457 | 0.654 | 0.682 | 0.671 |
| **Trial 8** | 0.432 | 0.449 | 0.431 | 0.713 | 0.714 | 0.674 |
| **Trial 9** | 0.472 | 0.475 | 0.465 | 0.669 | 0.709 | 0.659 |
| **Trial 10** | 0.387 | 0.456 | 0.478 | 0.806 | 0.688 | 0.672 |
| **Mean** | **0.447** | **0.459** | **0.466** | **0.678** | **0.713** | **0.667** |
| **Mean (all)** | **0.457** | | | **0.686** | | |

*This table shows the results of the force that individual 10cm strands of hair can withstand. The conditions in this table are swimmer hair and non-swimmer hair being soaked in tap water. The results show that the non-swimmer hair is much stronger than the swimmer hair. The results also show that there little effect on hair strength from soaking in tap water for 2, 4, and 6 days. The average of all tap water trials was used to determine percent change in hair strength.*

Table 2 (Manipulated Group): the maximum amount of force (in Newtons) that strand of hair can withstand after being soaked in pool water for 2, 4, and 6 Days

| | Swimmer Hair | | | Non-Swimmer Hair | | |
|---|---|---|---|---|---|---|
| | **2 Days** (±0.001 N) | **4 Days** (±0.001 N) | **6 Days** (±0.001 N) | **2 Days** (±0.001 N) | **4 Days** (±0.001 N) | **6 Days** (±0.001 N) |
| **Trial 1** | 0.337 | 0.319 | 0.298 | 0.531 | 0.413 | 0.259 |
| **Trial 2** | 0.345 | 0.324 | 0.347 | 0.572 | 0.397 | 0.267 |
| **Trial 3** | 0.356 | 0.342 | 0.313 | 0.546 | 0.395 | 0.258 |
| **Trial 4** | 0.331 | 0.307 | 0.290 | 0.551 | 0.427 | 0.280 |
| **Trial 5** | 0.381 | 0.319 | 0.296 | 0.593 | 0.391 | 0.237 |
| **Trial 6** | 0.365 | 0.316 | 0.307 | 0.496 | 0.404 | 0.249 |
| **Trial 7** | 0.364 | 0.305 | 0.304 | 0.485 | 0.402 | 0.233 |
| **Trial 8** | 0.342 | 0.310 | 0.299 | 0.557 | 0.389 | 0.241 |
| **Trial 9** | 0.355 | 0.318 | 0.311 | 0.548 | 0.376 | 0.252 |
| **Trial 10** | 0.359 | 0.300 | 0.309 | 0.596 | 0.406 | 0.263 |
| **Mean** | **0.353** | **0.316** | **0.307** | **0.548** | **0.400** | **0.254** |

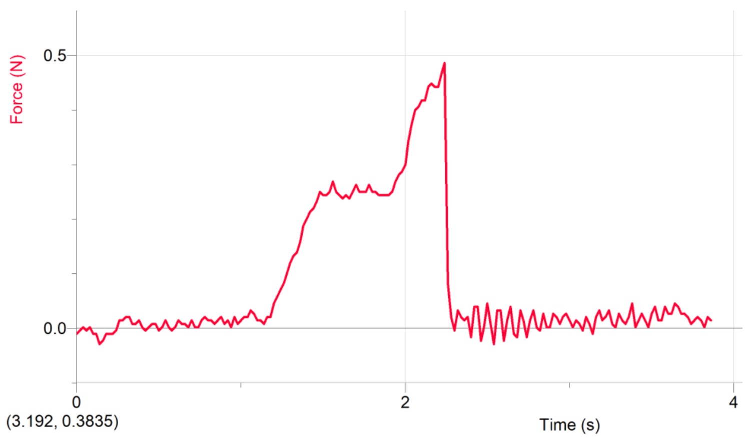
*This table shows the results of the force that individual 10cm strands of hair can withhold. The conditions in this table are swimmer hair and non-swimmer hair being soaked in chlorinated pool water with a chlorine concentration of 2.05ppm The results show that the non-swimmer loses strength faster than swimmer hair. Swimmer hair is weaker than non-swimmer hair after 2 and 4 days of soaking in chlorinated water but non-swimmer hair is weaker after 6 days of soaking.*

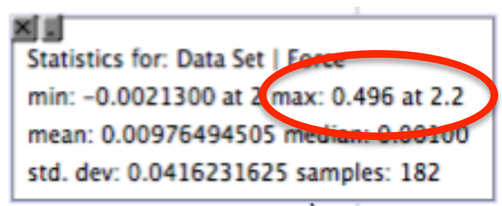
Qualitative Data:

- When placed in the water, the hair strands were straight and long. However, when the hair strands were removed from the chlorinated water after being soaked for a few days, the hair had become more brittle, and was slightly curled.
- After being soaked in the pool water, the hair strands had lightened significantly, turning from a brown to a light brown or dark blonde in color.
- The hair soaked in water changed minimally – there was little to no difference in color or shape.
- When the hair strands were pulled on the electronic spring scale, the hair seemed slightly more "elastic", stretching to become longer before breaking.
- Upon being removed from the water with tweezers, the hair strands stuck to each other, making it difficult to separate them.

<u>Sample Calculations:</u>

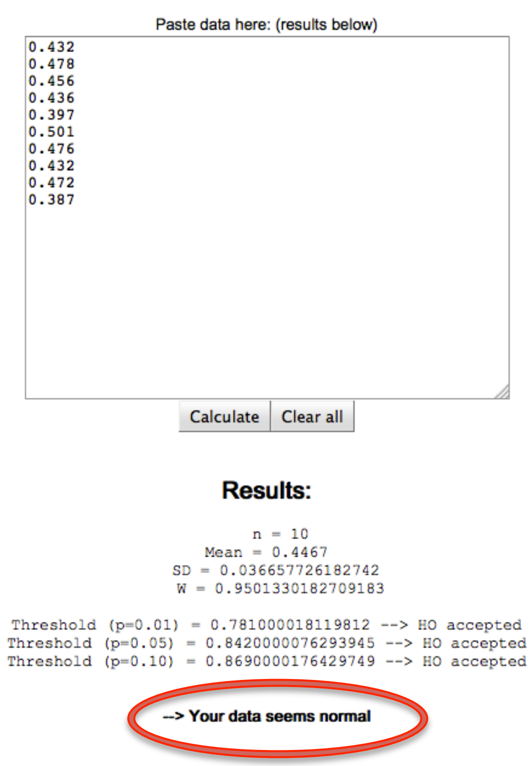
*Figure 1: Example Graph of Logger Pro to find the Trial Value and Statistics Box (Non-Swimmer, 2 Days, Trial 6)*



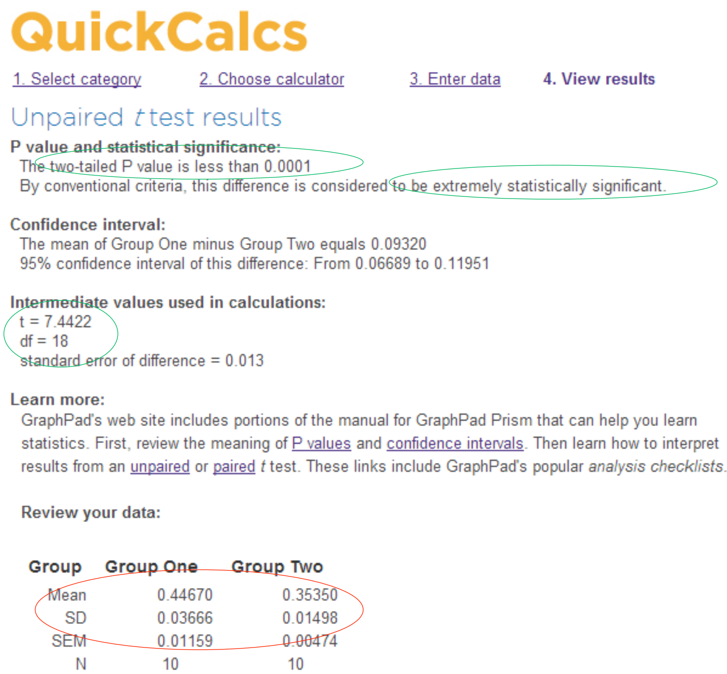

*Select the graph select "Statistics". Read the maximum value from "max".*

*Figure 2: Test for normal distribution of data.*




*To show that a t-test can be used, the data must show normal distribution. The Shapiro test for normality provided at [http://sdittami.altervista.org/shapirotest/ShapiroTest.html](http://sdittami.altervista.org/shapirotest/ShapiroTest.html) was applied to each set of 10 values. They all showed normal distribution.*

*Figure 3: Standard Deviation Calculation and T-Test Calculation:*



QuickCalcs

1. Select category 2. Choose calculator 3. Enter data **4. View results**

Unpaired *t* test results

**P value and statistical significance:**
The two-tailed P value is less than 0.0001
By conventional criteria, this difference is considered to be extremely statistically significant.

**Confidence interval:**
The mean of Group One minus Group Two equals 0.09320
95% confidence interval of this difference: From 0.06689 to 0.11951

**Intermediate values used in calculations:**
t = 7.4422
df = 18
standard error of difference = 0.013

**Learn more:**
GraphPad's web site includes portions of the manual for GraphPad Prism that can help you learn statistics. First, review the meaning of P values and confidence intervals. Then learn how to interpret results from an unpaired or paired *t* test. These links include GraphPad's popular *analysis checklists*.

**Review your data:**

| Group | Group One | Group Two |
|---|---|---|
| Mean | 0.44670 | 0.35350 |
| SD | 0.03666 | 0.01498 |
| SEM | 0.01159 | 0.00474 |
| N | 10 | 10 |

*When finding the t-value, the results of the QuickCalcs also calculate the means and standard deviations of the two sets of inputted data, as shown to the left circled in red.*

*The results of the t-test (the t-value, degrees of freedom, and the two-tailed p-value, as well as the degree of significance) is shown in the results, which are circled in green.*

Percent change in strength of hair:

((Mean of manipulated sample – mean of control sample) / mean of control sample) x 100

Swimmer Pool 2 Days v. **All** Swimmer Tap Water

((0.353 – 0.457) / 0.457) x 100 = -22.8%

Results:

Table 3: The Mean Values and the Standard Deviation for the trials of Swimmer and Non-Swimmer Hair found in Table 1 and Table 2.

| | **Swimmer** | | | | | |
|---|---|---|---|---|---|---|
| | **2 Days** | | **4 Days** | | **6 Days** | |
| | **Tap** | **Pool** | **Tap** | **Pool** | **Tap** | **Pool** |
| **Mean Value** | 0.447 | 0.353 | 0.459 | 0.316 | 0.466 | 0.307 |
| **Standard Deviation** | 0.037 | 0.015 | 0.026 | 0.012 | 0.024 | 0.016 |
| | **Non-Swimmer** | | | | | |
| | **2 Days** | | **4 Days** | | **6 Days** | |
| | **Tap** | **Pool** | **Tap** | **Pool** | **Tap** | **Pool** |
| **Mean Value** | 0.678 | 0.548 | 0.713 | 0.400 | 0.667 | 0.254 |
| **Standard Deviation** | 0.072 | 0.036 | 0.033 | 0.014 | 0.011 | 0.015 |

*The results above compare the mean value of each trial set to the standard deviation. These values are used in the graph below – the mean value is the height of each bar, while the standard deviation constitutes as the error bars.*

Table 4: The Percent Change in Strength of Hair By Length of Time Soaked in Pool Water as Compared to Average of All Control Trials.

| | **Swimmer Hair** | | | **Non-Swimmer Hair** | | |
|---|---|---|---|---|---|---|
| | Day 2 | Day 4 | Day 6 | Day 2 | Day 4 | Day 6 |
| **Change in Hair Strength (%)** | **-22.8** | **-30.9** | **-32.8** | **-20.1** | **-41.7** | **-63.0** |

*The loss of strength of the swimmer's hair is about the same after two days of soaking but thereafter the non-swimmer hair loses strength in a greater degree. There is little difference between day 4 and 6 for the swimmer unlike the non-swimmer.*

Table 5: Below are the t-values, p-values, and degrees of significance to compare the effects of length of chlorination for swimmers versus non-swimmers, effects of tap water and chlorinated pool water.

| | **Swimmer Hair in Tap Water** | | | **Non-Swimmer Hair in Tap Water** | | |
|---|---|---|---|---|---|---|
| | Day 2 to Day 4 | Day 4 to Day 6 | Day 2 to Day 6 | Day 2 to Day 4 | Day 4 to Day 6 | Day 2 to Day 6 |
| **T- Value** | 0.8782 | 0.6390 | 1.4042 | 1.4090 | 4.1902 | 0.4791 |
| **P-Value** | 0.3914 | 0.5309 | 0.1773 | 0.1759 | 0.0006 | 0.6376 |
| **Degree of Significance** | Not significant | Not Significant | Not Significant | Not Significant | Extremely Significant | Not Significant |

*The t-test above calculated differences based on length of time submerged in tap water. All the results showed that the tap water did not make a significant difference to the strength of the hair, with the exception of the anomalous result between Day 4 and Day 6 for the non-swimmer.*

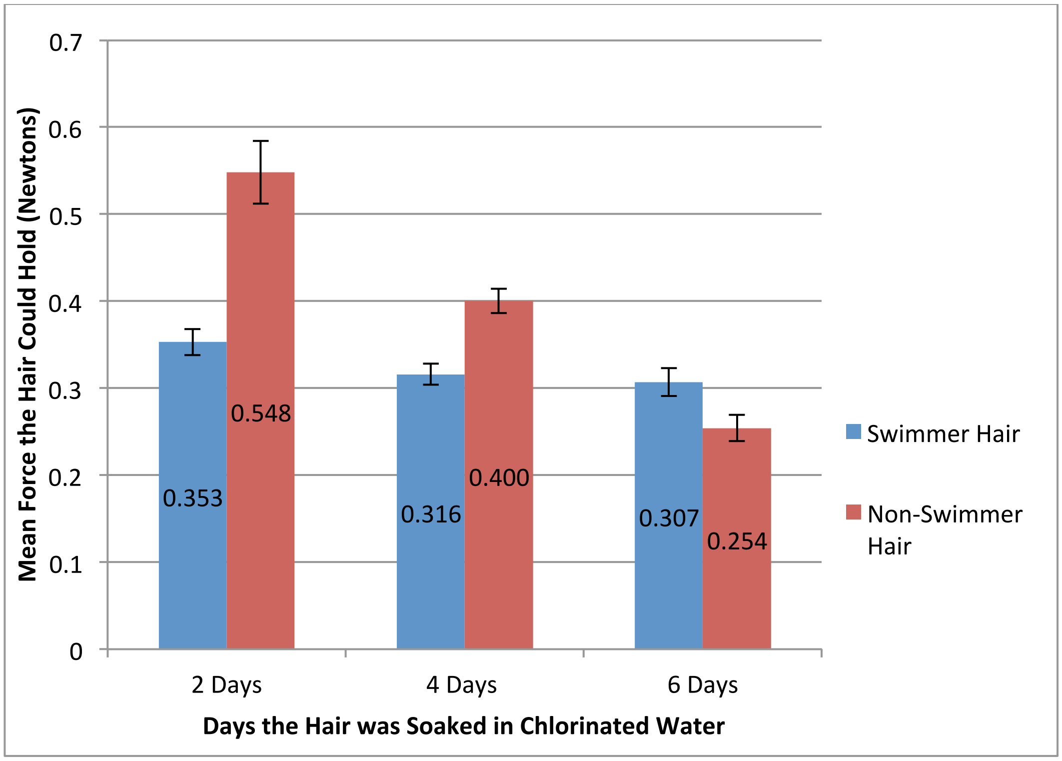
| | **Swimmer Hair in Pool Water** | | | **Non-Swimmer Hair in Pool Water** | | |
|---|---|---|---|---|---|---|
| | Day 2 to Day 4 | Day 4 to Day 6 | Day 2 to Day 6 | Day 2 to Day 4 | Day 4 to Day 6 | Day 2 to Day 6 |
| **T- Value** | 6.2521 | 1.3841 | 6.7137 | 11.9972 | 22.9075 | 23.7604 |
| **P-Value** | <0.0001 | 0.1832 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Degree of Significance** | Extremely significant | Not Significant | Extremely Significant | Extremely Significant | Extremely Significant | Extremely Significant |

*The t-test above calculated differences based on length of time submerged in tap water. All the results showed that length of time submerged in pool water caused a significant difference in the strength of the hair, with the exception of the anomalous results of Day 4 and Day 6 for the non-swimmer.*

| | **2 Days** | | **4 Days** | | **6 Days** | |
|---|---|---|---|---|---|---|
| | Swimmer: Tap Water and Pool Water | Non-Swimmer: Tap Water and Pool Water | Swimmer: Tap Water and Pool Water | Non-Swimmer: Tap Water and Pool Water | Swimmer: Tap Water and Pool Water | Non-Swimmer: Tap Water and Pool Water |
| **T- Value** | 7.4422 | 5.0769 | 16.1035 | 27.4054 | 17.4189 | 70.9297 |
| **P-Value** | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Degree of Significance** | Extremely significant | Extremely Significant | Extremely Significant | Extremely Significant | Extremely Significant | Extremely Significant |

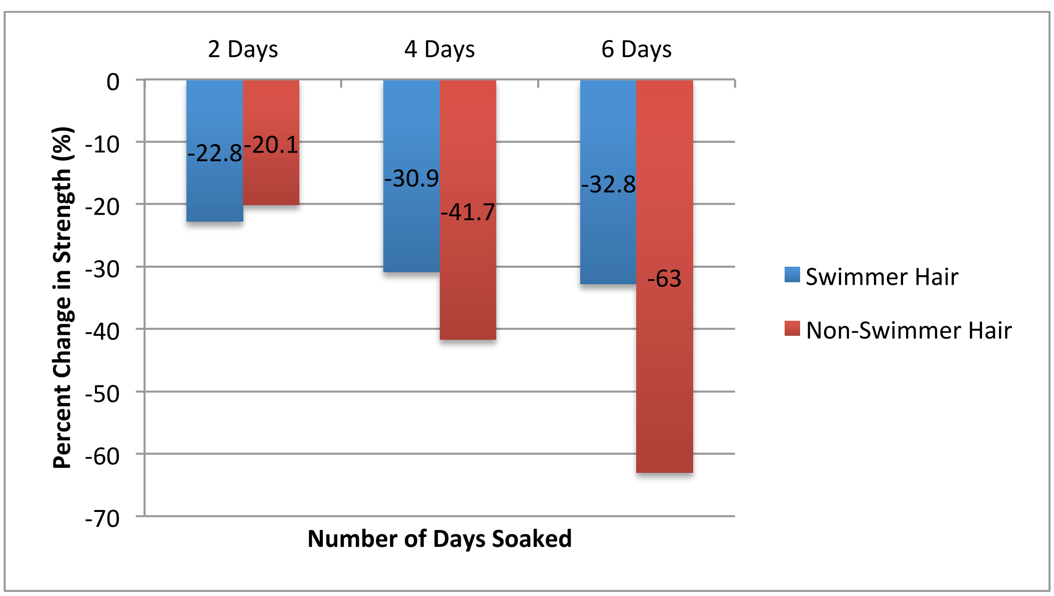
*The t-test above calculated the difference between the control groups of hair submerged in tap water and the manipulated groups with the hair submerged in pool water. All the results showed that the tap water and the chlorinated pool water have significant differences, showing that the strength of the hair was affected solely by the chlorine not the water itself. Therefore, it is now possible to compare the results of only the chlorinated water in the graph that follows.*

Figure 4: The Mean Values of the Force (in Newtons) that a Strand of Hair from Swimmers and Non-Swimmers Could Hold after being soaked in Chlorinated Water for Two, Four, and Six Days.




*The graph shows the mean breaking force of hairs with error bars showing ± one standard deviation. Hairs of both swimmers and non-swimmers showed a negative correlation between time spent in chlorinated water and mean breaking force. The non-swimmer's hair was much stronger in the beginning but lost strength much more rapidly than the swimmer's. By the end of six days, the non-swimmers hair had a lower mean breaking force than the swimmers.*

Figure 5: The Change in Strength of a Hair Soaked in Chlorinated Pool Water As a Percent of Control.




*The graph shows the loss of strength in hairs soaked in chlorinated pool water. Both swimmers and non swimmers show a correlation between time soaked an loss of hair strength. Although both swimmer and non-swimmer start with similar percent change in hair strength, the non-swimmer loses more pronounced and continued loss of strength in days 4 and 6.*

Interpretation:

Using the tap water as a control group, it was established that for the most part, length of soaking did not significantly change the strength of the hair. There were no significant differences in the control samples for swimmers. The mean tensile strength of swimmer hair soaked in tap water was 0.457 ± 0.029 N. Non-swimmer hair had an average strength of 0.686 ± 0.049 N, however there was a significant difference between the strength in days 4 and 6 (0.713 N and 0.667 N respectively). By using these values as a control group, it is possible to compare not just the strength, but the change in strength, of the hair in the manipulated trials.

The hair strands added to chlorinated water always showed significantly less strength than the control trials. By using a t-test for each section gave a p-value of less than 0.0001, showing that the difference between the sets of data were all extremely statistically significant. This suggests that the changes in the strength of the hair was likely due to the pool water, presumably the chlorine, and not the water itself.

For the swimmer hair, the mean force that the hair strand could hold after being soaked in chlorinated water was 0.353 after two days, 0.316 after four days, and 0.307 after six days. The strength decreased rapidly between the second and fourth day, decreasing by 0.037 N, but by the sixth day, it decreased only 0.009 N. Compared to the control, this represented a 22.8% loss of strength of day 2, a 30.9% loss of strength of day 4, and a 32.8% loss of strength on day 6.

For the non-swimmer hair, the mean force that the hair strand could hold after being soaked in chlorinated water was 0.548 after two days, 0.400 after four days, and 0.254 after six days. The strength decreased rapidly and consistently between the second and fourth day (-0.148 N) and between the fourth and sixth day (-0.416 N). Compared to the control, this represented a 20.1% loss of strength of day 2, a 41.7% loss of strength of day 4, and a 63.0% loss of strength on day 6.

For two and four days of soaking swimmer hair was weaker than non-swimmer hair, with swimmer hair having being able to withstand only 64% as much force as the non-swimmer hair. However after six day, the swimmer hair could withstand 121% of the force that non-swimmer hair could hold.

**EVALUATION:**

Conclusion:

The purpose of this investigation was to discover the relationship between the amounts of time hair was exposed to chlorinated water, and the force that the strand of hair could hold before breaking. In this experiment, the hair used was of two different types, from a person who swims often and has long-term exposure to chlorine and a person who swims rarely who has little exposure to chlorine. In all cases, the chlorine exposed hair showed a significant decrease in strength. This is likely due to the disruption of bonds in the protein structure of the hair, specifically sulfur-residues and peptide bonds (3, 4). Chlorine also breaks down the outermost layer of the hair strands, which contain oils. This removal of oils results the loss of shine and flexibility of the hair strand, as well as making the protein core of the hair more prone to damage (3).

The data suggest that both types of hair lose more strength when soaked longer in chlorinated water, but non-swimmer hair loses strength more rapidly after two days. Because swimmers are exposed to the effects of chlorine on a more frequent basis, the hair has developed in a way that decreases the effects of chlorine – more oils are produced by the body to protect the hair in response to the chlorine present, and the hair grown, although damaged, is more resistant to chlorine. Environmental changes are known to change the amount of oily coating secreted by the sebaceous glands on the scalp (6). These physiological changes may cause the hair to become less damaged by the chlorine exposure over a set period of time than non-swimmers. On the other hand, it is possible that there is only so much damage that chlorine will do to the hair and that since swimmer hair is already weakened by chlorine exposure it simply has less strength to lose. Further research is needed to determine the answer.

## Evaluation:

One weakness of this lab is that fata was only collected over three time periods and no data were collected prior to soaking (day 0). This produced insufficient points to make a line graph and determine the mathematical relationship between length of soaking time and loss of strength. The hair soaked in tap water served as a measure of starting strength but it would be better to also collect data after five minutes of soaking in tap and pool water to provide a starting point. More lengths of time should also be tested, such as every day for a week. Further, there was an anomalous result in the control hair strength for the non-swimmer. No reason could be found for this, and it is possible that having a much larger sample size would change the result. Instead of ten trials per condition, 100 or 1000 could be done, as time permitted.

Another concern was lack of control over the way the hair was pulled to exert force on the force meter. Although every effort way made to pull with a consistent strength and speed, there were no quantitative controls. This would increase the variability of the data. An automated method of pulling, perhaps using a motor to wind the hair around a spindle, would increase consistency and reliability of the data.

Other weaknesses have to do with the composition of the water. Tap water varies from place to place and may have chemical contaminants that could affect the strength of hair. For example, fluoridated water might cause a loss of hair strength because fluorine and chlorine, both being halogens, could act similarly on hair proteins (4). Distilled water would be a better control group in some ways, although the subject's hair would be primarily exposed to tap water when washing. The pH of the water also needs to be controlled. The effects of chlorine on keratin depend on pH, having less of an effect at alkaline pH value than neutral of acidic pH values (4). Tap water with a pH matching the pool water could be used, or the distilled water could have its pH altered to match the pool water. While this would change the distilled water composition, at least the specific amount and nature of the contaminant would be known.

Further research could be done on the effect of stirring the hair and water samples. It has been shown that stirring or mixing will increase chlorine damage (5), and this would more accurately mimic the constant motion of swimming in a pool. A magnetic stirrer could be added to agitate the samples.

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