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Are Isotonic Drinks Really Hydrating?

An investigation into the hydrating claims of isotonic drinks.

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Abstract

The aim of this study was to investigate the effect of different solutions advertised as 'hydrating' on horse red blood cells. The study focused on isotonic drinks and waters. Various isotonic drinks are sold as 'hydration solutions' but, given the commercial aspects of marketed products, it is questionable whether the manufacturers create them to be hydrating or whether the drinks are in fact dehydrating in order to make the customer thirstier and want to buy more drinks to quench their thirst.

Introduction

During the 2006 annual meeting of the North Eastern Association of Forensic Scientists, Wesley presented a study using gastric emptying as a method of quantifying the absorption of water in athletes. He postulated that the faster the rate of gastric emptying, the more hydrating the solution is. Wesley (2006)[1] stated that, 'Gatorade which contained a 4.5% sugar solution of glucose/fructose exhibited a 35% decrease in gastric emptying compared to drinks containing 1.0 or 2.5% sugar, and a 40% decrease when compared to plain water'. This intrigued me and I wanted to understand if this supported the advertised claims that all isotonic drinks were hydrating, and to investigate the effects of isotonic drinks on the body by using horse red blood cells as a model.

Different isotonic drinks may have differing effects on the body, and therefore I designed my study to investigate which drinks were better at hydrating the body and which were the least hydrating.

Method

Model used

The model used to investigate the hydrating effect of solutions was the horse red blood cell model. This model examines the effect of solutions on horse red blood cells, compared with a reference solution(horse physiological saline) – one that is as close as possible in overall salt concentration to horse plasma, the natural environment for horse red blood cells. Horse blood was diluted with the solution under investigation and the number and condition of red blood cells present after 4 minutes was noted. In order to draw conclusions about hydration, a comparison was made with the number and condition of red blood cells when they were diluted with horse physiological saline.

Adequate hydration was assumed if a similar number of cells were seen after dilution with the solution under investigation as those seen with horse physiological saline. Dehydration was assumed if crenation of the cells was observed, and over hydration was assumed if a significant decrease in the number of cells compared with horse physiological saline was observed, as the cells were presumed to have burst via osmosis.

Determination of horse physiological saline concentration

A range of different concentrations of saline was tested: 0%, 0.1%, 0.5%, 0.8%, 0.9%, and 1%. An Eppendorf tube was used to gently agitate 8 microlitres of horse blood with 1992 microlitres of each solution. This was then left for 4 minutes before placing on a haemocytometer for investigation.

Using an established method (Schoen S. 1988)[2] the red blood cells were mounted and counted on the haemocytometer grid (Figure 1).

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Fig. 1: The parts of the haemocytometer (as viewed from the side).

The percentage of saline solution deemed to result in no discernible bursting or crenation of red blood cells was determined to be the concentration of horse physiological saline solution, and was used as the reference solution.

Examination of hydration using the horse red blood cell model

Different dilutions of horse blood in horse physiological saline were made until a dilution was found that would allow cells to be easily counted using a haemocytometer.

Three different 'isotonic' drinks were then investigated: Powerade, Asda Sport, and Smart water. The horse blood (8 microlitres) was added to 1992 microlitres of each solution under investigation using an air displacement pipette for accuracy. The solutions were left for 4 minutes to allow osmosis to occur, and then each solution was mounted on a haemocytometer and the cells were examined and counted. The counts were made in four fields of view, and the whole process was repeated twice per solution. An average count was then taken. The number of cells that were intact and could be counted, and the number of cells that were crenated acted as an indicator of the hydrating properties of the different isotonic drinks.

Determination of the quantity of non-lysed red blood cells

A method was devised to quantify the amount of non-lysed red blood cells in each solution. The horse blood was diluted with each solution (8 microlitres of horse blood with 1992 microlitres of the solution), left for 4 minutes to allow osmosis to occur, and then placed into a centrifuge tube, before being spun for 2 minutes in a microfuge at 14.5×103 rpm. The supernatant was decanted and its absorbance at 700nm was measured using a colorimeter. The higher the absorbance, the more red blood cells were deemed to have lysed, allowing quantification of cell lysis.

Study controls

Controls were chosen to ensure that the red blood cells reacted in as natural a way as possible, proving that the results obtained were reliable and not caused by problems with the red blood cells themselves, or the study conditions.

Fifty percent glucose solution was used as a positive control for the study to induce crenation. Crenation is induced when the water potential inside the red blood cells is higher than the water potential in the glucose solution, causing the water to leave the red blood cells by osmosis, and subsequently causing a loss of turgor pressure in the cells.

Distilled water was used as a negative control for the study to induced lysis. Lysis is induced when the water potential of the distilled water is higher than inside the red blood cells, causing water to rapidly enter the cells, and increasing the turgor pressure to the extent that the cells burst.

Horse physiological saline (0.9% saline solution) was used as a control or 'reference solution' to check that the horse red blood cells were behaving in the correct way and had not been damaged in transit or storage. No change to the horse red blood cell condition or number was expected following dilution with horse physiological saline.

Results

Determination of horse physiological saline concentration

A range of different concentrations of saline was tested: 0%, 0.1%, 0.5%, 0.8%, 0.9%, and 1%. Of the various dilutions tested, 0.9% saline resulted in the most amount of cells counted after 4 minutes (Figure 2 and Figure 3). The standard deviations calculated were very large for all solutions tested, with the exceptions of the 0.9% saline solution.

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Fig. 2: Table 1 Cell counts in different saline solutions

SD = Standard Deviation

* Average taken of duplicate counts across all 4 fields of view

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Fig. 3: Cell counts in different saline solutions

1 = 0% saline solution

2 = 0.1% saline solution

3 = 0.5% saline solution

4 = 0.8% saline solution

5 = 0.9% saline solution

6 = 1% saline solution

Elucidation of horse physiological saline concentration using a colorimeter

Of the solutions tested, the 0.9% and 1.0% solutions showed a similar absorbance (Figure 4).

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Fig. 4: Absorbance of different solutions at 700nm

1 = 0.1% saline solution

2 = 0.5% saline solution

3 = 0.8% saline solution

4 = 0.9% saline solution

5 = 1% saline solution

Therefore, horse physiological saline concentration was determined by using these results in conjunction with the cell counts (Figure 5).

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Fig. 5: Table 2 Absorbance of different solutions at 700nm

SD = Standard Deviation

Investigation of cell condition

Severe crenation was noted in the 50% glucose solution (positive control), and no cells were visible in the distilled water solution (negative control; Figure 6). No crenation was observed in the 0.9% saline solution (horse physiological saline), and no cells were visible in the Smart Water solution.

The condition of the cells in the Powerade and Asda Sport solutions could not be assessed because the red blood cells had clumped significantly and formed a pellet at the bottom of the mixing tube (Figure 7, photos 1 and 2).

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Fig. 6: Table 3 Assessment of cell crenation in different diluents

* Please see photo 1

$ Please see photo 2

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Fig. 7: Red blood cell clumping

Photo 1. Cell clumping in Asda Sport

Photo 2. Cell clumping in Powerade

Investigation of cell count

The highest cell count was noted in the physiological saline solution (118.5 ± 8.51), followed by the 50% glucose solution (28.6 ± 5.36; Figure 8). No cells were visualised or counted in the distilled water or Smart water solutions, and it was not possible to count any cells in the Asda Sport or Powerade solutions because of cell clumping (Figure 6 and Figure 7). Small standard deviations were found in the cell counts taken (Figure 8 and Figure 9).

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Fig. 8: Table 4 Number of cells counted in different diluents

n/a – not applicable – cells clumped;

SD = Standard Deviation

* Average taken of duplicate counts across all 4 fields of view

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Fig. 9: Number of cells counted in different diluents

1 = 50% glucose solution

2 = Distilled water

3 = 0.9% saline solution

4 = Smart water

Analysis

All controls behaved as expected and therefore the study set-up can be considered to be valid.

Smart water was the only drink that could be adequately investigated in this model because if the unexpected clumping seen with the other solutions under investigation. All of the cells burst, and this is likely because the Smart water contains very few added ingredients (Smart water label viewed October 2015). According to the parameters set out from the model, it can be

concluded that of the isotonic drinks tested, Smart water had the most hydrating effect.

The counts from the other drinks varied greatly. These drinks contained a lot more salts and sugars than Smart water, which would be expected to affect the water potential dramatically, and could have resulted in the large differences in cell counts and reactions observed. The clumping seen with Powerade and Asda Sport was unexpected and could be due to severe crenation or a reaction caused by particular salts or sugars present in those drinks. This warrants further investigation in future studies.

This study indicates that isotonic drinks have definite effects on horse red blood cells. Bearing in mind the limitations of the model, and assuming that crenation equates with non-hydrating ability and bursting of the cells equates with over-hydrating ability, this study has shown that isotonic drinks do not all 'hydrate' as advertised, and may even have the opposite effect on red blood cells.

Limitations of the study

The main limitation of this study is that it is in vitro and does not replicate conditions inside the human body. Therefore, the study shows how hydrating the drinks are to individual red blood cells but fails to indicate their potential hydrating properties on the entire body. Furthermore, since it was necessary to refine the methodology of the model, a limited number of drinks could be tested in the time allotted.

Absorbance would be a supportive methodology for measuring red blood cell lysis, however, it couldn't be used with these isotonic drinks due to the colourings in the drinks. The equipment available was not sensitive enough to counteract this limitation.

Recommendations for future studies

Further studies should be conducted to investigate the clumping seen in two of the sports drinks. Additionally, experiments should be considered with counts in more fields of view; with different batches of horse blood (to check for batch variability), and with different species' blood (to look for a species effect). Different brands of isotonic drinks should be examined, and it would be possible to devise a study investigating the time that humans became thirsty (or measure the concentration of their urine) after consuming different sports drinks and completing a set amount of exercise. This would allow for an investigation into the hydrating effects of the drinks with a more real-world set of conditions.

Further studies should be conducted using more sensitive colorimeters able to account for any colourings present in the drinks.

Conclusions

Cell counts and absorbance readings taken showed that 0.9% saline solution caused the least amount of damage to the horse red blood cells. This concentration of saline had no discernible hydrating or dehydrating effect, and therefore was used as the reference solution throughout the rest of the study ('horse physiological saline') in order to act as a control for the red blood cells.

Smart Water was found to be over hydrating, as shown by the cell counts and amount of cell lysis. Powerade and Asda Sport caused the cells to clump together, and therefore it could not be determined whether they were crenated or not, and the hydrating/dehydrating effect of these solutions could not be assessed.

Acknowledgements

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References

1. J Wesley. Endurance sports, Rehydration, Cerebral Edema and Death. (2006), Northeastern Association of Forensic Scientists Annual Meeting, Rye Brook NY, Online
REFERENCE LINK

2. S Schoen & C.S. Lobban et al. Cell Counting in Experimental Phycology — a Laboratory Manual (1988)