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Taurine Supplementation Induces Cellular Dysfunction and Decreased Cell Viability

Over the past several years, there has been an increased incidence in the number of cases of Dilated Cardiomyopathy in dogs. It is thought that this increase may be, in part, due to the commercially available, grain free diets, which often times lack essential nutrients, such as amino acids. Taurine is an example of one such amino acid that is an important building block for proteins within the cells, particularly in cardiomyocytes. A deficiency in the free amino acid taurine is linked to heart diseases including Dilated Cardiomyopathy in many canine breeds. This causal relationship between nutrient deficiency and disease has led to supplementing with excess taurine.

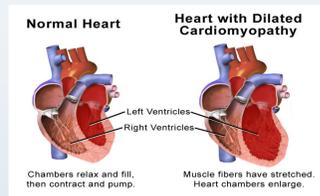
To date, little investigation has been done to show the cellular effects of excess taurine supplementation. We set out to assess the risk/benefit of taurine supplementation using an HL-60 (mammalian lymphocyte) cell culture model. We hypothesized that cells exposed to high levels of taurine would exhibit a decrease in overall cell viability. To investigate this, HL-60 cells were treated with increasing concentrations of taurine, and the cytotoxic effects were quantified using both Trypan Blue Staining and MTT Assays. The results presented herein provide initial evidence that too much taurine can be as damaging as too little taurine to mammalian cells. This work will serve to caution veterinarians of the risk associated with excessive taurine supplementation and will emphasize the need for stricter regulation in the pet food industries.

Taurine Supplementation Induces Cellular Dysfunction And Decreased Cell Viability

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Introduction

- In the past two years, the number of Dilated Cardiomyopathy cases in dogs has severely increased.
- Dilated Cardiomyopathy, or DCM, is a heart condition in which the heart cannot properly contract nor pump due to an enlargement of both the atria and ventricle.



- The diets suspected of causing DCM, the grain-free, boutique, and exotic protein diets, either lack the protein containing the amino acid taurine or the other ingredients such as lentils and chickpeas used to replace grains in grain-free diets are affecting the metabolism of taurine.
 - Genetic predisposition to low taurine is also playing a role.
 - These breeds include large breed dogs such as Cocker Spaniels, Boxers, Great Danes, and Golden Retrievers
- Taurine supplementation is not well understood and there is a lack in quality control.

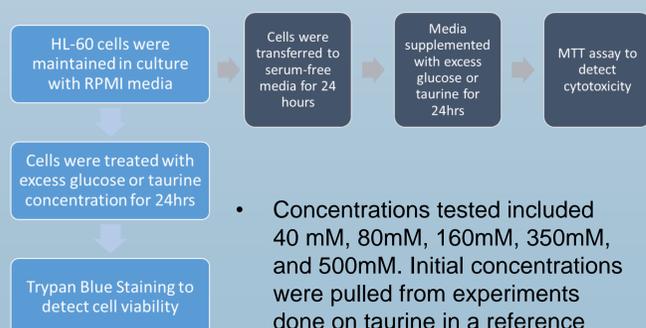
Objective

Due to the lack of studies pertaining to the supplementation safety of taurine, the current study looked to investigate the affects of large quantities taurine on cells.:

We hypothesize that cells exposed to high levels of taurine will exhibit a decrease in overall cell viability in a cell culture model.

The current study also investigates the affects of grain free and boutique diets on large breed dogs in a clinical setting. These dogs would be possible candidates for receiving taurine supplementation.

Methods Overview



- Concentrations tested included 40 mM, 80mM, 160mM, 350mM, and 500mM. Initial concentrations were pulled from experiments done on taurine in a reference journal article (5). From there we doubled each time the concentration for a more drastic affect.
- Absorbance₆₀₀ was read using ELISA. Higher the absorbance the more cell proliferation (less death).

Laboratory Results

Trypan Blue staining shows reduced cell viability with increasing glucose and taurine concentrations

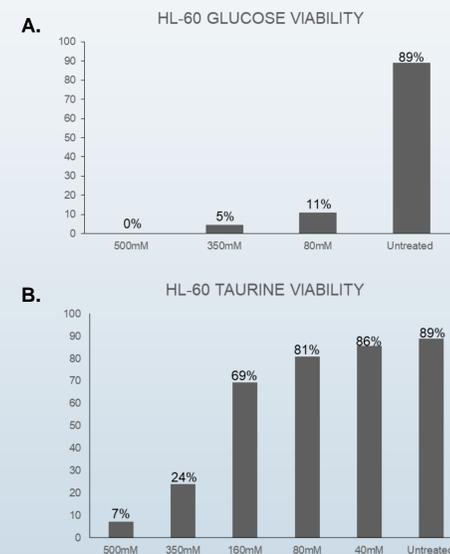


Figure 1. Trypan Blue staining shows reduced cell viability with increasing glucose and taurine concentrations. Qualitative analysis by Trypan blue stain was used to determine percent cell viability after glucose (a) and taurine (b) treatment for 24hrs. Glucose treatment included concentrations of 500mM (high), 350mM (medium), and 40mM (low). Taurine treatment included 500mM, 350mM, 160mM, 80mM, 40mM. In all experiments untreated cells were included as a control. As expected, glucose treatment of ≥ 80 mM reduced cell viability. Taurine treated cells showed the similar degrees of cell death (~7% surviving cells after treatment with 500mM). This indicates that taurine in excess may have a similar cytotoxic effect as excess glucose. Data presented is a representative sample of experiments conducted in duplicate (glucose) and triplicate (taurine).

Quantitative MTT assays demonstrate a positive correlation between cytotoxicity and increasing glucose and taurine concentrations.

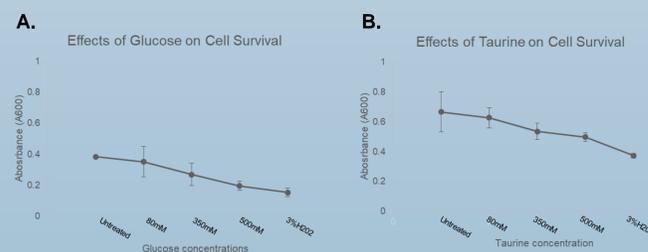


Figure 2. Excess taurine treatment results in cytotoxic effects on HL-60 cells. Briefly HL-60 cells were seeded at a concentration of $\sim 10^5$ cells/well in serum free media. 24hrs after serum starvation, serum-free media containing varied concentrations of either glucose or taurine were added for 24hrs. Trends remained similar to those seen by Trypan Blue staining; the higher the concentration of taurine, the more cell death. Taken together, these results suggest taurine is as damaging as the millimolar concentrations of glucose (NS difference between concentrations of glucose/taurine 80mM;p= 0.9, 350mM;p=0.7 and 500mM;p= 0.1. Student T-test, two-tailed, p<0.05).

Trypan Blue staining and MTT assay results suggest that excess taurine supplementation is cytotoxic to mammalian cells grown in culture.

Clinical Results

Clinical Veterinary Survey

- Research survey was conducted at Valley Cottage Animal Hospital in New York
- Sampling of the 15 Large breed dogs was performed at random
- Diet was correlated with cardiomyopathy conditions

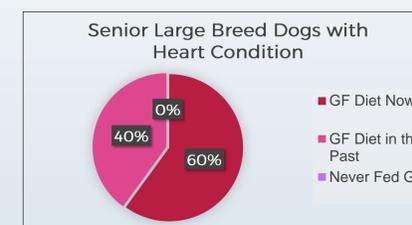


Figure 3. Prevalence of cardiomyopathy in dogs following introduction of grain free diets. All five dogs surveyed with cardiomyopathy either had previously been on, or are currently on, a grain free diet.

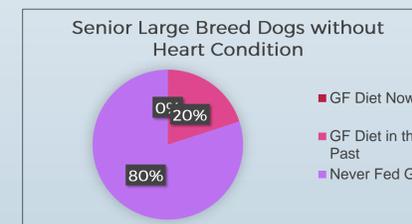


Figure 4. Dogs who have not been fed a Grain Free diet rarely display cardiomyopathy. Diet types of the dogs who had not been diagnosed with a heart condition. 8 out of 10 dogs had never been on a grain free diet. And none of the dogs are currently eating a grain free diet.

Vetmedin Dispensing:

- Data from the large breed, senior dogs currently on Vetmedin at the Valley Cottage Animal Hospital
- Vetmedin is the leading veterinary cardiology medication that helps the heart pump blood more strongly and steadily.
 - Used in the treatment of dilated cardiomyopathy.

Vetmedin Dosage	Breed	Age	Diet
2.5 mg	Sheltie	12	Rachel Ray GF
	Poodle	14	Home Cooked
	Cocker Spaniel	12	Purina GF
	Collie	10	Blue Buffalo and Merrick Lamb (Both GF)
10 mg	Sheltie	15	Blue Buffalo GF
	American Staff Terrier	14	Blue Buffalo GF
5 mg	German Shepherd	14	Fromm Dry GF
	Labrador Retriever	15	Rachel Ray GF
	Labrador Retriever	13	Purina Lamb GF
	Lab Mix	9	Natures Promise GF
	German Shepherd	11	Purina GF
	Pit Bull	13	Purina GF
English Bulldog	11	Blue Buffalo GF	

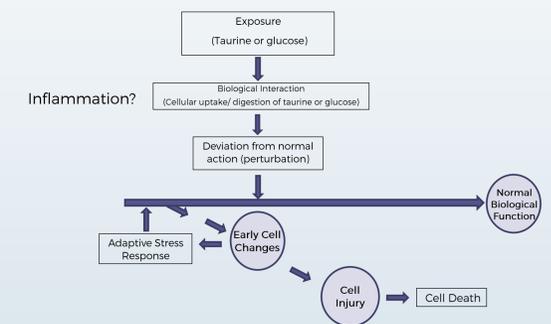
Figure 5. Clinical correlation between Vetmedin treatment and Grain Free diets in dogs. Data of large breed senior dogs currently on Vetmedin. All dogs, with the exception of one, are on a grain free diet.

Grain free and exotic diets were shown to correlate with heart disease in large breed dogs.

Results Continued

Toxicity Pathway

- Proposed model of cellular dysfunction due to inflammation from excess nutrient supplementation (taurine and glucose)



Conclusion

- Concentrations of taurine over 80mM induces cell death, showing the negative affects of over supplementing/dosing taurine.
- Grain free diets and exotic proteins have negative affects on the hearts of large breed, senior dogs such as the Golden Retriever.
- Treatment of DCM by taurine supplementation may be harmful in excessive quantities.

Future Directions

- Repeat experiment using canine cardiomyocytes to see the affects on heart cells in dogs.
- Next step would be to calculate mg/kg dosing based on the results of at what point is the amount of taurine more damaging than helpful.
- Work can be used by veterinarians to better understand dosing of taurine supplementation as to not overdose the patient and further damage the heart.

References

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