Research Proposal Summer 2011: 
The Effect of Energy Drinks on Cellular Development

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Energy drink consumption has grown exponentially since the introduction of Red Bull in the United States in 1997. Despite the widespread marketing of energy drinks, the United States does not regulate the ingredients found in energy drinks. The most common ingredients manufacturers include in energy drinks include caffeine, taurine, guaranã, and B vitamins.\textsuperscript{1} The FDA defines these ingredients to be “dietary ingredients” so energy drinks are defined to be “dietary supplements” under the Dietary Supplement Health and Education Act of 1994.\textsuperscript{2} This allows manufactures to include supplements in unspecified amounts even if some of the supplements are insufficiently researched.\textsuperscript{2,3}

The lack of regulation of energy drinks has raised concerns. Consumption of energy drinks has been associated with high-risk behaviors such as drug use, alcohol abuse, smoking, fighting, sexual risk taking, and accepting risky dares.\textsuperscript{1} Serious adverse effects of energy drinks have especially been linked to children, adolescents, and young adults diagnosed with conditions like seizures, diabetes, cardiac abnormalities, or behavioral disorders.\textsuperscript{4} Clinical studies show that energy drinks have been associated with cerebral vasculopathy, acute mania, and epilepsy.\textsuperscript{5} The ingredients found in energy drinks, such as caffeine and taurine, have been individually studied: Excess caffeine consumption leads to caffeine intoxication, which has symptoms of nervousness, anxiety, restlessness, insomnia, gastrointestinal upset, tremors, tachycardia, and psychomotor agitation.\textsuperscript{3} Taurine is involved in multiple metabolic processes such as, osmoregulation, antioxidation, and glycolysis.\textsuperscript{6,7} Taurine is used as a supplement because it is thought to promote biliary health, eye health, and prevent congestive heart failure.\textsuperscript{5} Even though some ingredients in energy drinks have been studied individually, the ingestion of high quantities of caffeine, taurine, and B-vitamins in combination has yet to be adequately researched.
The goal of this research project is to study energy drinks and their ingredients at the cellular level. The project this summer will continue research on cellular effects of energy drinks that was conducted last summer at Saint Mary’s College of California. Since energy drinks predominantly affect the cardiovascular system and nervous system, cellular effects of these energy drinks will be assessed on neurons and vascular endothelial cell lines. In addition, the effects of the energy drink ingredients on kidney cells will be evaluated because many of the ingredients are excreted through urine, suggesting potential deleterious effects on kidney cells. Also, kidney cells are a good model system for epithelial cells in general, which make the lining of our internal organs. The three cell types will be cultured using laminar flow hoods and maintained at 37°C. To examine effects of energy drinks on cellular development, cells will be treated with energy drinks and their ingredients individually and in combinations. The two energy drinks that I will focus our work on this summer are 5-hour Energy and Monster energy because of their popularity and widespread use. The effects will be evaluated by using phase contrast and fluorescent microscopy to examine cell structure, cellular processes, cell division, and cell death.

Previous results showed that when the neurons from the forebrain of chick embryos were treated with the 5-hour Energy and Monster Nitrous, they showed a decrease in the number of processes (axons and dendrites) they extended, compared to untreated cells. This suggests that there is change in neuronal shape and maybe cell survival following treatment with energy drinks. This summer, I plan to extend on the previous work by examining neurons in other regions. I will examine spinal motor neurons from chick embryos and sympathetic neurons to determine overall neuronal effects of these energy drinks. Since Monster Nitrous appears to have been discontinued, Monster energy will be used instead for further experiments because of its
similar ingredients. To address the question of whether there is increased cell death following treatment with energy drinks, I will examine the health of the spinal and sympathetic neurons. This will be executed by looking for markers of cell death and by looking for the presence of normal protein synthesis. Furthermore, I will evaluate the effects of ingredients such as caffeine, taurine, and B-vitamins individually and in combinations to determine the cause of the neuronal process loss previously observed following treatments of 5-hour Energy and Monster Nitrous. In addition, I plan to examine neuronal function by comparing the number of synapses in untreated neurons with those in neurons treated with energy drinks or their ingredients. In summary, our studies on neurons will allow me to understand the deleterious effects of energy drinks on neurological processes at the cellular level. This understanding will be extrapolated to the effects energy drinks have on the function of the nervous system as a whole.

Previous studies on kidney cells using a Madin-Darby canine kidney (MDCK) cell line showed that when MDCK cells were exposed to 5-hour Energy and Monster Nitrous, they caused a disruption of the actin cytoskeleton. The actin cytoskeleton is important for cell movement, division, and survival in culture. This summer, I plan to continue to look at MDCK cells in order to discover how the actin cytoskeleton, and possibly other cell structures, are compromised when they are treated with energy drinks and their ingredients. I will examine the ability of these cells to make kidney specific proteins. Also, I will look at cell death markers to determine if the cells are functioning normally. Since kidney cells being epithelial in nature have a polarity, I will evaluate the effects of energy drinks on cell polarity markers following the various treatments.

Vascular endothelial cells will be the third cell type that will be examined because of the effects of energy drinks on the cardiovascular system. A human vascular endothelial cell
line (purchased from ATCC) will be cultured in the presence of growth factors in order to keep the cells alive. The cells will be treated and evaluated using procedures similar to the methods described for the neuron and kidney cell lines. Since I know that caffeine has dramatic effects on vascular cells,\(^{39}\) I expect to see effects of energy drinks on these cells. I plan to set up a 3-D culture system, which will enable me to create clusters of cells that are similar to the formation of a blood vessel in an animal. Using the 3-D culture system, I will examine the effects of energy drinks and ingredients on blood vessel growth and blood vessel maintenance that would occur in an animal.

In summary, the project aims to study the effects of energy drinks and their ingredients on neurons, MDCK cells, and vascular endothelial cells. The facilities in Brousseau Hall will allow for the three cell types to be simultaneously cultured. One cell type can be studied and examined while the other cell types remain in the incubators. After examining the cell types, the data gathered from the treatments will be extrapolated to the various functions of the human body; specifically, the nervous system and cardiovascular system. Understanding the effects at a cellular level will allow me to discover the root cause of reported adverse effects of energy drinks related to the nervous system and cardiovascular system. The effects observed for the three types of cells will enable me to identify common themes between certain combinations of ingredients. Studying neurons, MDCK cells, and vascular endothelial cells will provide a strong foundation that can be used to assess the major concerns of energy drinks regarding the body. This cellular research will allow energy drink consumers to be informed of specific effects that unregulated ingredients have on different functions of the body.
References


