1015-1028; Nagaraju, G., and R. Scully 2007, DNA Repair 6:1018-1031; Pellegrini, L., and A. Venkitaraman 2004, Trends Biochem. Sci. 29: 310-316; Pellegrini, L., D.S. Yu, T. Lo, S. Anand, M. Lee *et al.* 2002, Nature 420: 287-293; Rabinow, L., 2002, Cancer Invest. 20: 531-56; Shivji, M.K., O.R. Davies, J.M. Savill, D.L. Bates, L. Pellegrini *et al.* 2006, Nucleic Acids Res. 34: 4000-4011; Venkitaraman, A.R. 2002, Cell 108: 171-182; Wang, W., 2007, Nat. Rev. Genet. 8: 735-748; Warren, M., A. Smith, N. Partridge, J. Masabanda, D. Griffin *et al.* 2002, Hum. Mol. Genet. 11: 841-851; Xia, F., D.G. Taghian, J.S. DeFrank, Z.C. Zeng, H. Willers *et al.* 2001, Proc. Natl. Acad. Sci. USA 98: 8644-8649.

Caffeine supplementation increases mortality in female *Drosophila melanogaster* without reproductive or metabolic impairment.

Bussel, Igor Irvin, Michael Joseph, Veronica Lewis, and Mahtab Jafari. Department of Pharmaceutical Sciences, University of California, Irvine.

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring stimulant substance that is extracted from over 60 sources and used in common comestibles. It is mainly found in substances such as coffee, tea, and energy drinks, but also may be found in cold medications, chocolate, and analgesics (Benowitz, 1990).

Caffeine antagonizes adenosine receptors that affect cellular concentration of cyclic AMP and triggers the release of norepinephrine, dopamine, and serotonin in the nervous system (Fredholm, 1985). Additionally, caffeine has been shown to stimulate thermogenesis by enhancing noradrenaline release, therefore, increasing metabolic rate in rodents and humans (Acheson *et al.,* 1980; Bukowiecki *et al.,* 1983; Cheung *et al.,* 1988; Astrup *et al.,* 1991). Furthermore, caffeine consumption results in an increased heart rate and blood pressure (Belza *et al.,* 2007). Caffeine has also been shown to possess antioxidant activity (Devasagayam *et al.,* 1996) along with ergogenic properties (Keisler and Armsey, 2006).

In a previous study, *Drosophila prosaltans* treated with caffeine demonstrated a dose-dependent decrease in fecundity (Itoyama and Bicudo, 1992). Furthermore, caffeine has been shown to inhibit mating frequency and copulation duration in the same model organism (Itoyama *et al.,* 1995). Additionally, after ten generations of caffeine treatment, longevity was significantly reduced in male and female *Drosophila prosaltans* (Itoyama *et al.,* 1998).

The main objective of this study was to evaluate the effect of caffeine on mortality, fecundity, and metabolic rate in *Drosophila melanogaster*.

Materials and Methods

Caffeine

Stock solution for each dose was prepared and mixed into yeast paste. The calculated doses reflect the final concentration of caffeine in yeast paste that flies consumed during the experiment. Caffeine was obtained from the Sigma-Aldrich manufacturer. For each assay, three doses of caffeine were compared to a control. We evaluated caffeine at the following doses: low (0.008mg/ml), medium (0.08 mg/ml) and high (0.8mg /ml). Adult flies, starting at day 1 were supplemented with caffeine at these doses.

Experimental Methods

The details of the experimental methods used in our mortality, fecundity, and metabolic rate assays are outlined in two recently published manuscripts (Jafari *et al.,* 2007a, b).

All *Drosophila melanogaster* stocks used in these experiments were derived from a sample (called "IV") of the Amherst, Massachusetts, Ives population that was collected in 1975 and studied extensively by Rose and colleagues (Rose *et al.,* 2004).

We evaluated the impact of caffeine on mortality using fraction dying as the proxy. For this assay, three doses of caffeine (0.008, 0.08, 0.8 mg/ml) were compared to a control group. For each dose, 320 males and 320 females were supplemented with caffeine. There were 4 males and 4 females in each vial, with a total of 80 vials per dose per sex. The flies were transferred to fresh food vials and survivors were counted, every 2 days. All assays were conducted on flies that had undergone two generations of controlled density rearing. When flies from different treatments were compared, all preliminary rearing was carried out in parallel.

Certain compounds may increase lifespan simply by substantially depressing fecundity (total number of eggs laid by each female fruit fly per day) (Jafari and Rose 2006). Thus, a fecundity assay is an important check for artifactual lifespan enhancement. We evaluated age-specific fecundities and the number of eggs laid each day by each individual female for a period of ten days.

As a further test for artifactual effects, the impact of caffeine on metabolic rate was evaluated. This assay was used to ascertain whether there had been an artifactual decrease in mortality due to hypometabolism. CO_2 production in drugged flies was compared to that of a control group handled in parallel and assayed simultaneously. We used flow-through respirometry to measure the rate of CO_2 release from groups of flies following the methods of Williams and colleagues (Williams *et al.,* 2004).

Results

Males supplemented with caffeine at 0.008, 0.08, and 0.8 mg/ml resulted in an increase in fraction dying of 6.3%, 4.3%, and 11.7%, respectively. There were no statistically significant differences in fraction dying with any dose relative to control (Figure 1). Females supplemented with caffeine at 0.008, 0.08, and 0.8 mg/ml resulted in an increase in fraction dying of 25.6%, 25.1%, and 3.7%, respectively. However, statistical significance was only observed at 0.008 mg/ml and 0.08 mg/ml (Figure 1).

Consequently, we proceeded with further testing of fecundity. There were no statistically significant differences in fecundity with any dose relative to control. Furthermore, we evaluated metabolic rate; there were no statistically significant differences in metabolic rate at any dose with either sex.

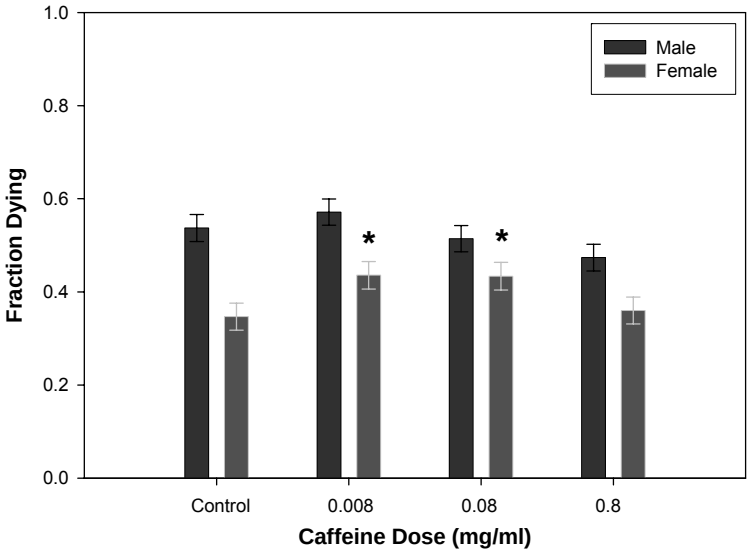

Figure 1. Fraction dying during aging phase with caffeine supplementation at 0.008, 0.08, 0.8 mg/ml. Caffeine did not decrease fraction dying in males with any dose. However, caffeine did increase fraction dying in females at 0.008 mg/ml and 0.08 mg/ml. $P < 0.05$.

Discussion

The present study demonstrates that caffeine increased mortality of female *Drosophila* without significantly affecting two major physiological mechanisms that can artifactually modulate mortality. In females, the observed increased mortality at 0.008 mg/ml and 0.08 mg/ml was not associated with any statistically significant reductions in fecundity or metabolic rate. This indicates that while caffeine does not negatively impair reproduction or metabolism, there is an underlying negative health effect in females that results in increased mortality.

Females supplemented with the highest dose, 0.8 mg/ml, did not demonstrate any significant change in mortality. We cannot explain this mortality observation; however, caloric restriction can be ruled-out due to the lack of depressed fecundity.

We evaluated the impact of caffeine on the metabolic rate of both sexes and did not observe any significant changes at any dose. While caffeine is a thermogenic stimulant and was presumed to increase the metabolic rate, no such change was observed. This can be attributed to the fact that *Drosophila melanogaster* naturally have a prominently constant metabolic rate that cannot easily be altered (Promislow and Haselkorn, 2002).

We have presented evidence that caffeine can significantly increase mortality in female *Drosophila melanogaster* without impacting reproductive and metabolic mechanisms. Further research is needed to investigate the primary effect of caffeine on female mortality.

References: Acheson, K.J., B. Zahorska-Markiewicz, P. Pittet, K. Anantharaman, and E. Jequier 1980, Am. J. Clin. Nutr. 5: 989-997; Astrup, A., S. Toubro, S. Cannon, P. Hein, and J. Madsen 1991, Metabolism 3: 323-329; Belza, A., S. Toubro, and A. Astrup 2007, Eur. J. Clin. Nutr.; Benowitz, N. L., 1990, Annu. Rev. Med. 277-288; Bukowiecki, L.J., J. Lupien, N. Follea, and L. Jahjah 1983, Am. J. Physiol. 4: R500-507; Cheung, W.T., C.M. Lee, and T.B. Ng 1988, Pharmacology 5: 331-339; Devasagayam, T.P., J.P. Kamat, H. Mohan, and P.C. Kesavan 1996, Biochim. Biophys. Acta 1: 63-70; Fredholm, B.B., 1985, Acta Med. Scand. 2: 149-153; Itoyama, M.M., and H.E. Bicudo 1992, Rev. Bras. Genet. 15: 303-321; Itoyama, M.M., H.E. Bicudo, and A.J. Manzato 1998, Cytobios 382: 81-93; Itoyama, M.M., H.E. de Campos Bicudo, and A.J. Manzato 1995, Cytobios 335: 245-248; Jafari, M., J.S. Felgner, I.I. Bussel, T. Hutchili, T., B. Khodayari, M.R. Rose, and L.D. Mueller 2007, Rejuvenation Res.; Jafari, M., B. Khodayari, J. Felgner, I.I. Bussel, M.R. Rose, and L.D. Mueller 2007a. Biogerontology 6: 639-651; M. Jafari, and M.R. Rose 2006, Aging Cell 1: 17-22; Keisler, B.D., and T.D. Armsey 2006, Curr. Sports Med. Rep. 4: 215-219; Promislow, D.E., and T.S. Haselkorn 2002, Aging Cell 1: 66-74; Rose, M.R., H.B. Passananti, and M. Mato 2004, *Methuselah Flies A Case Study in the Evolution of Aging*. Singapore, World Scientific Publishing; Williams, A.E., M.R. Rose, and T.J. Bradley 2004, Physiol. Biochem. Zool. 1: 10-17.

Deficiency screen reveals genomic region required for tumorigenesis and metastasis of *lethal (2) giant larvae* brain tumors.

Woodhouse, Elisa C., and Kathleen Kelly. Cell and Cancer Biology Branch, CCR, NCI, NIH, Bethesda, MD; e-mail: woodhousee@mail.nih.gov.

Lack of function mutations in the *lethal giant larvae (lgl)* gene cause neoplasia of the larval brain and imaginal discs (Gateff, 1978). The LGL protein has been shown to be important for the