Katy Stuparu 12/13/2020

Project Notes: The effects of ethanol metabolism on the reproductive system of *D. melanogaster*

Table of Contents:

Knowledge Gaps

Knowledge Gap	Resolved By	Information is located	Date resolved
How does ethanol affect the body?	doing more research about the effects of ethanol	- The Risks Associated with Alcohol Use and Alcoholism - Alcohol Metabolism - Oxidative stress, metabolism of ethanol and alcohol-related diseases	07/28/2020
Which part of the body should be focused on for the effect of ethanol?	doing more research about what is relevant in this field	- Metabolism of ethanol to acetaldehyde by rat uterine horn subcellular fractions - Metabolism of ethanol could play a role in the ovarian tissue cell injury	Decided upon at the end of Sept. Article read: 10/06/2020
How should research be done (lab or with animals (what species))?	talking to Dr. C. about what is possible for this STEM project	- STEM Meeting 09/16/2020	09/16/2020
How can this research project be done with *Drosophila?*	reading articles using a *Drosophila* model, Dr. C. teaching me how to culture *Drosophila*	- Developmental Ethanol Exposure Leads to Oxidative Stress in Drosophila - Sexual Deprivation Increases Ethanol Intake in Drosophila - STEM Meeting 10/15/2020	Article read: 10/08/2020 STEM meeting: 10/15/2020

Literature Search Parameters - 7/28/2020 - 11/23/2020

Database/search engine	Keywords	Summary of search
NCBI - 07/28/2020	effects of alcohol	This search was done to find an area in the very broad subject to focus on, or at least get some ideas for what to focus on.
WPI Ex Libris - 09/03/2020	metabolism ethanol enzyme inhibitor	This search was done to find journal articles relevant to the topic that was narrowed down from the last search and brainstorming.
Google Scholar - 09/21/2020	ethanol metabolism patent	This search was done to find any patents that may be useful in research and finding a direction in the project.
WPI Ex Libris - 09/23/2020	gerardo d. castro	This search was done to find articles related to this corresponding author, who has a lot of experience in the effect of the metabolism of ethanol. The goal was to learn more about the author's previous research to be able to write an email with meaningful questions.
WPI Ex Libris - 10/08/2020	ethanol drosophila	This search was done to get a better understanding of how to do research with *Drosophila*.
WPI Ex Libris - 11/23/2020	drosophila reproductive system	This search was done to find some background information for the literature review about the reproductive system of *Drosophila*

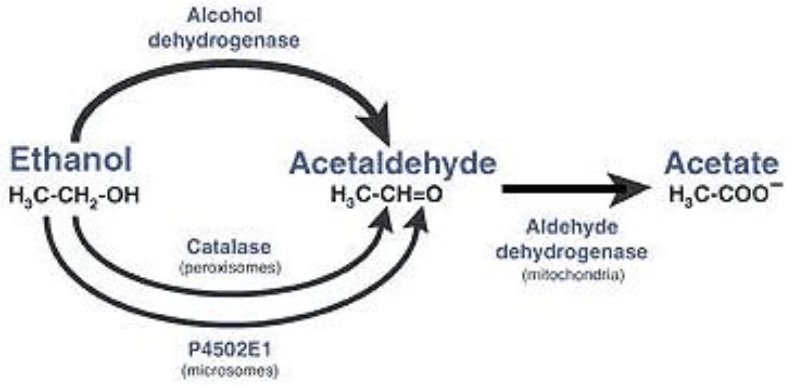
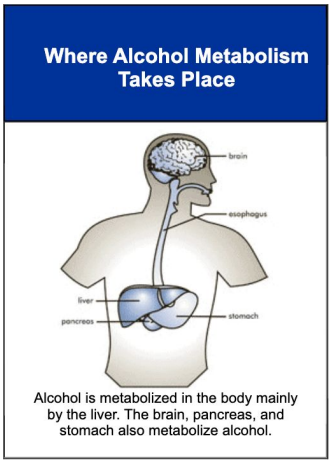
1a: The Risks Associated with Alcohol Use and Alcoholism

07/28/2020

Source Title	The Risks Associated with Alcohol Use and Alcoholism
Source citation	Rehm, J. (2011). The Risks Associated With Alcohol Use and Alcoholism. *Alcohol Research & Health*, *34*(2), 135–143.
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3307043/
Source type	Database: National Center for Biotechnology Information
Keywords	alcohol and other drug (AOD) use, alcohol use disorders, alcoholism, heavy drinking, AOD induced risk, AOD effects and consequences, health, disease cause, disease factor, disease risk and protective factors, burden of disease, health care costs, injury, social harm, drinking guidelines, prevention
Summary of key points (include methodology)	Alcohol consumption over a period of time is associated with many physical and mental diseases, such as infectious, carcinogenic, diabetic, neuropsychiatric, cardiovascular, hepatic, and pancreatic. Some of these effects can be traced directly to the consumption of ethanol, including specific types of cancer, epilepsy, and heart disease. Other risks may have other attributing factors but have an association with drinking, of which examples include contracting HIV and injuring others or oneself.
Research Question	What effects does consuming alcohol have on the physical and mental health of the human body?
Important Figures	This article does not include important figures, as it summarizes the general findings of other researchers in this broad field.
Notes	This article is an overview of the effects that alcohol can have on the body. It does not go very much in depth. This article is also a summary of the research of others, and does not provide new data.
Cited references to follow up on	Irving HM, Samokhvalov AV, Rehm J. Alcohol as a risk factor for pancreatitis. A systematic review and meta-analysis. Journal of the Pancreas. 2009;10(4):387–392. Rehm J, Room R, Graham K, et al. The relationship of average volume of alcohol consumption and patterns of drinking to burden of disease: An overview. Addiction. 2003b;98(9):1209–1228.
Follow up Questions	How is ethanol processed by certain parts of the body and why does this cause harm? How can ethanol be stopped from being diffused or transported to certain parts of the body and damaging those organs?

1b: Alcohol Metabolism

10/13/2020

Source Title	Alcohol Metabolism: An Update
Source citation	NIAAA Publications. https://pubs.niaaa.nih.gov/publications/aa72/aa72.htm. Accessed 13 Oct. 2020.
Original URL	https://pubs.niaaa.nih.gov/publications/aa72/aa72.htm#:~:text=The%20most%20common%20of%20these,eliminate%20it%20from%20the%20body.
Source type	National Institute on Alcohol Abuse and Alcoholism publication
Keywords	acetaldehyde, acetate, alcohol dehydrogenase (ADH), aldehyde, cytochrome P450 2E1, ethanol, ethyl esters
Summary of key points (include methodology)	First, ethanol is broken down by alcohol dehydrogenase (ADH) into acetaldehyde, which is very toxic. The acetaldehyde is then metabolized into acetate, and later water and carbon dioxide. Cytochrome P450 2E1 (CYP2E1) helps to break down ethanol if there is a high concentration of ethanol. Catalase also works to break down ethanol to acetaldehyde, but only a small amount. Ethanol can also be broken down by fatty acids called ethyl esters (FAEEs), which are very harmful. The size of the liver, body mass, and genetics are some factors that can contribute to how much alcohol an individual can break down in an hour. The article also goes on to summarize common effects of alcohol use, as in the previous article (less in-depth).
Research Question	How is ethanol broken down, and how does that affect the body?
Important Figures	 
Notes	This article is an overview of the effects that alcohol can have on the body. It does not go very much in depth. This article is also a summary of the research of others, and does not provide new data.
Cited references to follow up on	Edenberg, H.J. The genetics of alcohol metabolism: Role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Research & Health 30(1):5–13, 2007. Vonlaufen, A.; Wilson, J.S.; Pirola, R.C.; and Apte, M.V. Role of alcohol metabolism in chronic pancreatitis. Alcohol Research & Health 30(1):48–54, 2007.
Follow up Questions	How does each step in the process of metabolizing ethanol contribute to long-term alcohol diseases? Can these steps be inhibited or changed so that acetaldehyde does not damage the body?

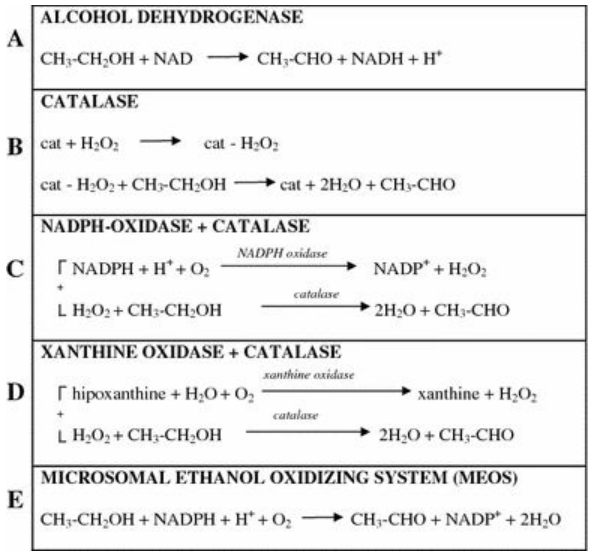
1c: Oxidative stress, metabolism of ethanol and alcohol-related diseases

09/03/2020

Source Title	Oxidative stress, metabolism of ethanol and alcohol-related diseases
Source citation	Zima, T., Fialová, L., Mestek, O., Janebová, M., Crkovská, J., Malbohan, I., Stípek, S., Mikulíková, L., & Popov, P. (2001). Oxidative stress, metabolism of ethanol and alcohol-related diseases. *Journal of Biomedical Science*, *8*(1), 59–70. https://doi.org/10.1007/BF02255972
Original URL	https://pubmed-ncbi-nlm-nih-gov.ezpxy-web-p-u01.wpi.edu/11173977/
Source type	Database: PubMed
Keywords	alcohol, antioxidant, atherosclerosis, blood vessel, body, endothelium, enzyme, ethanol, free radical, homeostasis, neutralization, nitric oxide (NO), oxidative stress, oxidized low-density lipoproteins (oxLDL)
Summary of key points (include methodology)	Oxidative stress is invoked when the body loses the natural balance of antioxidants and toxins called radicals, and therefore the radicals are not neutralized and can become harmful. Some of the enzymes that break down ethanol are alcohol dehydrogenase, microsomal ethanol oxidation system, and catalase. When they break down ethanol, they also produce many free radicals. This results in elevated levels of radicals in those who consume alcohol. There are many different radicals that have an increased concentration due to the body breaking down ethanol, and they cause the body to react in different ways. Nitric oxide (NO) is a molecule that helps regulate blood vessel wall size. When in moderation, it contributes to homeostasis, but if the levels of this radical are too high, then blood vessel walls can expand too much. If levels of NO are too high, the endothelium of vessels and platelets can be damaged. Some studies have found that when the body makes NO, it correlates with dependency on alcohol. The breakdown of ethanol also leads to higher concentrations of oxidized low-density lipoproteins (oxLDL), which builds up plaque on blood vessel walls, this study finds. Now, alcoholic patients are at a higher risk for atherosclerosis, tying heart disease to alcoholism. By understanding the increase in different free radicals, scientists can understand more about how the process of breaking down ethanol can lead to imbalance and dysfunction. Since this article included just the abstract, they did not include methodology. They mentioned testing on rats, and comparing the levels of certain molecules between groups with and without alcohol.
Research Question	How do the free radical byproducts of the body processing ethanol lead to an imbalance of antioxidants and the dysfunction of bodily processes?
Important Figures	Levels of nitrites and nitrates were increased in alcoholics: 34.3 +/- 2.6 vs. 22.7 +/- 1.2 micromol/l, $P < 0.001$ oxLDL levels were higher in alcoholics: 71.6 +/- 4.1 vs. 44.2 +/- 2.7 micromol/l, $P < 0.001$. (no tables or graphs were displayed showing data, but P-values shown)
Notes	This article begins to get more in-depth with my topic and is an interesting subject to explore.
Cited references to follow up on	https://pubmed-ncbi-nlm-nih-gov.ezpxy-web-p-u01.wpi.edu/10052604/ https://pubmed-ncbi-nlm-nih-gov.ezpxy-web-p-u01.wpi.edu/8895826/ https://pubmed-ncbi-nlm-nih-gov.ezpxy-web-p-u01.wpi.edu/15554233/
Follow up Questions	What is some more information about the enzymes that break down ethanol and the free radical byproducts? Can oxidative stress lead to all dysfunctions in the body relating to ethanol? How else is the body affected?

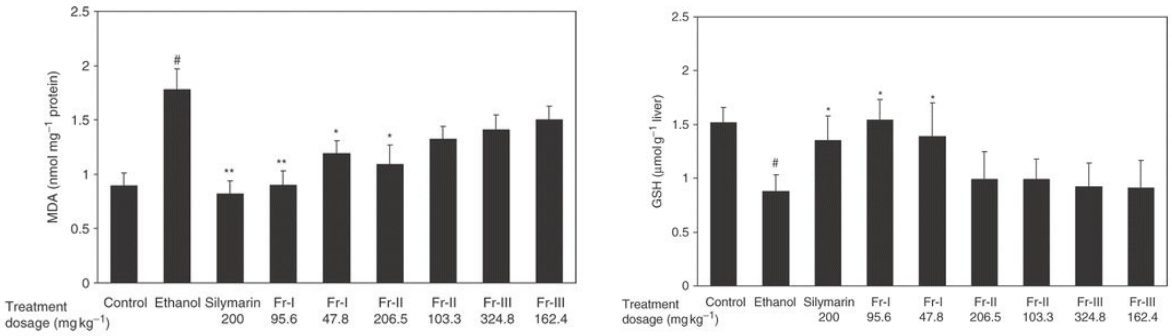
1d: Ethanol-induced oxidative stress: basic knowledge

11/09/2020

Source Title	Ethanol-induced oxidative stress: basic knowledge
Source citation	Comporti, M., Signorini, C., Leoncini, S., Gardi, C., Ciccoli, L., Giardini, A., Vecchio, D., & Arezzini, B. (2010). Ethanol-induced oxidative stress: Basic knowledge. Genes & Nutrition, 5(2), 101–109. https://doi.org/10.1007/s12263-009-0159-9
Original URL	https://link.springer.com/article/10.1007/s12263-009-0159-9#:~:text=Several%20endogenous%20radicals%20are%20known,during%20its%20metabolism%20in%20MEOS.&text=Ethanol%20oxidation%20results%20in%20the,both%20oxygen%20and%20ethanol%20itself
Source type	Database: Springer Link
Keywords	CYP2E1 isoform, ethanol metabolism, hydroxyethyl radicals, liver-free non-protein bound iron, oxidative stress, plasma isoprostanes
Summary of key points (include methodology)	Ethanol is produced in the body naturally, making it different from other hepatotoxic chemicals. However, in large amounts, it can be dangerous. In adult males, the rate of metabolism of ethanol is between 50 and 180 mg/h/kg body weight. Rats can metabolize ethanol two to three times faster. Ethanol is metabolized primarily by alcohol dehydrogenase to acetaldehyde. The acetaldehyde is broken down into acetate by aldehyde dehydrogenase. Acetaldehyde is a toxic substance that inhibits mitochondrial respiration and fatty acid oxidation (due to the inhibition of β-oxidation, citric acid cycle and oxidative phosphorylation). Acetaldehyde also impairs its own mitochondrial metabolism.
Research Question	How does oxidative stress work when induced by ethanol?
Important Figures	
Notes	This is a good article to further my understanding for the effects of acetaldehyde and how oxidative stress works
Cited references to follow up on	Cederbaum AI, Lieber CS, Rubin E (1975) Effect of acetaldehyde on fatty acid oxidation and ketogenesis by hepatic mitochondria. Arch Biochem Biophys 169:29–41
Follow up Questions	What are these processes like in different parts of the body?

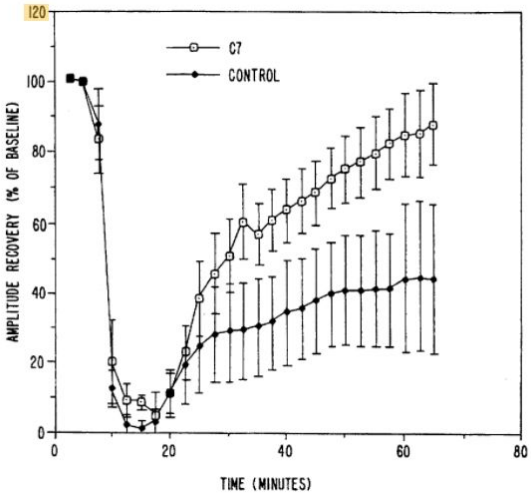
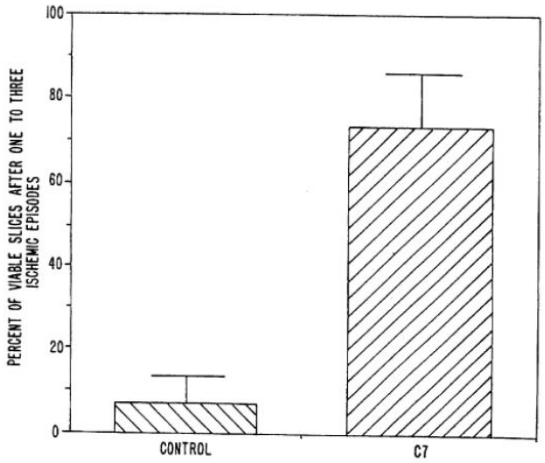
2a: The of *Antrodia camphorata* on ethanol-induced acute liver injury in rats

09/21/2020

Source Title	Further studies on the hepatoprotective effect of *Antrodia camphorata* in submerged culture on ethanol-induced acute liver injury in rats
Source citation	Lu, Z.-M., Tao, W.-Y., Xu, H.-Y., Ao, Z.-H., Zhang, X.-M., & Xu, Z.-H. (2011). Further studies on the hepatoprotective effect of Antrodia camphorata in submerged culture on ethanol-induced acute liver injury in rats. *Natural Product Research*, *25*(7), 684–695. https://doi.org/10.1080/14786410802525487
Original URL	https://www.tandfonline.com/doi/full/10.1080/14786410802525487
Source type	Journal: Taylor & Francis Online
Keywords	*Antrodia camphorata,* hepatoprotective, ethanol, silymarin, lipid peroxidation
Summary of key points (include methodology)	Alcoholic liver disease arises from reactive oxygen species-mediated oxidative stress. This process involves the breaking down of molecules that the liver needs to function. Antioxidants can negate the free radicals produced when alcohol dehydrogenase functions. *Antrodia camphorata*, native to Taiwan, may hold a property as an antioxidant. The scientists separated rats into nine groups of eight. These included control, ethanol, silymarin and ethanol, Fr-I and ethanol, Fr-II and ethanol, and Fr-III and ethanol. Fr (fractions) I-III are variations of the powdered *A. camphorata.* The purpose of the silymarin, Fr-I, Fr-II, and Fr-III was to test if they negated the effects of the alcohol. The researchers looked for fatty changes, infiltration of lymphocytes, and deformation in hepatocytes. They found that silymarin, Fr-I, and Fr-II worked the best as antioxidants, since the results were similar to the control group. They observed similar results in the Fr-III and ethanol group and the ethanol group. The scientists concluded that *A. camphorata* has hepatoprotective traits.
Research Question	Can *Antrodia camphorata* serve as an antioxidant and detoxify the liver from the free radicals produced by breaking down alcohol?
Important Figures	A pretreatment of Fr-I or silymarin worked well as an antioxidant to inhibit lipid peroxidation ($P < 0.01$), keep GSH levels low ($P < 0.05$), and increase levels of glutathione peroxidase and glutathione reductase ($P < 0.05$). A pretreatment of Fr-II also worked well to inhibit lipid peroxidation ($P < 0.05$). 
Notes	This article (patent) was about the function of a plant as an antioxidant.
Cited references to follow up on	Balkan, J, Oztezcan, S, Kucuk, M, Cevikbas, U, Kocak-Toker, N and Uysal, M. 2004. The effect of betaine treatment on triglyceride levels and oxidative stress in the liver of ethanol-treated guinea pigs. *Experimental and Toxicologic Pathology*, 55(6): 505–509. Ishii, H, Kurose, I and Kato, S. 1997. Pathogenesis of alcoholic liver disease with particular emphasis on oxidative stress. *Journal of Gastroenterology and Hepatology*, 12: 272–282.
Follow up Questions	Are there any other plants that can function as an antioxidant in this case? Are there antioxidants for parts of the body (minor) that break down alcohol other than the liver?

2b: Synthetic catalytic free radical scavengers useful as antioxidants for prevention and therapy of disease

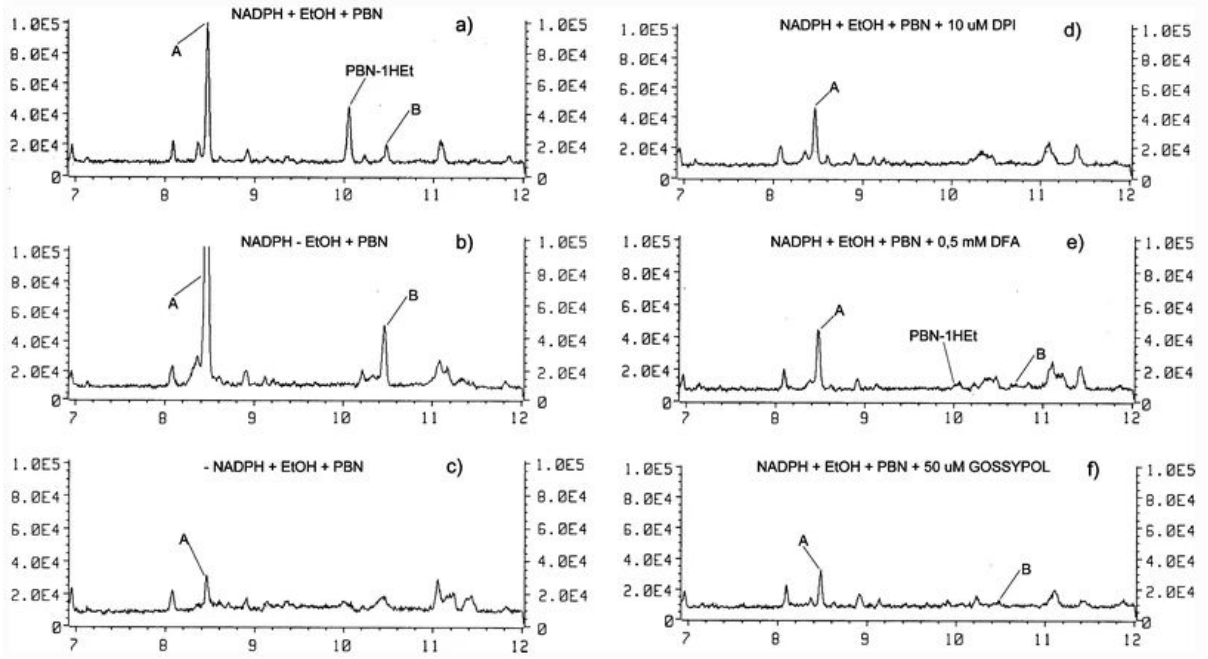
09/21/2020

Source Title	Synthetic catalytic free radical scavengers useful as antioxidants for prevention and therapy of diseases
Source citation	Malfroy-Camine, B., & Doctrow, S. R. (1997). *Synthetic catalytic free radical scavengers useful as antioxidants for prevention and therapy of disease* (United States Patent No. US5696109A). https://patents.google.com/patent/US5696109A/en
Original URL	https://patents.google.com/patent/US5696109A/en
Source type	Database: Google Scholar
Keywords	antioxidant, free radical
Summary of key points (include methodology)	Scientists created a compound to imitate antioxidants, which could help to suppress free radicals such as superoxides, catalses, and peroxides which can damage cells and tissues. The excess amount of free radicals is how many diseases form, so balancing it out with antioxidants can prevent and treat these diseases. The applications of this include preventing and treating diseases, preserving hydrocarbons, protecting tissues, and protecting cells exposed to free radicals. The scientists made many combinations of elements to form compounds, and tested them. They created some salen-Mn(III) complexes and combined Mn, Cu, and Zn with antioxidant enzymes such as SOD. The scientists administered the drugs they created orally on humans, and also on various animals. They measured heart rates, systolic pressures, diastolic pressures, and free radicals in the cell. They found that the drug they created could help prevent neuronal and other cell damage.
Research Question	Can a drug be created to prevent cell and tissue damage that leads to diseases and to act as an antioxidant against free radical byproducts?
Important Figures	 
Notes	This study discussed the development of a drug in the same topic that I am looking into (patent), but I am not sure how useful this article will be.
Cited references to follow up on	Czapski and Goldstein, "Superoxide Scavengers and Sod or Sod Mimics," in Antioxidants in Therapy and Preventive Medicine, Eds. Emerit, et al., Plenum Press, New York, pp. 45-50 (1990). Nagano, et al., "Superoxide Dismutase Mimics Based on Iron in Vivo," J. Biol. Chem., 264(16):9243-9249 (1989)
Follow up	What effects can free radicals have that this study did not encompass? What is unknown about these effects?

Questions	What enzymes produced the free radicals that the drug acted against?

2c: Oxidation of ethanol to acetaldehyde and free radicals by rat testicular microsomes

09/23/2020

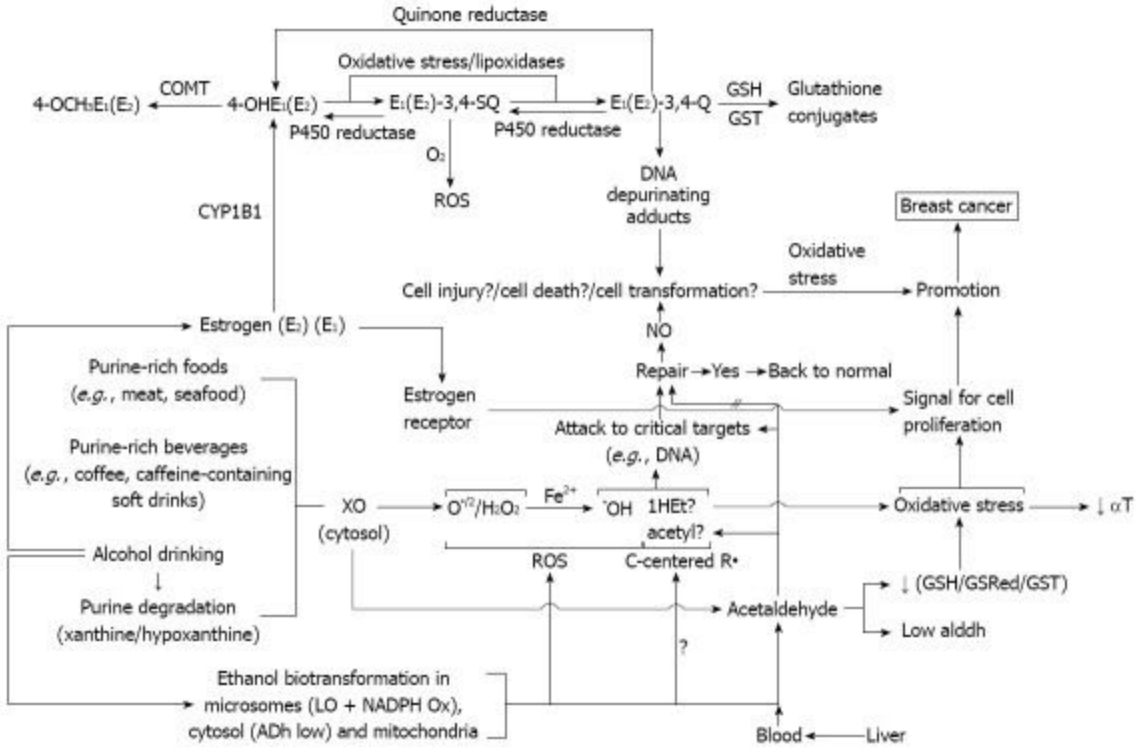
Source Title	Oxidation of ethanol to acetaldehyde and free radicals by rat testicular microsomes
Source citation	Quintans, L. N., Castro, G. D., & Castro, J. A. (2005). Oxidation of ethanol to acetaldehyde and free radicals by rat testicular microsomes. *Archives of Toxicology*, *79*(1), 25–30. https://doi.org/10.1007/s00204-004-0609-5
Original URL	https://link-springer-com.ezpxy-web-p-u01.wpi.edu/article/10.1007/s00204-004-0609-5#Abs1
Source type	Database: SpringerLink
Keywords	acetaldehyde, alcohol, ethanol, microsomes, radicals, testes, 1-hydroxyethyl
Summary of key points (include methodology)	This article explained why testosterone production is lower when more alcohol is consumed. Scientists tested the amount of acetaldehyde and 1-hydroxyethyl in centrifuged rat testes. Enzymes such as CYP2E1 and P450 reductase (lipoxygenase- or peroxidase-like behavior) break down ethanol in testes, producing acetaldehyde and the free radical 1-hydroxyethyl. 1-hydroxyethyl is also formed in the mitochondrion with NADPH. Some chemicals such as diphenyleneiodonium, gossypol, and deferoxamine were able to regulate the productions since they function as enzyme inhibitors. This study suggests that the toxic compounds acetaldehyde and 1-hydroxyethyl cause the lower level of testosterone production. They also concluded that reactive oxygen species can play a role in lowered fertility rates.
Research Question	Does the production of acetaldehyde and 1-hydroxyethyl from enzymes breaking down ethanol have an effect on the production of testosterone?
Important Figures	 Caption: Selected-ion current profile obtained from GC–MS–SIM analysis of a sample of incubation containing microsomes and PBN. a NADPH and 150 mM ethanol. Peaks: A and B, hydroxyl-derived PBN adducts, PBN-1HEt, 1-hydroxyethyl-PBN adduct. b The same as in a but in the absence of ethanol. c The same as in a but in the absence of NADPH. d The same as in a with 10 μM DPI. e The same as in a with 0.5 mM DFA. f The same as in a with 50 μM gossypol

Notes	This article helped me narrow down my research question, and brings me to questions that I would like to look into next. I'm sending an email to Gerardo D. Castro.
Cited references to follow up on	Degen GS, Vogel C, Abel J (2002) Prostaglandin synthases. In: Ioannides C (ed) Enzyme systems that metabolise drugs and other xenobiotics. Wiley, Chichester, pp 189–229 Díaz Gómez MI, Fanelli SL, Castro GD, Costantini MH, Castro JA (1999) A liver nuclear ethanol metabolizing system. Formation of metabolites that bind covalently to macromolecules and lipids. Toxicology 138:19–28
Follow up Questions	How does consumption of ethanol by the parents (production of sex cells or gestation) affect the offspring? Is the offspring deformed in any way?

2d: Alcohol drinking and mammary cancer

09/28/2020

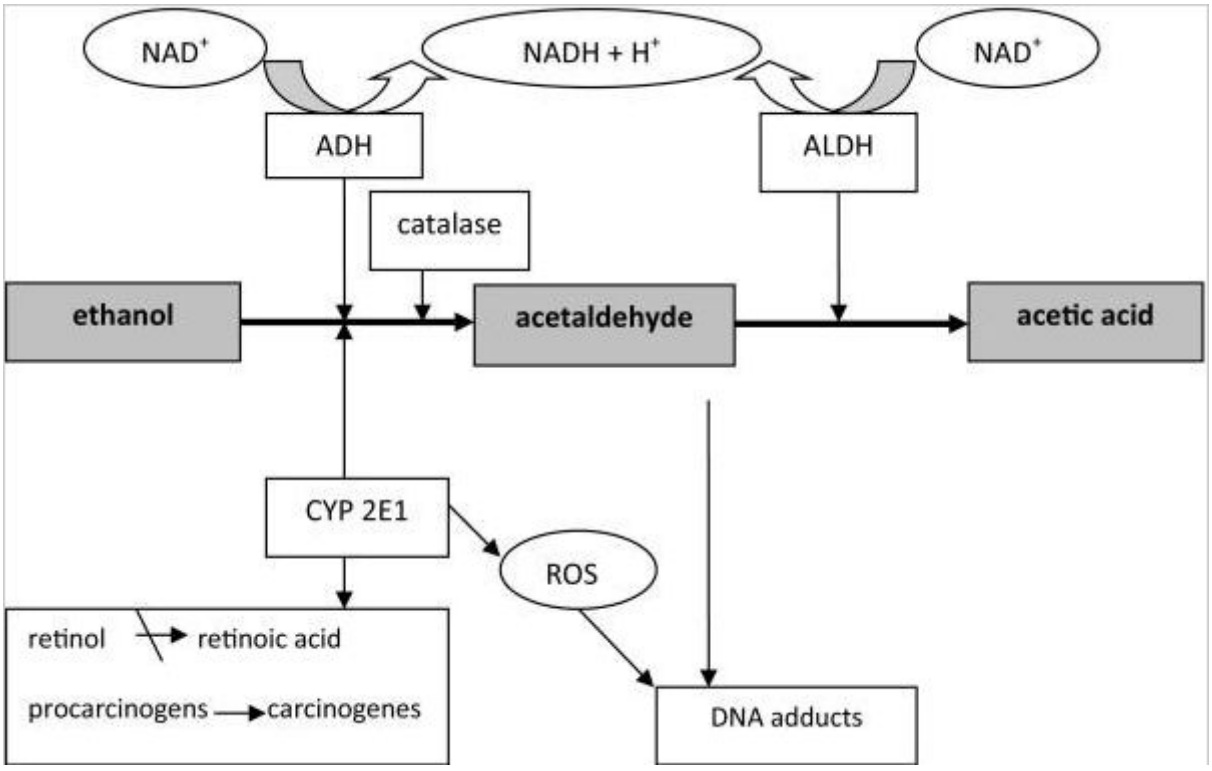
Source Title	Alcohol drinking and mammary cancer: Pathogenesis and potential dietary preventative alternatives
Source citation	Castro, G. D., & Castro, J. A. (2014). Alcohol drinking and mammary cancer: Pathogenesis and potential dietary preventive alternatives. *World Journal of Clinical Oncology*, *5*(4), 713–729. https://doi.org/10.5306/wjco.v5.i4.713
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4129535/
Source type	Database: NCBI
Keywords	acetaldehyde, alcohol, estrogens, ethanol, free radicals, mammary cancer, oxidative stress, polyphenols
Summary of key points (include methodology)	Introduction: In the past, scientists have found a correlation between alcohol consumption and the development of breast cancer. This correlation was not associated with other potential risk factors. In addition to this, it is known that estrogen levels can increase after drinking alcohol. The estrogen may act as a carcinogen. Methodology: (No section on methodology in the article; throughout the article, the researchers mentioned some details, mainly about rat mammary tissue.) Rat mammary tissue was observed for… - Acetaldehyde - researchers looked for cytosolic fractions (xanthine oxidoreductase) and microsomes (1-hydroxyethyl radicals) - Free radicals - researchers looked xanthine oxidoreductase and lipoxygenase - Estrogen - rats treated with 4-hydroxyestradiol and estrogen quinones, human breast tumors were examined with LC/MS-MS procedures Conclusion: When ethanol is broken down in the mammary glands, more acetaldehyde, free radicals, and estrogen are produced. They accumulate there, promoting poor detoxifying methods and increasing oxidative stress. This creates a higher risk for cancerous cells to develop.
Research Question	How does the metabolism of ethanol increase the risk of mammary cancer?

Important Figures	
Notes	Gerardo D. Castro is also an author here.
Cited references to follow up on	Castro GD, Castro JA. Metabolism of ethanol to acetaldehyde in the rat mammary tissue. Inhibitory effects of plant polyphenols and folic acid. In: Watson RR, Preedy VR, Zibadi S, editors. Alcohol, Nutrition and Health Consequences. Nutrition and Health. New York: Springer Science Business Media; 2013. pp. 145–154. Castro GD, Quintans LN, Maciel ME, Castro JA. Preventive effects of plant polyphenols in the promotion of mammary cancer and testicular damage induced by alcohol drinking. In: Watson RR, Preedy VR, Zibadi S, editors. Polyphenols in Human Health and Disease. San Diego: Elsevier-Academic Press; 2014. pp. 1181–1190.
Follow up Questions	Does the metabolism of ethanol also relate to other types of cancers? Similar to estrogen, what are some other imbalances of hormones during alcohol metabolism that can lead to diseases or oxidative stress?

2e: Alcohol dehydrogenase and aldehyde dehydrogenase in the cancer diseases

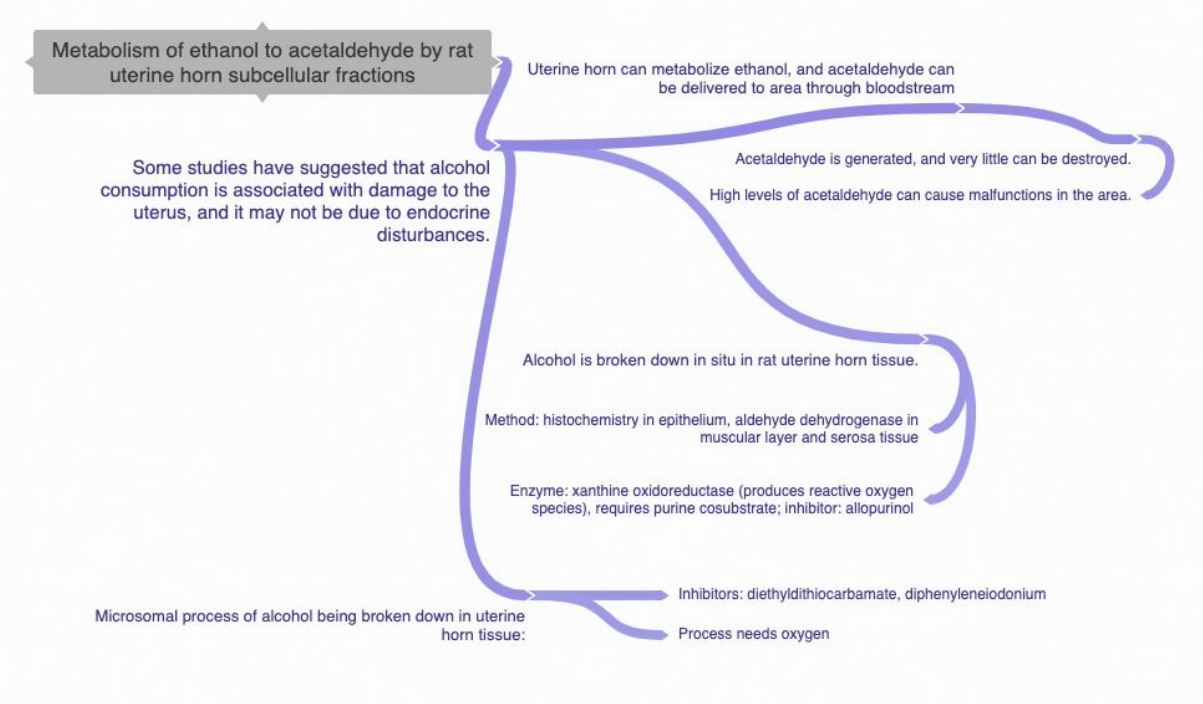
09/28/2020

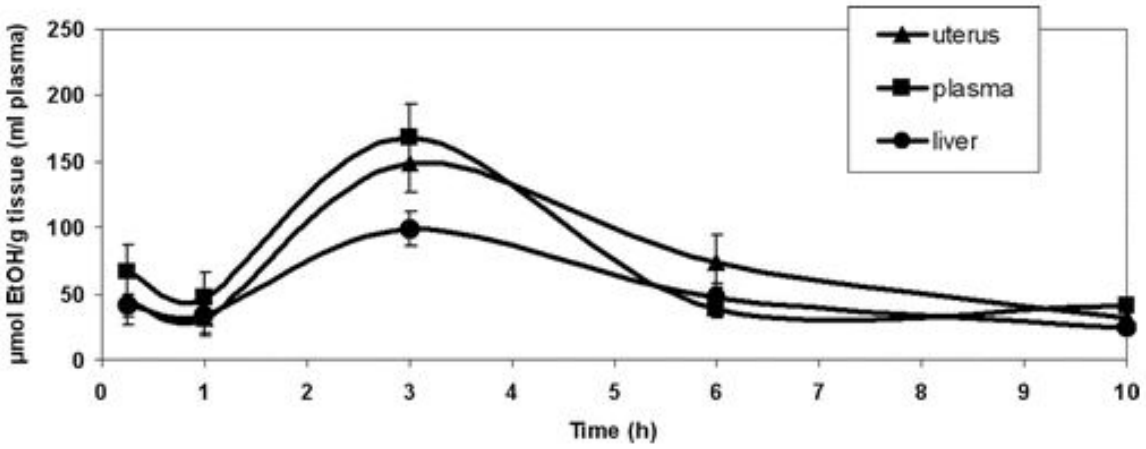
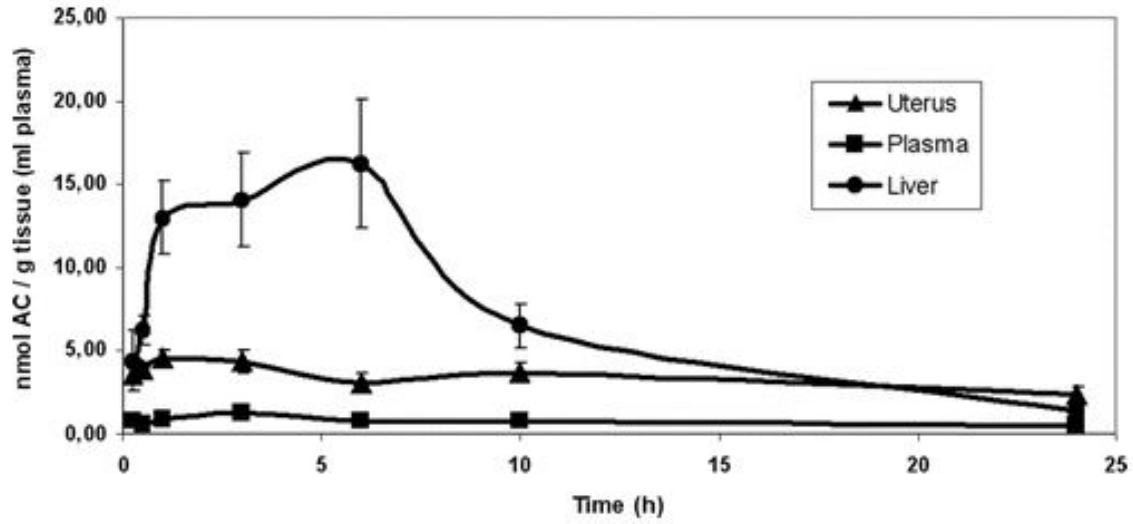
Source Title	Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the cancer diseases
Source citation	Jelski, W., & Szmitkowski, M. (2008). Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the cancer diseases. Clinica Chimica Acta; International Journal of Clinical Chemistry, 395(1–2), 1–5. https://doi.org/10.1016/j.cca.2008.05.001
Original URL	https://www.sciencedirect.com/science/article/pii/S0009898108002258?via%3Dihub

Source type	Database: Science Direct
Keywords	alcohol dehydrogenase, aldehyde dehydrogenase, cancer
Summary of key points (include methodology)	When ethanol is metabolized, it is first broken down by alcohol dehydrogenase to acetaldehyde. The enzyme that breaks down the acetaldehyde into less toxic substances is called aldehyde. There are fewer aldehyde enzymes than alcohol dehydrogenase, so when ethanol is metabolized, there is a lot of toxic acetaldehyde waiting to be broken down further. The toxic effects of acetaldehyde have been found to be correlated with a high risk of cancer. The scientists found that cancerous tissues have higher alcohol dehydrogenase activity than healthy organs, and therefore the activity of alcohol dehydrogenase is higher than the activity of aldehyde dehydrogenase. Therefore, levels of acetaldehyde are increased. This suggests that cancerous cells can metabolize alcohol faster, but not acetaldehyde. This may be useful in the diagnosis of cancer.
Research Question	How is ethanol metabolism different in cancerous tissue than in healthy tissue?
Important Figures	
Notes	This article tells more about the effect of the metabolism of ethanol on cancer/disease.
Cited references to follow up on	J.O. Höög, S. Svensson Mammalian class II alcohol dehydrogenase. A highly variable enzyme Adv Exp Med Biol, 414 (1997), pp. 303-311 J.O. Höög, J.J. Hedberg, P. Stromberg, S. Svensson Mammalian alcohol dehydrogenases — functional and structural implications J Biomed Sci, 8 (2001), pp. 71-76
Follow up Questions	Can the activity of alcohol dehydrogenase be lowered in cancerous tissue? What is the activity of alcohol dehydrogenase like in tissues with other diseases?

2f: Metabolism of ethanol to acetaldehyde by rat uterine horn subcellular fractions

10/06/2020

Source Title	Metabolism of ethanol to acetaldehyde by rat uterine horn subcellular fractions
Source citation	Buthet, L. R., Bietto, F. M., Castro, J. A., & Castro, G. D. (2011). Metabolism of ethanol to acetaldehyde by rat uterine horn subcellular fractions. Human & Experimental Toxicology, 30(11), 1785–1794. https://doi.org/10.1177/0960327110396537
Original URL	https://journals.sagepub.com/doi/10.1177/0960327110396537?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed
Source type	Database: SAGE Journals
Keywords	uterus, ethanol, alcohol, acetaldehyde, reproductive toxicity
Summary of key points (include methodology)	
Research Question	How does the metabolism of ethanol and the production of acetaldehyde affect the functionality of the uterine horn in female rats?

Important Figures	 Shows ethanol levels in uterine horn tissue, plasma, and liver; after single dosage of 3.8 g/kg alcohol  Shows acetaldehyde levels in uterine horn tissue, plasma, and liver; after single dosage of 3.8 g/kg alcohol
Notes	The research question is similar to my research question and it focuses on analyzing the uterine horn in rats.
Cited references to follow up on	Fernandez-Solá, J, Nicolás, JM, Estruch, R, Urbano-Márquez, A. Gender differences in alcohol pathology. In: Preedy, VR, Watson, RR (eds) Comprehensive handbook of alcohol related pathology, Volume 1 London: Elsevier Science Ltd-Academic Press 2005, p.261–278. Emanuele, MA, Wezeman, F, Emanuele, NV. Alcohol's effects on female reproductive function. Alcohol Res Health 2002; 26: 274–281.
Follow up Questions	How are other parts of the female reproductive system affected by the metabolism of ethanol? How does oxidative stress and the free radicals produced affect the uterine horn?

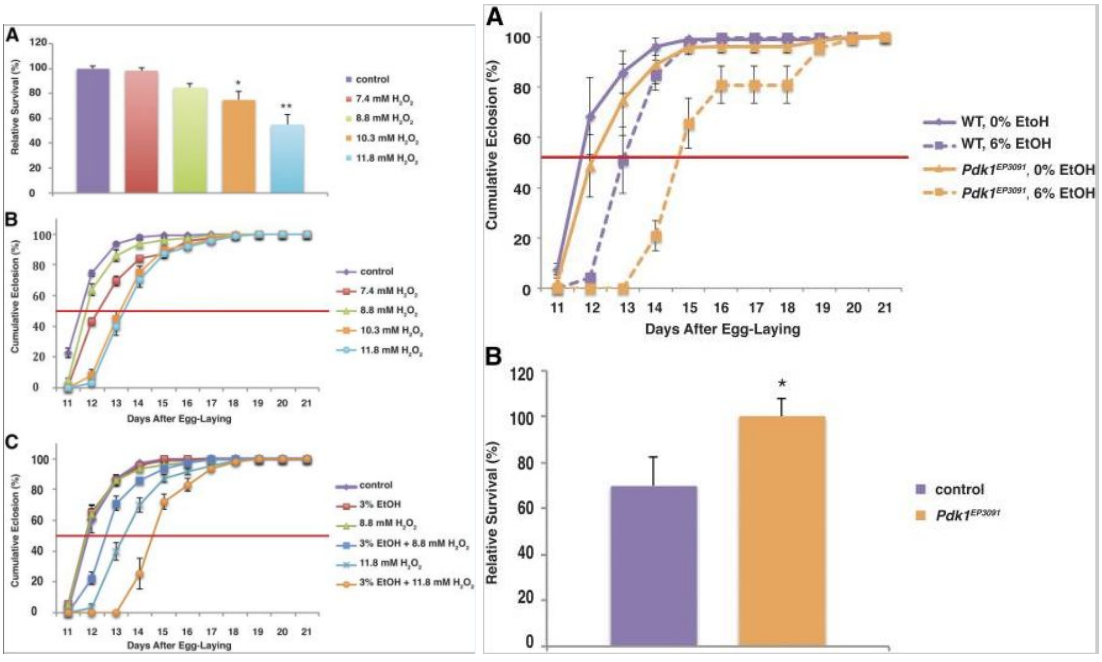
2g: Metabolism of ethanol could play a role in the ovarian tissue cell injury

10/16/2020

Source Title	Metabolism of ethanol to acetaldehyde and increased susceptibility to oxidative stress could play a role in the ovarian tissue cell injury promoted by alcohol drinking
Source citation	Faut, M., Rodríguez de Castro, C., Bietto, F. M., Castro, J. A., & Castro, G. D. (2009). Metabolism of ethanol to acetaldehyde and increased susceptibility to oxidative stress could play a role in the ovarian tissue cell injury promoted by alcohol drinking. Toxicology and Industrial Health, 25(8), 525–538. https://doi.org/10.1177/0748233709345937
Original URL	https://journals.sagepub.com/doi/abs/10.1177/0748233709345937
Source type	Database: SAGE Publications
Keywords	acetaldehyde, alcohol, ethanol, ovary, reproductive toxicity
Summary of key points (include methodology)	Studies have shown that alcohol can affect the reproductive system in women. This study found that ethanol oxidation in situ can cause it to malfunction. The enzymatic process required NADPH, was sensitive to oxygen, and was inhibited by sodium diethyldithiocarbamate, 4-methylpyrazole, and diphenyleneiodonium. They found that aldehyde dehydrogenase functioned when there was no alcohol dehydrogenase detected. There was some metabolism of ethanol in the ovaries of rats, but it was mainly in the liver. When they exposed the rats to ethanol multiple times, the activity of alcohol dehydrogenase (not in the cytosolic fraction) and t-butyl hydroperoxide-promoted chemiluminescence were increased. The scientists also found differences in the structure of the ovary, particularly a dilation of the endoplasmic reticulum and mitochondria. They also found broken or absent cell processes. This could all explain why the ovary function was decreased with an increase of the metabolism of ethanol.
Research Question	How does the metabolism of ethanol change the functionality of the ovaries in rats?
Important Figures	Just the abstract was included, no figures.
Notes	This is another article that was about the effect of the metabolism of ethanol on the reproductive system with a specific example of studying the ovaries in this case.
Cited references to follow up on	Davis BJ , Heindel JJ (1998) Ovarian toxicants: multiple mechanisms of action . In: Korach KS (ed.) Reproductive and Developmental Toxicology. New York: Marcel Dekker, pp.373-396. Díaz Gómez MI , Tamayo D. , and Castro JA (1988) Nitrosodimethylamine metabolism in rat ovaries. Interactions of its metabolites with nucleic acids and proteins. Cancer Letters 41: 257-263. Mendelson JH , Mello NK (1988) Chronic alcohol effects on anterior pituitary and ovarian hormones in healthy women. Journal of Pharmacology and Experimental Therapeutics 245: 407-412.
Follow up Questions	What other parts of the reproductive system does the metabolism of ethanol affect? Does it change the tissue similarly to how it was found to change the ovaries?

3a: Developmental Ethanol Exposure Leads to Oxidative Stress in *Drosophila*

10/08/2020

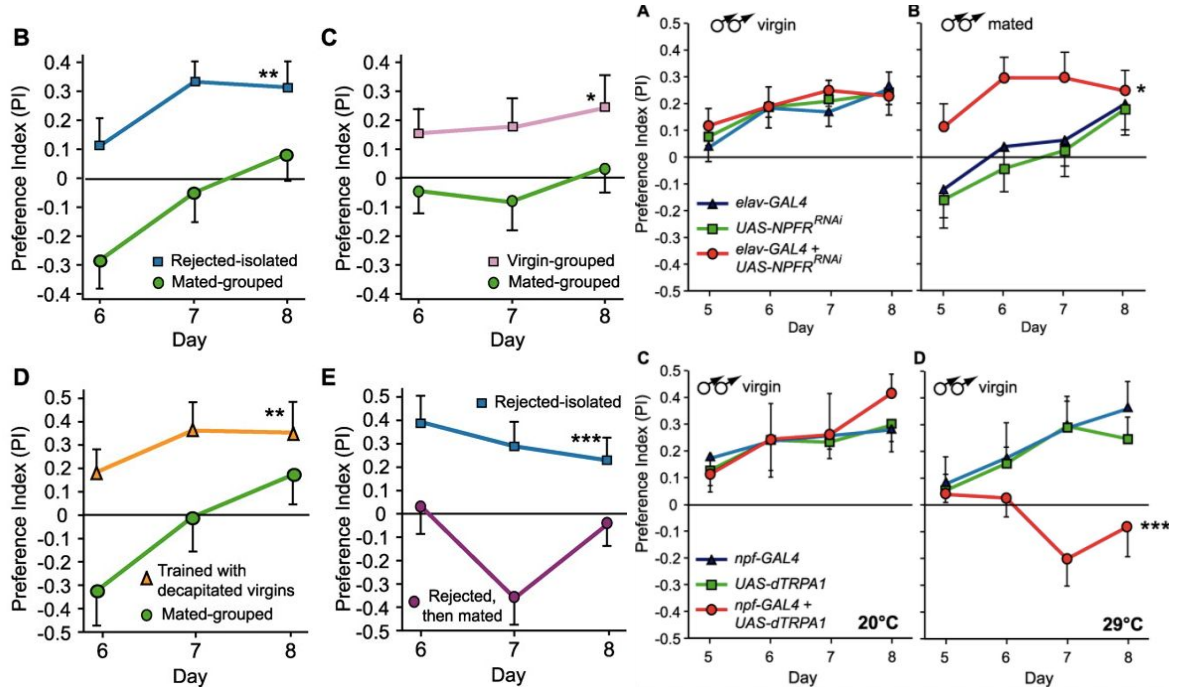
Source Title	Developmental Ethanol Exposure Leads to Dysregulation of Lipid Metabolism and Oxidative Stress in *Drosophila*
Source citation	Logan-Garbisch, T., Bortolazzo, A., Luu, P., Ford, A., Do, D., Khodabakhshi, P., & French, R. L. (2014). Developmental Ethanol Exposure Leads to Dysregulation of Lipid Metabolism and Oxidative Stress in Drosophila. *G3: Genes|Genomes|Genetics*, *5*(1), 49–59. https://doi.org/10.1534/g3.114.015040
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4291469/
Source type	Database: NCBI
Keywords	fetal alcohol syndrome, reactive oxygen species, lipid accumulation, withered, carnitine transporter
Summary of key points (include methodology)	Scientists developed a model of fetal alcohol effects (FAE) or fetal alcohol spectrum disorder (FASD) using *Drosophila melanogaster.* They had already found that developmental ethanol exposure leads to the inhibition of insulin-like peptides (dILPs). In this study, they found that developmental ethanol exposure leads to the dysregulation of lipid metabolism. The unusual process of fatty acid metabolism then leads to oxidative stress, a cause of many developmental issues. They added various levels of H_2O_2 to invoke oxidative stress, which reduced survival rate and eclosion time. The scientists also altered some genes. With a mutation of Pdk1 and ethanol exposure compared to just ethanol exposure, it took longer for development, but there was a higher survival rate. There were no effects of ethanol on the developmental stage. Methodology: - Fly stocks kept at 25℃, fed cornmeal/molasses medium, alleles and transgenes introgressed for 5 generations - Egg collections were kept for 16-20 hrs with fly food, then transferred to vials with ethanol (and exposed to ethanol bath, 3-8% (matches concentration in food)), peroxide, or control food - Adult flies counted after 9-21 days of egg laying, survival rate
Research Question	How does developmental ethanol exposure affect lipid metabolism and oxidative stress?
Important Figures	

	Quantitation of development time for data in Figure 2 **Condition** | **Time to Median Eclosion (d)** | **Difference (d)** control | 11.67 ± 0.08 | N/A 3% ethanol | 11.75 ± 0.05 | +0.08 (not significant) 7.4 mM H2O2 | 12.28 ± 0.08 | +0.61 ($P < 0.01$) 8.8 mM H2O2 | 11.78 ± 0.06 | +0.11 (not significant) 3% ethanol + 8.8 mM H2O2 | 12.57 ± 0.09 | +0.9 ($P < 0.01$, all comparisons) 10.3 mM H2O2 | 13.15 ± 0.14 | +1.48 ($P < 0.01$) 11.8 mM H2O2 | 13.33 ± 0.17 | +1.66 ($P < 0.01$) 3% ethanol + 11.8 mM H2O2 | 14.48 ± 0.17 | +2.81 ($P < 0.01$, all comparisons)
Notes	The methodology has important information for using a *Drosophila* model.
Cited references to follow up on	Broughton S. J., Piper M. D., Ikeya T., Bass T. M., Jacobson J., et al. , 2005. Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. USA 102: 3105–3110. McClure K. D., French R. L., Heberlein U., 2011. A Drosophila model for fetal alcohol syndrome disorders: role for the insulin pathway. Dis. Model. Mech. 4: 335–346.
Follow up Questions	How does ethanol metabolism relate to the metabolism of other substances besides lipids? Does ethanol metabolism after the fetal stage also dysregulate the metabolism of lipids?

3b: Sexual Deprivation Increases Ethanol Intake in *Drosophila*

10/16/2020

Source Title	Sexual Deprivation Increases Ethanol Intake in *Drosophila*
Source citation	Shohat-Ophir, G., Kaun, K. R., Azanchi, R., Mohammed, H., & Heberlein, U. (2012). Sexual Deprivation Increases Ethanol Intake in Drosophila. Science, 335(6074), 1351–1355. https://doi.org/10.1126/science.1215932
Original URL	https://science.sciencemag.org/content/335/6074/1351.full
Source type	Database: Science
Keywords	alcohol, drosophila, ethanol, neuropeptide F (NPF)
Summary of key points (include methodology)	Scientists found that the inhibition of the neuropeptide F (NPF) system increased the intake in alcohol for male *Drosophila*. This can be generalized to find a connection between physical activity, sexual experience, and the NPF system activity.

	They used a model with *Drosophila melanogaster*. The model had two groups of male flies. The first were sexually rejected by female flies which had already mated (3 times a day for 4 days). The other male flies experienced sexual activity (6 hours a day for 4 days, ratio of male to virgin female was 1:5). The scientists later manipulated the genes of flies and used artificial simulation of NPF neurons. The male flies from both groups were allowed to voluntarily choose food with 15% ethanol or none for 4 days.
Research Question	How does the sexual activity of *Drosophila* affect voluntary ethanol intake?
Important Figures	
Notes	This article had a lot of good information about methodology while using *Drosophila.* The scientists ran many trials with different methods. This article may be good to read again if these methods need to be referenced.
Cited references to follow up on	A. V. Devineni, U. Heberlein, Preferential ethanol consumption in Drosophila models features of addiction. Curr. Biol. 19, 2126 (2009). doi:10.1016/j.cub.2009.10.070 pmid:20005106 J. C. Billeter, J. Atallah, J. J. Krupp, J. G. Millar, J. D. Levine, Specialized cells tag sexual and species identity in Drosophila melanogaster. Nature 461, 987 (2009). doi:10.1038/nature08495 pmid:19829381
Follow up Questions	How well do *Drosophila melanogaster* represent the human reproductive system (is this a way for the results to be generalized)?

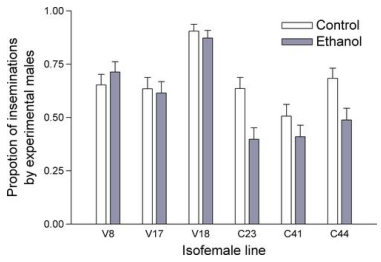
3c: A comment on "Sexual Deprivation Increases Ethanol Intake in *Drosophila"*

12/12/2020

Source Title	Male sexual behaviour and ethanol consumption from an evolutionary perspective: A comment on "Sexual Deprivation Increases Ethanol Intake in *Drosophila"*
Source citation	Guevara-Fiore, P., & Endler, J. A. (2015). Male sexual behaviour and ethanol consumption from an evolutionary perspective: A comment on "Sexual Deprivation Increases Ethanol Intake in Drosophila." Fly, 8(4), 234–236. https://doi.org/10.1080/19336934.2015.1045694
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4594573/
Source type	Database: NCBI
Keywords	alcohol, ethanol, NPF system, sexual behaviour, sexual deprivation
Summary of key points (include methodology)	This article explains the applications from the previous article in a broader way. The previous article connected their findings to the neuropeptide F system (physiological). This article gives an ecological and an evolutionary perspective instead. *Drosophila* encounter ethanol in nature often and are attracted to it. Low concentrations of ethanol can be beneficial for higher calorie intakes and can protect against parasites. Thus, the neuropeptide F system has evolved. In nature, the concentration of ethanol is 6%, but in the previous article, 15% was used. However, *Drosophila* are capable of managing how much ethanol they consume. It can be easy to associate the findings from the previous article with humans, but the ecological and evolutionary aspects of both species in this regard is different, so that should be taken into consideration.
Research Question	How does the ecological and evolutionary aspect of *Drosophila* affect the connection to humans in "Sexual Deprivation Increases Ethanol Intake in *Drosophila"*?
Important Figures	None, since this article was a discussion.
Notes	Gives a different perspective of the previous article, has important information for connecting the results with *Drosophila* in this subject area with humans.
Cited references to follow up on	Hewes RS, Taghert PH. Neuropeptides and neuropeptide receptors in the *Drosophila melanogaster* genome. Genome Research 2001; 11:1126-42. Fujita M, Tanimura T. *Drosophila* Evaluates and Learns the Nutritional Value of Sugars. Current Biology 2011; 21:751-5.
Follow up Questions	How might ethanol affect the mating behavior of female *Drosophila?* What other factors are important to consider when connecting results with *Drosophila* back to humans?

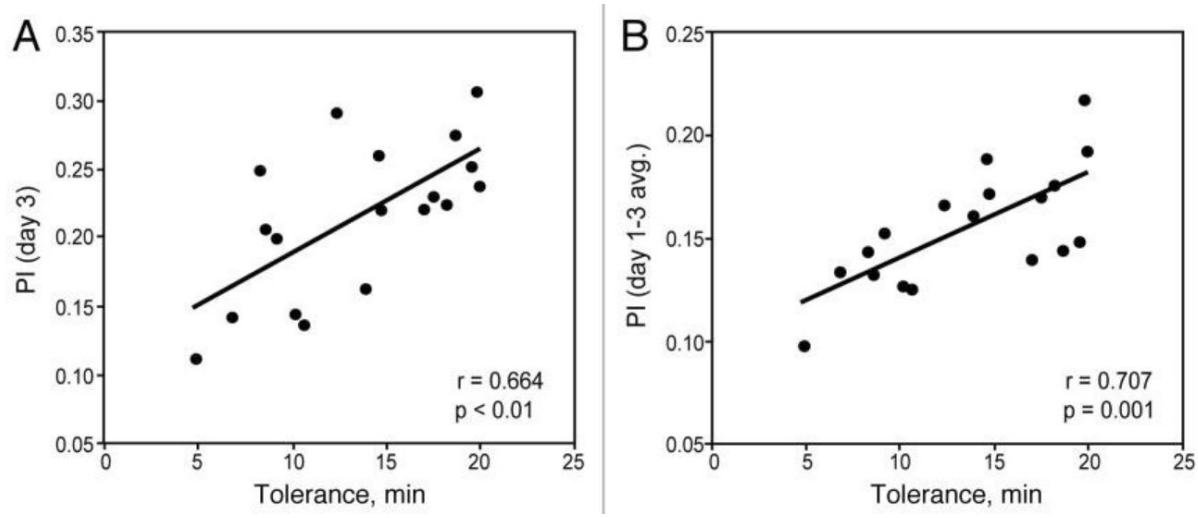
3d: Effects of a low dose of ethanol on mating success of *Drosophila* males

12/12/2020

Source Title	Effects of a low dose of ethanol on mating success of *Drosophila melanogaster* males: implications for the evolution of ethanol resistance?
Source citation	Zhu, J., & Fry, J. D. (2018). Effects of a low dose of ethanol on mating success of Drosophila melanogaster males: Implications for the evolution of ethanol resistance? Entomologia Experimentalis et Applicata, 166(10), 801–809. https://doi.org/10.1111/eea.12714
Original URL	https://onlinelibrary-wiley-com.ezpxy-web-p-u01.wpi.edu/doi/full/10.1111/eea.12714
Source type	Database: Wiley Library
Keywords	adaptation, courtship, genetic variation, geographic variation, sexual selection, sub-lethal effects, toxin resistance, Diptera, *Drosophilidae*
Summary of key points (include methodology)	As discussed in the previous article, *Drosophila* have adapted to benefit from ethanol and have a high ethanol resistance. The scientists in this article studied the effects of ethanol on flies from temperate climates and non-temperate climates. They discovered that the flies from the temperate climates were not equipped to live in an environment with high ethanol concentrations. This may be because in temperate climates, there is less ethanol found naturally that is accessible to *Drosophila.*
Research Question	How does the climate in which species of *Drosophila* have evolved affect their tolerance to ethanol?
Important Figures	 Caption: "Mean (+ SE) male mating success of Drosophila melanogaster isofemale lines from Vienna (V8, V17, and V18) and Cameroon (C23, C41, and C44), in the presence or absence of a non-lethal concentration of ethanol. Males from each line competed for mating with males from a genetically marked stock."
Notes	This study used 0%, 4%, and 8% ethanol concentration in *Drosophila* medium and also used ethanol vapor.
Cited references to follow up on	Devineni AV & Heberlein U (2012) Acute ethanol responses inDrosophilaare sexually dimorphic. Proceedings of theNational Academy of Sciences of the USA 109: 21087–21092. Fry JD (2014) Mechanisms of naturally evolved ethanol resistanceinDrosophila melanogaster. Journal of Experimental Biology217: 3996–4003.
Follow up Questions	What are the differences between the effects of ethanol in medium and ethanol vapor? What other differences are there between *Drosophila* of different climates?

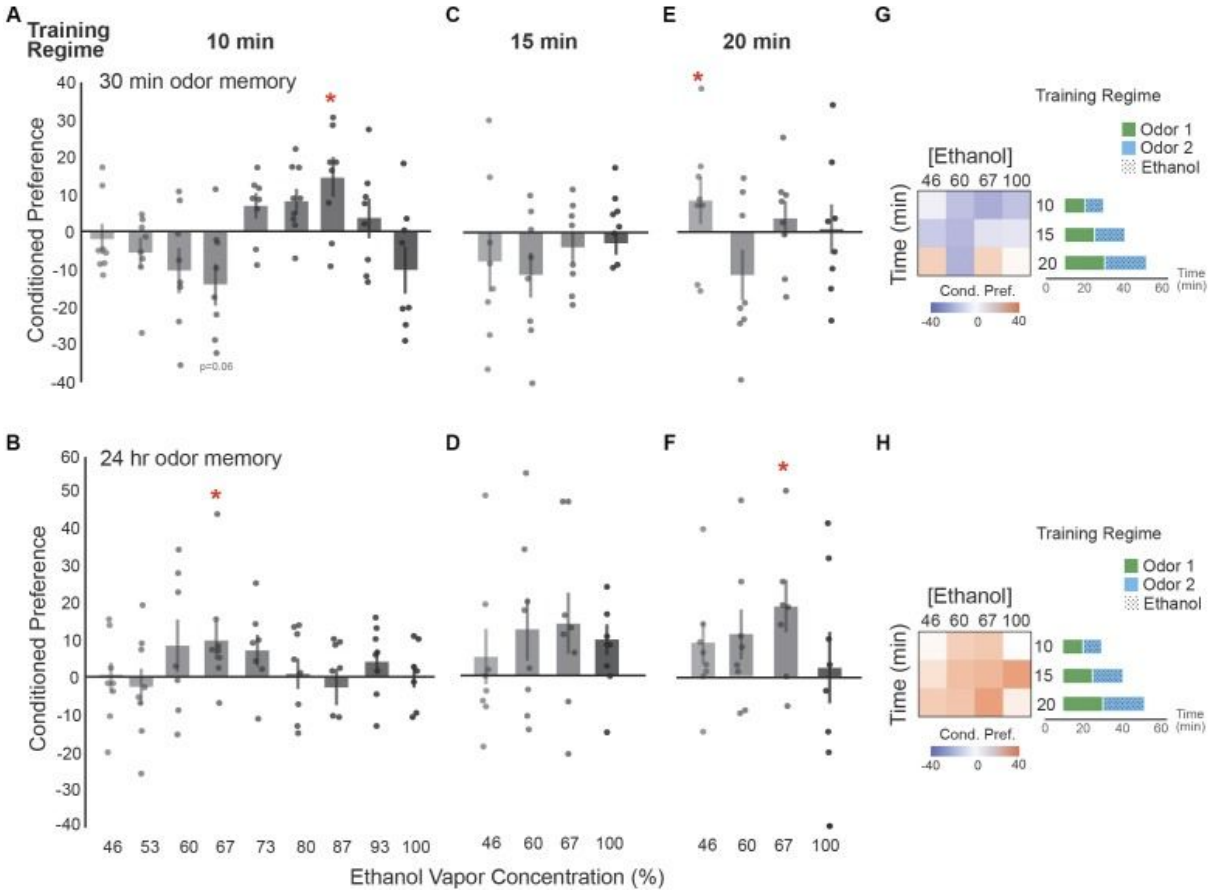
3e: The genetic relationships between ethanol preference, acute ethanol sensitivity and ethanol tolerance in *Drosophila melanogaster*

12/12/2020

Source Title	The genetic relationships between ethanol preference, acute ethanol sensitivity and ethanol tolerance in *Drosophila melanogaster*
Source citation	Devineni, A. V., McClure, K. D., Guarnieri, D. J., Corl, A. B., Wolf, F. W., Eddison, M., & Heberlein, U. (2011). The genetic relationships between ethanol preference, acute ethanol sensitivity and ethanol tolerance in Drosophila melanogaster. Fly, 5(3), 191–199. https://doi.org/10.4161/fly.5.3.16987
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3225762/
Source type	Database: NCBI
Keywords	ethanol consumption, ethanol sensitivity, tolerance, correlation, *Drosophila*
Summary of key points (include methodology)	The scientists studied the ethanol-induced hyperactivity, ethanol sedation, sedation tolerance and ethanol consumption preference of *Drosophila*. They discovered that ethanol preference and ethanol tolerance had a strong relationship. However, there were no relationships found with the other factors.
Research Question	How are ethanol-induced hyperactivity, ethanol sedation, ethanol tolerance, and ethanol consumption related in *Drosophila melanogaster* and in humans?
Important Figures	
Notes	This article described some important factors of *Drosophila* to consider when giving them ethanol.
Cited references to follow up on	Moore MS, DeZazzo J, Luk AY, Tully T, Singh CM, Heberlein U. Ethanol intoxication in Drosophila: genetic and pharmacological evidence for regulation by the cAMP signaling pathway. Cell. 1998;93:997–1007. Le AD, Kiianmaa K. Characteristics of ethanol tolerance in alcohol drinking (AA) and alcohol avoiding (ANA) rats. Psychopharmacology (Berl) 1988;94:479–483
Follow up Questions	How do the factors described relate to the reproductive system specifically? What other factors may influence the results of experimenting with *Drosophila* and ethanol?

3f: Cue-Induced Ethanol Seeking in *Drosophila melanogaster* Is Dose-Dependent

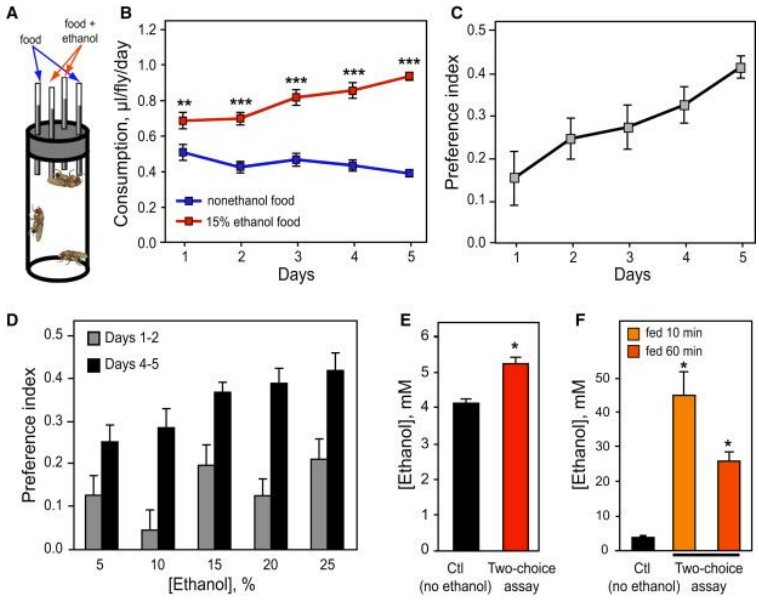
12/12/2020

Source Title	Cue-Induced Ethanol Seeking in *Drosophila melanogaste*r Is Dose-Dependent
Source citation	Nunez, K. M., Azanchi, R., & Kaun, K. R. (2018). Cue-Induced Ethanol Seeking in Drosophila melanogaster Is Dose-Dependent. Frontiers in Physiology, 9. https://doi.org/10.3389/fphys.2018.00438
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5925608/
Source type	Database: NCBI
Keywords	*Drosophila*, ethanol, alcohol-use disorder, memory, addiction, reward
Summary of key points (include methodology)	Researchers discovered that the amount of ethanol, the amount of time of exposure, the number of exposures, and the amount of time between each exposure determine whether the flies view ethanol positively or negatively. It also determines how long the flies remember a certain scent. The study found that *Drosophila melanogaster* have olfactory memory of ethanol intoxication.
Research Question	How does ethanol affect the memory of *Drosophila melanogaster?*
Important Figures	 Ethanol preference is dose-dependent
Notes	This experiment used various doses of ethanol vapor (46%, 53%, 60%, 67%, 73%, 80%, 87%, 93%, and 100%).
Cited references to	Heberlein U., Wolf F. W., Rothenfluh A., Guarnieri D. J. (2004). Molecular genetic analysis of ethanol intoxication in *Drosophila melanogaster*. *Integr. Comp. Biol.* 44 269–274. 10.1093/icb/44.4.269

follow up on	Carrara-Nascimento P. F., Olive M. F., Camarini R. (2014). Ethanol pre-exposure during adolescence or adulthood increases ethanol intake but ethanol-induced conditioned place preference is enhanced only when pre-exposure occurs in adolescence. Dev. Psychobiol. 56 36–48. 10.1002/dev.21089
Follow up Questions	How would this experiment have been different if the ethanol was in the medium rather than in vapor? How does the olfactory memory carry over longer periods of time, such as days or weeks?

3g: Preferential Ethanol Consumption in *Drosophila* Models Features of Addiction

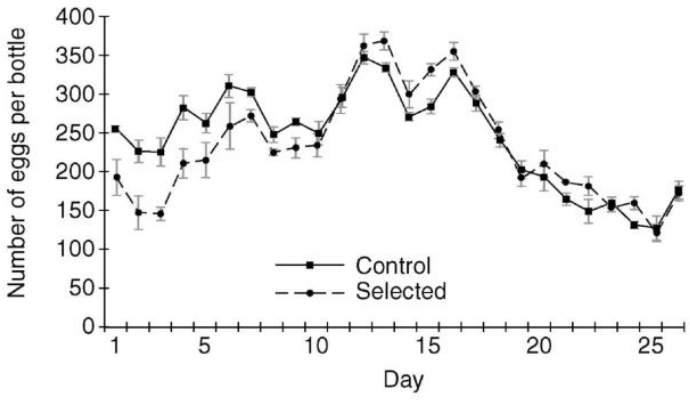
12/12/2020

Source Title	Preferential Ethanol Consumption in *Drosophila* Models Features of Addiction
Source citation	Devineni, A. V., & Heberlein, U. (2009). Preferential Ethanol Consumption in Drosophila Models Features of Addiction. Current Biology, 19(24), 2126–2132. https://doi.org/10.1016/j.cub.2009.10.070
Original URL	https://www.cell.com/current-biology/fulltext/S0960-9822(09)01942-3?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0960982209019423%3Fshowall%3Dtrue
Source type	Website: www.cell.com
Keywords	addiction, *Drosophila,* ethanol
Summary of key points (include methodology)	The scientists in this study discovered that *Drosophila* can show aspects of alcohol addiction, showing that they may be used to model humans in this regard. They found that the flies overcome obstacles to reach the ethanol and that they consume it after abstaining from it for some time. This may be due to olfactory or caloric attraction. Interestingly, *Drosophila* are not attracted to the taste of ethanol but are nevertheless attracted to it.
Research Question	How do *Drosophila melanogaster* act with ethanol?
Important Figures	

Notes	This article described how fruit flies react to ethanol, which is important to consider when experimenting. For my experiment, these findings are beneficial since it may make it more applicable to humans.
Cited references to follow up on	Berger K.H., Kong E.C., Dubnau J., Tully T., Moore M.S., Heberlein U. Ethanol sensitivity and tolerance in long-term memory mutants of *Drosophila melanogaster*. Alcohol. Clin. Exp. Res. 2008; 32: 895-908
Follow up Questions	What effects do these findings have on experimenting with *Drosophila?* How is ethanol addiction different in humans and in *Drosophila?*

3h: Environmental stress and reproduction in *Drosophila melanogaster*

12/12/2020

Source Title	Environmental stress and reproduction in *Drosophila melanogaster*: starvation resistance, ovariole numbers and early age egg production
Source citation	Wayne, M. L., Soundararajan, U., & Harshman, L. G. (2006). Environmental stress and reproduction in Drosophila melanogaster: Starvation resistance, ovariole numbers and early age egg production. BMC Evolutionary Biology, 6(1), 57. https://doi.org/10.1186/1471-2148-6-57
Original URL	https://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-6-57
Source type	Database: BMC Evolutionary Biology
Keywords	control line, wing length, selection experiment, line type, selection regime
Summary of key points (include methodology)	When female *Drosophila* are starved, they produce eggs later in life. The ovariole numbers increased with starvation, so they did not have an effect on early age egg production. This might show an evolutionary trait. The flies were starved for 24 hours after 3-4 days after eclosion to allow for sexual maturity and mating to occur. About half of the flies died.
Research Question	How does starvation of female *Drosophila* change the number of eggs produced?
Important Figures	

Notes	This article highlights the importance of giving *Drosophila* an equal amount of food. It also shows that the flies can produce eggs for 26 days.
Cited references to follow up on	Delpuech J-M, Moreteau B, Chiche J, Pla E, Vouidibio J, David JR: Phenotypic plasticity and reaction norms in temperate and tropical populations of *Drosophila melanogaster*: ovarian size and developmental temperature. Evolution. 1995, 49: 670-675. 10.2307/2410320.
Follow up Questions	How does starvation affect the male reproductive system of *Drosophila?* How does starvation affect the sexual behavior of *Drosophila?*

3i: Changes in enzymatic activity and behavioural responses during *Drosophila melanogaster* development

12/12/2020e

Source Title	Changes in enzymatic activity and behavioural responses during *Drosophila melanogaste*r development: effects of environmental ethanol and acetic acid
Source citation	ELAMRANI, A., & IDAOMAR, M. (2001). Changes in enzymatic activity and behavioural responses during *Drosophila melanogaster* development: Effects of environmental ethanol and acetic acid. Invertebrate Reproduction & Development, 40(2–3), 171–180. https://doi.org/10.1080/07924259.2001.9652717
Original URL	https://www.tandfonline.com/doi/abs/10.1080/07924259.2001.9652717
Source type	Database: Taylor and Francis Online
Keywords	*Drosophila melanogaster,* olfactory response, pupation height, ADH activity, ethanol, acetic acid
Summary of key points (include methodology)	Testing done with a French (tolerant of ethanol) and Congolese population (very sensitive to ethanol) found that the former group had higher ADH activity typically but not when larvae were enriched with acetic acid, indicating that the acetic acid is lethal to the flies at that developmental stage (more larvae died than pupa or adults). The olfactory behavior of just larvae was also affected.
Research Question	How do ADH levels differ amongst *Drosophila* in different life stages and how does it affect their olfactory response?
Important Figures	Only the abstract was included.
Notes	This article showed that when fruit flies are in the larval stage, they are very sensitive.
Cited references to follow up on	David, J. R., Bocquet, C., Arens, M. F. and Fouillet, P. 1976. Biological role of alcohol dehydrogenase in the tolerance of *D. melanogaster* to aliphatic alcohols: Utilization of an Adh-null mutant. *Biochem. Genet.*, 14: 989–997.
Follow up Questions	How does ethanol affect flies at the larval stage compared to other stages?