Project Notes:

Project Title:

Name: Ila Chakravarthy

Note Well: There are NO SHORT-cuts to reading journal articles and taking notes from them. Comprehension is paramount. You will most likely need to read it several times, so set aside enough time in your schedule.

Contents:

Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved and where to find the information.

Knowledge Gap	Resolved By	Information is located	Date resolved
Volcano plots	Article reading		08/30/2024
Glycolysis	Textbook reading	Article #5	10/09/2024
Pyruvate Oxidation	Textbook reading	Article #6	10/09/2024
Citric Acid Cycle	Textbook reading	Article #7	10/09/2024
Heme Groups			

Literature Search Parameters:

These searches were performed between 08/26/2024 and XX/XX/2025.
List of keywords and databases used during this project.

Database/search engine	Keywords	Summary of search
Science.org	Prions; Neurodegeneration	"prions and neurodegeneration" ■ 6th result found
Google Scholar	Mitochondrial disease; Anesthesia; Metabolism	

Tags:

Tag Name	
#OXPHOS	#ETC
#neurodegeneration	#metabolism
#propofol	#isoflurane
#anesthesia	#mitochondria
#KrebsCycle	#metabolicorgandamage

Article #example Notes: Title

Article notes should be on separate sheets

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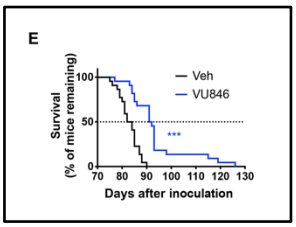
Source Title	
Source citation (APA Format)	
Original URL	
Source type	
Keywords	
#Tags	
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #1 Notes: "M_1 muscarinic receptor activation reduces the molecular pathology and slows the progression of prion-mediated neurodegenerative disease"

Source Title	Science Signaling
Source citation (APA Format)	Dwomoh, L., Rossi, M., Scarpa, M., Khajehali, E., Molloy, C., Herzyk, P., Mistry, S. N., Bottrill, A. R., Sexton, P. M., Christopoulos, A., Conn, P. J., Lindsley, C. W., Bradley, S. J., & Tobin, A. B. (2022). M_1 muscarinic receptor activation reduces the molecular pathology and slows the progression of prion-mediated neurodegenerative disease. *Science Signaling*, *15*(760). https://doi.org/10.1126/scisignal.abm3720
Original URL	https://www.science.org/doi/10.1126/scisignal.abm3720
Source type	Journal
Keywords	Prions; Neurodegeneration; Neurodegenerative disease; GPCRs; Allosteric modulators
#Tags	#neurodegeneration
Summary of key points + notes (include methodology)	Neurodegeneration in patients with prion and prion-like diseases often manifests through cognitive decline, a symptom associated with reduced acetylcholine activity. Positive allosteric modulators (PAMs), drugs that increase agonist efficiency, have been used to stimulate acetylcholine signaling in mouse models of prion disease. PAM treatment was shown to significantly reduce biomarkers for prion disease, like neuroinflammation, thereby preserving the cognitive function of the mice; prion disease's pathological similarities to other neurodegenerative diseases such as Alzheimer's suggest that similar treatments may be effective for both prion and prion-like conditions.
Research Question/Problem/ Need	Can chemical activation of acetylcholine receptors in the brain alleviate cognitigve neurodegeneration in patients with prion or prion-like diseases?
Important Figures	

	 *Figure 1E*: In which survival rates of mice, pictured using Kaplan-Meier survival plots, treated with VU846 (the PAM used in this study) are shown to significantly outlast those treated with the vehicle. *See "Knowledge Gaps"*
VOCAB: (w/definition)	*Proteomic* – The study of the fundamental structures, functions, and interactions of proteins on the cellular level. *Transcriptomic* -- "Transcriptomics is the analysis of the RNA transcripts produced by the genotype at a given time that provides a link between the genome, the proteome, and the cellular phenotype." (sourced from ScienceDirect.com) *Allosteric Regulator* – A substance that binds to a non-active site on an enzyme in order to either accentuate or diminish its function. *Positive Allosteric Modulator* – Increase the affinity or efficiency of an agonist, a chemical that creates a biological response when reacting with a receptor. *Cholinergic* – pertaining to the neurotransmitter acetylcholine
Cited references to follow up on	P. T. Francis, A. M. Palmer, M. Snape, G. K. Wilcock,The cholinergic hypothesis of Alzheimer's disease: A review of progress. *J. Neurol. Neurosurg. Psychiatry***66**,137–147 (1999). S. J. Bradley, J. M. Bourgognon, H. E. Sanger, N. Verity, A. J. Mogg, D. J. White, A. J. Butcher, J. A. Moreno, C. Molloy, T. Macedo-Hatch, J. M. Edwards, J. Wess, R. Pawlak, D. J. Read, P. M. Sexton, L. M. Broad, J. R. Steinert, G. R. Mallucci, A. Christopoulos, C. C. Felder, A. B. Tobin,M1 muscarinic allosteric modulators slow prion neurodegeneration and restore memory loss. *J. Clin. Invest.***127**,487–499 (2017). G. J. Digby, M. J. Noetzel, M. Bubser, T. J. Utley, A. G. Walker, N. E. Byun, E. P. Lebois, Z. Xiang, D. J. Sheffler, H. P. Cho, A. A. Davis, N. E. Nemirovsky, S. E. Mennenga, B. W. Camp, H. A. Bimonte-Nelson, J. Bode, K. Italiano, R. Morrison, J. S. Daniels, C. M. Niswender, M. F. Olive, C. W. Lindsley, C. K. Jones, P. J. Conn,Novel allosteric agonists of M_1 muscarinic acetylcholine receptors induce brain region-specific responses that correspond with behavioral effects in animal models. *J. Neurosci.***32**,8532–8544 (2012).
Follow up Questions	1. Are there any other neurotransmitters associated with cognitive function that could be manipulated in a similar way as described in the study?

	2. Are there methods opposite of PAMs through which the aggregation of proteins in the brain that *contribute* to neurodegeneration, like alpha-synuclein, can be slowed down or stopped altogether? 3. Does PAM therapy offer a significant change in disease prognosis or patient longevity? How permanent or temporary of a fix might this be, in practice?

Article #2 Notes: "Mitochondrial Disease and Anesthesia"

Source Title	SageJournals
Source citation (APA Format)	Hsieh V. C., Krane E. J., & Morgan P.G. (2017) Mitochondrial Disease and Anesthesia. *Journal of Inborn Errors of Metabolism and Screening*. https://doi:10.1177/2326409817707770
Original URL	https://journals.sagepub.com/doi/10.1177/2326409817707770#:~:text=1-3,for%20their%20long-term%20care.
Source type	Online Journal
Keywords	Anesthesia; Metabolism; Metabolic byproducts; Surgery
#Tags	#metabolism, #propofol, #ETC, #anesthesia, #mitochondria

Summary of key points + notes (include methodology)	▪ **INTRODUCTION**
	▪ Mitochondrial dysfunction first affects
	  ○ CNS
	  ○ Heart
	  ○ GI tract
	  ○ Muscular system
	▪ Increasingly common for pediatric MDs to undergo surgery requiring general anesthetic (GA)
	  ○ Ex. Muscle biopsy
	  ○ Belief that MD increases risk of perioperative complications like organ damage
	▪ Advanced sequela of MD present
	  ○ Respiratory failure
	  ○ Cardiac depression
	  ○ Conduction defects
	  ○ Dysphagia
	▪ Anesthesia can exacerbate the above problems, particularly respiratory issues
	▪ Better not to require pre-anesthetic fasting (fasting in general)
	▪ **HYPERSENSITIVITY TO ANESTHETICS**
	▪ Respiratory chain defects
	  ○ When MD impairs breathing/swallowing
	▪ Fatty acid metabolism defects
	  ○ ". . . deficiency of specific enzyme activities or transport proteins involved in the mitochondrial catabolism of fatty acids, leading to tissue accumulation of characteristic fatty acids and L-carnitine derivatives"
	▪ Complex I respiratory chain
	  ○ "exquisite hypersensitivity" to sevoflurane as observed through

	"monitoring the depth of anesthesia using a processed [EEG], such as the bispectral index (BIS). ▪ Fatty acid defects (defects in acylcarnitine) seem relatively undisturbed (in terms of anesthesia sensitivity) to volatile anesthetics o May, however, increase the cardiotoxicity of bupivacaine, and theoretically, propofol ▪ *research tricarboxylic acid cycle* ▪ **VOLATILE ANESTHETICS** o Isoflurane o Sevoflurane o Desflurane ▪ Suppress oxidative phosphorylation @ Complex I, Coenzyme Q, Complex V ▪ C. Elegans & mice used (w/mutations in mitochondrial proteins in Complex I) to show increased sensitivity o No severe repercussions given careful monitoring ▪ Modern volatile anesthetics are largely excreted by the lungs; minimally metabolized o Parenteral anesthetics undergo hepatic metabolism ▪ **PARENTERAL ANESTHETICS** o Propofol o Etomidate o Ketamine o Barbiturates o Midazolam ▪ Inhibit oxidative phosphorylation ▪ Familiarity with MD pathology on the anesthesiologist's part often mitigates the negative side effects of anesthetic ▪ Propofol affects at least 4 separate mechanisms o In vitro, uncouples oxi. phospho. o Inhibits Complexes I, II, IV o Propofol inhibits L-carnitine transferase, therefore the transportation of L-carnitine esters o Propofol infusion syndrome as a result of long term infusion ▪ **LOCAL ANESTHETICS** o Bupivacaine o Ropivacaine o Lidocaine ▪ Avoid effect of parenteral on respiratory drive and upper airway tone
Esw34Research Question/Problem/ Need	What are some reasons that MD patients may display negative side-effects from anesthesia, and how might these effects be mitigated in medical practice?

Important Figures

Table 1. Listed below are Common Anesthetic Agents and the Sites Affected by Each. The References Match those in the Manuscript.

Medication	Mitochondrial Effects	References
Barbiturates	Complex I inhibition	33
Etomidate	Complex I inhibition, mild inhibition complex III	32
Propofol	Acylcarnitine transferase, complexes I/II/IV inhibition	25,37,38
Benzodiazepines	Complex I/II/III inhibition	34
Ketamine	Increase energy consumption +/– reports of complex I	35,36
Dexmedetomidine	None reported	None
Fentanyl/remifentanil	Minimal	39
Morphine	Mild complex I inhibition	39,40
Volatile Anesthetics	Complex I inhibition	20,21,27
Bupivacaine (Etidocaine)	Acylcarnitine translocase Mild complex I	24

Table 1: In which Common Anesthetic Agents are displayed next to the parts of the Electron Transport Chain that are (most commonly) most affected by them

VOCAB: (w/definition)

Perioperative – all the time "around" a patient's surgical procedure (including pre-op, during, post-op, etc.)

Myopathy – disease of the muscles

Hemodynamics – how the blood flows through the blood vessel

Sequela -- consequence of a previous disease or injury (like *sequel*)

Conduction defects – affect how cardiac electrical impulses travel (heartbeat), causing arrythmias

Dysphagia – difficulty swallowing (esophageal or oropharyngeal)

L-carnitine – substance (produced from essential amino acid lysine) that helps the body turn fat to energy; made in the liver and kidneys, stored in the skeletal muscles, heart, brain, and sperm

Phosphorylation – the addition of a PO_3 group to a molecule; cellular storage and transfer of free energy using energy carrier molecules

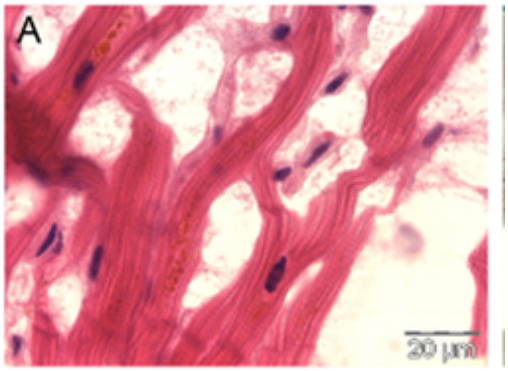
****Electron Transport Chain (ETC) is comprised of:*

Complex I – ubiquinone oxidoreductase, made of NADH dehydrogenase,

	flavin mononucleotide (FMN) and eight iron-sulfur (Fe-S) clusters *Complex II* – succinate dehydrogenase *Coenzyme Q* – ubiquinone (CoQ), functions as an electron transporter *Complex III* – cytochrome c reductase *Cytochrome c oxidase* – AKA: Complex IV, oxidizes cytocrhome c and transfers electrons to oxygen to complete aerobic cellular respiration *Complex V* – AKA: Complex V, formation of ATP using proton gradient across inner mitochondrial membrane *Anesthetic Depth* – the degree to which the CNS is depressed *Parenteral Anesthetics* – mostly act through ligand-gated ion channels in the CNS
Cited references to follow up on	Miyamoto, Y., et al. (2016). Perioperative considerations in adult mitochondrial disease: A case series and a review of 111 cases. *Mitochondrion*, *26*, 26–32. https://doi.org/10.1016/j.mito.2015.11.004 Vanlander, A. V., et al. (2012). Inborn oxidative phosphorylation defect as risk factor for propofol infusion syndrome. *Acta Anaesthesiologica Scandinavica*, *56*(4), 520–525. https://doi.org/10.1111/j.1399-6576.2011.02628.x
Follow up Questions	■ Can the organ failure/dysfunction implied in a condition like MD be a contributing factor to the patient's inability to metabolize anesthesia? ■ Identify specific metabolites in propofol to potentially work with ■ What is used for surgical patients who are allergic to anesthesia (alternatives to traditional anesthesia)? ■ When side-effects are present, what is done to treat them?

Article #3 Notes: "Inborn oxidative phosphorylation defect as risk factor for propofol infusion syndrome"

Source Title	Wiley Online Library
Source citation (APA Format)	Vanlander, A. V., et al. (2012). Inborn oxidative phosphorylation defect as risk factor for propofol infusion syndrome. *Acta Anaesthesiologica Scandinavica*, *56*(4), 520–525. https://doi.org/10.1111/j.1399-6576.2011.02628.x
Original URL	https://onlinelibrary.wiley.com/doi/full/10.1111/j.1399-6576.2011.02628.x
Source type	Case Study from Journal
Keywords	Oxidative Phosphorylation, Electron Transport Chain, PRIS, Propofol, Anesthesia, Mitochondria
#Tags	#propofol, #OXPHOS, #metabolism, #metabolicorgandamage, #anesthesia
Summary of key points + notes (include methodology)	**Abstract** ■ Propofol is generally for pediatric use ■ Propofol Infusion Syndrome (PRIS) ○ Implicated in mitochondrial dysfunction ■ Case study on patient w/ Leber hereditary optic neuropathy (LHON) ○ Severe deficiency in Complex I of the oxidative phosphorylation (OXPHOS) in skeletal muscle ■ Proposes that PRIS can occur in adult patients with preexisting OXPHOS deficiencies ○ And that PRIS is caused by OXPHOS inhibition **Case Report** ■ 40-year-old blind Caucasian male ■ Analgosedation w/remifentanil and propofol ■ 88 h propofol infusion @ about 4.8 mg/kg/h ■ post-craniotomy systemic hypotension ⇾ increase of vasopressor ○ arterial lactate concentration increase ○ rhabdomyolysis symptoms ○ nodal bradyarrhythmia ○ *all above symptoms indicate PRIS* ■ propofol stopped, renal replacement started (carnitine, thiamine, B12) ■ patient died of multiorgan failure, metabolic disequilibrium ○ congestion of the liver, lungs, brain ○ atrophied optic nerve ○ myocytolysis in diaphragm, skeletal muscle, cardiac muscle ○ accumulation of fat in skeletal muscle fibers ■ spectrophotometric analysis of skeletal muscle biopsy showed deficient

	Complex I and increased (other) OXPHOS Complexes II, III, IV, and citrate synthase ○ gelelectrophoresis confirmed up-regulation, did not confirm Complex I deficiency ■ propofol concentration measured using liquid chromatography ○ propofol metabolites (quinol, quinone . . .) detected **Discussion** ■ PRIS pathology historically observed ○ Widened arrhythmia ○ Hepatomagaly ○ Hyperlipemic plasma ○ Metabolic acidosis ○ Rhabdomyolysis symptomology ■ Propofol is lipophilic and has small MW ○ Diffuses easily across membranes ■ "Propofol can induce impairment of mitochondrial function" ■ A study using rats showed lowered ATP production at high doses (1mM) ■ LHON ○ more than 90% of patients show one of three point mutations in mitochondrial genes coding for Complex I ■ "As such, propofol could accept all electrons released from complex I without undergoing a new oxidation and preventing in this way further transfer of electrons through the OXPHOS system." ■ High (in some cases, *extremely* high) concentrations of propofol observed in skeletal muscle, liver tissue, etc. ■ Since propofol can induce mitochondrial toxicity, patients with underlying mitochondrial disorders are at higher risk for lactic acidosis and other negative side-effects when exposed to it, even in a non-pediatric context.
Research Question/Problem/ Need	■ Is the onset/presence of PRIS more likely in patients who have underlying mitochondrial defects?
Important Figures	 *Figure 1A:* The disappearance of the traditional striated pattern characteristic of cardiac muscle indicates vacuolar degeneration. Observed through hematoxylin and eosin staining – a very commonly used tissue stain, where hematoxylin is used to stain nuclei, and eosin stains the extracellular matrix.

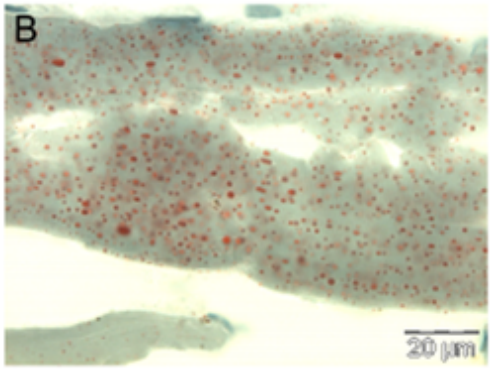


Figure 1B: Oil red staining shows massive lipid accumulation in gastrocnemius muscle tissue (the calf)

Tissue	Fraction	Complex I/CS	Complex II/CS	Complex III/CS	Complex IV/CS	CS [a]
Skeletal muscle	Homogenate	**0.21 (–7.15)**	0.74 (1.41)	0.81 (–1.12)	0.90 (–1.64)	660
	Controls (*n* = 30)	0.64 ± 0.06	0.68 ± 0.04	0.89 ± 0.07	1.00 ± 0.06	174 ± 70

Table 1: In which z-scores (in parentheses) less than -1.96 indicate significantly depressed respiratory chain enzyme activity in the patient's skeletal muscle sample. Results obtained through spectrophotometric analysis.

VOCAB: (w/definition)

Propositus – the subject

Analgosedation – targeting pain in ICU before sedation

Myocytolysis – sublethal injury of cardiac muscle cells

Cited references to follow up on

Sasano, N., Fujita, Y., So, M., Sobue, K., Sasano, H., & Katsuya, H. (2007). Anesthetic management of a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) during laparotomy. *Journal of Anesthesia*, *21*(1), 72–75. https://doi.org/10.1007/s00540-006-0449-y

(a lot of the references on this case study were around 20 years old)

Follow up Questions

- Is renal replacement an effective strategy to counter PRIS? Did it fail to work due to the severity of this specific patient's condition, due to his pre-existing disease, or due to inefficacy of treatment (or some combination of all of the above)?
  - How is PRIS currently treated?
- Since anesthesia is dosed partially based upon weight – and since MD patients are theorized to be more sensitive to anesthesia – can PRIS and other anesthetic-related-toxicities be prevented simply by reducing the

	dose? ■ Can perioperative CoQ supplementation prevent anesthesia-toxicicity?

Article #4 Notes: "Anesthetic management of a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) during laparotomy"

Source Title	Journal of Anesthesia (Sourced through Springer Link)
Source citation (APA Format)	Sasano, N., et al. (2007). Anesthetic management of a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) during laparotomy. *Journal of Anesthesia*, *21*(1), 72–75. https://doi.org/10.1007/s00540-006-0449-y
Original URL	https://link.springer.com/article/10.1007/s00540-006-0449-y
Source type	Case Study from Journal
Keywords	Mitochondria, Myopathy, Anesthetic, Respiratory Chain, Metabolism
#Tags	#metabolism, #propofol, #ETC, #metabolicorgandamage, #anesthesia, #mitochondria
Summary of key points + notes (include methodology)	**Abstract** ■ 53-year-old man with MELAS ○ mitochondrial myopathy ○ encephalopathy ○ lactic acidosis ○ stroke like episodes ■ Administered bicarbonated Ringer's solution ○ Stable serum lactate ○ No metabolic acidosis ■ "Aggressive warming was needed to maintain normothermia" due to nature of MELAS and danger of further mitochondrial metabolic depression **Introduction** ■ MELAS ○ Type of MD that affects multiple systems ○ Stable serum glucose levels, oxygen balance, cardiovascular function, gas exchange crucial ■ Study pertains to MELAS patient who underwent gastrectomy → received bicarbonated Ringer's solution as opposed to acetated Ringer's solution ○ MELAS patients have suppressed citric acid cycles (which metabolize acetate) **Case Report** ■ A host of issues

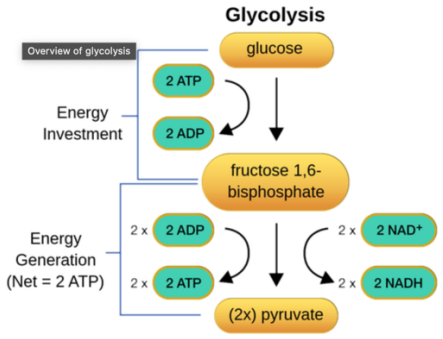
	○ Anemia (Hb 9.9) ○ Cachexia ○ Low CK ○ Etc. ■ Administered acetated Ringer's solution w/ 5% glucose ■ Pre-op bp 82/50 mmHG & 70 bmp ○ *(use bp and hr as metrics for study?)* ■ Then administered bicarbonated Ringer's solution ■ 2 ml 1% lidocaine ○ Then, continuous 0.375% ropivacaine at 5 $ml*h^{-1}$ and propofol infusion ■ Ringer's solution contained 5% glucose and insulin was administered to maintain blood glucose 120-200 md/dl ■ Dopamine at 2-8$\mu g*kg^{-1}*min^{-1}$ to keep systolic bp at 80-110 mmHg ■ Nerve block (neuromuscular blockade) reversed by 2mg neostigmine and 1mg atropine ■ Brief ICU stay due to mild hypercarbia and need for inotropic medication ■ Otherwise uneventful procedure, transferred to regular surgical ward in a day **Discussion** ■ MELAS is maternally inherited ○ 80% of cases caused by an A>G mutation in the t-$RNA^{LEU(UUR)}$ gene at position 3243 in the mitochondrial DNA ■ Avoid use of succinylcholine and volatile anesthetics generally avoided in MELAS patients due to risk of hyperthermia ○ Succinylcholine also runs the risk of hyperkalemia due to MELAS predisposition to peripheral neuropathy ■ IV fluid with alkalinizing agents are preferred over lactated solutions due to the impaired citric acid cycle – inability to metabolize acetate ○ In the case of severe lactic acidosis, even sodium bicarb cannot alleviate the symptoms ○ In *severe* cases, sodium bicarb can exacerbate hyperlactemia ■ Patients with MDs are more likely to develop hypothermia during anesthesia ■ *Temperature is a huge consideration in patients with MD* ○ Thermogenesis can be impacted by uncoupling oxidative phosphorylation – correlates to MDs
Research Question/Problem/ Need	■ How does the use of bicarbonated Ringer's solution, as opposed to (or in conjunction with small proportions of) acetated Ringer's solution during surgery affect prognosis in patients with MD?

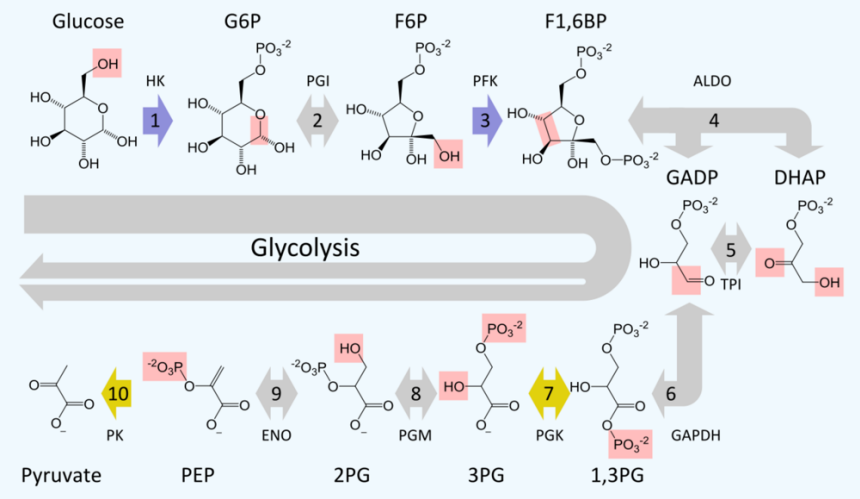
Important Figures	**Table 2.** Composition of the bicarbonated Ringer's solution ($mEq \cdot l^{-1}$) Na^+ 135 K^+ 4 Ca^{2+} 3 Mg^{2+} 1 Cl^- 113 HCO_3^- 25 $Citrate^-$ 5 *Table 2:* the chemical composition of bicarbonated Ringer's solution, which was used instead of acetated Ringer's solution due to predicted difficulties in metabolizing acetate. The measurements are taken in mEq/l – milliequivalents per liter. Note a distinct lack of lactate and acetate.
VOCAB: (w/definition)	*Cachexia* – altered metabolic activity leading to muscle protein loss *Ringer's solution* – a type of isotonic electrolyte solution *Lactic acidosis* – lactic acid build-up in the bloodstream *Ophthalmoplegia* – paralysis of eye muscles *Neuromuscular blockade* – state of paralysis induced by neuromuscular blocking agents to prevent the transmission of neuromuscular signals (nerve block) *Inotropes* – drugs that alter the force of the heart's contractions *Hypercarbia* – excess of carbon dioxide in the bloodstream *Hyperkalemia* – excessive potassium in the serum or plasma *Thermogenesis* – the process by which body heat is generated ▪ Either by rapid skeletal muscle contraction ▪ Or uncoupling oxidative phosphorylation in adipose tissues
Cited references to follow up on	The references for this article were all rather old (20+ years), so I thought it best not to follow up on *them* specifically. There were some research links I used to understand the paper, however, which I have listed here: Carter, S., & Lumen Learning. (2021, February 28). *8.12: Glycolysis*. Biology LibreTexts. https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6-_Metabolic_Pathways/8.12%3A_Glycolysis

	LibreTexts. (2021, February 28). *8.13: Pyruvate oxidation*. Biology LibreTexts. https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6-_Metabolic_Pathways/8.13%3A_Pyruvate_Oxidation LibreTexts. (2021b, February 28). *8.15: Electron Transport Chain*. Biology LibreTexts. https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6_Metabolic_Pathways/8.15%3A_Electron_Transport_Chain
Follow up Questions	1. Could a controlled study comparing acetated and bicarbonated Ringer's solutions provide insights into optimal fluid management? (What is – or, is there – the optimal combination of fluids for perioperative MD care?) 2. Can alterations in perioperative temperature mitigate the negative side effects of anesthetic (or of acetated Ringer's solution)? 3. Can metrics like blood pressure and body temperature be measured in a Drosophila model, or is that only possible with mammalian models?

Article #5 Notes: "8.12: Glycolysis"

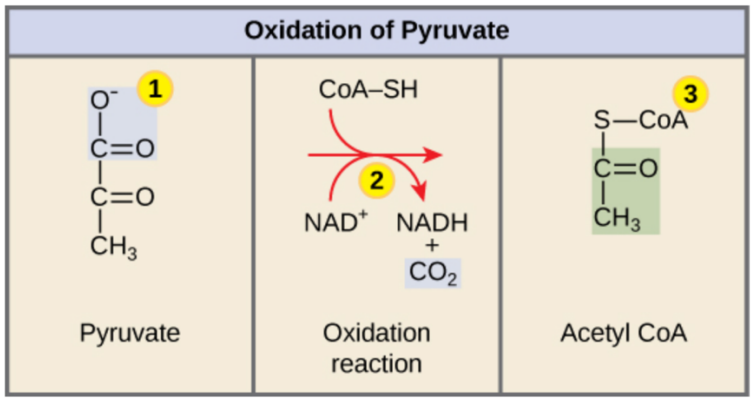
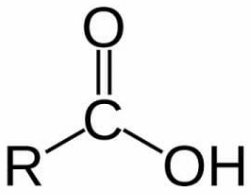
Source Title	Biology LibreTexts
Source citation (APA Format)	Carter, S., & Lumen Learning. (2021, February 28). *8.12: Glycolysis*. Biology LibreTexts. https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6-_Metabolic_Pathways/8.12%3A_Glycolysis
Original URL	https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6-_Metabolic_Pathways/8.12%3A_Glycolysis
Source type	Online Textbook
Keywords	Cellular Respiration, Glycolysis, Energy, ATP, Metabolism, Cellular Metabolism
#Tags	#KrebsCycle
Summary of key points + notes (include methodology)	<u>INTRODUCTION</u> ■ Glycolysis – step #1 in breakdown of glucose ○ Anaerobic ○ In cytoplasm of prokaryotic & eukaryotic cells ■ Two ways ○ Secondary active transport ○ OR facilitated diffusion using GLUT (glucose transporter proteins) proteins ■ Initial & end products: ○ START: x1 6-carbon glucose ○ END: x2 3-carbon pyruvate ■ Two halves: ○ Energy requiring steps, which split the glucose into two 3-carbon molecules ○ Energy releasing steps, which extract energy in the forms of ATP and NADH <u>ENERGY-REQUIRING STEPS (PREPARATORY PHASE)</u> 1. Hexokinase catalyzes the phosphorylation of glucose → *glucose-6-phosphate* a. Phosphate sourced rom existing ATP b. (–) charged phosphate prevents molecule from exiting hydrophobic interior of plasma membrane 2. Isomerase catalyzes the conversion of glucose-6-phospate → *fructose-6-phosphate* a. (Producing a phosphofructose) b. Enables eventual split into two molecules 3. Phosphofructokinase catalyzes the phosphorylation of fructose-6-

	phosphate → *fructose-1,6-biphosphate* a. Phosphofructokinase is rate-limiting, so enough ATP in the system allows the pathway to slow down (aka. When ADP is low) 4. Aldolase destabilizes fructose-1,biphosphate-6 → *dihydroxyacetone-phosphate* and *glyceraldehyde-3-phosphate* a. These are the two 3-carbon isomers prev. mentioned *5.* An isomerase transforms dihydroxyacetone-phosphate → *glyceraldehyde-3-phosphate* a. Now, we have two identical 3-carbon molecules <u>ENERGY-RELEASING STEPS (PAYOFF PHASE)</u> 1. Oxidation of the sugar extracts high-energy electrons a. Received by NAD^+, producing NADH i. Available oxygen: ATP production ii. No oxygen: fermentation 2. Sugar is phosphorylated → *1,3-biphosphoglycerate* 3. Phosphoglycerate kinase catalyzes the donation of a high-energy phosphate from 1,3-biphosphoglycerate to ADP a. This is ATP! b. Also, a carbonyl group is oxidized, making it a carboxyl group . . . → *3-phosphoglycerate* *4. 2-phosphoglycerate* a. Catalyzed by mutase 5. Enolase catalyzes the loss of water from 2-phosphoglycerate a. Double bond that is formed increases the potential energy in the new phosphate → phosphoenolpyruvate (PEP) 6. Pyruvate kinase catalyzes the production of another ATP a. Glycolysis ends with pyruvic acid
Research Question/Problem/ Need	On a molecular level, what are the steps involved in glycolysis?
Important Figures	 *Figure (not from article):* A simplified flowchart displaying the ultimate product of glycolysis, two pyruvate molecules (the salt form of pyruvic acid).

	 *Figure 4:* The entire process of glycolysis, in more detail than the previously listed figure. Note the double ended arrows, which occur because many of the enzymes involved in glycolysis can catalyze both ways (and are named accordingly).
VOCAB: (w/definition)	*Heterotrophic* – nourished by complex organic substances *Integral proteins* – a type of transport protein that is permanently embedded in the plasma membrane *Phosphorylation* – the addition of a PO_3 (phosphoryl) group to an existing molecule *Secondary active transport* – occurs against the concentration gradient *Isomerase* – catalyzes the conversion of a given molecule into an isomer *Isomer* – same formula, different structure
Cited references to follow up on	None available.
Follow up questions	1. If some of the enzymes can work "backwards", do they? When/why would this happen? 2. What impact does the position of the carbons on a molecule have on the process of glycolysis? For example, why must 3-phosphoglycerate be turned into 2-phosphoglycerate? 3. Patients with certain types of metabolic defects are at increased risk for lactic acidosis when exposed to substances like acetated Ringer's solution. What goes wrong in the glycolysis process in such cases, and can the introduction/stimulation of a specific enzyme prevent this (and potentially even allow for treatment using acetated Ringer's and similar substances)?

Article #6 Notes: "8.13: Pyruvate Oxidation"

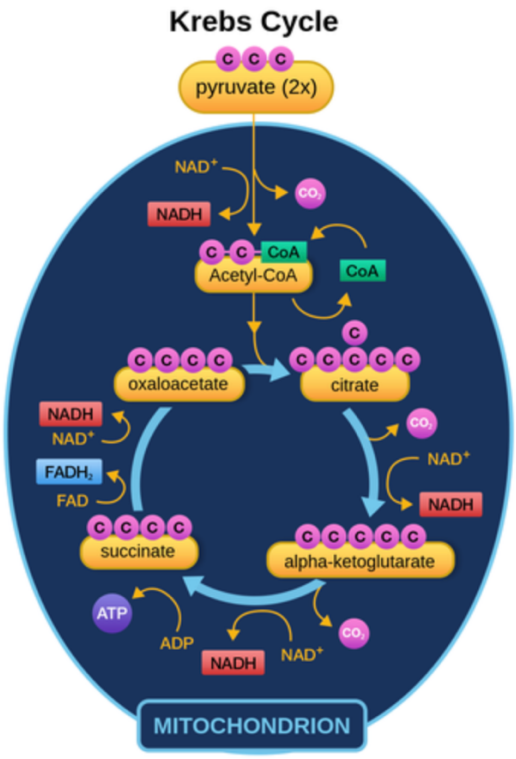
Source Title	Biology LibreTexts
Source citation (APA Format)	LibreTexts. (2021a, February 28). *8.13: Pyruvate oxidation*. Biology LibreTexts. https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6_Metabolic_Pathways/8.13%3A_Pyruvate_Oxidation
Original URL	https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6-_Metabolic_Pathways/8.13%3A_Pyruvate_Oxidation
Source type	Online Textbook
Keywords	Cellular Respiration; Citric Acid Cycle; Energy; ATP; Metabolism; Cellular Metabolism; Aerobic Respiration
#Tags	#KrebsCycle
Summary of key points + notes (include methodology)	<u>INTRODUCTION</u> ■ Pyruvate oxidation occurs when there is oxygen available to continue aerobic respiration after glycolysis. ■ Coenzyme A (CoA) is made of vitamin B5 ○ Explore supplementation as a possible treatment? <u>STEP 1</u> ■ Pyruvate loses a carboxyl group ○ A single CO_2 is released ○ → *two-carbon hydroxyethyl bound to pyruvate dehydrogenase* ■ Step 1 happens twice, once for each pyruvate ○ Therefore, one molecule of glucose → 2x (two-carbon hydroxyethyl bound to pyruvate dehydrogenase) ■ Net loss of two carbons <u>STEP 2</u> ■ Hydroxyethyl group oxidized → *acetyl group* ■ "left-over" electrons picked up by NAD^+ → NADH ○ Containing high-energy electrons that will eventually be used to synthesize ATP <u>STEP 3</u> ■ CoA acquires the acetyl group → *acetyl CoA* <u>OTHER NOTES</u> ■ Steps after step 1 occur in duplicate because there are two (2) enzyme bound two-carbon hydroxyethyl molecules ■ Every time carbon is removed from the molecule of interest, it binds to

	oxygen → CO_2, which is one of the main products of cellular respiration ■ *In the presence of oxygen* ○ Acetyl CoA delivers its acetyl to oxaloacetate (a 4-carbon molecule), forming *citrate* ○ The production of citrate initializes a final energy-harvesting pathway, the Citric Acid Cycle (AKA Krebs Cycle)!
Research Question/Problem/ Need	During aerobic respiration, what happens to the molecule(s) of interest in between glycolysis and the Krebs Cycle?
Important Figures	 *Figure 1:* Pyruvate oxidation described visually. Note the arrow in Step 2 that appears to move away from the main pathway – this is the visual representation of CO_2 being released. *Figure (not from article)*: the structure of a carboxyl group – see in VOCAB
VOCAB: (w/definition)	*Functional group* – a group of atoms in a molecule that possess a certain function regardless of the other atoms in the molecule *Carboxyl group* – a functional group that is comprised of a single carbon bonded to an oxygen and a hydroxyl. The "R" in the diagram represents the R group (the organic, rest of the molecule, since the carboxyl group is a functional group)  (Biology Dictionary)
Cited references to follow up on	None.
Follow up Questions	1. If a specific mitochondrial chain defect results from CoA deficiency, can it be treated with B5 supplementation? 2. The process of pyruvate oxidation is dependent upon there being sufficient oxygen. In the absence of oxygen, fermentation will occur and produce lactic acid. MD patients cannot metabolize lactic acid well, so

	what are the consequences of fermentation occurring in such cases? 3. Is there such a thing as “too much” cellular respiration, or too much energy? If so, what happens to the body?

Article #7 Notes: "8.14: Citric Acid Cycle"

Source Title	Biology LibreTexts
Source citation (APA Format)	LibreTexts. (2021c, February 28). *8.14: Citric acid cycle*. Biology LibreTexts. https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6_Metabolic_Pathways/8.14%3A_Citric_Acid_Cycle
Original URL	https://bio.libretexts.org/Courses/Lumen_Learning/Biology_for_Majors_I_(Lumen)/08%3A_Module_6-_Metabolic_Pathways/8.14%3A_Citric_Acid_Cycle
Source type	Online Textbook
Keywords	Cellular Respiration; Citric Acid Cycle; Krebs Cycle; Energy; ATP; Metabolism; Cellular Metabolism; Aerobic Respiration
#Tags	#KrebsCycle, #mitochondria
Summary of key points + notes (include methodology)	INTRODUCTION ■ Citric Acid Cycle also called ○ Krebs Cycle ○ TCA cycle (citric acid is a tricarboxylic acid) ■ In the mitochondrial matrix ■ All involved enzymes, except for succinate dehydrogenase, are soluble STEP 1 ■ CoA bonds with a sulfhydryl group (R-SH) and diffuses away ■ Exergonic ■ Negative feedback based upon ATP amount ○ That is, ↑ ATP, ↓ rate of reaction STEP 2 ■ Citrate loses one H_2O → *isocitrate* (isomer of citrate) STEP 3 ■ Isocitrate is oxidized → *α-ketoglutarate* (five-carbon) ■ CO_2 output ■ Two electrons that reduce NAD^+ to NADH ■ Negative feedback based upon ATP and NADH STEP 4 ■ CoA binds to a succinyl group → *succinyl CoA* ■ *To be researched later: succinyl groups & chitosan molecules* STEP 5 ■ Phosphate group substituted for CoA → *a high-energy bond that phosphorylates to form GTP or ATP* ○ Phosphate group replaces CoA; the bond between succinyl group and CoA is cleaved ○ Succinyl is converted to succinate ○ Energy from cleaving is used to phosphorylate GDP or ADP

	■ Two types of isoenzymes used here ○ One found in high-ATP-demand tissues, such as heart and skeletal muscle; produces ATP in Step 5 ○ One found in tissues with high number of anabolic pathways, like the liver; produce GTP in Step 5 ▪ Energetically equivalent to ATP, but more limited uses; largely used in protein synthesis <u>STEP 6</u> ■ Dehydration: succinate → *fumarate* ■ Two hydrogen atoms reduce FAD (flavin adenine dinucleotide) → $FADH_2$ ○ $FADH_2$ carries high-energy electrons to the ETC <u>STEP 7</u> ■ Fumarate receives water → *malate* ■ Malate is oxidized → *oxaloacetate* ■ NADH molecule produced
Research Question/Problem/ Need	What are the steps and products of the Citric Acid Cycle?
Important Figures	 *Figure (not from article):* A simplified diagram of the entire Krebs Cycle closed loop. Note that the outputs of a single cycle are two molecules of CO_2, three molecules of NADH, one molecule of $FADH_2$, and one molecule of ATP (or GTP, which isn't shown in the diagram)

VOCAB: (w/definition)	*Mitochondrial matrix* – the space within the mitochondrion, surrounded by the inner membrane, in which many of the enzymes crucial to cellular respiration are stored/steps take place *Exergonic* – a chemical process associated with the release of energy; the energy change in the substance/molecule is negative *Endergonic* – the opposite of exergonic; in which the energy change in the substance/molecule is positive *Anabolic* – a process that synthesize complex molecules from simple ones using energy-carrying molecules *Catabolic* – breakdown of complex molecules; releases energy into organism *Amphibolic* – a chemical process that is both catabolic and anabolic *Sulfhydryl group* – (AKA thiol group) functional group comprised of a single sulfur bonded to a hydrogen *Isoenzymes* – different forms and efficiencies, catalyze the same reaction
Cited references to follow up on	None.
Follow up Questions	1. What is a chitosan molecule (how does it work, what does it do, etc.)? 2. Are there any vitamins associated with the production of specific enzymes in the citric acid cycle and can supplementation be used to treat faulty cellular respiration in MD patients? 3. Are there any tissues/mechanisms in the body that rely *solely* on GTP and how are they impacted by mitochondrial chain defects?

Article #8 Notes: "Brain aging differs with cognitive ability regardless of education"

Source Title	Scientific Reports (Nature)
Source citation (APA Format)	Walhovd, K. B., et al. (2022). Brain aging differs with cognitive ability regardless of education. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-17727-6
Original URL	https://www.nature.com/articles/s41598-022-17727-6
Source type	Online Journal
Keywords	Cognitive ability; Neurodegeneration; Cortical atrophy; Neurodevelopment
#Tags	#neurodegeneration
Summary of key points + notes (include methodology)	ABSTRACT ■ High general cognitive ability (GCA) associated with lowered risk of neurodegeneration ■ Why? Hypothesized that high GCA can be a result of more cortical tissue ○ Measured by volume, area, thickness ■ Study involved 7002 MRIs from n=3327, ages 20-88 to observe cortical volume over time ■ Found that higher baseline GCA ➞ less atrophy ○ Results retained even when evaluated for education INTRODUCTION ■ GCA associated with all-cause mortality, disease risk, etc. ○ Associations retained even in the presence of other controlling factors like education ■ GCA ↔ Alzheimer's disease risk associations assumed, not corroborated by enough substantial evidence ■ Brain reserve ➞ preserved differentiation, differences are upheld through time ■ Brain maintenance ➞ less overall change due to higher baseline ability ■ 7002 MRIs from European cohorts sourced from Lifebrain consortium and UK Biobank ○ n=3327, ages 20-88 ■ to ensure that GCA is the variable at hand, controlled against PGS for education and GCA ■ Hypothesis: GCA is positively related to wide anatomical distribution of characteristics through the adult lifespan (intercept effect), but that association with differences in cortical aging trajectories (slope effects) less observed

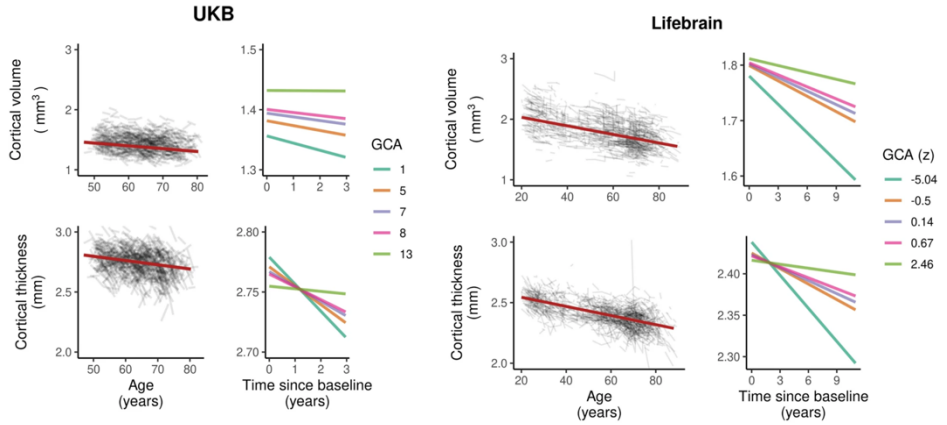
- That GCA is independent, to an extent, of education for both intercept and slope associations
- Method – graphical analysis (?)

RESULTS

- Lifebrain consortium
  - n=1129, 2606 MRIs
- UK Biobank
  - n=2198, 4396 MRIs
- GCA as explanatory variable
  - GCA * time as predictor
- Covariates
  - education * time
  - sex, baseline age, scanner, time (interval from baseline)
  - age * time
- Isolated, as much a possible, the GCA value
  - Did not include variables like intracranial volume (ICV)
- For Cortical Characteristics and GCA
  - Strong positive associations between GCA and both cortical volume and area across both hemispheres of brain . . . significant effects observed in nearly half of the cortical surface
  - Minor positive associations primarily in specific regions as shown through cortical thickness
- For Cortical Change and GCA
  - Higher baseline GCA ➔ reduced cortical thinning over time, particularly in areas corresponding to volume
  - no significant changes in cortical area were associated with GCA
  - A 1σ increase in GCA correlated with a 1.0% *larger cortical volume*
- PGS
  - Relationships between GCA and cortical characteristics remained significant even after controlling for PGSs
  - That is, evidence was retained after introduction of other variables
  - Some regional significance was slightly reduced, nothing huge
- Age
  - Association between GCA and cortical atrophy varied across ages, with stronger effects observed in > 60 years old
  - Older individuals with higher GCA exhibited less cortical decline!

DISCUSSION

- Study implies that GCA can be an accurate marker for brain health over time
  - GCA can be correlated with changes in larger neuroanatomical structures, and negative changes in GCA levels can be linked with cortical volume and thickness decline
- GCA ➔ cortical size metrics
- The interaction between GCA, time, and age was deemed significant,

	suggesting that higher GCA is associated with less atrophy in older adults ■ Notable limitations include the exclusion of subjects with preexisting neurodegenerative disorders <u>METHODS & MATERIALS</u> ■ Lifebrain and UK BioBank databases ■ Vertex-wise analysis ○ Analysis of statistical models on a point-wise basis (?) ■ MRI ■ Spatiotemporal modeling (modeling that takes place across time and space metrics; in this case, interval time and cortical area) ○ On MATLAB ■
Research Question/Problem/ Need	Can higher general cognitive ability (GCA) be associated with higher cortical volume and area, and consequently, be a marker for risk of neurodegeneration?
Important Figures	 *Figure 3:* Cortical change trajectories (age*cortical thickness *AND* age interval*GCA*cortical thickness). The graphs in red show a weak to moderate negative association between age and cortical thickness. The GCA trajectories indicate that a higher GCA yields less variability in cortical thickness over time. This graph exemplifies one of the main conclusions drawn at the end of the paper.
VOCAB: (w/definition)	*Brain reserve model* – the ability of the brain to withstand physical, emotional, social, chemical, etc. challenges; to maintain cognitive function through brain injury *Brain maintenance model* – relative absence of neurological changes over time *Polygenic score (PGS)* – value that reflects an individual's genetic predisposition to a trait or disease; result of multiple genetic variants *Meta-analyses* – statistical techniques that combine results from more than one study; synthesize evidence to draw more robust conclusions *Paucity* – the presence of something in small or insufficient quantities

Cited references to follow up on	Walhovd, K. B. *et al.* Neurodevelopmental origins of lifespan changes in brain and cognition. *Proc. Natl. Acad. Sci. USA.* **113**, 9357–9362. Cox, S. R., Ritchie, S. J., Fawns-Ritchie, C., Tucker-Drob, E. M. & Deary, I. J. Structural brain imaging correlates of general intelligence in UK Biobank. *Intelligence* **76**, 101376. https://doi.org/10.1016/j.intell.2019.101376 (2019). Yeo, R. A., Arden, R. & Jung, R. E. Alzheimer's disease and intelligence. *Curr. Alzheimer Res.* **8**, 345–353. https://doi.org/10.2174/156720511795745276 (2011).
Follow up Questions	1. Can the loss of cortical volume or thickness due to conditions like Alzheimer's Disease appear as a loss of "intelligence"? a. What is considered "intelligence"? 2. While baseline cortical characteristics are out of a patient's control, can cortical thickness be increased through a set of behavior changes or medical intervention? 3. How is GCA measured in a clinical setting? (The study evaluated GCA, but it did not explain how such values are obtained)

Article #9 Notes: "Mitochondrial Pathology"

Source Title	National Library of Medicine
Source citation (APA Format)	Davis, M., & Stroud, C. (2013a. Forum on Neuroscience and Nervous System Disorders; Board on Health Sciences Policy. In *Neurodegeneration: Exploring Commonalities Across Diseases: Workshop Summary*. Washington, D.C; National Academies Press. v
Original URL	https://www.ncbi.nlm.nih.gov/books/NBK208519/#_sec_038_
Source type	Report for Forum
Keywords	Mitochondrial Disease; Neurodegeneration; Antioxidants; OXPHOS
#Tags	#metabolism, #neurodegeneration, #mitochondria
Summary of key points + notes (include methodology)	NEUROBIOLOGY & MITOCHONDRIA ■ Neurons have high energy needs and are therefore vulnerable to injury from dysfunctional mitochondria ○ Mitochondrial dysfunction is commonly characteristic of many neurodegenerative diseases ■ It is uncertain, however, whether mitochondrial defects can be interpreted as a *cause* for neurodegeneration ■ Current treatments include: ○ Medically promoting mitochondrial synthesis ○ Antioxidants to counteract oxidative stress ○ Regulating intracellular calcium and redox potential ▪ Calcium ions are essential for enzyme activation in the Krebs cycle ▪ Calcium plays an important role in cell-signaling ▪ Reactive oxygen species (ROS) are largely sourced from the mitochondria; they can cause oxidative damage DISEASE-SPECIFIC FINDINGS & POTENTIAL TREATMENTS ■ Parkinson's Disease ○ Mitochondrial dysfunction in dopamine neurons ○ Mutations in Parkin and PINK1 ■ ALS ○ Mitochondrial defects precede symptoms in some ALS models ○ Mutations in the SOD1 gene ○ Targeting the pathway for the regulatory protein Bcl-2 as a potential treatment ▪ involved in the inhibition of (apoptosis) cell death ▪ Located in the outer mitochondrial membrane; stabilizes membrane and prevents permeation that can lead to apoptosis

	■ Huntington's Disease o Mutant huntingtin (mHTT) protein disrupts mitochondrial function and transport o Meclizine – potential neuroprotective effects & the potential to shift cellular energy metabolism from mitochondrial respiration to glycolysis ▪ In other words, reducing mitochondrial oxygen consumption, and therefore, oxidative stress ■ Near-Infrared Spectroscopy (NIRS) as a method to develop biomarkers o Light in the near-infrared spectrum penetrates tissues and measures light absorption o Different mitochondrial components, like proteins (think: cytochromes) have unique absorption spectra; oxidized and reduced forms absorb differently o Spectroscopy readings can help researchers infer the redox state of various mitochondrial components ■ Breath Analysis as a method to develop biomarkers o Detection of volatile organic compounds (VOCs), metabolic byproducts <u>GENETIC MUTATIONS & HYPOTHESES</u> ■ 3% of late-onset Alzheimer's cases were found to have a mutation in the mitochondrial tRNA genes ■ Increased mutation rates in the mitochondrial control region were observed in Alzheimer's cases ■ Hypothesized that amyloid-β accumulation (plaques) may not be a cause for neurodegeneration, but a compensatory response to protect dysfunctional mitochondria o In a counterintuitive outcome, Aβ aggregation is linked to the progression of neurodegeneration and the inhibition of mitochondrial function o Aβ collects in the mitochondria and disrupts the axonal transportation that is so crucial to neuron health o Perhaps aggregation starts as a protective response – since the mitochondrial dysfunction is not resolved, aggregation continues to a dangerous extent?
Research Question/Problem/ Need	Can mitochondrial dysfunction be a primary cause of neurodegenerative diseases? (What therapies can be employed to minimize mitochondrial damage, potentially slowing the progression of neurodegeneration?)
Important Figures	None.
VOCAB: (w/definition)	*Mitophagy* – the clearance of damaged mitochondria
Cited references to follow up on	None.
Follow up Questions	1. What specific biomarkers are considered indicators of mitochondrial dysfunction and how can these be consistently monitored in a clinical

	setting to monitor the progression of disease? 2. Is Aβ actually effective in protecting the mitochondria? If so, is there an alternative method that can implemented from a clinician's standpoint to protect the mitochondria without the negative consequences of Aβ plaque aggregation? 3. Since antioxidants can counter oxidative stress, can changes in diet (used alongside actual medical intervention) prevent/slow neurodegeneration?

Article #10 Notes: "Mammalian toxicity of trifluoroacetate and assessment of human health risks due to environmental exposures"

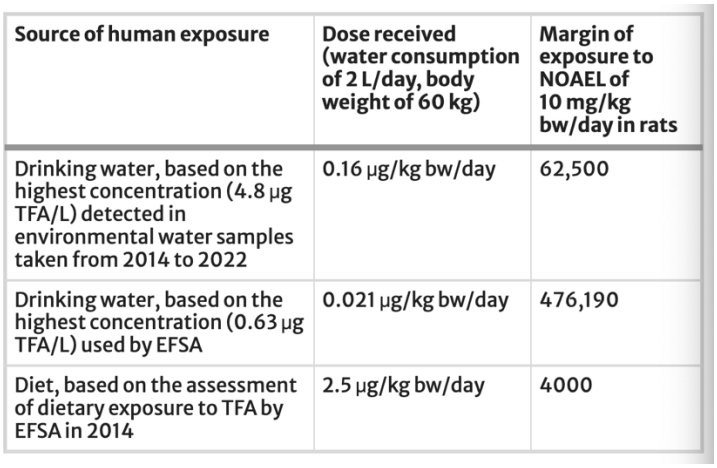
Source Title	Springer Link
Source citation (APA Format)	Dekant, W., & Dekant, R. (2023). Mammalian toxicity of trifluoroacetate and assessment of human health risks due to environmental exposures. *Archives of Toxicology*, *97*(4), 1069–1077. https://doi.org/10.1007/s00204-023-03454-y
Original URL	https://link.springer.com/article/10.1007/s00204-023-03454-y#Abs1
Source type	Journal
Keywords	Toxicology; Liver; Trifluoracetic acid
#Tags	#isoflurane, #metabolicorgandamage, #anesthesia
Summary of key points + notes (include methodology)	ABSTRACT ■ Trifluroacetate (TFA) found in low concentrations in water bodies ■ Mammalian toxicity of TFA evaluated to determine margin of exposures (MoE) ○ Observed liver hypertrophy ○ Peroxisome proliferation ■ MoEs do not indicate health risks when exposed to TFA in water bodies INTRODUCTION ■ Trifluoroacetic acid indicates no potential to bioaccumulate ■ TFA is a urinary metabolite of various inhalation anesthetics ○ Halothane ○ Desflurane ○ Isoflurane ■ TFA is a plant metabolite of various herbicides ■ Excretion of TFA from surgical patients and residual TFA from crops contribute to its presence in the environment MAMMALIAN TOXICITY OF TFA ■ Highly corrosive, typically handled diluted ■ Irritation/degradation of respiratory epithelium observed in rat study was reversible within 2 weeks, even at high exposure concentration (300 mg/mg^3) ■ Repeated inhalation (rats and guinea pigs) shown to cause significant negatives (although, described in a cursory study, so there is room for doubt?) ○ Sever irritation of respiratory path & eyes

- Liver and kidney dystrophy
- Weight loss

- Oral LD_{50} of free trifluoracetic acid is above 500 mg/kg of bw
- Very low acute toxicity
- 28-day diet assay spanning 0 to 16,000 ppm (maximum 1344 mg/kg bw/day in consumption) yielded no significant adverse physiological effects
  - Slight increase in alanine aminotransferase (ALT), changes in serum cholesterol and glucose (at highest dose level)
- Any observed liver enlargement was not accompanied by pathological changes
- 90-day study, sodium TFA with same dosage range (maximum 1216 mg TFA/kg bw/day in consumption)
  - Males showed weight loss
  - Females showed altered bloodwork levels at >160 ppm
  - Elevated clinical chemistry values (bilirubin, glucose, liver enzymes) and higher ketone bodies in urine
- Sodium TFA @ concentrations upwards of 2,400 ppm in drinking water opposite of Clofibric (control) acid @ 5,000 ppm
  - While being a weak peroxisome proliferator, high concentration TFA exposure can result in hepatomegaly and hepatocellular hypertrophy
  - The liver is the "target organ"
- Very loose, if not limited, evidence towards the physiological effects of TFA (at least from ECHA, as described in this paper)

<u>HUMAN EXPOSURES & RISK</u>

- TFA in the ocean has been radiocarbon dated as far as 1000 years back
- Generally, natural occurrence of TFA can be assumed anthropogenic
  - Refrigerants, aerosols, fertilizers, etc.
  - Of more importance (at least to my area of interest), inhalation anesthetics
- Experimental NOAEL of 10 mg/kg bw/day
  - Derived acceptable human intake of 0.05 mg TFA/kg bw/day
- Second experimental NOAEL of 1.8 mg/kg bw/day
  - Human 60µg/L
  - NOAEL results determined by observation of ALT liver enzyme, associated with hypertrophy at higher levels of TFA
  - In general, much more data needed to draw any conclusions – one altered liver enzyme in a random study cannot stand for much in terms of decisiveness/assurance
  - This one is still considered more accurate than the first despite its inapplicability to human pathology (human livers do not respond to the "proliferative effects of peroxisome proliferator-activated receptor α-agonists (α is a proliferated-activated receptor)
- Note: MoE is calculated by (NOAEL)/(EHE)
- MORE DATA NEEDED

Research Question/Problem/ Need	Does the Margin of Exposure of trifluroacetate in water sources and other natural environments pose a significant health risk to human beings exposed to it?
Important Figures	

Source of human exposure	Dose received (water consumption of 2 L/day, body weight of 60 kg)	Margin of exposure to NOAEL of 10 mg/kg bw/day in rats
Drinking water, based on the highest concentration (4.8 µg TFA/L) detected in environmental water samples taken from 2014 to 2022	0.16 µg/kg bw/day	62,500
Drinking water, based on the highest concentration (0.63 µg TFA/L) used by EFSA	0.021 µg/kg bw/day	476,190
Diet, based on the assessment of dietary exposure to TFA by EFSA in 2014	2.5 µg/kg bw/day	4000

Table 2: In which actual human TFA consumption is converted to MoE based upon a NOAEL of 10mg TFA/kg bw/day for 90-days in rats. The extremely high MoEs for drinking water imply a large gap between exposure and health risk of TFA, leading to the conclusion that natural TFA exposure does not pose a significant health risk to human beings.

VOCAB: (w/definition)	*Miscible* – capable of forming a homogenous solution when mixed together *NOAEC* – No-observed adverse-effect concentration *DNEL* – derived no-effect level LD_{50} – aka. Lethal Dose 50 – the dose of a substance that kills 50% of the population *Ketone bodies* – water soluble molecules produced by the liver to break down fat (instead of glucose) for energy [elevated ketone bodies in urine indicate that the body is breaking down fat instead of glucose] *Anthropogenic* – an environmental change as a result of human activity *POD* – point of departure *MoE* – margin of exposure *EHE* – estimated human exposure
Cited references to follow up on	Bayer C (2014) Summary of toxicological and metabolism studies for flurtamone. https://www.bayer.com/sites/default/files/M-482307-01-5.PDF

Follow up Questions	1. Does the MoE for TFA exposure via anesthesia indicate significant human health risk? 2. Since NOAECs were derived partially based upon bodyweight and we can assume that a population of people in the same area are being exposed to the same levels of TFA, do the risk factors associated with TFA increase with decreasing body weight? What implications might this have for the children in the population? 3. Can TFA effectively be filtered out of drinking water? Given the reassuring MoEs obtained from this study, would the risks and losses involved with implementing such a system even be worth it?

Article #11 Notes: "Role of oxidative stress in Alzheimer's disease"

Source Title	Biomedical Reports (Spanidos Publications)
Source citation (APA Format)	Wen-Juan, H., Xia, Z., & Wei-Wei, C. (2016). Role of oxidative stress in Alzheimer's disease. *Biomedical Reports*, *4*(5), 519–522. https://doi.org/doi.10.3892/br.2016.630
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4840676/
Source type	Journal
Keywords	Reactive oxygen species (ROS); Alzheimer's disease; oxidative stress;
#Tags	#mitochondria, #neurodegeneration
Summary of key points + notes (include methodology)	ABSTRACT ■ Alzheimer's Disease (AD) sees abnormal deposits of Aβ peptide ■ "intracellular accumulation of neurofibrillary tangles of hyperphosphorylated τ protein" ■ Initiated by/exacerbated by oxidative stress ■ ROS react with lipids, proteins, nucleic acids, etc. and therefore pose a great risk to tissues and organs (particularly one as sensitive as the brain) ■ *"The current review examined the role of oxidative stress in AD"* INTRODUCTION ■ Mitochondrial ETC consumes 98% of molecular oxygen at the cytochrome oxidase complex (IV), rest is reduced to H_2O_2 and HClO ■ Excessive $O_2^{\bullet -}$ and H_2O_2 → •OH → tissue damage ■ Metal catalyzes redox reactions, so a popular form of antioxidant defense involves storing and transporting iron in forms that do not catalyze reactive radicals ■ CSF cannot bind released iron ions ■ Other sources of oxidative stress include RNS like nitric oxide (NO) and peroxynitrite ○ Peroxynitrite can be especially detrimental to the brain given that it is extremely reactive with proteins, lipids, nucleic acids, and other molecules ■ *"Consequently, the considerable ROS formation increased by the electron transport system within the mitochondria under stressful conditions and in aging constitutes a risk for developing Alzheimer's disease (AD), when no efficient antioxidant system is available."*

- Mitochondrial dysfunction considered a means by which neuron degeneration occurs, through:
  - ROS generation
  - Mitochondrial permeability transition
  - Excitotoxicity
  - Impaired ATP production
  - Altered calcium homeostasis

OXIDATIVE STRESS

- "The reduction of oxygen by one electron produces fairly stable intermediates leading to the formation of a superoxide anion ($O2^{\bullet-}$), the precursor of most ROS and mediator in oxidative stress chain reactions."
  - Reduced by antioxidants to form $OH^{\bullet}$, one of the strongest oxidants
  - Repeating cycle
  - $O2^{\bullet-}$ also reacts with $NO^{\bullet}$, forming peroxynitrite, an extremely potent oxidant driving RNS
- In the case of a lack of antioxidant defenses, ROS and RNS contribute largely to oxidative stress
- Oxidative stress can go so far as to target DNA
- In vivo, $O2^{\bullet-}$ is produced by the mitochondria
- The major enzymatic sources of $O2^{\bullet-}$ include
  - NADPH oxidases, found in various cell membranes
  - Cytochrome $P450^{\bullet-}$
  - H_2O_2 dependent oxygenase
- Mitochondrial regulation/prevention of ROS in three mechanisms
  - Superoxide dismutase (SOD) dismutates $O2^{\bullet-}$ and produces H_2O_2 (hydrogen peroxide) and water
    - Manganese SOD (MnSOD, or SOD2) in the mitochondrial matrix
    - Copper-zinc SOD (SOD1) in the cytoplasm
  - Cytochrome c reduces $O2^{\bullet-}$ to regenerate oxygen; other enzymes can be involved in this mechanism
    - Glutathione peroxidase decomposes $O2^{\bullet-}$
    - $OH^{\bullet}$ (hydroxyl radicals) and catalase detoxifies peroxides in peroxisomes
  - Ubiquinol acts as a reducing agent, eliminating peroxides in the presence of succinate
- Mitochondria also contain DNA-repairing enzymes
- Under normal conditions, oxidative stress can be prevented/managed by inherent systems in the body

OXIDATIVE STRESS IN ALZHEIMER'S DISEASE

- Evidence suggests importance of biometals like iron, zinc, and copper in Aβ
- Corroborating evidence for [above] shows high-affinity binding sites for Cu and Zn on the N-terminal metal-binding domains of Aβ

	■ High Zn concentrations associated with memory and cognitive regions of the brain, neocortex and amygdala, hippocampus (most affected regions by AD) ■ Aβ releases hydrogen peroxide and ROS ■ Binding of Zn promotes toxic Aβ aggregates, disrupting zinc homeostasis ■ Consequently, increased oxidative stress and cytotoxicity exacerbated by accumulation of Zn and Aβ ■ Phospholipids of the brain's membrane are rich in polyunsaturated fatty acids, therefore highly vulnerable to ROS damage o Lipid peroxidation is a key feature in AD ■ Critical neuro proteins, like glutamine synthetase and CK, are affected by free radical oxidation as well, leading to altered glutamate levels and increased excitotoxicity o Note also: loss of energy due to CK impairment ■ Aggregation and hyperphosphorylation of τ protein → NFTs o Can lead to DNA damage like strand breaks and base modifications ■ All of the above suggests that AD is associated with oxidative stress <u>CONCLUSION</u> ■ AD is likely multifaceted in its causes and cannot be pinpointed to one ■ Existing trails support the use of antioxidant treatment in AD ■ Additional studies are required to gain a better understanding of the relationship between oxidative stress and neurodegeneration
Research Question/Problem/ Need	Can oxidative stress play a role in the onset and progression of Alzheimer's Disease, and how so?
Important Figures	None.
VOCAB: (w/definition)	*Neurofibrillary tangles (NFT)* – intracellular aggregation of τ protein inside neurons *Oligomer* – molecule consisting of repeating units derived from monomers *Tau-protein* – protein that serve to stabilize the skeletons of neurons *Free radicals* – molecules/atoms containing unpaired electrons, making them highly reactive; actively seek out other molecules to achieve stability, often leading to chain reactions that can cause cellular damage *Non-radicals* – do not have unpaired electrons and are therefore more stable than free radicals; less intense reactivity, but can still participate in red-ox reactions *Hydroxyl radical* – •OH, one of the most reactive species *Reactive oxygen species (ROS)* – highly reactive oxygen containing molecules that contain free radicals and/or hydroxyl radical and, sometimes, non-radical species;

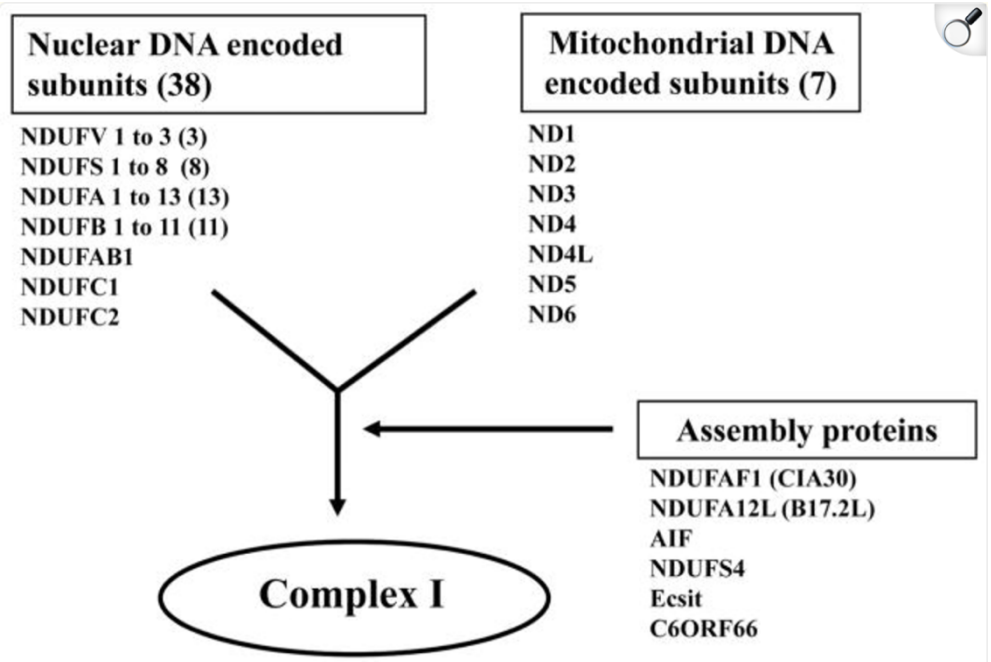
	produced during mitochondrial respiration *Reactive nitrogen species* – like ROS, characterized by the presence of nitrogen *Antioxidants* – neutralize free-radicals by donating electrons *Oxidants* – produce free radicals or react directly with cellular components (can be ROS) *Oxidative stress* – "imbalance between antioxidants and oxidants in favor of oxidants" *Excitotoxicity* – in which nerve cells are severely damaged/killed by excessive neurotransmitter stimulation (particularly glutamate) *Glutamate* – the most abundant excitatory neurotransmitter, plays a crucial role in learning and memory; too much glutamate in the synaptic cleft leads to excessive calcium influx into neurons, causing oxidative stress *Mitochondrial permeability transition (MPT)* – acute increase in the permeability of the mitochondrial membrane, leading to the loss of mitochondrial membrane potential; often triggered by calcium overload, oxidative stress, etc. *Oxygenase(s)* – enzymes that use molecular oxygen to add oxygen atoms to other organic molecules *Cytochrome* – a cytochrome is a redox-active protein that contains a heme group(s); involved in ETC and redox catalysis *Heme group* – a ring-shaped molecule containing iron; component of proteins like hemoglobin and myoglobin *Proteolytic* – referring to enzymes that break proteins down into amino acids or polypeptides (in short, smaller units) *Dismutation* – AKA disproportionation; simultaneous oxidation and reduction *Peroxidation* – chemical reaction that occurs when unsaturated fatty acids are exposed to ROS
Cited references to follow up on	Gelain DP, Antonio Behr G, de Oliveira Birnfeld R, Trujillo M. Antioxidant therapies for neurodegenerative diseases: mechanisms, current trends, and perspectives. *Oxid Med Cell Longev.* 2012;2012:895153. doi: 10.1155/2012/895153. Vignais PV. The superoxide-generating NADPH oxidase: Structural aspects and activation mechanism. *Cell Mol Life Sci.* 2002;59:1428–1459.

	doi: 10.1007/s00018-002-8520-9. Lee J, Koo N, Min D. Reactive oxygen species, aging, and antioxidative nutraceuticals. *Compr Rev Food Sci Food Saf.* 2004;3:21–33. doi: 10.1111/j.1541-4337.2004.tb00058.x.
Follow up Questions	1. Can the implementation of an antioxidant-heavy diet used to prevent Alzheimer's Disease? 2. Are there any lifestyle factors known or hypothesized to promote the production of ROS? 3. Many protection mechanisms that exist in the body originate in the mitochondria. Are patients with mitochondrial defects at a greater risk for neurodegenerative conditions? If so, can the enzyme therapies used to assist mitochondrial function in such cases also be used as supplementation in AD patients (to fortify their natural defenses)?

Article #12 Notes: "Mitochondrial Respiratory Complex I: Structure, Function and Implication in Human Diseases"

Source Title	Current Medicinal Chemistry
Source citation (APA Format)	Sharma, L., et al. (2009). Mitochondrial respiratory complex I: Structure, function and implication in human diseases. *Current Medicinal Chemistry*, *16*(10), 1266–1277. https://doi.org/10.2174/092986709787846578
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4706149/
Source type	Journal
Keywords	
#Tags	
Summary of key points + notes (include methodology)	ABSTRACT ■ LHON (this is the disease in the case study used for STEM Update #2) is caused by mutations in ND1, ND4, ND6 genes → Complex I impairment ○ Other MDs include MELAS (other case study), MERRF, Leigh Syndrome ■ Link between Complex I deficiency and spontaneous-onset neurodegeneration ■ MD & cancer linked through ROS generation and "altered apoptosis" INTRODUCTION ■ MDs caused my mutations in mtDNA (mitochondrial DNA) ■ Mutations in cancer likely secondary, contributions to tumor progression via increased ROS ○ ROS activate oncogenic pathways and genome instability NUCLEAR GENE MUTATIONS ■ Nuclear gene mutations such as NDUFS1-8 and NDUFV1-2 are associated with Leigh syndrome. Also: ○ Cardiomyopathy ○ Encephalomyopathy ○ Leukodystrophy NEURODEGENERATION AND AGE-RELATED DISORDERS ■ Complex I impairment/defects identified in Parkinson's disease patients ○ ND5 mutations impairing mitochondrial function in the substantia nigra → neuronal death ○ ND5 = mitochondrially encoded NADH dehydrogenase 5 → production of NADH dehydrogenase 5 (Complex I) ■ Establish link between MD and Type II Diabetes

	- And cardiac events - Dysfunction of the Electron Transport Chain has implications far beyond the scope of metabolic health <u>CANCER</u> ■ Deficient mitochondrial respiration and elevated ROS in cancer cells may enhance tumorigenesis ■ ROS can lend tumor cells resistance to radiation and chemotherapy o Some cancer therapies rely on ROS accumulation for targeted treatment; cancer cells can modulate ROS levels to "escape" ■ Premature apoptosis due to ROS potential to damage crucial biological material like proteins and lipids ■ ROS also used as cancer *treatments* in the case of xenobiotics, which disturb the redox balance in order to kill cancer cells selectively (premature apoptosis) <u>ANIMAL MODELS FOR COMPLEX-I DEFICIENCY</u> ■ MPTP-induced Parkinson's model demonstrates ROS-driven dopaminergic neuron death o MPTP is a neurotoxin that inhibits Complex I in dopaminergic neurons ▪ Affect sleep, sex drive, disposition, etc. o Rotenone is also a common Complex-I inhibitor in similar studies ■ Transgenic mice targeting NDUFA1 showed optic nerve degeneration, replicating LHON o NDUFA1 = NADH:ubiquinone oxidoreductase subunit A1 o These models showed significantly higher ROS levels than in similar studies run on cultured cells ■ Mutant ND4 in mice visual systems liked to visual loss with elevated ROS o ND4 = NADH dehydrogenase 4 → codes for production (Complex I) o Retinal ganglion cell degeneration o Optic nerve damage o Mitochondrial diseases and ETC dysfunction have strong, frequently observed consequences for eye-health (see also: LHON) <u>PERSPECTIVES (FURTHER)</u> ■ Continued research into post-translational regulation is needed to further understand processes like OXPHOS ■ Mass spectrometry has potential to identify new proteins liked to the electron complexes ■ Machine learning in the field of medicinal biochemistry? ■ Cellular and animal models are instrumental int the exploration of molecular afflictions and regulatory networks
Research Question/Problem/ Need	How can impairment of Complex I affect human health and how does mitochondrial dysfunction contribute to the onset/prognosis of other progressive

	diseases?
Important Figures	 *Figure 1:* Mammalian Complex I broken down into its subunits and assembly proteins. Assembly proteins contribute to the structural integrity of the protein complex. Mutations can occur in any one of these "pieces" and affect the functionality of the Complex.
VOCAB: (w/definition)	*mtDNA* – mitochondrial DNA *Encephalomyopathy* – group of diseases affecting the brain (encephalon) and muscles (myo) *Leukodystrophy* – a group of genetic disorders targeting the white (leuko) matter of the brain; characterized by abnormal white matter growth *White matter* – brain tissue constituted of nerve fibers, serves as the network for neuronal communication *Substantia nigra* – brain structure that mainly controls movement; also controls reward *Subunit* – in the context of the ETC, a subunit refers to a single protein/molecule in a larger complex
Cited references to follow up on	
Follow up Questions	1.

Article #13 Notes: "Bypassing the compromised mitochondrial electron transport with methylene blue alleviates efavirenz/isoniazid-induced oxidant stress and mitochondria-mediated cell death in mouse hepatocytes"

Source Title	
Source citation (APA Format)	Lee, K. K., & Boelsterli, U. A. (2014). Bypassing the compromised mitochondrial electron transport with methylene blue alleviates efavirenz/isoniazid-induced oxidant stress and mitochondria-mediated cell death in mouse hepatocytes. *Redox Biology*, *2*, 599–609. https://doi.org/10.1016/j.redox.2014.03.003
Original URL	https://www.sciencedirect.com/science/article/pii/S2212231714000500?via%3Dihub#ab3
Source type	
Keywords	
#Tags	
Summary of key points + notes (include methodology)	<u>ABSTRACT & INTRODUCTION</u> ■ Many therapeutic drugs (think: propofol, isoflurane) target mitochondria ○ Organ toxicity ○ Acute systems failure ○ Exacerbation of existing symptomology ■ Inhibition of mitochondrial electron transport at one or more ETC sites ○ For example, ubiquinone-binding site & rotenone ■ Minor ETC impairments are typically buffered by mitochondrial reserve capacity and functional thresholds ○ Non-existent or less effective in MD patients ○ MDs run the risk of amplifying drug-induced effects, impairing energy production ■ Complex I dysfunction → drug-induced mitochondrial and cellular toxicity ■ Rotenone or piericidin A (complex I inhibitors) with isoniazid (INH) cause hepatocyte damage ○ INH metabolite hydrazine inhibited complex II and increased ETC superoxide leakage ○ Joint inhibition of complexes I and II led to ATP depletion and necrotic cell death (this would be organ failure in a real patient) ■ EFV and INH often used to treat HIV-1/tuberculosis co-infections

- EFV induces mitochondrial stress; INH linked to liver injury in susceptible patients.
- Methylene blue, an alternative electron carrier, bypasses the proximal ETC to restore energy production
    - Methylene blue as an alternative electron carrier to prevent/reduce the exacerbation of drug-induced mitochondrial dysfunction in MD patients

<u>METHODOLOGY</u>

Mitochondria Isolation:

- Mitochondria isolated from untreated mice via standard methods
- Protein content determined using the BCR protein assay
- Mitochondria stored at −80 °C until analysis

Complex I Activity Measurement:

- Measured as NADH: ubiquinone oxidoreductase activity in potassium phosphate buffer.
- Monitored NADH oxidation via absorbance decrease at 340 nm

Complex II Activity Measurement:

- Measured as succinate: ubiquinone oxidoreductase activity using DCPIP reduction
- Monitored reduction at 600 nm

Measurement of Mitochondrial ROS/RNS Generation

- Used MitoSOX Red probe for detecting mitochondrial superoxide generation.
- Fluorescence measured at 396/580 nm (excitation/emission)

Drug Treatment:

- Hepatocytes exposed to efavirenz (EFV) and isoniazid (INH).
- Methylene blue (MB) added in post-treatment experiments.

<u>RESULTS & CONCLUSION</u>

- EFV inhibited complex I activity in isolated liver mitochondria (IC50 ~30 µM)
    - Increased mitochondrial superoxide production detected with MitoSOX Red.
- MB successfully bypassed proximal ETC inhibition, feeding electrons directly to cytochrome c
    - Enhanced NADH oxidation, even with complex I inhibition.
    - Prevented mitochondrial permeability transition pore opening and necrotic cell death.
    - Protected against LDH release and ATP depletion during EFV/INH co-exposure.
- EFV/INH co-exposure causes toxicity through severe inhibition of ETC,

	leading to oxidative and nitrosative stress ■ Methylene blue effectively bypasses ETC block, preventing hepatocyte injury
Research Question/Problem/ Need	Can methylene blue serve as an alternative electron carrier in the case of Complex I-inhibitory exposure?
Important Figures	
VOCAB: (w/definition)	*Superoxide* – a type of ROS formed when Oxygen is reduced by a single electron
Cited references to follow up on	
Follow up Questions	

Article #14 Notes: "Isolation and Functional Analysis of Mitochondria from the Nematode *Caenorhabditis elegans*"

Source Title	Mitochondria Practical Protocols
Source citation (APA Format)	Grad, L. I., Sayles, L. C., & Lemire, B. D. (2007). Isolation and Functional Analysis of Mitochondria From the Nematode Caenorhabditis elegans. In D. Leister & J. M. Herrmann (Eds.), *Mitochondria Practical Protocol* (1st ed., Ser. 1064-3745, pp. 51–66). essay, Humana.
Original URL	https://link.springer.com/protocol/10.1007/978-1-59745-365-3_4#Sec2
Source type	Protocol
Keywords	Mitochondria; C. Elegans
#Tags	
Summary of key points + notes (include methodology)	ABSTRACT ■ C. elegans as a thorough model for mitochondria dysfunction due to their ability to mimic deleterious mutations o Specifically useful for Complex I mutations for this reason INTRODUCTION ■ Full development in about 3 days at 25° C o L1-L4 before reproductive age ■ 300 progeny per generation ■ 959 total somatic cells, 302 of which neuronal ■ Possess differentiated tissue ■ Orthologs for approx. 50% of human diseases ■ "It is worth noting that the *C. elegans* complex I (NADH-ubiquinone oxidoreductase), which consists of at least 36 subunits, resembles the complex I of higher eukaryotes and is sensitive to the inhibitor rotenone." MATERIALS (only copied relevant ones) **2.3 Harvesting *C. elegans* Cultures** • M9 buffer o 3 g KH_2PO_4 o 6 g Na_2HPO_4 o 5 g NaCl o water to a final volume of 1 L o 1 mL 1*M* $MgSO_4$. **2.6 Isolation of Purified Mitochondria**

- 1 *M* Sucrose
    - 1 *M* sucrose
    - 10 m*M* Tris-HCl
    - pH 7.4, 1 m*M* EDTA.
- 2 *M* Sucrose
    - 2 *M* sucrose
    - 10 m*M* Tris-HCl
    - pH 7.4, 1 m*M* EDTA.

METHODS

- Isolation through centrifugation and washing, several times over to ensure no bacterial contamination
- Shake worms after isolation to ensure full digestion of *E. coli* before assay
- Keep worms in 0-4°C for proper flotation; work quickly – prolonged high-concentration sucrose exposure is lethal
- Glass bead homogenization

3.3 Harvesting *C. elegans* Cultures

1. The culture is harvested by centrifugation in 50-mL polypropylene tubes in a swinging bucket rotor at 1100*g* for 5 min
2. The supernatant is either carefully poured off or removed by aspiration; the worm pellet is soft. The worm pellets are pooled and washed several times in M9 buffer until the supernatant is clear.
3. The final worm pellets are resuspended in M9 and allowed to shake on an orbital shaker for 30 min. The worms are centrifuged, and the supernatant is removed. The yield ranges from 10 to 17 mL of soft packed worms per 150 mL of culture.
4. If the worms are to be used to isolate mitochondria, then it is best to continue without freezing them. Otherwise, the worm pellets can be frozen at −20°C until needed.

3.4 Cleaning *C. elegans* by Sucrose Flotation

1. Wash worm pellets once in ice-cold 0.1 *M* NaCl and resuspend in 100 mL of 0.1*M* NaCl. Aliquot 25 mL into four 50-mL polypropylene tubes and place on ice for several minutes to chill.
2. Add an equal volume of ice-cold 60% sucrose and invert several times. Centrifuge the worms for 5 min at 1100*g*. It is important to work quickly because the high osmolarity of the sucrose will kill the worms if exposed for too long.
3. The worms will float to the top, and the debris will pellet to the bottom of the tube. Quickly remove the worms using a glass Pasteur pipet and dilute at least fourfold in 0.1 *M* NaCl. Wash worms twice in 0.1 *M* NaCl.
4. Resuspend the final worm pellet in 2 volumes of worm lysis buffer with added protease inhibitor cocktail. At this stage, the worms can be frozen in liquid N_2 or, preferably, used directly for mitochondrial isolation.

3.5 Isolation of Crude Mitochondria

	1. A Bead-Beater (Biospec Products) is assembled, and the chamber is filled one-half to two-thirds full with acid-washed glass beads. The worms (in worm lysis buffer with protease inhibitor cocktail) are added to the chamber, and the chamber is filled to the top with cold worm lysis buffer. The rotor assembly is lowered into the chamber, displacing a small amount of liquid. It is important to exclude all air during the operation of the Bead-Beater. 2. The assembled chamber is surrounded with ice. Grinding proceeds with three pulses of 1 min each interspersed with 1-min intervals to allow for heat dissipation. A small aliquot of the supernatant is examined to assess the extent of breakage. 3. The supernatant is recovered and homogenized by hand in a glass-Teflon homogenizer for 30 s. Recovery is increased by rinsing the glass beads in worm lysis buffer and pooling the supernatants. 4. Wash the beads several times with water (until the water is clear) between samples. After all the samples are processed, soak the beads in lab detergent overnight and rinse thoroughly with water. Dry the beads overnight in an oven. 5. Centrifuge the lysate at 2500*g* for 10 min at 4°C to pellet debris. 6. Centrifuge the supernatant at 15,000*g* for 10 min at 4°C. Resuspend the pellet in cold worm lysis buffer and centrifuge again at 15,000*g* for 30 min at 4°C. 7. Resuspend the pellet in a small volume of worm lysis buffer and briefly homogenize in a glass-Teflon homogenizer. 8. Aliquot the crude mitochondria into microcentrifuge tubes, freeze in liquid N_2, and store at −80°C. **3.6 Isolation of Purified Mitochondria** 1. Pour a 10-mL, 1 *M* to 2 *M* sucrose gradient in a 15-mL tube for a swinging bucket rotor such as the Beckman SW27. Up to 4 mL of crude mitochondria in worm lysis buffer can be layered onto the gradient. 2. Centrifuge at 80,000*g* for 90 min at 4°C. Intact mitochondria will be found in the brown band in the middle of the gradient. 3. Remove the mitochondria with a glass Pasteur pipet and dilute with 3 volumes of cold worm lysis buffer. Centrifuge at 30,000*g* for 30 min at 4°C to pellet the mitochondria. 4. Gently resuspend the pellet in a small volume of worm lysis buffer and homogenize with a glass-Teflon homogenizer. Aliquot the purified mitochondria into microcentrifuge tubes, freeze in liquid N_2, and store at −80°C.
Research Question/Problem/ Need	How can the mitochondria of *C. elegans* be isolated for use in an experiment?
Important Figures	None pertaining to *my* experiment

VOCAB: (w/definition)	*Somatic cells* – non-reproductive cells in a multicellular organism
Cited references to follow up on	None
Follow up Questions	1. What alternatives exist for a glass-Teflon homogenizer to lyse the mitochondria from cells? 2. Will reducing the centrifuge speed (therefore, presumably, reducing the purity of the isolated sample) skew the spectrophotometric analysis greatly? Will the relationships between variables still count for something? 3. Is there a way to skip the crude-isolation process and skip straight to obtaining a purified sample using a different buffer concentration or alternative centrifugal process?

Article #15 Notes: “An improved spectrophotometric method for a more specific and accurate assay of mitochondrial complex III activity”

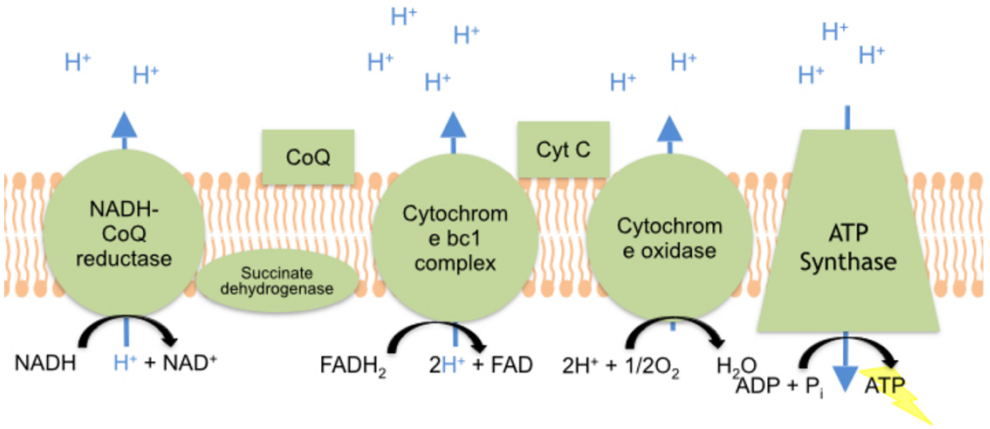
Source Title	
Source citation (APA Format)	
Original URL	https://www.sciencedirect.com/science/article/abs/pii/S0009898108002192?via%3Dihub#aep-section-id16
Source type	
Keywords	
#Tags	
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #16 Notes: “Cytochrome *c*: functions beyond respiration”

Source Title	
Source citation (APA Format)	
Original URL	https://www.nature.com/articles/nrm2434
Source type	
Keywords	
#Tags	
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #17 Notes: "Cytochrome C"

Source Title	
Source citation (APA Format)	
Original URL	https://chem.libretexts.org/Courses/Saint_Marys_College_Notre_Dame_IN/CHEM_342%3A_Bioinorganic_Chemistry/Readings/Metals_in_Biological_Systems_(Saint_Mary's_College)/Cytochrome_C
Source type	E-Textbook/Collection
Keywords	
#Tags	
Summary of key points + notes (include methodology)	<u>INTRODUCTION</u> ■ Proton gradient (matrix → membrane) powers ATP synthesis ■ 34 molecules of ATP per cycle of Electron Transport Chain ■ 4 Complexes o NADH dehydrogenase o Succinate dehydrogenase o Cytochrome bc_1 o Cytochrome c oxidase <u>CYTOCHROME C</u> ■ Water soluble ■ Heme iron metal center o Converts between Fe^{3+} and Fe^{2+} oxidation states during the electron transfer process depending on whether receiving/donating ■ ETC Complexes are ordered by increasing redox potential "(each complex has higher affinity for electrons than the previous)" ■ Electron transfer via: Complex III (cytochrome bc_1) → Complex IV (cytochrome c oxidase) <u>OTHER IMPORTANT INFO</u> ■ ATP powers high-energy bodily processes ranging from tissue repair and movement to DNA synthesis
Research Question/Problem/ Need	What are the underlying mechanisms behind Complex III of the Electron Transport Chain and how do properties of cytochrome c contribute to its overall functionality?

Important Figures	 *Figure 1:* A simplified diagram of the Electron Transport Chain broken down into its individual complexes and byproducts.
VOCAB: (w/definition)	*Heme group* – ring-shaped, iron-containing molecule that is not a protein; component of hemoglobin *Redox potential* – literally, the potential to reduce/oxidize as expressed in volts (V); AKA oxidation-reduction potential *Cytochrome bc_1* – alternative name for cytochrome reductase
Cited references to follow up on	none
Follow up Questions	1. Is there such a thing as an over-accumulation of protons in the intermembrane space, and if so, what are the medical consequences? 2. Does a dysfunctional Electron Transport Chain simply produce < 34 molecules of ATP, or is the body's ATP impaired by other factors like protein "quality"? 3. How does it work that the same amount of ATP is produced regardless of the body's age/size?

Article #18 Notes: "4.1: Myoglobin, Hemoglobin, and their Ligands"

Source Title	
Source citation (APA Format)	
Original URL	https://chem.libretexts.org/Courses/University_of_Arkansas_Little_Rock/Chem_4320/Chem_4320%2F%2F5320%3A_Biochemistry_1/04%3A_Overview_of_Hemoglobin_and_Myoglobin/4.1%3A_Myoglobin%2C_Hemoglobin%2C_and_their_Ligands
Source type	E-Textbook/Collection
Keywords	
#Tags	
Summary of key points + notes (include methodology)	<u>INTRODUCTION</u> ■ Myoglobin (Mb) and Hemoglobin (Hb) serve as critical models for understanding protein-ligand interactions. ■ Mb & Hb bind small ligands like dioxygen (O_2), CO_2, and H^+ o covalent interactions rather than the intermolecular forces typically seen in substrate binding (e.g., hydrogen bonds) <u>HEMOGLOBIN</u> ■ tetramer of two α and two β subunits, each containing a heme group ■ Dioxygen binding is modulated by allosteric factors (H^+, CO_2, bisphosphoglycerate), which bind far away from the oxygen-binding site ■ Solutions of Hb become bright red upon O_2 binding due to changes in the heme group's electronic structure <u>MYOGLOBIN</u> ■ Mb is a monomeric protein with a single heme group o compact ■ Found in muscle, Mb stores O_2 transported by Hb, especially under low oxygen conditions (think about oxidative stress) ■ interior predominantly nonpolar o except for two polar histidines at the active site critical for oxygen binding ■ higher oxygen affinity than Hb, enabling efficient oxygen storage. <u>HEME GROUP & LIGAND BINDING</u> Heme Group: ■ Contains a porphyrin ring (protoporphyrin IX) with an Fe^{2+} ion at the center, like cytochrome c oxidase ■ Deoxy-heme: Square pyramidal, Fe^{2+} lies above the porphyrin plane.

	■ Oxy-heme: Octahedral geometry, Fe^{2+} pulled into the plane upon O_2 binding. Ligand Binding Mechanism: ■ Dioxygen forms a coordinate covalent bond with Fe^{2+} ■ Mb has a higher affinity for O_2, allowing it to serve as an oxygen reservoir. o Hb's affinity is regulated allosterically and adapts to tissue oxygen demand ■ Fetal Hemoglobin (HbF): o Composed of two α and two γ subunits o Higher affinity for O_2 than adult Hb, facilitating oxygen transfer from mother to fetus.
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Patent #1 Notes: "Method for reducing the effects of general anesthetics"

Source Title	Google Patents
Source citation (APA Format)	Orr, J. A., Westenskow, D. R. (2011). *Method for reducing the effects of general anesthetics* (U.S. Patent No. US 7891365B2). U.S. Patent and Trademark Office. https://patents.google.com/patent/US7891356B2/en?q=(anaesthetic)&oq=anaesthetic
Original URL	https://patents.google.com/patent/US7891356B2/en?q=(anaesthetic)&oq=anaesthetic
Source type	Patent
Keywords	Anesthetics; Inhaled anesthesia; CO_2
#Tags	#anesthesia
Summary of key points + notes (include methodology)	ABSTRACT ■ A device for reversing the effects of inhaled anesthesia ■ Aims to increase ventilation of patient and cause the inhalation of CO_2 free of anesthetic agent (via filtration) BACKGROUND ■ Ventilators are commonly used, currently ○ Sensors that detect breathing circuits can indicate vital signs, anesthetic, etc. (generally provide information) ■ Circle systems, for example, aim to minimize the amount of expired, or rebreathed, CO_2 ○ Typically for use in adults ■ Bain systems involve a simple system of linear inspiratory/expiratory gas flow tubes ■ Optimal to reverse anesthetic effects ASAP post-op ○ Time efficiency – frees up hospital space ○ Safer to be under anesthesia for less time ○ Better cognitive prognosis in geriatric patients ■ Activated charcoal as gas removal method – preexisting ○ Not fast enough ■ Hyperventilation ○ Reduced CO_2 levels → dependence on ventilation from artifical respiration ■ Rebreathing processes ○ Good idea in theory ○ Current applications do not filter the anesthetic from the "rebreathed" material

	INVENTION
	■ Breathing apparatus that increases rate/volume of inhalation via ventilator
	■ Induces periodic inhalation of elevated CO_2 gas
	  o Via rebreathing
	■ Filter membrane selectively removes anesthetic from gas made of any known material to filter a significant portion of anesthetic products. Ex:
	  o Activated charcoal
	  o Activated carbon
	  o Crystalline silica molecular sieve
	  o Lipid-based absorption
	  o "Bonus points" if the filter is antimicrobial, like an electrostatic polypropylene fiber
	■ Retains ins/expiratory limbs
	■ Accessible to mask or mouthpiece, connects to endotracheal tube

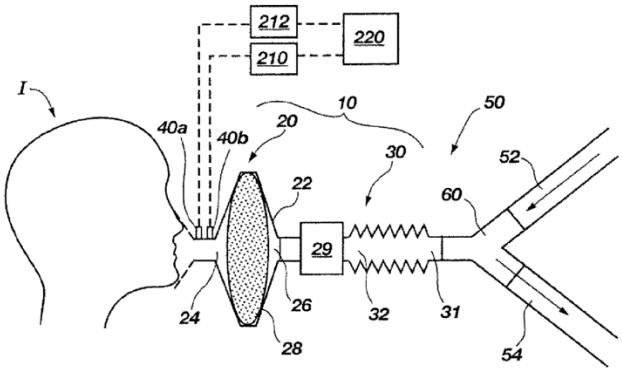
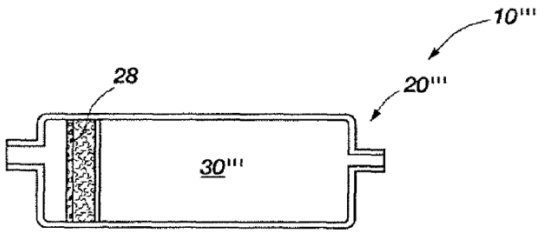
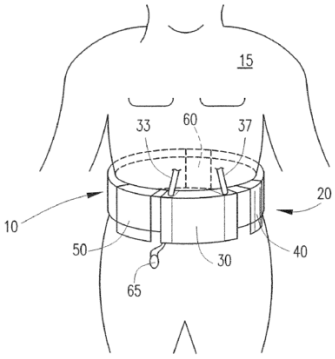
Research Question/Problem/ Need	An anesthesia reversal apparatus that is time-efficient, compensates for CO_2 loss, and filters out anesthesia post-operation.
Important Figures	 FIG. 1 *Figure 1:* An example of a reversal breathing circuit. Note the retention of the Y-connector and inspiratory/expiratory tubes, features preserved from previous designs. Components 40a and 40b are of special interest – they are gas ports that can be connected to sensors allowing for closer monitoring of gas composition inhaled/exhaled, and therefore, more adaptive treatment.  FIG. 4

	Figure 4: One of several possible configurations of the reversal system (system 10’’’) – filter 20’’ exists to provide space of vol. 30’’’ for CO_2 rich gasses to collect from exhalation. Anesthetic membrane filter 28 filters exhaled gas for reuptake.
VOCAB: (w/definition)	None.
Cited references to follow up on	None.
Follow up Questions	1. Is the entire unit of the system that houses the filtration membrane disposable, or can the membrane be replaced? 2. In the case that gas sensors indicate *elevated* CO_2 concentration in the gas sample, does this device adapt accordingly? Will it respond and stop pushing high CO_2 gas, or does it need human input in order to do so? 3. Can a similar (or the same) system be utilized for the infusion of other inhaled drugs?

Patent #2 Notes: "Wearable continuous renal replacement therapy device"

Source Title	Google Patents
Source citation (APA Format)	Gura, V. & Rambod, E. (2011). *Wearable continuous renal replacement therapy device* (U.S. Patent No. US7896829B2). U.S. Patent and Trademark Office. https://patents.google.com/patent/US7896829B2/en?q=(renal+replacement)&oq=renal+replacement&page=3
Original URL	https://patents.google.com/patent/US7896829B2/en?q=(renal+replacement)&oq=renal+replacement&page=3
Source type	Patent
Keywords	Kidneys; Renal replacement; Dialysis systems
#Tags	#metabolicorgandamage
Summary of key points + notes (include methodology)	ABSTRACT ■ Continuous renal replacement therapy (CRRT) ■ Wearable, rechargeable ○ Battery life > 5h ○ Dialysate refreshed by filter BACKGROUND ■ Hemodialysis removes hemotoxins using a dialyzer ○ Sessions lasting 3-4h, 2-3 times/week (inefficient) ○ Daily dialysis theoretically improves patient prognosis ■ CRRT affords convenience, increased quality of care ○ Increased quality of life for terminal patients (end-stage renal disease [ERSD]) ■ Current CRRT machines are also inefficient and, for lack of a better word, bad ○ Large, cumbersome ○ High energy demand – need to be plugged in ○ Require fresh water ○ Constant disruption of arteriovenous shuts at each reconnection (as in, for each session) greatly increases risk of infection INVENTION ■ Dialyzers can be arranged in various configurations ■ Variations include dialyzers made of cylindrical hollow fibers or parallel sheets of semiporous material, enabling blood and dialysate to flow in opposite directions for effective filtration. ■ Dialyzers can be flexible or semi-rigid, adapting to the body shape of the patient for optimal fit and comfort – also accommodating differing dialysis

	prescriptions ■ blood inlet tube w/side ports for electronic control of additive infusions from multiple reservoirs ■ The device incorporates sorbent devices, either connected in series or parallel, for regenerating dialysate. These may consist of replaceable cartridges containing materials like activated charcoal or zirconium phosphate.
Research Question/Problem/ Need	A CRRT that is portable, wearable, durable, comfortable, and efficient.
Important Figures	 *FIG. 1* *Figure 1*: a frontal view of the new CRRT. Note the distinct lack of cables and cumbersome extensions to the device. The dialyzer is located at 30, the sorbent at 40, and additive pump at 50. TABLE 1 Amount of Fluid Removed (in ml.) from each Animal in Eight Hours | | Pig C (g) | Pig D (g) | Pig E (g) | Pig F (g) | Pig G (g) | Pig H (g) | |---|---|---|---|---|---|---| | 1 hr. | 400 | 100 | 100 | 100 | 150 | 180 | | 2 hrs | 700 | 200 | 200 | 200 | 220 | 200 | | 3 hrs | | 300 | 200 | 300 | 380 | 350 | | 4 hrs | 800 | 400 | 250 | 400 | 500 | 700 | | 5 hrs | | 500 | 300 | 500 | 600 | 710 | | 6 hrs | | 500 | 500 | 800 | 690 | 1410 | | 7 hrs | | 620 | 600 | 1000 | 700 | 1400 | | 8 hrs | | 800 | 1000 | 1150 | 800 | 1400 | | Average | 100 | 100 | 124 | 144 | 100 | 175 | TABLE II Experimental Data Acquired from Six Pigs, Using the Exemplary CRRT Device | | Creatinine Clearance (mL/min) | Total Creatinine Removed (g) (8 hrs) | Urea Clearance (mL/min) | Total Urea Removed (g) (8 hrs) | Weekly std (Kt/V) Urea | Phosphorus (grams) (24 hrs) | Potassium (mmole) (24 hrs) | |---|---|---|---|---|---|---|---| | Pig C | 20.10 | 0.91 | 29.40 | 7.61 | 6.50 | 2.30 | 266.11 | | Pig D | 21.10 | 0.76 | 26.80 | 5.75 | 6.20 | 2.60 | 259.91 | | Pig E | 23.50 | 1.14 | 27.30 | 5.37 | 6.10 | 2.67 | 303.54 | | Pig F | 23.50 | 1.14 | 27.30 | 5.37 | 6.00 | 2.44 | 270.50 | | Pig G | 22.30 | 0.95 | 25.70 | 6.46 | 5.20 | 2.41 | 236.97 | | Pig H | 22.30 | 1.02 | 26.30 | 6.24 | 5.80 | 2.42 | 227.01 | | Mean | 22.13 ± 1.34 | 0.99 ± 0.15 | 27.13 ± 1.27 | 6.13 ± 0.85 | 5.97 ± 0.44 | 2.47 ± 0.14 | 260.67 ± 27.05 | *Tables 1 & 2:* Testing of the CRRT on 6 farm raised pigs yielded promising results in various dialysis requirements. No complications during the 8h dialysis period (attributed largely to low flow rates as shown in Table 2) and remainder of life imply that the CRRT is a safe device without high risk of complications. Sufficient elimination of creatine and urea, coupled with the high dialysis dose experimented with, suggest that the CRRT can achieve just as much as the widely used, intermittent daily dialysis regimen can.
VOCAB: (w/definition)	*Dialyzer* – filters waste products, toxins, and excessive fluid from the blood. Contains a semi-permeable membrane that retains larger, vital molecules such as the blood and some proteins; filters out smaller substances

	Dialysate – mixture of (mostly) electrolytes, glucose, and water that facilitates the exchange of waste through the process of diffusion *Sorbent Device* – contain materials that bind to waste products and toxins, removes them from dialysate so that it can be reused
Cited references to follow up on	None.
Follow up Questions	1. Can a patient safely encounter water (say, a spill) while wearing the CRRT? 2. If part of the criteria for this design is convenience and efficiency, how accessible would the CRRT be to the average user in terms of cost? 3. Does the CRRT have potential for pediatric use, or is it more suited to the adult body (that is, among other differences, done growing and maturing)?