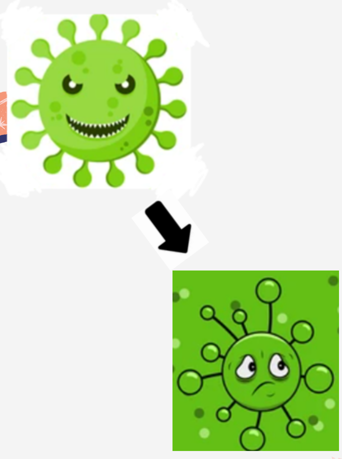
How Do Attenuated Viral Vaccines work?

Addressing common questions and concerns about attenuated viral vaccines.

WHAT IS AN ATTENUATED VIRAL VACCINE?

An attenuated vaccine is a viral vaccine that contains a weakened virus that gets injected into a person to help them build immunity without the risk of getting sick.



HOW DO THESE VACCINES WORK?

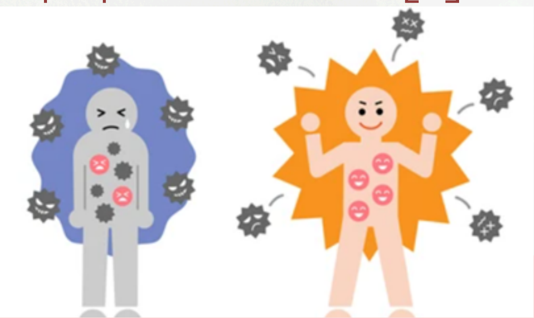
Attenuated viral vaccines contain a weakened version of the virus. The virus is not in its most active form and will pose no threat to the person injected. This weakened version of the virus is obtained through attenuation. The virus loses its strength and makes it safe for people to receive it [1].

WHAT IS ATTENUATION?

Attenuation is editing a virus or bacterium to make it less dangerous. In its weakened state, the virus cannot cause serious illness or disease. Attenuation is done by isolating the wild-type virus and repeatedly culturing it until the virus has lost its strength. Weakening the virus makes it harmless to people [2].

HOW ATTENUATED VACCINES AFFECT THE IMMUNE SYSTEM

The body recognizes the injected weakened virus as an antigen, which is a substance that triggers an immune response that produces antibodies. This response is recorded by memory cells so that the next time the body encounters the virus, it will have a conditioned response and prepared antibodies [3].



COMMON MISCONCEPTIONS ABOUT ATTENUATED VIRAL VACCINES

A lot of misinformation is spread about attenuated viral vaccines and how they work. People often believe that these vaccines contain a living virus which could make people sick. This is a common misunderstanding, and it must be made clear that attenuated viral vaccines do not contain the virus in its most active state. They are 100% safe.

STILL CONFUSED? DON'T WORRY.

An attenuated viral vaccine is like soccer practice. Before playing in a tournament, you must first practice in scrimmages which help you get better at playing. Keep in mind these scrimmages are not meant to be played full out, they are at lower stakes.

This is how attenuated viral vaccines work, before fighting the wild-type virus, the vaccine gives you a weaker version of the virus for your body's immune system to practice fighting it off.

References

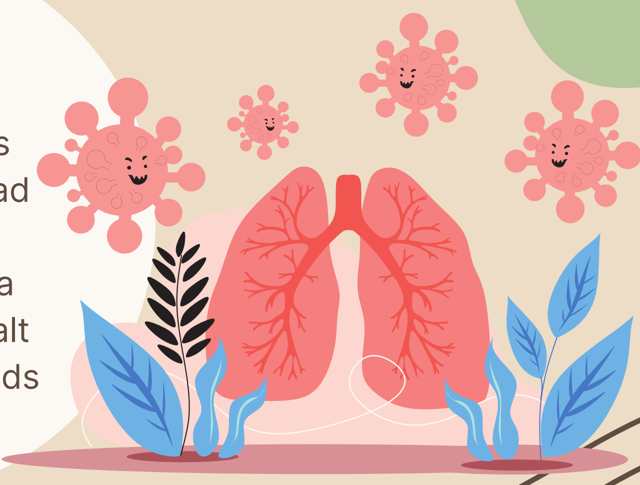
1. Vos, A., Neumann, G., Hundt, B., & Neubert, A. (2014). Attenuated Vaccines for Veterinary Use. Current Laboratory Techniques in Rabies Diagnosis, Research and Prevention, 1, 237–244. https://doi.org/https://doi.org/10.1016/B978-0-12-800014-4.00022-6
2. Wodi, P., & Morelli, V. (2021). Immunology and Vaccine-Preventable Diseases. Principles of Vaccination, 1–7. Retrieved March 21, 2022, from https://www.cdc.gov/vaccines/pubs/pinkbook/downloads/prinvac.pdf.
3. Clem, A. S. (2011). Fundamentals of Vaccine Immunology. Journal of Global Infectious Diseases, 3(1), 73–78. https://doi.org/10.4103/0974-777X.77299

Cystic Fibrosis: Trikafta

"The 5-feet apart rule"-Rachael Lippincott

What is Cystic Fibrosis (CF)?

Cystic Fibrosis (CF) is an autosomal recessive disease caused by a mutation in the CFTR gene. [1] This mutation causes the body to produce thick, sticky mucus that blocks airways, digestive tracts, and other organs. [1,2] CF can lead to respiratory problems, infections, malnutrition, weak immune system, and delays in growth. [1] CF is caused by a malfunction or lack of the CFTR protein, which maintains salt and water balance in the body. [3] Without this protein, fluids secreted in the body become more viscous, causing the symptoms associated with CF. [3]



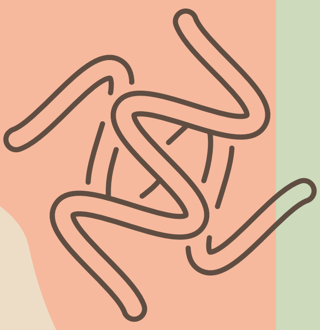
CFTR (cystic fibrosis transmembrane conductance regulator)

How does Trikafta treat CF patients?

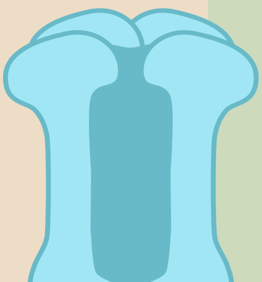
Trikafta is a triple combination drug consisting of tezacaftor, elexacaftor, and ivacaftor. [4] Tezacaftor and elexacaftor function as folding correctors, while ivacaftor acts as a gating potentiator. [4] These drugs are used to correct the misfolding of the CFTR protein caused by the F508del mutation of the CFTR gene.[4]

The science behind it

Tezacaftor and elexacaftor are folding correctors that work at different binding sites to facilitate the proper folding and presentation of mature CFTR protein on the cell surface. [5] This results in the improvement of CFTR protein function for the F508del mutation, which is a common mutation in CF patients that causes the protein to be misfolded. [5]



Ivacaftor, on the other hand, is a gating potentiator that acts by increasing the protein's ability to hold the gate open, thus creating a sodium channel to allow for chloride to flow through. [6] It works synergistically with elexacaftor to improve the ion conductance through mutant CFTR. Ivacaftor binds to the CFTR protein to increase its open-state probability, which leads to an increase in chloride secretion. [7]



Effective treatment

The combination of two correctors and a potentiator has created an additive and synergistic effect that makes Trikafta very effective at treating CF. [7] It corrects the folding of the CFTR protein and increases its open-state probability, leading to improved chloride secretion and mucus clearance in CF patients. By targeting multiple mechanisms of the CFTR protein, Trikafta addresses the complex nature of the disease and has revolutionized the treatment of CF.[7]

References

1. Davies, J. C., & Alton, E. W. F. W. (2006). Gene therapy for cystic fibrosis: Successes and challenges. Cystic Fibrosis, 79–87. https://doi.org/10.1183/1025448x.00035006
2. NIH. (2022). What is cystic fibrosis? National Heart Lung and Blood Institute. Retrieved November 14, 2022, from https://www.nhlbi.nih.gov/health/cystic-fibrosis
3. Cystic Fibrosis Foundation. (n.d.). Basics of the CFTR protein. Cystic Fibrosis Foundation. Retrieved November 14, 2022, from https://www.cff.org/research-clinical-trials/basics-cftr-protein#:~:text=people%20with%20CF.-,The%20cystic%20fibrosis%20transmembrane%20conductance%20regulator%20(CFTR)%20protein%20helps%20to,salt%20%E2%80%94%20becomes%20trapped%20in%20cells.
4. Veit, G., Roldan, A., Hancock, M. A., Da Fonte, D. F., Xu, H., Hussein, M., Frenkiel, S., Matouk, E., Velkov, T., & Lukacs, G. L. (2020). Allosteric folding correction of F508del and rare CFTR mutants by elexacaftor-tezacaftor-ivacaftor (Trikafta) combination. JCI Insight, 5(18). https://doi.org/10.1172/jci.insight.139983
5. Ridley, K., & Condren, M. (2020). Elexacaftor-Tezacaftor-Ivacaftor: The first triple-combination cystic fibrosis transmembrane conductance regulator modulating therapy. The Journal of Pediatric Pharmacology and Therapeutics, 25(3), 192–197. https://doi.org/10.5863/1551-6776-25.3.192
6. Editorial Team. (2019, November 18). Types of CFTR modulators for CF: Correctors, potentiators, and amplifiers. Cystic Fibrosis . Retrieved November 14, 2022, from https://cystic-fibrosis.com/clinical/cftr-modulators
7. Shaughnessy, C. A., Zeitlin, P. L., & Bratcher, P. E. (2021). Elexacaftor is a CFTR potentiator and acts synergistically with ivacaftor during acute and chronic treatment. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-99184-1

FAH

FUMARYLACETOACETATE HYDROLASE

EC 3.7.1.2

3 Hydrolases

3.7 Acting on C-C bonds

3.7.1 In ketonic sunstances

3.7.1.2 Fumarlacetoacetase (3)

Accession # P16930

Properties:

- 419 amino acids (1)
- molecular weight: 46374 Da (1)
- pI ~6.7 (12)
- 1 sequence, 14 exons (13)

Isomers

P16930-1

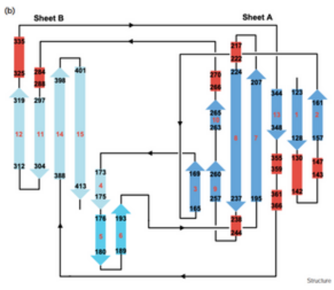
P16930-2*

*Produced by alternative splicing (1)

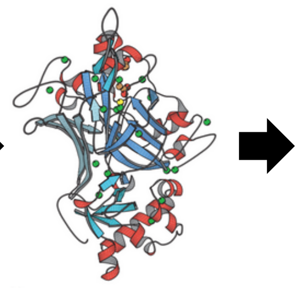
What does it do?

FAH conducts the final step in the break down of tyrosine, which is found in many foods. The series includes five reactions to obtain this protein building block. (2)

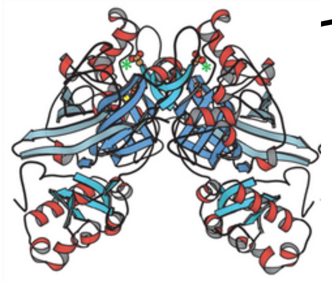
Structures



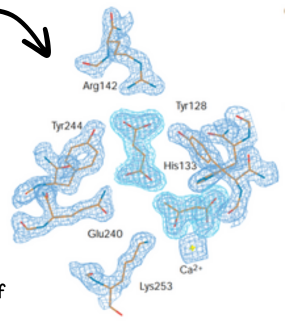
Secondary Structure = mixture of mainly β-sheets (blue) and a few alpha-helices (red). (8)



Tertiary Structure = Folded secondary structure into mixed β-sandwich role. Possible point mutations (green) and Ca2+ ion cofactor added (yellow). (8)



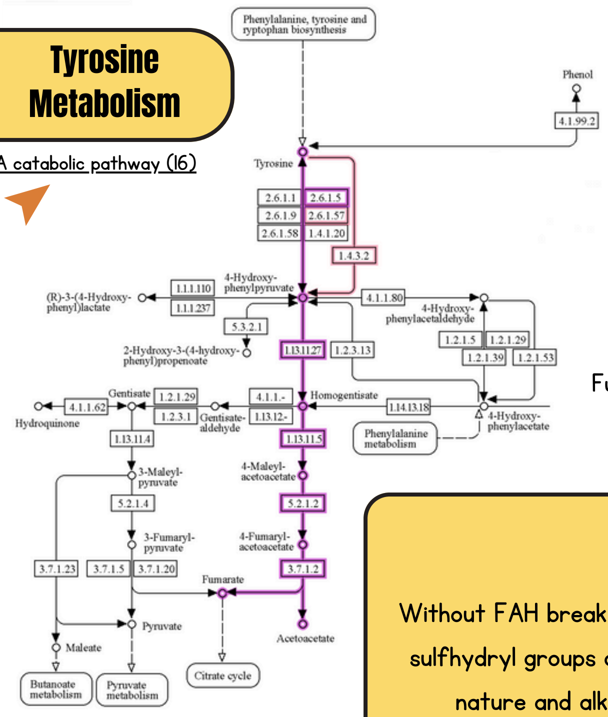
Quaternary Structure = Horseshoe dimer of FAH with active sites (green stars) containing acetate carbon (orange), oxygen (red), and calcium ion (yellow). (8)



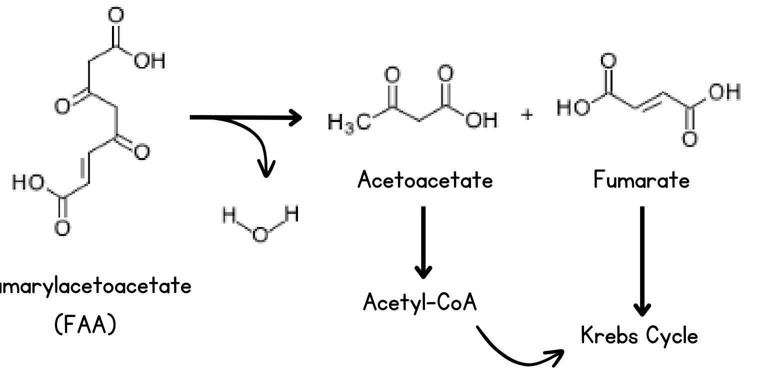
ACTIVE SITE (8)

Tyrosine Metabolism

A catabolic pathway (16)



Mechanism



Reaction Importance

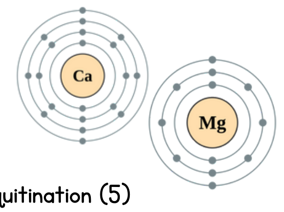
Without FAH breaking FAA down, it builds up in the body and reacts with glutathione and sulfhydryl groups on proteins, causing oxidative damage to cells due to the electrophilic nature and alkylation proficiency. This is the basis for Tyrosinemia Type I. (7)

Competitive Inhibitor:

4-(hydroxymethylphosphinoyl)-3-oxo-butanoic acid (HMPOBA). (9)

Most phosphinate mimics can inhibit FAH. (10)

Co-factors:
Ca2+ and Mg2+ (1)



Lindblad et al. were the first to link FAH to the breakdown of tyrosine and identify it as the cause for Tyrosinemia. (15)

Negative regulation of FAH = polyubiquitination (5)

Location

Throughout the body, the highest concentrations are within the liver, kidneys and fat tissue. because this enzyme converts fumarylacetoacetate (a tyrosine by-product) into smaller molecules which can be excreted by the kidneys. (2, 6)

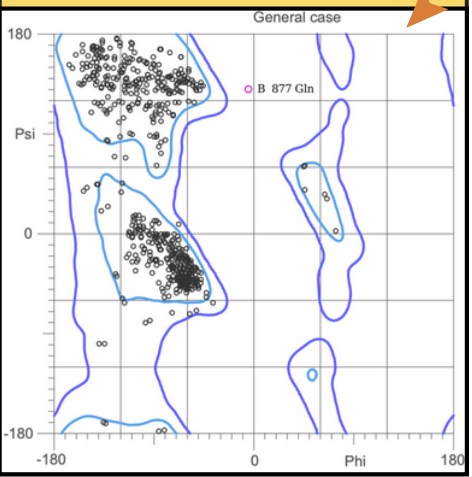
Subcellular Locations:

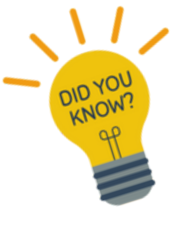
Cytosol (5)

Nucleus (5)

The FAH coding gene is on chromosome 15 (2)

Ramachandran Plot (9, 17)

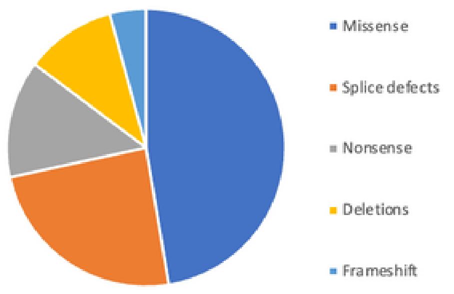




FAH is found across many species of bacteria, yeast, nematodes, plants, and mammals. Any inhibition of this enzyme can result in organ failure and cell death (4).

FAH Mutations

Of 100 identified mutations...



70% of the missense mutations that result in this disease are IVS6-1(G>T). (11) These mutations alter the FAH gene, rendering the resulting protein unable to complete its function. (13)

References

(1) P16390: FAAA_Human. (2002-2023). UniProt. [accessed Oct 10, 2023] https://www.uniprot.org/uniprotkb/P16930/entry

(2) FAH gene: MedlinePlus Genetics. Medline Plus. (2015). National Library of Medicine. [accessed Oct 10, 2023] https://medlineplus.gov/genetics/gene/fah/

(3) Information on EC 3.7.1.2 - fumarylacetoacetase. (2023). BRENDA. [accessed Oct 10, 2023] https://www.brenda-enzymes.org/enzyme.php?ecno=3.7.1.2

(4) Liu, Y., Xia, W., Yang, P., Zhang, S., Shi, Z., Tang, H., & Zhang, L. (2015). Cloning and expression of fumarylacetoacetate hydrolase derived from marine yeast Rhodosporidium diobovatum. J Basic Microbiol. 55(9):1082-93.

(5) Kaushal, K., Woo, S. H., Tyagi, A., Kim, D. H., Suresh, B., Kim, K.S., & Ramakrishna, S. (2020). E3 ubiquitin ligase APC/CCDH1 negatively regulates fah protein stability by promoting its polyubiquitination. Int J Mol Sci. 21, 8719.

(6) FAH fumarylacetoacetate hydrolase [Homo sapiens(human)]. (2023). NCBI-NLM.[accessed Oct 10, 2023] https://www.ncbi.nlm.nih.gov/gene/2184

(7) Chinsky, J. M., Singh, R., Ficicioglu, C., Van Karnebeek, C. D. M., Grompe, M., Mitchell, G., Waisbren, S. E., Gucsavas-Calikoglu, M., Wasserstein, M. P., Coakley, K., & Scott, C. R. (2017). Diagnosis and treatment of tyrosinemia type I: a US and Canadian consensus group review and recommendations. GIM, 19(12), 1380-1395.

(8) Timm, D. E., Mueller, H. A., Bhanumoorthy, P., Harp, J. M., & Bunick, G. J. (1999). Crystal structure and mechanism of a carbon–carbon bond hydrolase. Structure, 7(9), 1023–1033.

(9) Bateman, R. L., Bhanumoorthy, P., Witte, J. F., McClard, R. W., Grompe, M., & Timm, D. E. (2001). Mechanistic Inferences from the Crystal Structure of Fumarylacetoacetate Hydrolase with a Bound Phosphorus-based Inhibitor. J Biol Chem, 276(18), 15284–15291.

(10) Bateman, R. L., Ashworth, J., Witte, J. F., Baker, L.-J., Bhanumoorthy, P., Timm, D. E., Hurley, T. D., Grompe, M., & Mcclard, R. W. (2007). Slow-onset inhibition of fumarylacetoacetate hydrolase by phosphinate mimics of the tetrahedral intermediate: kinetics, crystal structure and pharmacokinetics. Biochem. J. 402(2), 251–260.

(11) Arranz, J.A., F. Piñol, L. Kozak, C. Pérez-Cerdá, B. Cormand, M. Ugarte, & E. Riudor. (2002). Splicing mutations, mainly IVS6-1(G>T), account for 70% of fumarylacetoacetate hydrolase (FAH) gene alterations, including 7 novel mutations, in a survey of 29 tyrosinemia type I patients. Human Mutation. 20:180–188.

(12) Mahuran, D. J., Angus, R. H., Braun, C. V., Sim, S. S., & Schmidt Jr., D. E. (1977). Characterization and substrate specificity of fumarylacetoacetate fumarylhydrolase. Can J Biochem. 55, 1–8.

(13) Dreumont, N., Poudrier, J. A., Bergeron, A., Levy, H. L., Baklouti, F., & Tanguay, R. M. (2001). A missense mutation (Q279R) in the fumarylacetoacetate hydrolase gene, responsible for hereditary tyrosinemia, acts as a splicing mutation. BMC Genetics. 2(1), 9–9.

(14) Gil-Martínez, J., Macias, I., Unione, L., Bernardo-Seisdedos, G., Lopitz-Otsoa, F., Fernandez-Ramos, D., Lain, A., Sanz-Parra, A., Mato, J. M., & Millet, O. (2021). Therapeutic Targeting of Fumaryl Acetoacetate Hydrolase in Hereditary Tyrosinemia Type I. Int J Mol Sci. 22(4), 1789.

(15) Lindblad, B., Lindstedt, S., & Steen, G. (1977). On the Enzymic Defects in Hereditary Tyrosinemia. PNAS. 74(10), 4641–4645.

(16) Tyrosine metabolism - Reference pathway. ND. KEGG. [accessed Oct 10, 2023] https://www.genome.jp/pathway/map00350

(17) Ramachandran Plot for 1QCN. 2023. MolProbity, Duke Biochemistry. [accessed Oct 12, 2023] http://molprobity.biochem.duke.edu/data/gc5hceg8e5vdcsgjns5empgqf5/ charts/1qcn-rama.pdf

*Images were obtained from the references listed above, and the icons used were from Canva.

Curb the Burn: Acid Reflux

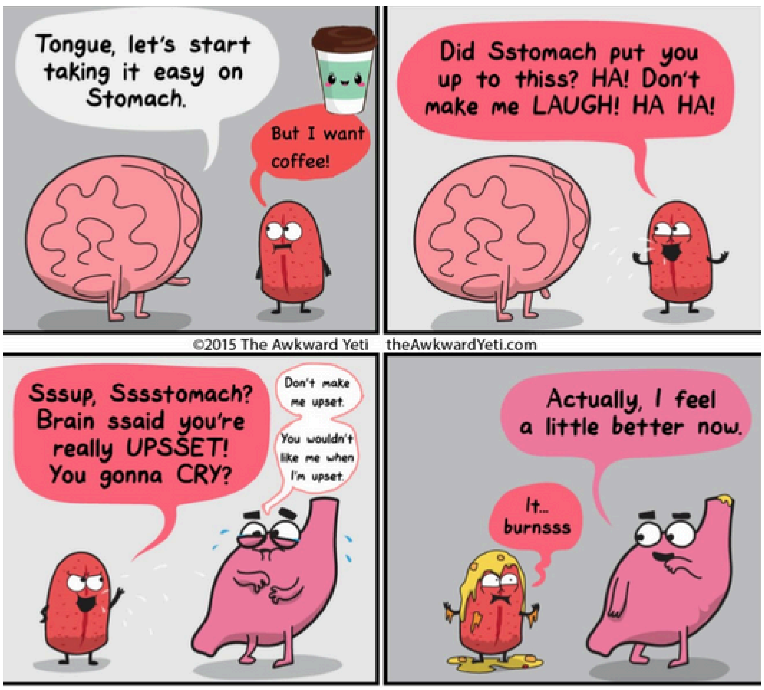
By: Nadine Kamm-Ramirez, Anya Patel, Hannah Doyle, Hunter Brzezinski

WHAT IS ACID REFLUX?

When stomach acid escapes into your esophagus, it can cause a burning sensation in your chest or throat, known as heartburn. Sometimes, it can also lead to other symptoms like chest pain, coughing, or a sour taste in your mouth. While occasional acid reflux is normal and can happen to anyone, frequent or severe cases may indicate a condition called gastroesophageal reflux disease (GERD). [3]

For a typical person, a nice warm cup of coffee is the perfect way to start off the day. But not if your breakfast begins bubbling in your stomach minutes later and you have to choke down the bile that has made it's way up your throat. Acid reflux is a common gastrointestinal disorder that goes undiagnosed in approximately 41% of Canadians [1]. Even when diagnosed and prescribed medication, 30% of patients continue to have symptoms [2].

So what is acid reflux? Think about it this way, your stomach is like a container with a lid that is supposed to keep everything inside where it belongs. But sometimes, this lid doesn't close properly, allowing stomach acid and food to come back up into the throat. That's acid reflux. It happens when the muscle at the bottom of your esophagus, the lower esophageal sphincter, weakens or relaxes when it shouldn't. This can be due to various reasons, like eating acidic foods, being overweight, or even just lying down after eating.



Beyond Quick Fixes

Addressing the gap in the industry for long-term treatment of acid reflux is crucial, particularly considering the ineffectiveness of short-term interventions for many individuals. Although the availability of over-the-counter and prescription medications to manage acid reflux symptoms on a short-term basis, a noteworthy proportion of patients continue to experience persistent or recurrent issues. Around one-third of individuals who initially seek relief through short-term treatments still struggle with symptoms over time [4].

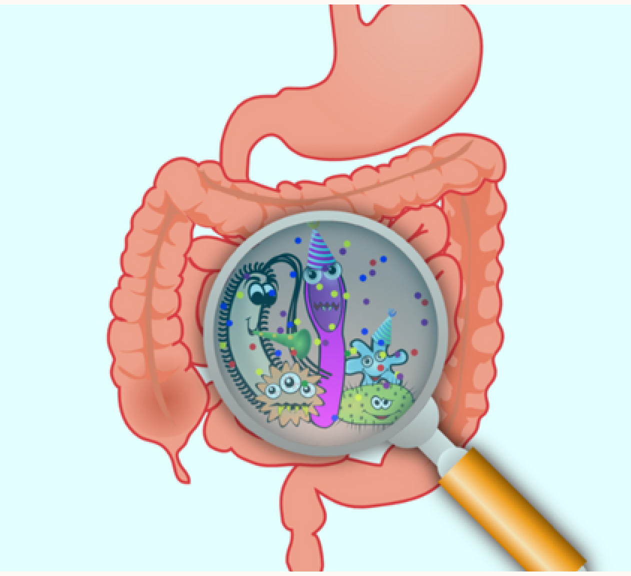
The most commonly used over-the-counter drugs for acid reflux are antacids and H2 receptor antagonists (H2 blockers). Antacids, such as Tums, Peptol Bismol, or Rolaids, provide quick relief by neutralizing stomach acid [5]. However, their effects are short-lived and do not adequately address the underlying cause of acid reflux. Similarly, H2 blockers like famotidine (Pepcid) or ranitidine (Zantac) reduce the production of stomach acid, offering temporary relief but often proving insufficient for long-term management [5].

Prescription medications such as proton pump inhibitors (PPIs), are frequently prescribed for more severe or persistent cases of acid reflux. PPIs, including omeprazole (Prilosec), esomeprazole (Nexium), and lansoprazole (Prevacid), work by blocking the enzyme responsible for acid production in the stomach [5]. Whilst PPIs are highly effective in easing symptoms and promoting healing of the oesophagus, concerns have arisen regarding their long-term safety and potential adverse effects [6]. Despite their widespread use, these "quick fix" short-term treatments such as antacids, H2 blockers, and PPIs often fail to provide lasting relief for individuals with chronic acid reflux.

"If you have nausea, heartburn, indigestion, upset stomach, diarrhea, here's Pepto Bismol" - Pepto-Bismol

Nurturing the Gut Garden

The gap in the industry lies in developing more effective and sustainable long-term treatments for acid reflux. This involves addressing the underlying mechanisms driving reflux symptoms, such as impaired esophageal motility or dysfunction of the lower esophageal sphincter [6]. Microbiology is a field of research that has attempted to explain these mechanisms through the content of microorganisms in our digestive systems (gut flora). This ecosystem is an essential driver for several important digestive processes including the breakdown of complex carbohydrates, production of essential vitamins to aid in digestion, and even immune system regulation [7]. While these floras can be beneficial to the individual, it has been suggested that dysbiosis, or the imbalance of these bacterial populations, is associated with irritable bowel syndrome and gastroesophageal reflux disease (GERD)[7]. As such, having more accessible methods to measure gut flora content as a metric for health has the potential to improve our long-term treatment options.



Biomesense is a company that looks to address this issue, through the development of its products: GutLab and Metabiome. The former specializes in providing a fully automated, self-contained laboratory attachment for bathrooms that can measure microbiome content. Similarly, Metabiome leverages analytical technology to compare any pending results to their microbiome database.

The end result is a metric that the consumer can use to track the health of their microbiome. Most importantly, this allows for consumers to perform tests from the comfort of their homes and remove the extensive process that is often required. GutLab also places emphasis on non-pharmacological treatments. While traditional methods are effective for short term goals, long term impacts can sometimes be devastating. Having precision medicine at your disposal can direct the consumer on preventative measures, such as dietary modifications and lifestyle changes, which show promise in promoting long-term symptom control [8]. Given these prospects, it is clear that developing screening technology for microbiome content can assist in providing a more sustainable long-term treatment plan.

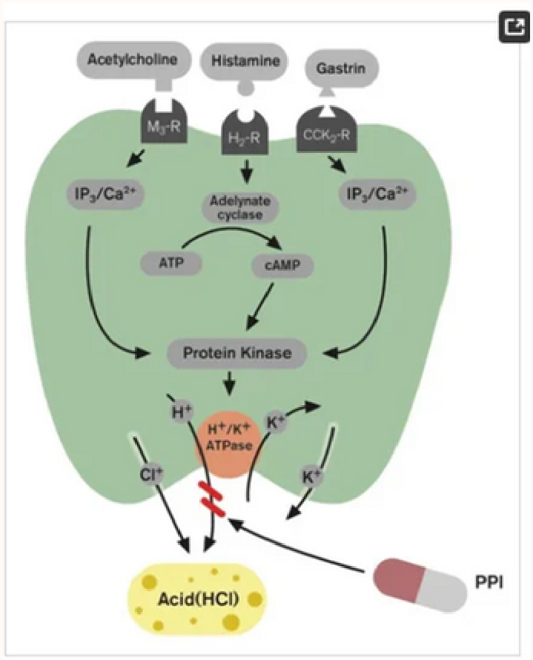
BIOMESENSE™

Reflux Rescue Technologies

The current technologies mentioned above, either prescribed or over-the-counter treatments, that are actively used to treat those who suffer from Acid Reflux or Reflux Diseases (GERD) are not truly addressing the large issue of long-term treatments but rather only glaze the surface of endless possibilities. Addressing this large gap in treatment follows two paths which must intertwine: modernized Screening Technologies for Acid Reflux Research and novel Therapeutic Treatment/Methods. Now focusing in on novel therapeutic treatments, there are a few innovations that appear promising with diminishing the large barrier acid reflux has built. Both technologies shed light on treating Acid Reflux back in 2023, the two being discussed are a NoReflux Device Implant and Chitosan Nanoparticles Delivery. The NoReflux device, a unique biodegradable valve, gets inserted into the esophageal junction, using a balloon and FDA approved glue, to allow for a minimally invasive way to treat reflux of stomach aid into the esophagus [9].

This device was created by Patrick F. Leahy and staff employed by the Laser Center Clinic located in Dublin Ireland. [9] Your local physician can administer this device and the patient can swallow the capsulated valve; this valve is encased inside of a balloon that inflates just below the beginning of the esophagus tract. This device is patented in the United States of America and Europe. The evidence from clinical trials shows to be promising in treating the unmet needs of those suffering from acid reflux [9].

The next technology was proposed by Yedi Herdiana, employed by Universitas Padjadjaran's Department of Pharmaceutics and Pharmaceutical Technology. This technology is making Nanoparticles from seafood's skeleton. Chitosan is a sugar, linear polysaccharide, that is found within the outer skeleton of shellfish, crab, lobster as well as shrimp [12]. These proposed nanoparticles allow for a targeted drug delivery in line with the already existing Proton Pump inhibitors pathways and technology. It is well known that targeting specific areas for drug delivery proves to be quite difficult although the use of Chitosan Nanoparticles can regulate acidity levels through the gradual release of pH mechanisms [11].



Research is currently being done to find the optimal pH of these nanoparticles so that when passing the esophagus, the capsule containing Chitosan Nanoparticles will biodegrade and coat the lower esophageal junction as it passes the site where NoReflux Device is located [10,11]. The goal of this technology is to minimize inflammation, burning sensations and allow for those who experience nightly acid reflux a greater chance at feeling comfortable, the study acknowledges that although this is a common disorder, more research is required among essential lifestyle changes.

Farewell to Acid Reflux

As we look upon the future, we envision a world with new technologies and methods to provide simple and effective solutions to acid reflux. From the biodegradable marvel of the NoReflux Device to the precision of Chitosan Nanoparticles, science is forging pathways toward a future where the simple joys of daily life are no longer overshadowed by discomfort. With continued research and development, coupled with essential lifestyle adjustments, savoring that morning cup of coffee without the unwelcome intrusion of acid reflux will be restored to comfort and ease. Ensuring that every sip of coffee brings pleasure, and not bile, here's to mornings filled with warmth and flavor, unmarred by the unpleasantness of your last meal.

References

[1] Fedorak, R. N., Veldhuyzen van Zanten, S., & Bridges, R. (2010). Canadian Digestive Health Foundation Public Impact Series: gastroesophageal reflux disease in Canada: incidence, prevalence, and direct and indirect economic impact. Canadian journal of gastroenterology = Journal canadien de gastroenterologie, 24(7), 431–434. https://doi.org/10.1155/2010/296584

[2] Ang, D., How, C. H., & Ang, T. L. (2016). Persistent gastro-oesophageal reflux symptoms despite proton pump inhibitor therapy. Singapore medical journal, 57(10), 546–551. https://doi.org/10.11622/smedj.2016167

[3] Antunes, C., Aleem, A., & Curtis, S. A. (2023, July 3). Gastroesophageal Reflux Disease. In StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK441938/

[4] Ang, D., How, C., & Ang, T. (2016). Persistent gastro-oesophageal reflux symptoms despite proton pump inhibitor therapy. Singapore Medical Journal, 57(10), 546–551. https://doi.org/10.11622/smedj.2016167

[5] Research, C. for D. E. and. (2021). Over-The-Counter (OTC) Heartburn Treatment. FDA. https://www.fda.gov/drugs/information-consumers-and-patients-drugs/over-counter-otc-heartburn-treatment

[6] Abbas, M. K., Zaidi, A. R. Z., Robert, C. A., Thiha, S., & Malik, B. H. (2019). The Safety of Long-term Daily Usage of a Proton Pump Inhibitor: A Literature Review. Cureus, 11(9). https://doi.org/10.7759/cureus.5563

[7]Antunes, C., Curtis, S. A., & Aleem, A. (2023, July 3). Gastroesophageal reflux disease. National Library of Medicine; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK441938/

[8] Okereke, I., Hamilton, C., Wenholz, A., Jala, V., Giang, T., Reynolds, S., Miller, A., & Pyles, R. (2019, August). Associations of the microbiome and esophageal disease. Journal of thoracic disease. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6702393/#:~:text=Gut%20dysbiosis%20has%20been%20associated,irritable%20bowel%20syndrome%2C%20and%20colitis.

[9] Gastroenterology & Hepatology, 11(5), 343–345. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4962685/#:~:text=VV%20GERD%20is%20usually%20first

[10] Leahy, P. F. (2023). A Novel Therapeutic Method in Gastro-esophageal Reflux Disease. Surgical Innovation. https://doi.org/10.1177/15533506231223913

[11] Yedi Herdiana. (2023). Chitosan Nanoparticles for Gastroesophageal Reflux Disease Treatment. Polymers, 15(16), 3485–3485. https://doi.org/10.3390/polym15163485

[12] WebMD. (n.d.). Chitosan: Uses, Side Effects, Interactions, Dosage, and Warning. Www.webmd.com. https://www.webmd.com/vitamins/ai/ingredientmono-625/chitosan