POMPE WARRIOR FOUNDATION

RESEARCH EDUCATE EMPOWER

Research Hypothesis

**Updated as of May 19th, 2023*

The Problem

If you are "normal" your body has the ability to clear your cells of waste, miss folded proteins, and other organelles. If you have a lysosomal storage disease (LSD) you do not have this ability or it is severely reduced. The result is a buildup of waste, miss folded proteins, and so on. This is known as a buildup of the autophagic process. In normal cell function, the autophagic process aids the lysosome with its work. So is the LSD the problem or the buildup of everything else? We believe the answer is both. The first paper listed below does a great job of explaining the issues. Once the problem has been established we intend on showing potential solutions to the problems that exist with LSDs.

Autophagy in lysosomal storage disorders

https://www.tandfonline.com/doi/full/10.4161/auto.19469#.XJ9zInYTUw4.email

From the Abstract

Lysosomes also play a fundamental role in the autophagic pathway by fusing with autophagosomes and digesting their content. Considering the highly integrated function of lysosomes and autophagosomes it was reasonable to expect that lysosomal storage in LSDs would have an impact upon autophagy. The goal of this review is to provide readers with an overview of recent findings that have been obtained through analysis of the autophagic pathway in several types of LSDs, supporting the idea that LSDs could be seen primarily as "autophagy disorders."

From the third paragraph in the introduction. Pay attention to the term TFEB!

The lysosomal system has thus emerged from being considered only an end-organelle, to being at the very hub of metabolic regulatory control. Perhaps the best example of the interconnection of the lysosome with other cellular systems is autophagy. Lysosomes play a fundamental role in the autophagic pathway by fusing with autophagosomes and digesting their content. Recent evidence of the cooperative and integrated roles of lysosomes and autophagosomes comes from the discovery of an overarching regulatory gene network (CLEAR) and a master gene, transcription factor EB (TFEB), controlling the biogenesis and function of both lysosomes and autophagosomes.[8]Sardiello M, Ballabio A. Lysosomal enhancement: a CLEAR answer to cellular degradative needs. Cell Cycle 2009; 8:4021 - 2; http://dx.doi.org/10.4161/cc.8.24.10263; PMID: 19949301[Taylor & Francis Online], [Web of Science ®], [Google Scholar][-10]

This Table shows the Type of LSD and the types of the autophagic buildup that each disease has. They all have multiple forms of buildup. NOTICE INCREASED P62 IN EVERY DISEASE THAT THEY TESTED

Table 1 of 1

Table 1. The main results of the studies on autophagy in LSDs

Disease	AV accumulation[7]	Defective AV degradation[7]	Increased AV formation[9]	Increased poly-ub proteins[1]	Increased dysfunctional mitochondria[5]	Increased p62[4]	
GLYCOGENOSES							
Pompe disease	Y	Y	Y	Y	NT	Y	11Raben N, Hill V, Shea L, Takikita S, Baum R, Mizushima N, et al.Suppr autophagy in skeletal muscle uncovers the accumulation of ubiquitinated prot their potential role in muscle damage in Pompe disease. Hum Mol Gen 17:3897 - 908; http://dx.doi.org/10.1093/hmg/ PMID: 18782848[Crossref], [PubMed], [Web of Science ®],

Table 1. The main results of the studies on autophagy in LSDs

Disease	AV accumulation[†]	Defective AV degradation[‡]	Increased AV formation[°]	Increased poly-ub proteins[¶]	Increased dysfunctional mitochondria[§]	Increased p62[•]	
							Scholar],12Raben N, Schreiner C, Baum R, Takikita S, Xu S, Xie T, et al.Suppr autophagy permits successful enzyme replacement therapy in a lysosoma disorder--murine Pompe disease. Autophagy 2010; 6:1(http://dx.doi.org/10.4161/auto.6.8.13378; PMID: 20861693[Taylor Online], [Web of Science ®], , [Google
Danon disease	Y	Y	NT	NT	NT	NT	13Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lüllmann-Ra al. Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficie Nature 2000; 406:902 - 6; http://dx.doi.org/10.1038/35 PMID: 10972293[Crossref], [PubMed], [Web of Science ®], , [Google
POLYSACCHARIDOSES							
MSD	Y	Y	N	Y	Y	Y	14Settembre C, Fraldi A, Jahreiss L, Spampanato C, Venturi C, Medina D, et al. A autophagy in lysosomal storage disorders. Hum Mol Genet 2008; 17:1 http://dx.doi.org/10.1093/hmg/ddm289; PMID: 17913701[Crossref], [PubMed] Science ®], , Scholar],15Fraldi A, Annunziata F, Lombardi A, Kaiser HJ, Medina DL, Spampan al. Lysosomal fusion and SNARE function are impaired by cholesterol accumu lysosomal storage disorders. EMBO J 2010; 29:36 http://dx.doi.org/10.1038/emboj.2010.237; PMID: 20871593[Crossref], [PubMe of Science ®], , [Google
MPSIII A	Y	Y	N	Y	Y	Y	14Settembre C, Fraldi A, Jahreiss L, Spampanato C, Venturi C, Medina D, et al. A autophagy in lysosomal storage disorders. Hum Mol Genet 2008; 17:1 http://dx.doi.org/10.1093/hmg/ddm289; PMID: 17913701[Crossref], [PubMed] Science ®], , Scholar],15Fraldi A, Annunziata F, Lombardi A, Kaiser HJ, Medina DL, Spampan al. Lysosomal fusion and SNARE function are impaired by cholesterol accumu lysosomal storage disorders. EMBO J 2010; 29:36 http://dx.doi.org/10.1038/emboj.2010.237; PMID: 20871593[Crossref], [PubMe of Science ®], , [Google
MPS VI	Y	Y	NT	Y	Y	Y	16Tessitore A, Pirozzi M, Auricchio A.Abnormal autophagy, ubiquitination, infla and apoptosis are dependent upon lysosomal storage and are useful biom mucopolysaccharidosis VI. Pathogenetics 20 http://dx.doi.org/10.1186/1755-8 PMID: 19531206[Crossref], [PubMed], , [Google
SPHINGOLIPIDOSES							
NPC1, NPC2	Y	Y	Y	Y	Y	Y	17Ko DC, Milenkovic L, Beier SM, Manuel H, Buchanan J, Scott MP. Cell-aut death of cerebellar purkinje neurons with autophagy in Niemann-Pick type C PLoS Genet 2005; 1:81 - 95; PMID: 16103921[Crossref], [PubMed], [Web o ®], , [Google Scholar],18Pacheco CD, Elrick MJ, Lieberman AP.Tau deletion exa the phenotype of Niemann-Pick type C mice and implicates autophagy in patho Hum Mol Genet 2009; 18:956 - 65; PMID: 19074461[Crossref], [PubMed] Science ®], , [Google
Gaucher disease	Y	NT	NT	NT	NT	Y	19Sun Y, Liou B, Ran H, Skelton MR, Williams MT, Vorhees CV, et al.Neuro Gaucher disease in the mouse: viable combined selective saposin C defici mutant glucocerebrosidase (V394L) mice with glucosylsphingosine and glucosyl accumulation and progressive neurological deficits. Hum Mol Genet 2010; 19:1(http://dx.doi.org/10.1093/hmg/ddp580; PMID: 20047948[Crossref], [PubMed] Science ®], , [Google
Fabry disease	Y	Y	NT	Y	NT	Y	20Chévrier M, Brakch N, Céline L, Genty D, Ramdani Y, Moll S, et al.Autoph maturation is impaired in Fabry disease. Autophagy 2010; 6:5 http://dx.doi.org/10.4161/auto.6.5.11943; PMID: 20431343[Taylor Online], [Web of Science ®], , [Google
GM1 gangliosidosis	Y	NT	Y	NT	Y	NT	21Takamura A, Higaki K, Kajimaki K, Otsuka S, Ninomiya H, Matsuda J, et al. E autophagy and mitochondrial aberrations in murine G(M1)-gangliosidosis. Biophys Res Commun 2008; 367: http://dx.doi.org/10.1016/j.bbrc.2007 PMID: 18190792[Crossref], [PubMed], [Web of Science ®], , [Google
MUCOLIPIDOSES							
MLII	Y	NT	N	Y	Y	Y	22Otomo T, Higaki K, Nanba E, Ozono K, Sakai N. Inhibition of autophagosome f restores mitochondrial function in mucolipidosis II and III skin fibroblasts. M Metab 2009; 98:393 - 9; http://dx.doi.org/10.1016/j.ymgme.2009 PMID: 19656701[Crossref], [PubMed], [Web of Science ®], , [Google
MLIII	Y	NT	N	Y	Y	Y	22Otomo T, Higaki K, Nanba E, Ozono K, Sakai N. Inhibition of autophagosome f restores mitochondrial function in mucolipidosis II and III skin fibroblasts. M Metab 2009; 98:393 - 9; http://dx.doi.org/10.1016/j.ymgme.2009 PMID: 19656701[Crossref], [PubMed], [Web of Science ®], , Scholar],23Kobayashi H, Takahashi-Fujigasaki J, Fukuda T, Sakurai K, Shimada a K, et al. Pathology of the first autopsy case diagnosed as mucolipidosis typ suggesting autophagic dysfunction. Mol Genet Metab 2011; 102 http://dx.doi.org/10.1016/j.ymgme.2010 PMID: 21051253[Crossref], [PubMed], [Web of Science ®], , [Google

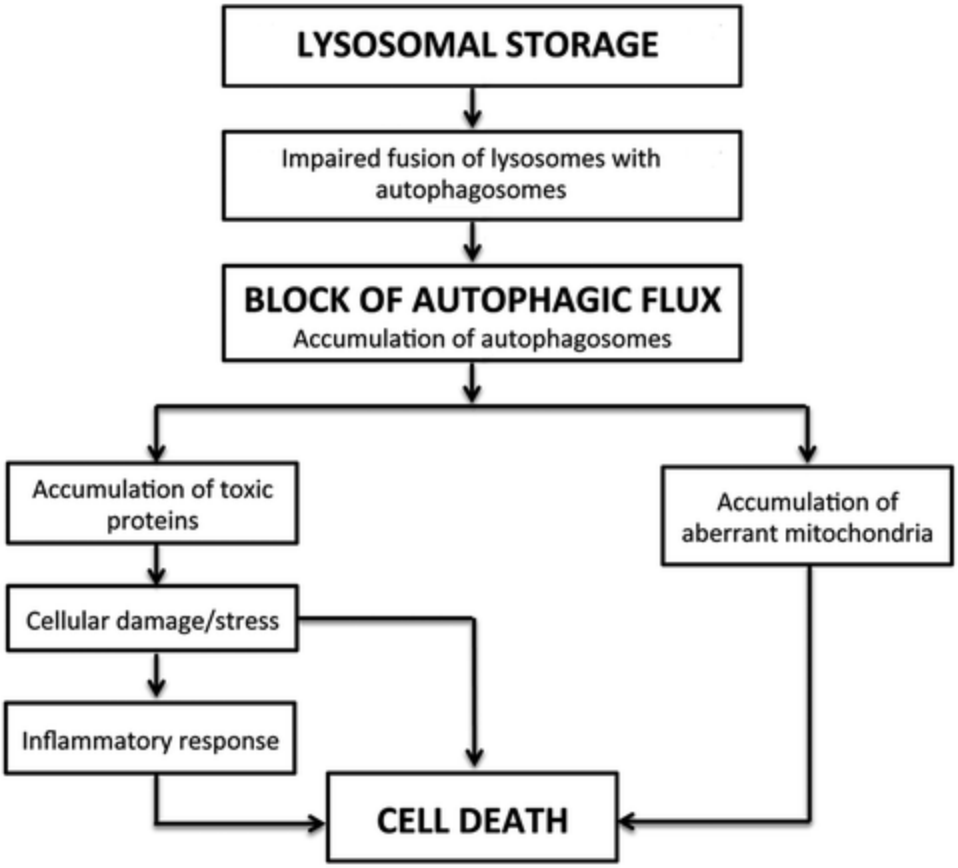
Table 1. The main results of the studies on autophagy in LSDs

Disease	AV accumulation†	Defective AV degradation‡	Increased AV formation°	Increased poly-ub proteins¶	Increased dysfunctional mitochondria§	Increased p62•	
MLIV	Y	Y	Y	Y	Y	Y	24Vergarajauregui S, Connelly PS, Daniels MP, Puertollano R. Autophagic dysfu mucolipidosis type IV patients. Hum Mol Genet 2008; 17:27 http://dx.doi.org/10.1093/hmg/ddn174; PMID: 18550655[Crossref], [PubMed], Science ®], , Scholar]–26Curcio-Morelli C, Charles FA, Micsenyi MC, Cao Y, Venugopal B, Brow et al. Macroautophagy is defective in mucolipin-1-deficient mouse neurons. M Dis 2010; 40:370 - 7; http://dx.doi.org/10.1016/j.nbd.2010 PMID: 20600908[Crossref], [PubMed], [Web of Science ®], , [Google
OID LIPOFUSCINOSES							
CLN10	Y	NT	NT	NT	NT	NT	27Koike M, Shibata M, Waguri S, Yoshimura K, Tanida I, Kominami E, et al. Par of autophagy in storage of lysosomes in neurons from mouse models of ceroid-lipofuscinoses (Batten disease). Am J Pathol 2005; 167:17 http://dx.doi.org/10.1016/S0002-9440(10)6 PMID: 16314482[Crossref], [PubMed], [Web of Science ®], , [Google
CLN 3	Y	NT	Y	NT	NT	NT	28Cao Y, Espinola JA, Fossale E, Massey AC, Cuervo AM, MacDonald ME, et al.Au is disrupted in a knock-in mouse model of juvenile neuronal ceroid lipofuscinos Chem 2006; 281:20483 - 93; http://dx.doi.org/10.1074/jbc.M602 PMID: 16714284[Crossref], [PubMed], [Web of Science ®], , [Google

†Number of autophagic vesicles (AV) quantified by electron microcopy or LC3-immunofluorescence, amounts of LC3-II by western blotting. ‡Impaired autophagosome-lysosome fusion, defective degradation of long-lived proteins. °MTOR downregulation, BECN1 activation ¶Poly-ubiquitinated proteins (poly-ub) revealed by immunofluorescence or western blotting using anti-ubiquitin antibodies. §Dysfunctional mitochondria revealed by western blotting using mitochondrial markers. •p62/SQSTM1 protein revealed by immunofluorescence or western blotting using anti-p62 antibodies.

This next figure does a good job of showing the progression to cell death

Figure 2. Model depicting disease pathogenesis in LSD's



This excerpt is the first paragraph from the papers conclusion

Autophagy has been analyzed in a variety of LSDs with different severities of the phenotype, different tissues involved, and different types of storage molecules. Table 1 shows the main findings obtained and Figure 1 displays some examples of the results obtained by these studies. In spite of all the above-mentioned differences among the diseases and samples analyzed, a common theme can be recognized. In most cases there is an impairment of autophagic flux, causing a secondary accumulation of autophagy substrates such as polyubiquitinated proteins, p62/SQSTM1 and dysfunctional mitochondria, on one end, and an increase in factors involved in autophagosome formation, such as BECN1, as an attempt to compensate for the impaired autophagic flux, on the other. Accordingly, LSDs can be seen primarily as "autophagy disorders.". Interestingly, a defect in autophagic lysosome formation, due to abnormal MTOR activation, is reported in several LSDs.[3]

[10]Settembre C, Fraldi A, Rubinsztein DC, Ballabio A. Lysosomal storage diseases as disorders of autophagy. Autophagy 2008; 4:113 - 4; PMID: 18000397[Taylor & Francis Online], [Web of Science ®], , [Google Scholar]

This is from the last paragraph in their conclusion

Finally, a potentially attractive possibility would be to induce a global enhancement of both the lysosomal and autophagic pathways by acting on the master gene TFEB.[9] Further studies are needed to explore the effects of modulators of autophagy, which operate at different steps of the autophagic flux, on the LSD phenotype in cell

culture and in animal models. Hopefully, these studies will lead to the development of effective treatments for several LSDs.

In the above paper, they do a good job of showing the complexity of having a LSD. They point out the many problems within the autophagic process like the accumulation of a particular protein called p62/SQSTM1. This protein is an issue with all LSD known to us today. At the very end of the paper, they hypothesize that the activation of TFEB might be a solution to some or all of the autophagic problems. In the remainder of this email, I intend to show how we get rid of p62/SQSTM1 and how we activate and aid TFEB in the clearance of unwanted substrates.

The Solution

Below I will try and show how we at Pompe Warrior Foundation have attacked the Autophagy problem in our son who has infantile Pompe. First I will show how the Ketogenic diet suppresses a master growth hormone called mTor. The suppression of mTOR leads to an upregulation of TFEB which is the master regulator of the autophagic process.

What is the Ketogenic Diet?

This is from Webster's Dictionary: Medical Definition of *ketogenic diet*
: a diet supplying a large amount of fat and minimal amounts of carbohydrate and protein

Here is the first study in our argument:

The ketogenic diet inhibits the mammalian target of rapamycin (mTOR) pathway

This study clearly states the suppression of mTOR thru the Ketogenic diet. Below is an excerpt from this study:

Abstract
The ketogenic diet (KD) is an effective treatment for epilepsy, but its mechanisms of action are poorly understood. We investigated the hypothesis that the KD inhibits mammalian target of rapamycin (mTOR) pathway signaling. The expression of pS6 and pAkt, markers of mTOR pathway activation, was reduced in hippocampus and liver of rats fed KD. In the kainate model of epilepsy, KD blocked the hippocampal pS6 elevation that occurs after status epilepticus. Because mTOR signaling has been implicated in epileptogenesis, these results suggest that the KD may have anticonvulsant or antiepileptogenic actions via mTOR pathway inhibition.

Here is a link to the full study:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3076631/

The next few studies will show why the suppression of mTOR is so important for the upregulation of TFEB (the master regulator of autophagy).

The role of mTOR in Glucose and Lipid Metabolism

Below is taken from the above study. You can see how the upregulation of mTOR blocks TFEB and the autophagic process.

mTORC1 activation suppresses lysosome pathway through inhibiting the activity of the master regulator of lysosomal biogenesis, transcription factor EB (TFEB). Nutrient deprivation or inhibition of mTORC1 activates TFEB by promoting its nuclear translocation, thus initiating the expression of lysosomal and autophagic genes

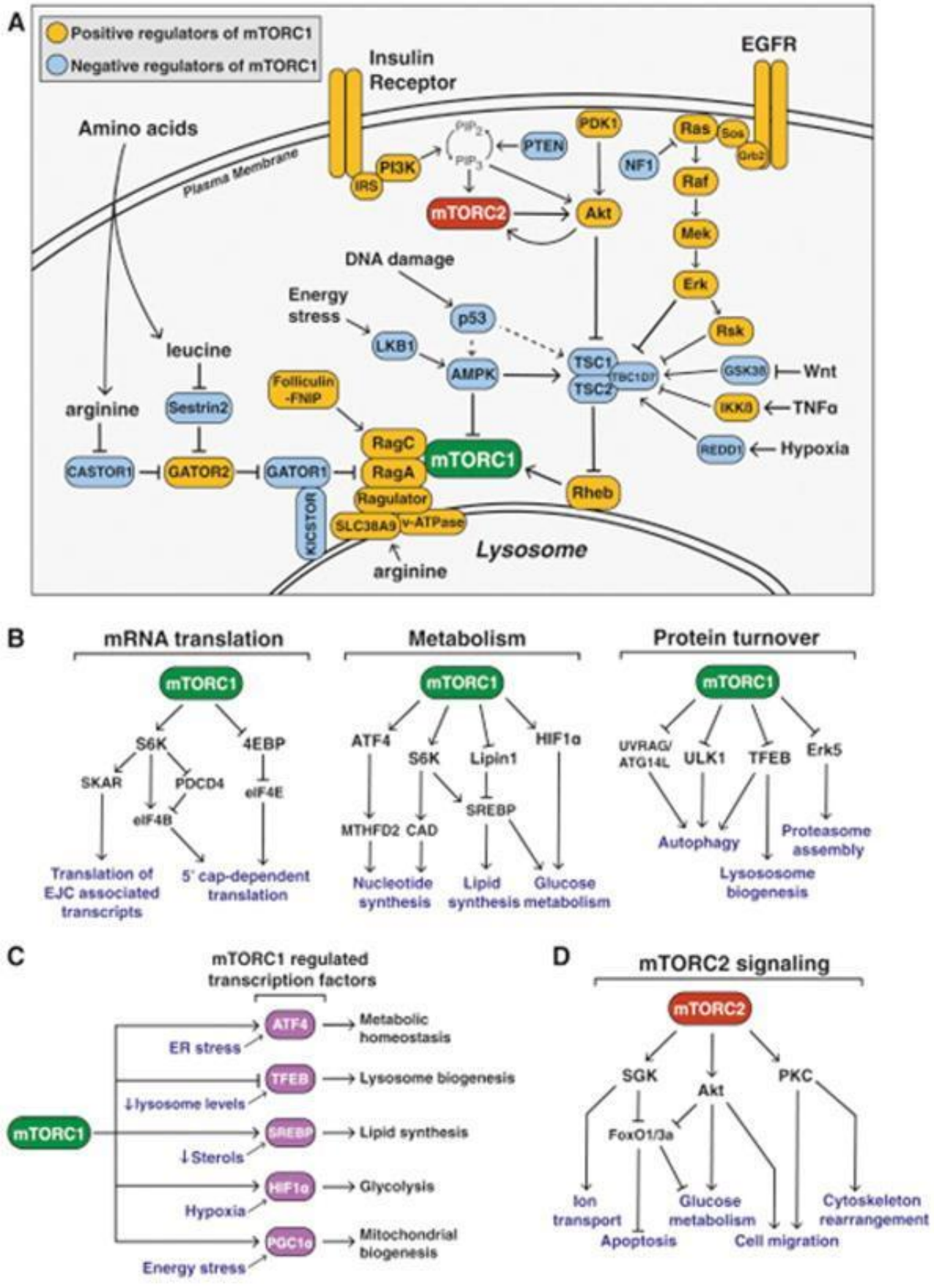
[13]. Recently, two reports have demonstrated that the ubiquitin-proteasome system (UPS) in mammalian cells is increased when mTORC1 signaling pathway is inactivated

Here is a link to the full study:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6073766/

mTOR Signaling in Growth, Metabolism, and Disease

Here is a graph from the above study that clearly shows that mTor blocks TFEB. If you look at the last graphic in the B section you will see this demonstrated. It is similarly shown in the first graphic in section C.



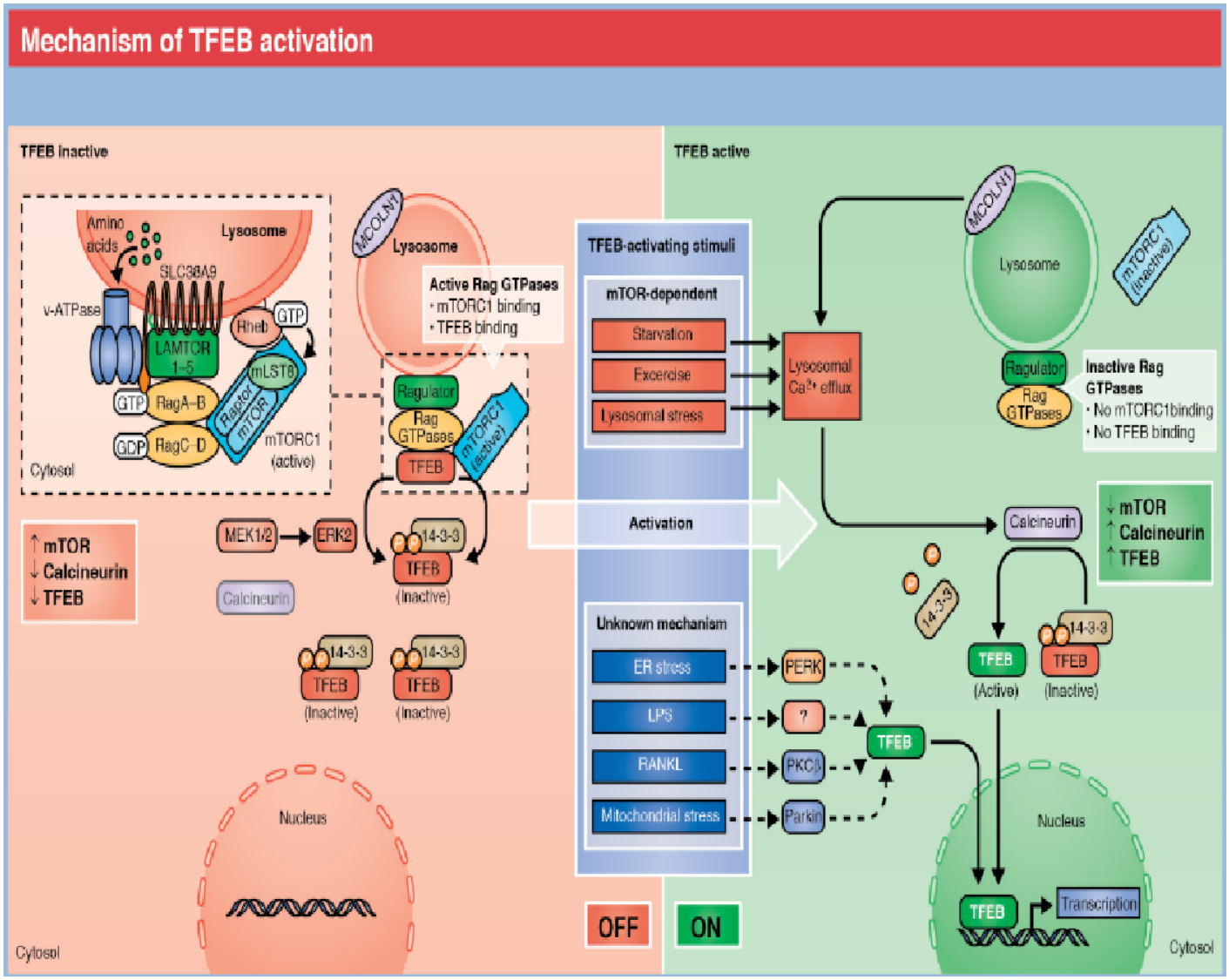
Here is a link to the above study and graph:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5394987/

The next study will confirm TFEB as the master regulator of the autophagic process and the relationship between mTOR and TFEB.

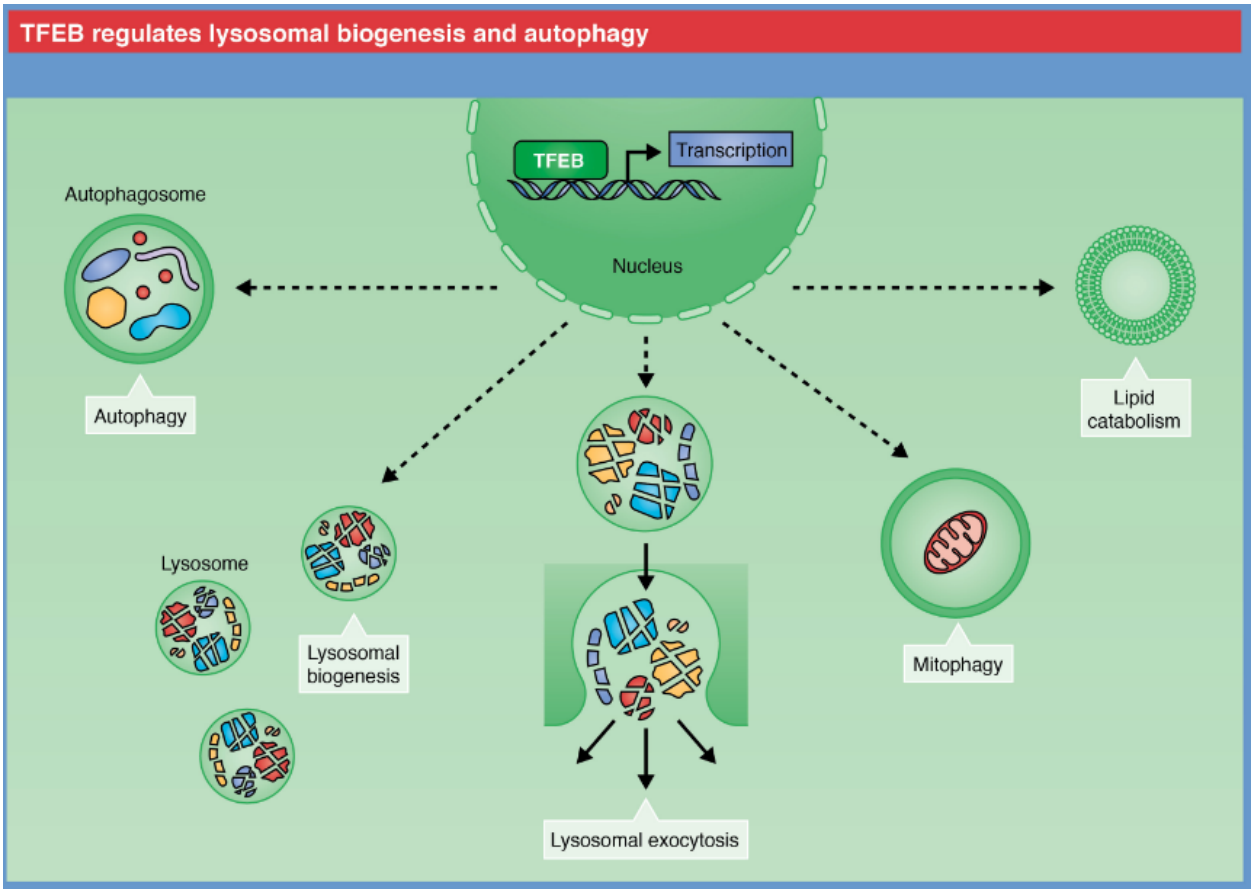
TFEB at a glance.

The following graphic, Mechanism of TFEB activation, shows one side is red and has an up arrow next to the word mTOR and a down arrow next to the word TFEB. The other side is green and shows a down arrow next to mTOR and an up arrow next to TFEB. We are now testing the idea that a properly formulated ketogenic diet has the ability to shift a person's metabolism over to the green side of this graphic more often. This allows TFEB to move down to the nucleus and express itself.



http://jcs.biologists.org/content/joces/suppl/2016/06/23/jcs.146365.DC2/JCS146365supp3.jpg

The next graphic is also from the paper TFEB at a glance, and it shows the processes that happen when TFEB moves to the nucleus and is expressed.



http://jcs.biologists.org/content/joces/suppl/2016/06/23/jcs.146365.DC2/JCS146365supp2.jpg

By using a properly formulated Ketogenic Diet, we think it's possible to over express TFEB and limit lysosomal-glycogen buildup in the body. PWF is hypothesizing that the over-expression of TFEB is a treatment that would improve the standard of care in LSD's.

This is a great paper and here is the link:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4958300/

The next studies will focus on the activation of the NRF2 pathway thru sulforaphane:

What is the NRF2?

Nrf2 is a powerful protein that is latent within each cell in the body, unable to move or operate until it is released by an Nrf2 activator. Once released it migrates into the cell nucleus and bonds to the DNA at the location of the Antioxidant Response Element (ARE) or also called hARE (Human Antioxidant Response Element) which is the master regulator of the total antioxidant system that is available in ALL human cells.

Heoverexpressr on how you activate NRF2 thru sulforaphane:

Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality?

Here is an excerpt from the above study:

Notably, the CD value of sulforaphane is 13.5-fold greater than that of curcumin, 18-fold greater than silymarin, and 105-fold greater than resveratrol, all phytochemicals which are extensively promoted for their claimed health-promoting properties

The CD value is the measurement they use for the activation of NRF2. Here is a link to the full study:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4736808/

The next paper will discuss the relationship between the p62 gene and the NRF2 pathway. If you remember from the first half of this email entailed The Problem the accumulation of p62 was seen across all LSD's.

p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription.

Here is an excerpt from this paper:

Expression of the *p62* gene is induced by NRF2 upon exposure to electrophiles, reactive oxygen species, and nitric oxide (38, 43, 49). Furthermore, the p62 protein has been reported to stimulate the expression of genes containing an ARE in their promoter regions (39). From these previous studies, it appeared that a positive interrelationship of some sort exists between NRF2 and p62, which influences the ARE-gene battery, but the exact mechanism was unresolved. Herein we have shown that NRF2 can induce *p62* expression by binding directly to a conserved ARE in its promoter/enhancer and that the p62 protein is able to augment its own expression via this element. Specifically, we found p62 stimulated NRF2 activity by binding to KEAP1, sequestering it, and directing its degradation by autophagy.

The p62 gene acts as a shuttle and a docking station for part of the autophagic process. The accumulation of the p62 gene is seen in every LSD because the autophagic process doesn't work. This paper demonstrates how the NRF2 gene can get rid of excess p62 and utilizes it in the autophagic process. Here is a link https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903417/

Below are two videos that go in-depth about the NRF2 pathways. They are long but informative:

https://www.youtube.com/watch?v=zz4YVJ4aRfg

This next video is with a Dr. Jed Fayhe from John Hopkins. He is the one who developed the research around the NRF2 pathways and sulforaphane:

https://www.youtube.com/watch?v=V8IjvUVL3tw

The next study will focus on Vitamin C and its role in authophagy.

Vitamin C plays a big role in stabilizing the PH levels within the cell which helps promote normal function of cell operations. It also helps in the degradation of molecules that have been hanging around the cell to long. Both of these functions are critical in the autophagic process.

Stimulatory effect of vitamin C on autophagy in glial cells

Here are a few excerpts from the above study:

Supplementation of the culture medium with physiological concentrations of vitamin C did not affect protein synthesis, but did increase the rate of protein degradation by lysosomes. Vitamin C accelerated the degradation of intra- and extracellular proteins targeted to the lysosomal lumen by autophagic and heterophagic pathways

We then compared the total rates of protein degradation in HA cells treated or not with VC. Supplementation of cells with 200 lM VC significantly increased the degradation rate of proteins with a long half-life (20% increase in percentage protein degraded per hour; Fig. 3a) but did not change the degradation rate of short half-life proteins (Fig. 3b). When the vitamin was removed from the cultured medium, protein degradation rates returned to values close to non-treated cells

. VC supplementation did not modify the degradation of proteins with a half-life shorter than 4 h, but accelerated, in a dose-dependent manner, the degradation of proteins still present inside the cell 20 h after synthesis (long half-life proteins).

These results suggest that VC stimulates macroautophagy but also other lysosomal pathways active under these conditions, such as chaperone-mediated autophagy.

These results suggest that though VC moderately increases the uptake of cytosolic proteins into lysosomes, its most striking effect is to accelerate their degradation once in the lysosomal lumen.

Our results suggest that supplementation of HA with VC stimulates intralysosomal protein degradation, probably by lowering and stabilizing the intralysosomal pH, at values at which the lysosomal proteases reach their maximal proteolytic activity.

The current recommended dosage for vitamin C is at a very low level. It is the level at which it will keep scurvy away. This paper along with others shows that vitamin C does a lot more than just keep away scurvy and we should be using it at higher volumes. Here is a link to the above study.

http://www.einstein.yu.edu/cuervo/images/vitaminc.pdf

Final Thoughts

Anyone who is a researcher in the Lysosomal field knows that the autophagic process or the buildup of the autophagic process is a huge problem for someone with an LSD. If your lysosome is stuck on a particular molecule it cannot clean up the rest of the cell which leads to cell death and ultimately the death of someone with LSD. The medical community is starting to talk about this but they avoid already published research to activate these systems. Pompe Warrior Foundation believes we have put together a few of the pieces to this puzzle. **It is the synergy of the Ketogenic diet, activation of the NRF2 pathway, use of vitamin C, and proper supplementation.** This will lead to clearance of the cellular debris, genesis and bio-genesis of the lysosome, creation of autophagosomes, down-regulation of mTOR, up-regulation of TFEB, and finally lead to as normal function as possible for the lysosome and the environment in which it lives.

We are currently working with the Mayo Clinic on a study that will put children with Pompe on the Ketogenic diet. The lead author of this is Dr. Marc Patterson. Here is a link to his profile - https://www.mayo.edu/research/faculty/patterson-marc-c-m-d/bio-00026606. You will notice he is very involved with the Lysosomal Disease Network. He is the Chief of Pediatric and Adolescent Neurology among many other things. Dr. Patterson will not let us activate the NRF2 pathway or use vitamin C without more research in this area. The problem with this is there is no research that puts all of these strategies together.

We are currently working with Dr. Dom D'Agostino from the University Of Southern Florida. He is one of the leading researchers in the world on the Ketogenic Diet. Here is a link to his profile - https://health.usf.edu/medicine/mpp/faculty/24854/D'Agostino. Dr. D'Agostino currently has a study looking at the activation of the autophagic process through the ketogenic diet and supplementation.

Pompe Warrior Foundation is a Mom and a Dad fighting for their son and for others with LSD. We are reaching out to the public and others in the medical community in the hopes that you will know an avenue for us to take, to obtain funding for more of Dr. D'Agostino's work and others like it. We know the problem, we think we have a part of the solution, and now we need to prove it!