MENTAL HEALTH DISORDER OR NEUROTOXICITY: A BINARY CONCLUSION

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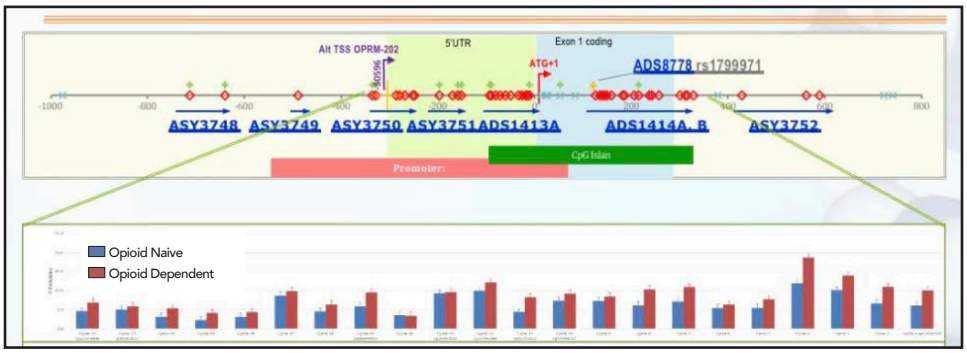
Abstract

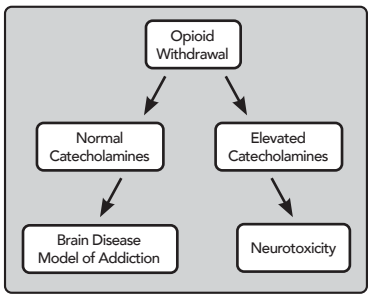
It is a fundamental principle of Medicine that any and all underlying physiological pathology is eliminated as the diagnosis prior to the application of a mental health diagnosis. If a mental health diagnosis is inappropriately applied and the underlying physiological pathology remains undisclosed, the patient is at risk of loss of life and limb.

It is our hypothesis that the diagnosis of Opioid Addiction/Opioid Use Disorder has been improperly applied and the true underlying pathology has been overlooked.[1] This has resulted in great harm, suffering, and death. It is our hypothesis that the true pathology is a neurotoxicity as a result of the methylation within the promoter region of the OPRM1 gene and due to repetitive opioid exposure. This neurotoxicity occurs after some yet to be determined threshold of methylation and when opioid abstinence is attempted. A neurotoxicity occurring secondary to a methylation within the DNA is a form of a genotoxicity.[2] Neurotoxicity as a result of methylations are common in the heavy metals and have been very well described.[3, 4, 5, 6, 7] (Figure 1)

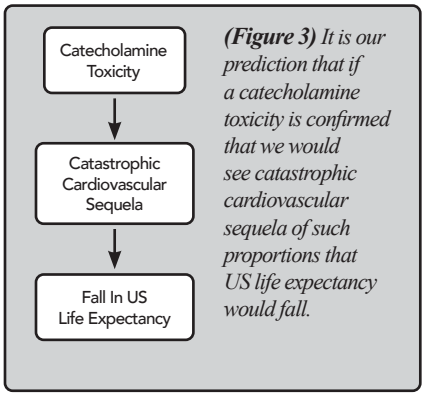


(Figure 1) *OPRM1 gene: This gene encodes for the main opioid receptor, the mu-opioid receptor. Hypermethylation is easily identified in the opioid dependent population and on multiple CpG Islands.*

This is an important question of our time and one that needs an urgent answer. Are millions of our citizens suffering from a mental health disorder driving a craving to get high on opioids? Or has genetic damage due to repetitive opioid exposure damaged the body in such a manner that symptoms of a neurotoxicity occur when opioid abstinence is attempted? This is a binary conclusion. It is one or the other. It cannot be both. And an answer is urgently needed. It is our hypothesis that the answer will be found in a simple blood test done on human volunteers and during an episode of opioid withdrawal due to an opioid abstinence. The blood test is a measure of the catecholamine levels: epinephrine, norepinephrine, and dopamine. If a loss of control within the nervous system is occurring, these levels will be abnormally elevated. If the catecholamine levels are elevated, this would establish the diagnosis of a neurotoxicity. If the catecholamine levels are normal, this would lend credence to the more traditional Brain Disease Model of Addiction. It cannot be both. This became the first aim of our clinical trial, determine the catecholamine levels during opioid withdrawal and give a binary conclusion to the question: is this a mental health disorder or is this a deadly genotoxicity? (Figure 2)

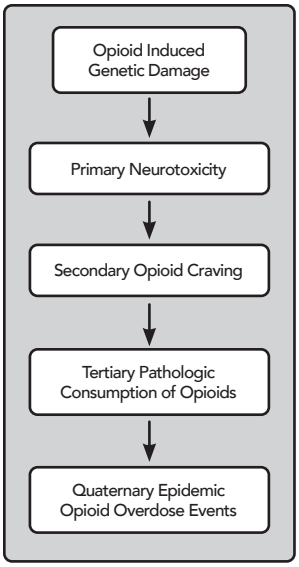


(Figure 2) *Are people suffering from a mental health disorder or a neurotoxicity? A simple blood test for catecholamines should resolve this question.*



(Figure 3) *It is our prediction that if a catecholamine toxicity is confirmed that we would see catastrophic cardiovascular sequela of such proportions that US life expectancy would fall.*

Catecholamine toxicity is not without sequela. If widespread catecholamine toxicity, as predicted by our hypothesis, is confirmed, then the hypothesis predicts that a Public Health Emergency of near unprecedented proportions will be found in cardiovascular morbidity and mortality. According to the hypothesis, cardiovascular morbidity will include such rare and obscure clinical entities such as Takotsubo Cardiomyopathy. The cardiovascular mortality will be of such a magnitude that U.S. life expectancy will fall until either the vulnerable population receives proper diagnosis and treatment or the vulnerable population is driven towards extinction. This would be the first modern Public Health Emergency with the capability to reverse national life expectancy trends. (Figure 3)



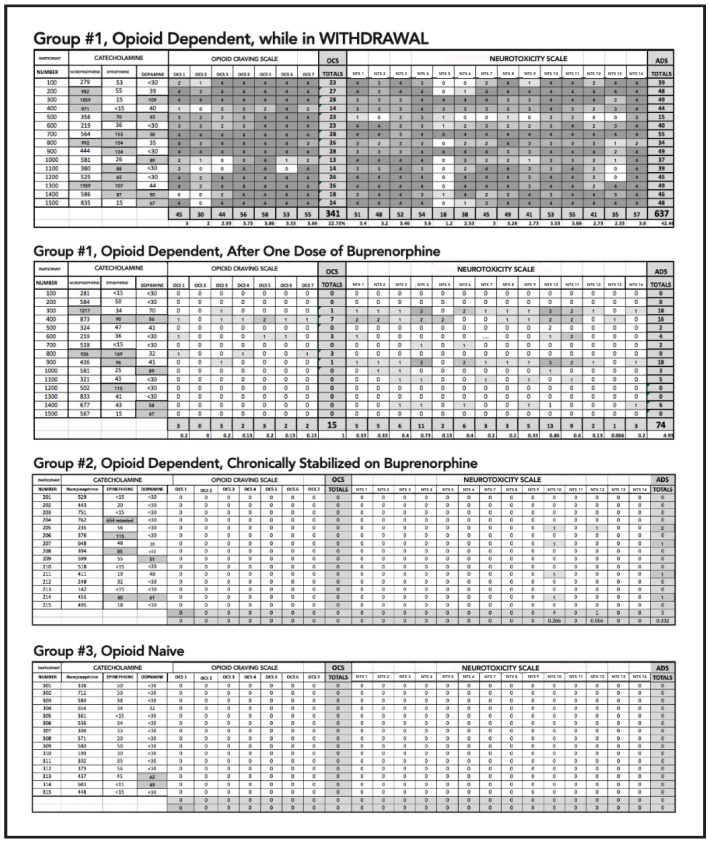
(Figure 4) *This pathologic consumption of the opioids was misinterpreted by the mental health community as an addiction.*

This epidemic of cardiovascular morbidity and mortality would mean that a DNA based clinical test to detect those at risk for cardiovascular catastrophe would need to be urgently developed. This test would involve the ability to measure hypermethylation on the CpG islands within the promoter region of the OPRM1 gene. This became the second aim of our clinical trial, determine likely CpG islands within the promoter region of the OPRM1 gene suitable for a clinical test. Furthermore, it is our hypothesis that a primary neurotoxicity, and with associated widespread catecholamine toxicity, is driving a secondary opioid craving as the neurotoxicity is unbearable to humans and it is quickly learned that the full agonist opioids provide a temporary, but sometimes dangerous, respite from the agony of the neurotoxicity. It is our theory this secondary opioid craving is driving a tertiary pathological consumption of often illegal opioids leading to the quaternary epidemic of opioid overdose deaths. Fortunately, we hypothesized that buprenorphine would provide a safer resolution of the primary neurotoxicity and thus the resolution of the secondary opioid craving. Establishing buprenorphine as an antidote for the symptoms of the DNA toxicity became the third aim of our study. Lastly, we were not able to find either a neurotoxicity scale nor an opioid craving scale that would meet the needs of our clinical trial. Therefore we developed our own Neurotoxicity Scale and our own Opioid Craving Scale. Establishing the validity and reliability of these two scales became the fourth and final aim of our clinical trial. It is noted that as the individual is in a life threatening situation due to the poisoning, and as buprenorphine is an antidote for the symptoms of the poisoning, the provider/patient relationship is now of an implied nature. No further action is required to establish the provider/patient relationship than the existence of the emergency due to a poisoning and the existence of an effective and well tolerated antidote. By simply asking for the antidote, the afflicted has the right to obtain the antidote and the provider has the obligation to provide the antidote. (Figure 4)

The clinical trial itself was an interventional case control study. Three groups were studied. The first group, under written informed consent, were opioid dependent and allowed themselves to go into opioid withdrawal. The second group, also under written informed consent, were opioid dependent but stabilized chronically on buprenorphine. The third group were not opioid dependent but were also given written informed consent.

Emergency Halt

We determined the need to call an emergency halt to the clinical trial after only the first 15 participants in opioid withdrawal. The catecholamine levels we detected were judged to be incompatible with life. Even a more in-depth informed consent could not offset the risk represented by the catecholamine levels. Thirteen of the first fifteen participants had at least one elevated catecholamine (norepinephrine, epinephrine, or dopamine). Six of the participants had two elevated catecholamines. Two of the participants had both elevated norepinephrine and elevated epinephrine levels. In animal models, the combination of both norepinephrine toxicity and epinephrine toxicity were associated with rapid death.[8] An emergency halt was determined under HHS CFR 45 part 46 and the Institutional Review Board was notified.[12]



(Figure 5) *Widespread catecholamine toxicity was detected in participants experiencing an episode of opioid withdrawal. Catecholamine toxicity carries a high morbidity and mortality. 87% of the participants had at least one elevated catecholamine. An emergency halt to the clinical trial was implemented. The mental health diagnosis of Opioid Addiction/Opioid Use Disorder is now an impossibility.*

A literature review confirmed a current epidemic of Takotsubo Cardiomyopathy as predicted by our hypothesis.9 Further literature review revealed a current epidemic of cardiovascular deaths had stalled U.S. life expectancy, again as predicted by the hypothesis.[10,11] (Figure 5, previous page)

The first aim of the study was met, widespread catecholamine toxicity during opioid withdrawal was detected. This confirms the proper diagnosis of a neurotoxicity. Opioid Addiction/Opioid Use Disorder are not a proper diagnosis for those who have suffered a genetic damage due to the opioids. The second aim of the study was met, several excellent CpG candidates for a clinical test to detect the presence of the methylation due to opioid exposure were determined. The third aim of the study was met, buprenorphine provided an excellent resolution of the symptoms of the primary neurotoxicity and, therefore, a resolution of the secondary opioid craving. Unfortunately, the fourth aim of the study was not met. Due to the emergency halt required by the severity of the catecholamine toxicity, statistical analysis for the validation of the two scales we had created was not achieved.

1). Introduction

This study had the primary aim of determining whether or not widespread catecholamine toxicity was a component of the clinical entity known as opioid withdrawal. If widespread catecholamine toxicity is present, then the proper diagnosis to be applied would be a neurotoxicity as opposed to a mental health diagnosis such as Opioid Use Disorder. Catecholamine toxicity had been predicted by our hypothesis. This hypothesis was the result of an in-depth analysis of four separate bodies of scientific evidence. The four separate bodies of scientific evidence that led to the hypothesis are:

1). Scientific Evidence for a neurotoxicity during opioid withdrawal.

2). Scientific Evidence for a methylation within the promoter region of the OPRM1 gene due to repetitive opioid exposure.

3). Catastrophic cardiovascular sequela of catecholamine toxicity.

4). Inadequate Scientific Evidence to support the Brain Disease Model of Addiction as it pertains to the opioids.

Each of these four separate bodies of scientific evidence will be presented including how each contributed to the formation of the hypothesis.

1.1). Scientific Evidence For A Dysfunction Within the Autonomic Nervous System During Opioid Withdrawal

A plethora of scientific evidence supporting the concept of a dysfunction within the autonomic nervous system during opioid withdrawal goes back well over 50 years. Dysfunction within the autonomic nervous system is a type of neurotoxicity. Here we will highlight several of the key studies with an emphasis on what each study contributed to the subject.

Gunne (1963) - "The content of adrenaline (Epi) in adrenal glands was depleted in chronic morphine-treated rats which experienced withdrawal symptoms 48 hr after abrupt morphine withdrawal." (Figure 6)

Gunne 1963	Depleted Adrenal Glands after two days "Opioid Withdrawal"
Akera/Brody 1968	Increased levels of Epi/Norepi in urine during "Opioid Withdrawal"
Delle et al 1990	400% increase sympathetic nerve activity to adrenal glands and twenty fold surge in plasma epinephrine during "Opioid Withdrawal"
Chang et al 1990	No surge in epinephrine when adrenal glands surgically removed
Keinbaum et al 1998	Thirty fold surge in plasma epinephrine in humans during "Opioid Withdrawal"

(Figure 6)

Gunne had stated that withdrawal from morphine, "shows many features indicative of a gross disturbance of the autonomic

nervous system." While Gunne focused on the autonomic nervous system, it simply wasn't clear at the time the fundamental etiology behind the, "gross disturbance of the autonomic nervous system," and the depletion of the adrenal glands. The disturbance within the autonomic nervous system was noted. But the etiology behind the disturbance was unknown. What Gunne did note, however, is that during an episode of opioid withdrawal, the content of the adrenal glands are depleted.

Akera and Brody (1968) - "During withdrawal after chronic (opioid) drug treatment, larger amounts of epinephrine and norepinephrine were excreted, epinephrine being the primary free amine excreted."
So where Gunne had noted a depletion of the adrenal glands during opioid withdrawal, Akera and Brody had noted an increase in the amount of epinephrine and norepinephrine excreted in the urine during opioid withdrawal. But the fundamental question remained- what was stimulating the adrenal glands to secrete their catecholamine contents during opioid withdrawal?

Delle et al (1990) - "Although renal SNA (sympathetic nerve activity) was inhibited by 50%, adrenal SNA and lumbar SNA increased by approximately 400% and 80% respectively." "The arterial plasma level of norepinephrine was doubled and epinephrine increased almost 20-fold."

It was Delle et al in their landmark 1990 study that added evidence from direct measurements taken at various points throughout the sympathetic nervous system and simultaneous plasma levels of norepinephrine and epinephrine. Delle was studying morphine dependent rats given intravenous injections of naloxone to induce opioid withdrawal. It is important to emphasize and understand that Delle demonstrated direct scientific evidence for a 400% increase in sympathetic nerve activity and in the nerve innervating the adrenal glands. Measurement of this 400% surge in sympathetic nerve activity following the administration of naloxone to opioid dependent rats is a direct observation of the opioid related neurotoxicity. This 400% increase in sympathetic nerve activity to the adrenal glands coincided with a doubling of the plasma norepinephrine level and a twenty-fold increase in plasma epinephrine level. Measurement of this doubling of the plasma norepinephrine level and twenty-fold surge in plasma epinephrine due to the 400% surge in sympathetic nerve activity is a direct observation of the consequences of the opioid related neurotoxicity. And this occurred in response to the administration of naloxone to morphine dependent mice. While Gunne (1963) had noted a depletion of the adrenal glands during opioid withdrawal and while Akera and Brody (1968) had noted the increase in norepinephrine and epinephrine in the urine, it was Delle et al (1990) that demonstrated that the surge in catecholamine release was in response to a massive and abnormal increase in activity within the sympathetic nerve innervating the adrenal glands. Delle went on to say, "This study shows that marked differentiation of the SNA response occurs during morphine withdrawal in rats, which suggests an interaction between opioid receptors and the control of regional sympathetic output." This was a novel concept and one worthy of additional research.

Delle had voiced a concept that the opioid receptor had a role in the control of regional sympathetic output. This would mean that the opioid receptor is involved in the management of the autonomic nervous system. This was a scientific concept that should have resulted in a flurry of further study. At the very least, blood levels for the catecholamines should have been drawn on opioid dependent humans while in opioid withdrawal. This study that we did in 2022 should have been done over thirty years ago. But it wasn't. What changed?

We do know that in 1992, the National Institute on Drug Abuse (NIDA) became a component of the National Institute of Health (NIH).[34] NIDA oversaw the NIH research funds dedicated towards drug abuse and addiction. NIDA has had two Executive Directors since becoming an arm of the NIH, Dr. Alan Leshner and Dr. Nora Volkow. As such, NIDA "funds more than 85 percent of the world's research about the health aspects of drug abuse and addiction."[35] Both Dr. Leshner and Dr. Volkow are vocal advocates for the Brain Disease Model of Addiction. What role this played in why the questions raised by the Delle study were never appropriately addressed is an unknown.

Chang et al (1990) - "After removal of the adrenal glands from morphine-dependent rats, naloxone injection produced no change in BP or plasma Epi."

The Delle and Chang studies were done in the same year, 1990. What Chang added was to clarify that the surge in epinephrine noted during opioid withdrawal was indeed originating in the adrenal glands. Chang accomplished this by surgically ablating the adrenal glands in opioid dependent rats prior to the injection of the naloxone. Without the adrenal glands, no increase in epinephrine occurred in response to the injection of naloxone. Again, follow up to this line of inquiry would have been proper.

Keinbaum et al (1998) - "Administration of naloxone induced a thirty-fold increase in concentration of epinephrine in plasma and a three-fold significant increase in concentration of norepinephrine in plasma."
Delle and Chang had been working with rats. But Keinbaum was working with humans. Keinbaum was a German anesthesiologist. Keinbaum was looking into the safety of a relatively new treatment for opioid dependency called ultrarapid opioid detoxification. The opioid dependent individual is anesthetized prior to the administration of naloxone, thus unaware of the acute discomfort. A discussion of the ultrarapid opioid detoxification is beyond the scope of this manuscript. But what is of interest is the strong similarities between the findings of Delle and Keinbaum. Delle gave naloxone to opioid dependent rats and measured a twenty-fold surge in plasma epinephrine. Keinbaum gave naloxone to opioid dependent humans and measured a thirty-fold surge in plasma epinephrine. It is noted scientifically that an episode of opioid withdrawal induced by naloxone is not identical to an episode of opioid withdrawal due to opioid abstinence. Therefore, the next obvious study was to simply evaluate catecholamine levels in human volunteers and while experiencing an episode of opioid withdrawal due to an opioid abstinence. While this is the study that we undertook in 2022, the need for this study had it's origins decades previously. The proper study at the proper time could have significantly impacted and possibly have prevented some of the carnage and devastation that has occurred over the past three decades and due to the opioids.

The scientific evidence for a dysfunction within the autonomic nervous system during opioid withdrawal spans decades and multiple researchers. It is simply a body of scientific evidence that cannot be refuted. It could be ignored, however. This body of scientific evidence was a source of inspiration for our hypothesis. And perhaps no study was more persuasive than the Delle study from 1990 with its concept of the opioid receptor playing a role in the control of the regional sympathetic output. If the opioid receptor controlled regional sympathetic output, and if this opioid receptor was somehow damaged genetically, then, theoretically, genetic damage could lead to a dysfunction within the autonomic nervous system. Thus, we can understand a mechanism by which genetic damage can result in a neurotoxicity. We now will look at the scientific evidence for a genetic damage to the gene that encodes for the main opioid receptor, the *mu*-opioid receptor. It is the OPRM1 gene that encodes for the *mu*-opioid receptor.

1.2). Scientific Evidence For A Methylation Within the Promoter Region of the OPRM1 Gene

We have reviewed a number of scientific studies that give support to the concept of a dysfunction within the autonomic nervous system during opioid withdrawal. We have seen scientific evidence that the opioid receptor may be involved in the control of the regional sympathetic nervous system. Now we will examine scientific evidence for a methylation within the promoter region of the OPRM1 gene and due to repetitive opioid exposure. Taken together, these two seemingly unrelated bodies of scientific evidence gave rise to the part of our hypothesis that methylation within the promoter region of the OPRM1 gene results in the formation of an abnormal *mu*-opioid receptor. When some unknown threshold of methylation has occurred, and when the individual suffering from the methylation attempts opioid abstinence, the individual experiences a primary neurotoxicity. Somehow, the receptors spawned from damaged, methylated DNA are unable to perform normally when opioid abstinence is attempted. The neurotoxicity is described by the individuals in our study as unbearable. All individuals had learned that a full agonist opioid, often obtained illegally, was the most common respite available for them. This gave rise to a secondary opioid craving. This secondary opioid craving gave rise to a tertiary

Nielsen 2008 Chorbov 2014	Scientific evidence for an association between opioids and DNA methylation
Wachman 2014 & 2018	Scientific evidence for a correlation between opioids and DNA methylation
Sandoval/Sierra 2020	Scientific evidence for a causation between opioids and DNA methylation (Opioids cause DNA methylation).

(Figure 7)

pathological consumption of an often illegal opioid. It was this secondary opioid craving and tertiary opioid consumption that was improperly interpreted as an addiction. According to our hypothesis, this process begins with a methylation within the promoter region of the OPRM1 gene. Let's look now at some of the scientific evidence supporting this methylation within the promoter region of the OPRM1 gene. (Figure 7, previous page)

1.21). Association Between the Opioids and DNA Methylation Within the Promoter Region of the OPRM1 Gene

Nielsen et al (2008) - "Direct sequencing of bisulfate-treated DNA showed that the percent methylation at two CpG sites was significantly associated with heroin addiction."

While association is a type of scientific evidence, association is not the strongest of scientific evidence. The first step when an association is detected is to replicate the results.

Chorbov et al (2011) - "Increased DNA methylation in the OPRM1 gene is associated with opioid dependence. Hypermethylated CpG sites located in the OPRM1 promoter may potentially block the binding of SP1 and other transcription activators, thus leading to OPRM1 silencing."

Chorbov had replicated the findings of Nielsen. An association between opioid dependence and hypermethylation within the promoter region of the OPRM1 gene had been established and replicated. Chorbov further espoused that the methylation may result in a down regulation of the *mu*-opioid receptor population known as gene silencing. As this down regulation of the *mu*-opioid receptor has not been found to have occurred,[31] this led to our hypothesis that the *mu*-opioid receptor was being transcribed but the transcription was resulting in the formation of an abnormal *mu*-opioid receptor. It is this abnormal *mu*-opioid receptor that is unable to maintain proper control over the autonomic nervous system when opioid abstinence is attempted. We coined the term partial gene silencing to describe the formation of a normal population of *mu*-opioid receptors but with abnormalities present within the individual receptors. As an association between opioid dependence and hypermethylation within the promoter region of the OPRM1 gene had been established, the next scientific step was to determine if a correlation exists between opioid dependence symptoms and this hypermethylation. Of note, as an aside hypothesis, our concept of a partial gene silencing that produces a normal population of abnormal receptors is recognized as a viable hypothesis for the development of opioid tolerance and the escalation of opioid dosing that has, to date, escaped explanation. It would make sense that a population of receptors experiencing an increasing disarray would require an escalation in stimulation to function. This discussion, however important, is beyond the scope of this manuscript. But we present it as a separate hypothesis for future study.

1.22 Correlation Between the Opioids and DNA Methylation Within the Promoter Region of the OPRM1 Gene

Wachman et al (2014) - "Increased methylation within the OPRM1 promoter is associated with worse NAS outcomes, consistent with gene silencing."

What Wachman has accomplished here is the establishment of not just the existence of an association, but more specifically, the existence of a correlation between the methylation within the promoter region of the OPRM1 gene and the severity of symptoms experienced by the individual. Higher levels of methylation correlated with higher severity of the withdrawal symptoms. Wachman was working with infants born to opioid dependent mothers and whom manifested Neonatal Abstinence Syndrome (NAS). NAS has long been difficult for the Brain Disease Model of Addiction to explain coherently. NAS, like other inconvenient science, is largely ignored by those advocating for the Brain Disease Model of Addiction. Wachman next replicated her own study.

Wachman et al (2018) - "These results suggest an association of higher levels of OPRM1 methylation at specific CpG sites and increased NAS severity, replicating prior findings."

Wachman had again shown a correlation between the level of methylation and the severity of the symptoms experienced by the individual. And the symptoms experienced by the individual are consistent with a neurotoxicity. We examined this data and arrived at the hypothesis that the methylation was resulting in the formation of an abnormal *mu*-opioid receptor. This abnormal *mu*-opioid receptor could no longer maintain control within the autonomic nervous system. The more severe the methylation, the more severe the damage to the *mu*-opioid receptor population, the more severe the dysfunction within the autonomic nervous system. That is our interpretation of this scientific evidence. Now that association and correlation have been established, the next step is to establish a causation between an exposure to the opioids and a methylation within the promoter region of the OPRM1 gene.

1.23). Causation Between the Opioids and DNA Methylation Within the Promoter Region of the OPRM1 Gene

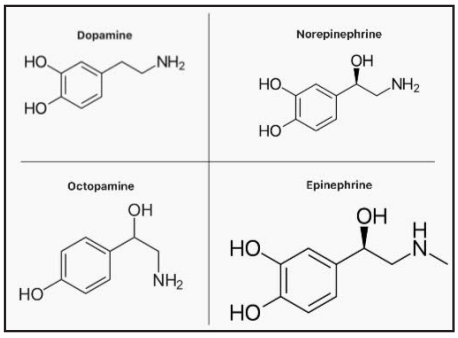
Sandoval-Sierra et al (2020) - "The present study provides evidence that the hypermethylation of the OPRM1 promoter is in response to opioid use and that epigenetic differences in OPRM1 and other sites are associated with a short-term use of therapeutic opioids."

What Sandoval-Sierra accomplished was simple but important. The study took opioid-naive individuals undergoing a dental procedure and followed their DNA while the individual utilized the opioids for acute pain management. Hypermethylation within the promoter region of the OPRM1 gene was detected and with minimal doses of the opioids. This study establishes a causation between opioid exposure and damage within the DNA. In other words, taking opioids causes a toxicity within the DNA as methylation due to a drug is considered a form of toxicity.[38]

We have now reviewed a number of scientific studies that establishes association, correlation, and causation between the opioids and DNA methylation within the promoter region of the OPRM1 gene. Simply put, a drug, a medication, is causing a change in the epigenetics of those who are taking the medication. It is our hypothesis that this change in the epigenetics of the individual results in a disease state. A change in epigenetics resulting in a disease state is an accepted notion.[36] Methylation resulting in a disease state is recognized and accepted by the medical community. Cancer is perhaps the best studied example.[37] Moshe Szyf stated in his 2011 article titled *The Implications of DNA Methylation for Toxicology: Toward Toxicomethylomics, the Toxicology of DNA Methylation*, "The realization that long-ranged damage could be caused without changing the DNA sequence has important implications on the way we assess the safety of chemicals, drugs, and food and broadens the scope of definition of toxic agents." The opioids, it turns out, are simply a toxic agent.

1.3). Catastrophic Cardiovascular Sequela of Catecholamine Toxicity

We have just reviewed two bodies of scientific evidence. First, we reviewed the scientific evidence for a neurotoxicity within the autonomic nervous system and during the clinical entity widely known as opioid withdrawal. And we noted catecholamine toxicity was a component of this neurotoxicity within the autonomic nervous system. Secondly, we reviewed the scientific evidence for a type of poisoning to the DNA known as methylation and due to repetitive opioid exposure. Combining these two bodies of scientific evidence together, we derived the hypothesis that the methylation within the promoter region of the OPRM1 gene results in the formation of an abnormal *mu*-opioid receptor that is no longer able to maintain balance and homeostasis within the autonomic nervous system. This now raises the question, what are the consequences of an episode of catecholamine toxicity? So we now examine our third body of scientific evidence: the cardiovascular sequela of catecholamine toxicity.



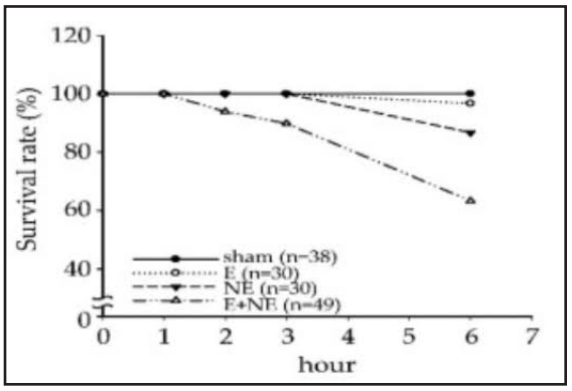
The Catecholamines

(Figure 8) *The catecholamines are among the most powerful organic molecules known. The half-lives are measured in minutes. Octopamine is the catecholamine found in invertebrates. These molecules can even be found in single-cell organisms. As a group, these organic molecules are involved in the "fight or flight" response.*

The catecholamines are among the most powerful organic molecules known. When the invertebrate analog known as octopamine is included, the

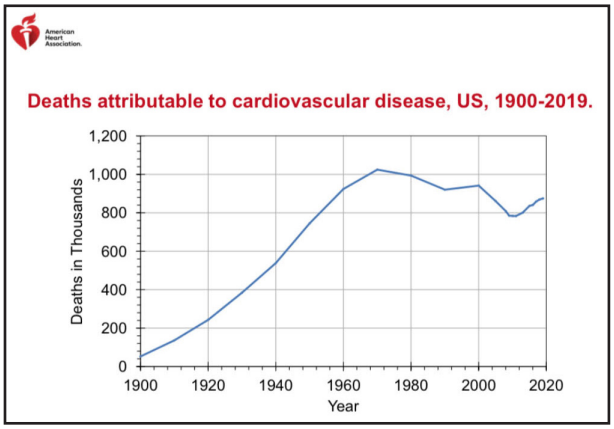
catecholamines are ubiquitous to animal life on this planet. Interwoven into the "fight or flight response", these compounds do not cross the blood brain barrier and have a half-life of approximately two minutes. The short half-life is of importance. In a toxic or prolonged state, the catecholamines are strongly linked to cardiovascular morbidity and mortality. (Figure 8, previous page)

Wu et al 2021 - "Catecholamine surge causes cardiomyocyte necroptosis…". Cardiomyocyte necroptosis is part of the process that occurs at the cellular level with a myocardial infarction. The current thinking is that the heart, under excessive stimulation by the catecholamines, simply exceeds the available oxygen supply resulting in cell damage and death. This is part of the scientific evidence that led to our hypothesis that an epidemic of cardiovascular deaths will be occurring within the population of genetically damaged individuals improperly diagnosed and treated. It is our hypothesis that individuals are dying from a secretion of catecholamines from their own adrenal glands and due to a loss of control of the adrenal gland from an episode of autonomic dysfunction.



(Figure 9) *Lu et al (2020) found that catecholamine toxicity led to significant cardiopulmonary dysfunction and death.*

Lu et al (2020) - "Catecholamine overdose induces acute lung injuries and ventricular cardiomyopathy, and epinephrine plus norepinephrine is associated with a more severe outcome." (Figure 9)

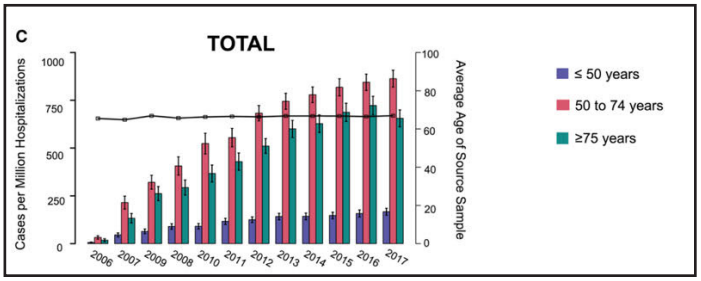


(Figure 10) *American Heart Association Cardiovascular deaths pre-COVID-19. Our hypothesis had predicted this epidemic of cardiovascular deaths.*

In this study, the morbidity and mortality of the rats exposed to catecholamine toxicity was rapid and widespread. It was this study that led us to declare an emergency halt to our clinical trial. The neurotoxicity and with the associated catecholamine toxicity produces a toxic state that is incompatible with life. No informed consent process, no matter how accurate and detailed, could offset the risk associated with the catecholamine toxicity. It was this study that led to our hypothesis that the cardiovascular deaths would be so numerous that the life expectancy in this country would be negatively impacted. The life expectancy would remain negatively impacted until the vulnerable population, those genetically damaged by repetitive opioid exposure, either received a proper diagnosis and treatment or were driven towards extinction. (Figure 10)

Pelliccia et al (2017) - "catecholamine surge leads…to myocardial damage, which has a functional counterpart of transient apical left ventricular ballooning. The relative preponderance among postmenopausal women suggests that estrogen deprivation may play a facilitating role, probably mediated by endothelial dysfunction".

Takotsubo Cardiomyopathy is pathognomonic for a catecholamine surge.[24] Takotsubo Cardiomyopathy is normally a relatively rare and obscure clinical entity. But as Takotsubo Cardiomyopathy is pathognomonic for a catecholamine surge, we hypothesized that an epidemic of Takotsubo Cardiomyopathy would be found in the vulnerable population genetically damaged by repetitive opioid exposure. We further hypothesized that this epidemic of Takotsubo Cardiomyopathy would be in predominantly older women due to the endothelial dysfunction noted in estrogen deprivation. (Figure 11)



Pattisapu et al (2020)

(Figure 11) *An epidemic of the otherwise rare Takotsubo Cardiomyopathy is occurring in parallel to the Opioid Crisis as predicted by our hypothesis. Takotsubo Cardiomyopathy is pathognomonic for catecholamine toxicity.*

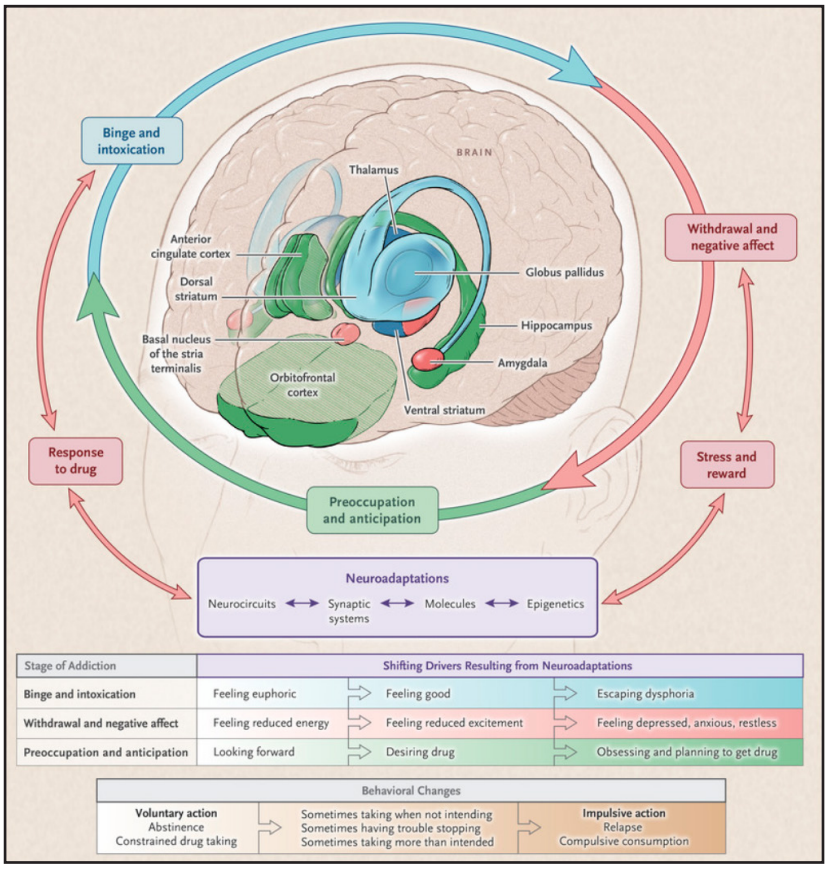
It would be difficult to imagine that widespread methylation within the promoter region of the OPRM1 gene is not going to have some impact on the *mu*-opioid receptor. It is equally difficult to imagine that widespread undiagnosed and untreated catecholamine toxicity is not going to have a significant impact on cardiovascular morbidity and mortality. While it's not difficult to envision the impact epidemic catecholamine toxicity is going to have on the population, what is difficult to comprehend is that the medical community remained oblivious to the obvious and for years.

We have now examined three of the four separate bodies of scientific evidence that gave rise to our hypothesis. We have looked at the scientific evidence for a neurotoxicity during opioid withdrawal. We have looked at the scientific evidence for a methylation, a toxicity, within the promoter region of the OPRM1 gene and due to repetitive opioid exposure. And we have looked at the scientific evidence for the catastrophic cardiovascular sequela resulting from catecholamine toxicity. The fourth and final body of scientific evidence that contributed to the creation of our hypothesis is the scientific evidence, or more correctly, the lack of scientific evidence, supporting the Brain Disease Model of Addiction as pertaining to the opioids.

1.4). Inadequate Scientific Evidence to Support the Brain Disease Model of Addiction As It Pertains to the Opioids

The Brain Disease Model of Addiction has been promoted for decades. It has become dogmatic. But when attention is directed to the underlying science as how this Brain Disease Model of Addiction pertains to the opioids, an immediate discrepancy arises. This discrepancy is of such a magnitude, that it brings into doubt whether or not the Brain Disease Model of Addiction can even be applied in the case of the opioids.

Volkow et al (2016) - "All known addictive drugs activate reward regions in the brain by causing sharp increases in the release of dopamine." Three separate studies are cited by Volkow et al to support this statement: Di Chiara et al. (2002), Koob et al (1992), and Wise et al (2008). But, on review, none of these references give any scientific evidence that a "sharp increase in the release of dopamine" occurs in the brain in response to the administration of an opioid in either animal models or human clinical trials. In fact, when the subject of an increase in brain dopamine in response to the administration of an opioid is researched, three studies are found. The first study is Di Chiara et al (1988). This is an older study using a primitive and now largely abandoned method known as microdialysis. Tiny holes are drilled into the brain of laboratory rats. Tiny glass tubes are then glued into presumably the proper location within the rat's brain. Samples are taken for analysis after the administration of the opioid. And the 1988 study by Di Chiara did report an increase in brain dopamine. But subsequent researchers using the more advanced and accurate methodology of the Positron Emission Tomography Scan (PET Scan) failed to show any evidence of a surge in brain dopamine and in response to the administration of an opioid. Daglish et al (2008) injected an opioid into former heroin addicts. There was no increase in brain dopamine in response to the administration of



Volkow et al (2016)

(Figure 12) *According to the Brain Disease Model of Addiction, opioid withdrawal is due to "Shifting drivers resulting from Neuroaptations" and is characterized by "Feeling reduced energy" and "Feeling reduced excitement". Instead we found widespread and deadly catecholamine toxicity. The presence of the catecholamine toxicity negates the Brain Disease Model of Addiction as it pertains to the opioids and makes a mental health diagnosis such as Opioid Addiction/Opioid Use Disorder an impossibility.*

the opioid. Watson et al (2014) injected opioid into opioid dependent humans. And again, there was no detectable increase in brain dopamine in response to the administration of an opioid. Volkow et al (2016) stated clearly, "All known addictive drugs activate reward regions in the brain by causing sharp increases in the release of dopamine." But replicating modern studies, and utilizing the superior technique of the PET Scan, found no evidence of an increase in brain dopamine in humans in response to the administration of an opioid. Nutt et al (2015), in a very well written and researched manuscript, made note of how these actions may be inconsistent with modern Popperian Science. To avoid the possibility for hyperbole, we will close this discussion with the statement that these facts are of a most troubling nature. History has well recorded the typical outcome when science is replaced with opinion. (Figure 12, previous page)

1.5). Implied Provider/Patient Relationship in the Emergency Setting

The Federal Law known as the Emergency Medical Treatment And Active Labor Act (EMTALA) provides the following definition of an emergency medical condition[41]:

(A) a medical condition manifesting itself by acute symptoms of sufficient severity (including severe pain) such that the absence of immediate medical attention could reasonably be expected to result in—

(i) placing the health of the individual (or, with respect to a pregnant woman, the health of the woman or her unborn child) in serious jeopardy,

(ii) serious impairment to bodily functions, or

(iii) serious dysfunction of any bodily organ or part; or

(B) with respect to a pregnant woman who is having contractions–
That there is inadequate time to effect a safe transfer to another hospital before delivery, or

(ii) that transfer may pose a threat to the health or safety of the woman or the unborn child.[41]

A neurotoxicity with associated catecholamine toxicity with its strong correlation with cardiovascular morbidity and mortality would be included as an emergency medical condition. And failure to stabilize the neurotoxicity with associated catecholamine toxicity would be a violation of EMTALA. Furthermore, the American College of Emergency Physicians (ACEP), has adopted the following policy in regards to any interference with the Provider/Patient relationship:

The American College of Emergency Physicians (ACEP) believes that emergency physicians must be able to practice high quality, objective evidence-based medicine without legislative, regulatory, or judicial interference in the physician-patient relationship.[42]

In the setting of a medical emergency, the provider/patient relationship is of an implied nature. The implied relationship is built by actions, not by a dialogue. In some emergencies, a dialogue is not even a possibility.

2). Methods & Materials

2.1). Setting

The clinical trial took place in the medical office setting. Institutional Review Board approval was obtained. A written informed consent process was established.

2.2). Subjects

All subjects were over 18 years of age. All subjects were capable of understanding the Informed Consent. All subjects gave written consent. The study consisted of three separate groups. Of note, the collection devices for the saliva samples used for DNA analysis were on back order. DNA sample collection was completed at a later date and via the US Postal Service and with no special transport/refrigeration requirements. Initial contact and call for volunteers for this study was in late 2021. Informed consent and study protocol began in early 2022 and was completed by June of 2022 with the last of the saliva samples collected for DNA analysis.

2.21). Group #1

This was the main study group. This group was recruited as volunteers from our Medication Assisted Treatment Program. After voluntarily stopping the buprenorphine and under written Informed Consent, this group each came into the office on Day #3, as close to 72 hours after their last dose of buprenorphine as possible. These individuals were in a state of opioid abstinence withdrawal. Each individual was placed in an examination room. Blood was drawn per the protocols of LabCorp for the catecholamines. Each participant completed both a Neurotoxicity Scale and an Opioid Craving Scale. After completion of the first blood draw and the first set of questionnaires, buprenorphine at 16 mgms was administered sublingually. This dosage was chosen as this dosage is recognized as the Blockade Dose, achieving approximately 80% opioid receptor saturation.[39] Two hours after the absorption of the buprenorphine, a second blood draw for catecholamines and both questionnaires were repeated. This completed the clinical trial participation except for the later DNA sample collection.

2.22). Group #2

This group was recruited as volunteers from our Medication Assisted Treatment Program. This group came into the office when it was convenient. This group was not in withdrawal as they were chronically stabilized on buprenorphine. They were not asked to stop taking the buprenorphine. This is how they differed from the first group. Each individual was placed in an examination room. Blood was drawn per the protocols of LabCorp for catecholamines. Each participant completed both an Autonomic Dysfunction Scale and an Opioid Craving Scale. No medication was given in this office encounter. This completed the clinical trial participation except for the later DNA sample collection.

2.23). Group #3

This group was recruited by word of mouth from the community at large. This group came into the office when it was convenient. This group was not in withdrawal as they were not opioid dependent. Each individual was placed in an examination room. Blood was drawn per the protocols of LabCorp for catecholamines. Each participant completed both a Neurotoxicity Scale and an Opioid Craving Scale. This completed the clinical trial participation except for the later DNA sample collection.

2.3). Neurotoxicity Scale

The Autonomic Dysfunction Scale was created to meet the needs of this study. The questions were reviewed by clinicians familiar with the clinical presentations. An emergency halt was called prior to the establishment of the reliability and validity of the scale had been completed.

2.4). Opioid Craving Scale

The Opioid Craving Scale was created to meet the needs of this study. The questions were reviewed by clinicians familiar with the clinical presentations. An emergency halt was called prior to the establishment of the reliability and validity of the scale had been completed.

2.5). Statistical Methods

2.51). Catecholamine Levels

The differences in catecholamine values between the general population and subjects in opioid withdrawal, as well as the differences between the general population and those stabilized on buprenorphine, were evaluated using a two-sided t-test and pooled standard deviation. For the former comparison, at a significance level of 0.05, significant differences were found for dopamine ($p = 0.0194$), epinephrine ($p=0.0083$), and norepinephrine values ($p = 0.0454$). For the latter comparison, none of the values were significant. The main limitation with this analysis is that many of the levels were at the edge of detection, so precise values were not available for many of the observations. For dopamine values, this was recorded as "< 30". For epinephrine values, this was recorded as " < 15". For analysis purposes, these values were replaced with 30 and 15, respectively.

2.52). Neurotoxicity Scale

An emergency halt was called to the study before a statistical relevance had been obtained.

2.53). Opioid Craving Scale

An emergency halt was called to the study before a statistical relevance had been obtained.

2.54). DNA Methylation Analysis

Statistical analyses were performed using R (R version 4.2.1), the program for statistical computing. Descriptive statistics of the DNA methylation data of CpG sites was summarized, comparatively grouped by the Control and Experimental groups. All boxplots, density plots, bar plots, and heatmaps were created using ggplot2 (version 3.3.6). All tables were produced by "kable" in the kableExtra package (version 1.3.4)

Spearmen's correlations comparing each CpG site were explored, connecting those relationships with the Promoter and non-Promoter regions of the OPRM gene. Correlations and visualizations were constructed by the "ggcorr" function in GGally (version 2.1.2). Principal Component Analysis (PCA) and Non-Metric Multidimensional Scaling (nMDS) were implemented to visualize group separation of the samples. nMDS was built using the "metaMDS" function in the vegan package (version 2.6-2) specifying for Euclidean distance, and PCA used the "prcomp" function in base stats package. Biplots and scree plots were produced from the "fviz" function in factoextra package (version 1.0.7), as well as ggplot2.

The analysis of DNA methylation percentages of the OPRM gene were evaluated at a CpG site basis, stratified by the two groups of interest: Control and Experiment. Multiple tests for comparison of the groups were analyzed using the non-parametric Wilcoxon-Mann-Whitney (WMW) U-test, also known as the Wilcox Rank Sum Test. The WMW U-test was performed using wilcox_test function in the rstatix package (version 0.7.0), specifying the test as a one-sided, unpaired test. The 16 tests (for the 16 CpG sites) were corrected using the Benjamini-Hochberg correction. Additionally, the Kruskal-Wallace test ("kruskal_test") and one-way ANOVA test ("anova_test") were implemented as weaker alternatives to the WMW U-test, both from the rstatix package.

A PERMANOVA model (non-parametric) was built using the "adonis2" function in the vegan package (version 2.6-2) to investigate the multivariate relationship of the CpG sites by group. Measures of dissimilarity, or distances, were calculated using Euclidean distances. Additionally, a MANOVA model (parametric, unbalanced) was created as an alternative utilizing the "manova" function in the base stats packages, and tested using the "Manova" function in rstatix. The test specified for a type III test for unbalanced design, to account for the unequal sample sizes between groups.

All assumptions for statistical methods were checked using the "mvn" function in the MVN package (version 5.9), and "levene_test" function for equal covariance from rstatix.

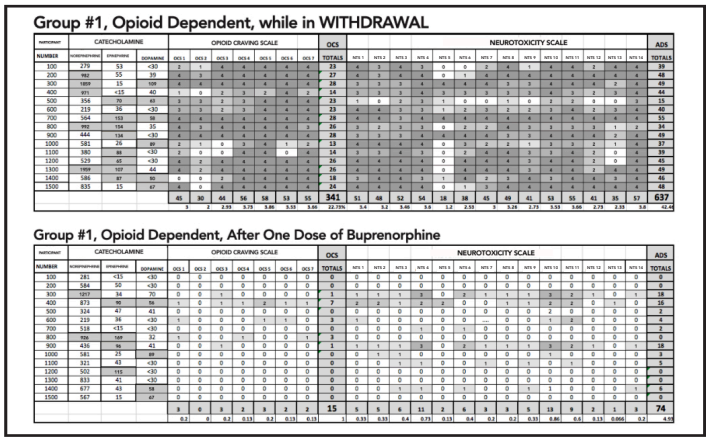
3). Results

3.1). The Catecholamines

Catecholamine toxicity in humans during opioid withdrawal due to an opioid abstinence had previously been undetected and unknown. This is the first such study to detect this catecholamine toxicity during an opioid abstinence withdrawal. The catecholamine toxicity was severe and widespread. Many individuals exhibited multiple catecholamine toxicities simultaneously. The prevalence and severity of the previously undetected catecholamine toxicity led us to declare an emergency halt to the study. Even a more detailed informed consent process was deemed inadequate to offset the risk posed by the prevalence and severity of the catecholamine toxicity.

3.11). Group #1

This is the group that exhibited the severe catecholamine toxicity. But the primary aim of this study had been obtained. The question of whether or not elevated catecholamine levels were a component of an opioid abstinence withdrawal had been answered. The answer was an overwhelming "yes", catecholamine toxicity is a component of the neurotoxicity that occurs when an opioid dependent individual attempts opioid abstinence. The emergency halt was determined when the first results of the catecholamines began to be available. Ultimately, catecholamine results on 15 individuals in a state of opioid withdrawal due to an opioid abstinence were obtained. Only 2 of the 15 participants had normal catecholamine levels. Thirteen out of fifteen had abnormal catecholamine levels. Seven out of fifteen had one elevated catecholamine level. Six out of fifteen had two elevated catecholamine levels. Two individuals had both a norepinephrine toxicity and an epinephrine toxicity. This was the combination that worried us the most. In the Lu et al (2020) study, the combination of norepinephrine toxicity combined with epinephrine toxicity produced rapid cardiovascular death in the mice. Of note, for all three catecholamines, when compared to the catecholamine levels of the opioid naive control group, all three catecholamines had p-values of less than the cutoff of 0.05. This is significant as a pushback to this science is the expectation. If the p-values had been higher than 0.05, the pushback would be effective based on statistical significance. But even with only 15 participants, the p-values for all three catecholamines, norepinephrine (p=0.0454), epinephrine (p=0.0083), and dopamine (p=0.0194), were below the cutoff of 0.05. This catecholamine toxicity we detected is real. (Figure 13)


Group #1, Opioid Dependent, while in WITHDRAWAL

PARTICIPANT NUMBER	CATECHOLAMINE			OPIOID CRAVING SCALE							OCS	NEUROTOXICITY SCALE													ADS
	NOREPINEPHRINE	EPINEPHRINE	DOPAMINE	OCS 1	OCS 2	OCS 3	OCS 4	OCS 5	OCS 6	OCS 7	TOTALS	NTS 1	NTS 2	NTS 3	NTS 4	NTS 5	NTS 6	NTS 7	NTS 8	NTS 9	NTS 10	NTS 11	NTS 12	NTS 13	NTS 14	TOTALS
100	279	53	<30	2	1	4	4	4	4	4	23	4	3	4	3	0	0	2	4	1	4	4	2	4	4	39
200	782	55	35	4	3	4	4	4	4	4	27	4	3	4	4	0	1	0	4	0	4	4	4	4	4	48
300	1809	15	109	4	4	4	4	4	4	4	28	3	3	3	4	4	4	4	3	3	4	4	4	2	4	49
400	871	<15	40	1	0	2	3	4	4	4	18	3	3	3	4	3	3	4	3	3	4	4	3	3	4	44
500	356	70	60	3	2	2	3	4	4	4	23	1	0	2	3	1	0	0	1	0	2	2	0	0	3	15
600	219	36	<30	3	3	2	3	4	4	4	23	4	4	3	3	1	2	3	2	2	3	4	2	3	4	40
700	564	150	98	3	4	4	4	4	4	4	28	4	4	3	4	4	4	4	4	4	4	4	4	4	4	55
800	992	154	35	4	3	4	4	4	4	3	26	3	2	3	3	0	2	2	4	3	3	3	3	1	2	34
900	444	134	<30	4	4	4	4	4	4	4	28	3	3	3	4	0	4	4	3	3	4	4	4	2	4	49
1000	581	26	87	2	1	0	3	4	1	2	13	4	4	4	4	4	3	3	2	2	3	3	2	1	4	37
1100	380	88	<30	2	0	0	4	4	0	4	14	3	3	4	3	0	2	4	4	3	3	4	2	0	4	39
1200	529	65	<30	4	2	4	4	4	4	4	26	4	4	4	4	0	4	4	4	3	4	4	2	0	4	45
1300	1909	107	44	4	2	4	4	4	4	4	26	4	4	4	4	0	4	4	4	3	4	4	2	4	4	49
1400	586	87	50	0	0	2	4	4	4	4	18	3	4	4	3	1	4	2	3	4	3	4	4	3	4	46
1500	835	15	67	0	0	2	4	4	4	4	18	4	4	4	4	4	0	1	2	4	4	4	4	4	4	48
				45	30	44	56	58	53	55	341	51	48	52	54	38	38	45	49	41	53	55	41	35	57	637
				3	2	2.93	3.73	3.86	3.53	3.66	22.73%	3.4	3.2	3.46	3.6	2.53	2.53	3	3.26	2.73	3.53	3.66	2.73	2.33	3.8	42.46

Group #1, Opioid Dependent, After One Dose of Buprenorphine

PARTICIPANT NUMBER	CATECHOLAMINE			OPIOID CRAVING SCALE							OCS	NEUROTOXICITY SCALE													ADS
	NOREPINEPHRINE	EPINEPHRINE	DOPAMINE	OCS 1	OCS 2	OCS 3	OCS 4	OCS 5	OCS 6	OCS 7	TOTALS	NTS 1	NTS 2	NTS 3	NTS 4	NTS 5	NTS 6	NTS 7	NTS 8	NTS 9	NTS 10	NTS 11	NTS 12	NTS 13	NTS 14	TOTALS
100	281	<15	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
200	584	50	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
300	1207	34	70	0	0	1	0	0	0	0	1	1	1	1	3	0	2	1	1	1	3	2	1	0	1	18
400	873	90	56	1	0	1	1	2	1	1	7	2	2	1	2	2	0	0	1	1	2	2	0	1	0	16
500	324	47	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
600	219	36	<30	1	0	0	0	1	1	0	3	1	0	0	0	0	0	–	0	0	1	2	0	0	0	4
700	518	<15	<30	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
800	926	169	32	1	0	0	1	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
900	436	96	41	0	0	1	0	0	0	0	1	1	1	1	3	0	2	1	1	1	3	2	1	0	1	18
1000	581	23	69	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	3
1100	321	43	<30	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	1	0	0	5
1200	502	116	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1300	833	41	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1400	677	43	58	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	1	6
1500	567	15	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3	0	3	2	3	2	2	15	5	5	6	11	2	6	3	3	5	13	9	2	1	3	74
				0.2	0	0.2	0.13	0.2	0.13	0.13	1	0.33	0.33	0.4	0.73	0.13	0.4	0.2	0.2	0.33	0.86	0.6	0.13	0.066	0.2	4.93

(Figure 13) *Group #1 exhibited widespread catecholamine toxicity, severe neurotoxicity symptoms, and severe opioid cravings. The catecholamine toxicity was improved and both the neurotoxicity symptoms and the opioid cravings were dramatically improved with a single dose of buprenorphine 16 mgms sublingual.*

There was some improvement following the administration of the 16 mgm buprenorphine sublingually. But catecholamine toxicity persisted even two hours after the buprenorphine dosage. The significance of this will require further study.

3.12). Group #2

Oddly enough, this group also exhibited some catecholamine toxicity, although no p-values were below the cutoff of 0.05. This group will require additional study before the significance, if any, of these abnormal catecholamine levels can be determined. (Figure 14)

Group #2, Opioid Dependent, Chronically Stabilized on Buprenorphine

PARTICIPANT	CATECHOLAMINE			OPIOID CRAVING SCALE							OCS	NEUROTOXICITY SCALE														ADS
NUMBER	Norepinephrine	EPINEPHRINE	DOPAMINE	OCS 1	OCS 2	OCS 3	OCS 4	OCS 5	OCS 6	OCS 7	TOTALS	NTS 1	NTS 2	NTS 3	NTS 4	NTS 5	NTS 6	NTS 7	NTS 8	NTS 9	NTS 10	NTS 11	NTS 12	NTS 13	NTS 14	TOTALS
201	529	<15	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
202	443	20	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
203	751	<15	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
204	762	654 retested	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
205	235	56	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	2
206	376	115	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
207	648	48	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
208	394	85	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
209	599	55	51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
210	518	<15	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
211	411	19	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
212	248	32	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
213	142	<15	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
214	455	80	61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
215	495	18	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	0	0	5
				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.266	0	0.066	0	0	0.332

(Figure 14) *Group #2 were opioid dependent, chronically stabilized on buprenorphine. The neurotoxicity symptoms and the opioid cravings both remained low.*

3.13). Group #3

Group #3, Opioid Naive

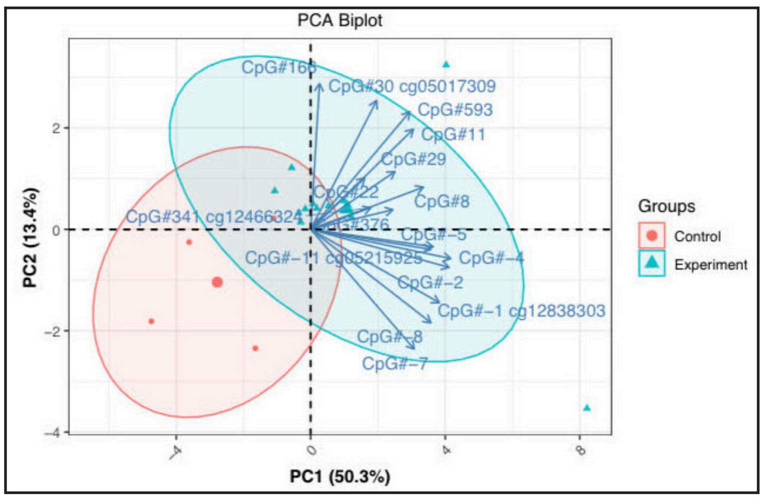
PARTICIPANT	CATECHOLAMINE			OPIOID CRAVING SCALE							OCS	NEUROTOXICITY SCALE														ADS
NUMBER	Norepinephrine	EPINEPHRINE	DOPAMINE	OCS 1	OCS 2	OCS 3	OCS 4	OCS 5	OCS 6	OCS 7	TOTALS	NTS 1	NTS 2	NTS 3	NTS 4	NTS 5	NTS 6	NTS 7	NTS 8	NTS 9	NTS 10	NTS 11	NTS 12	NTS 13	NTS 14	TOTALS
301	338	50	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
302	712	50	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
303	580	38	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
304	654	34	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
305	361	<15	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
306	536	34	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
307	304	33	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
308	571	20	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
309	580	50	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
310	190	30	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
311	302	35	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
312	379	56	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
313	437	45	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
314	683	<15	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
315	448	<15	<30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(Figure 15) *Group #3 were opioid naive and exhibited neither neurotoxicity symptoms or opioid cravings.*

The opioid naïve group showed little, if any, abnormalities in the catecholamine levels. (Figure 15)

3.2). DNA Methylation Analysis

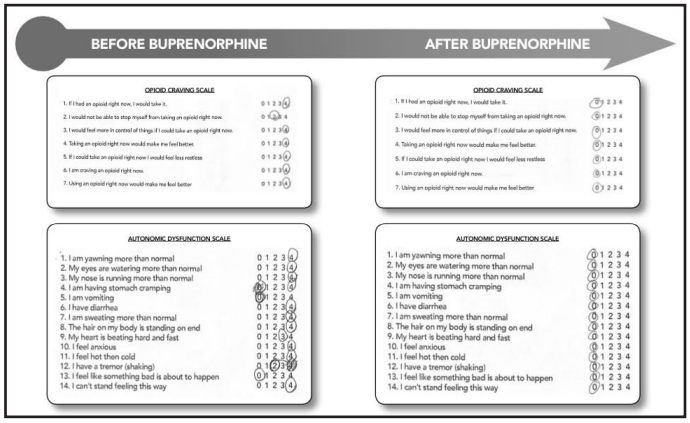
Our hypothesis had predicted that widespread catecholamine toxicity would be found in association with an episode of opioid abstinence withdrawal in an opioid dependent individual. Albeit, the severity of the catecholamine levels took us by surprise. But the first aim of the study had been met. This places emphasis on the second aim of the study which was to determine specific CpG sites within the promoter region of the OPRM1 gene that may be good candidates for a clinical test for opioid induced hypermethylation. A clinical test would also forever end the question in any given individual- does the patient have a mental health disorder and is craving opioids to get high, or does the patient have genetic damage due to repetitive opioid exposure and is experiencing a neurotoxicity when opioid abstinence is attempted? For this reason, methylation levels were compared between two groups: the opioid dependent and the opioid naïve. Multiple CpG sites showed statistically significant hypermethylation in the opioid dependent group as opposed to the opioid naïve group. These sites will require further statistical validation prior to clinical use. But the second aim was met. A clinical test for DNA damage due to repeated opioid exposure is a relatively straightforward test to create and validate. This will be an important test for those individuals who have been poisoned by the opioids. The DNA methylation and the significant differences between the opioid naive and the opioid dependent are explored further in a follow up article. (Figure 16)



(Figure 16) *Artificial Intelligence was able to discern two distinct populations- the opioid naive and the opioid dependent.*

3.3). Effectiveness of Buprenorphine in Alleviating the Primary Neurotoxicity and the Secondary Opioid Craving

The third aim of this study was to begin the process of evaluating the effectiveness of buprenorphine in first resolving and then preventing the recurrence of the primary neurotoxicity and thus the secondary opioid craving. Ultimately, it is our goal to demonstrate that the resolution of the primary neurotoxicity and the secondary opioid craving prevents the tertiary pathological opioid consumption. In this manner, quaternary opioid overdose events and deaths should be reduced. For this reason, individuals were assessed during an episode of acute opioid withdrawal brought on due to an opioid abstinence. Once assessed, these individuals were given a single dose of buprenorphine 16 mgms sublingually. Two hours after the dosage had been absorbed, the individuals were assessed a second time. Each assessment consisted of a blood draw for catecholamines and both the Neurotoxicity Scale and the Opioid Craving Scale. The results were universal for all 15 participants. The widespread catecholamine toxicity has previously been discussed. While in an opioid abstinence induced withdrawal state, all 15 participants scored excessively high on both the Neurotoxicity Scale and the Opioid Craving Scale. These results are available for review. But after just one single dosage of buprenorphine, every participant experienced a sharp drop in both the Neurotoxicity Scale and the Opioid Craving Scale. On average, the Neurotoxicity Scale fell from 42.9 to 4.9. This represented an average drop of 88% in the Neurotoxicity Scale. On average, the Opioid Craving Scale fell from 22.7 to 1.0. This represented an average drop in the Opioid Craving Scale of 96%. There were no outliers. Buprenorphine was effective in all 15 participants. These dramatic results will need to be followed over time and to ensure that buprenorphine is effective both in preventing the recurrence of neurotoxicity/opioid craving and in the prevention of further aberrant opioid purchase and consumption. In this manner, accidental opioid overdose events and death should be reduced. (Figure 17)



(Figure 17) *Even a single dose of buprenorphine was highly effective at relieving both the neurotoxicity symptoms and the opioid cravings.*

3.4). Neurotoxicity Scale

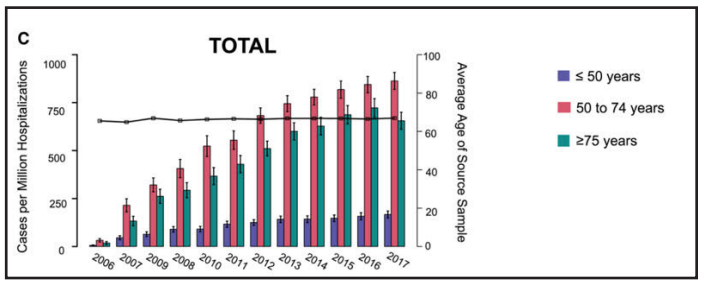
It is unfortunate that an emergency halt was called prior to the completion of the statistical analysis for the validity and reliability of Neurotoxicity Scale. But while a statistical significance of the Neurotoxicity scale was not possible to obtain, anecdotally, the scale exceeded expectations. This scale appears to be capable of measuring neurotoxicity and in measuring rapid changes in neurotoxicity. Further study should provide statistical proof of the validity and reliability of the Neurotoxicity Scale.

3.5). Opioid Craving Scale

Likewise, it is also unfortunate that an emergency halt was called prior to the completion of the statistical analysis for the validity and reliability of the Opioid Craving Scale. But while a statistical significance of the Opioid Craving Scale was not possible to obtain, anecdotally, the scale exceeded expectations. This scale appears to be capable of measuring opioid craving and in measuring rapid changes in opioid craving. Further study should provide statistical proof of the validity and reliability of the Opioid Craving Scale.

3.6). Literature Review for Evidence of an Epidemic of Takotsubo Cardiomyopathy

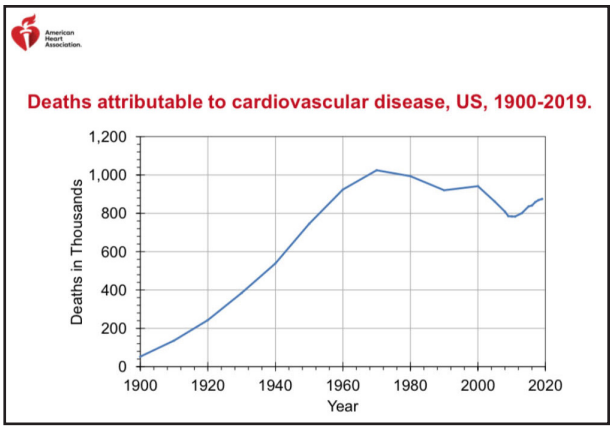
The hypothesis had predicted an epidemic of Takotsubo Cardiomyopathy would be detected due to widespread and untreated catecholamine toxicity. Takotsubo Cardiomyopathy is pathognomonic for catecholamine toxicity.[24] Unfortunately, the hypothesis was accurate. A review of the literature revealed a massive epidemic of Takotsubo Cardiomyopathy in the United States and occurring in a parallel timeframe to the opioid crisis and prior to the COVID-19 pandemic. As seen in Pattisapu et al (2021), Takotsubo Cardiomyopathy exploded in the study years from 2006 to 2017 with a total of 135,463 cases reported. Unfortunately, almost 90% of the 135,463 cases of Takotsubo Cardiomyopathy were in women, mostly women over the age of 50. It was hypothesized this would be the outcome and due to endothelial dysfunction in the setting of estrogen deprivation. These women were the unfortunate collateral damage of an unrecognized and untreated epidemic of catecholamine toxicity and due to the genetic damage done by repetitive opioid exposure. (Figure 18)



(Figure 18) *As seen previously, Pattisapu et al (2020) had detected an epidemic of Takotsubo Cardiomyopathy as had been predicted by our hypothesis.*

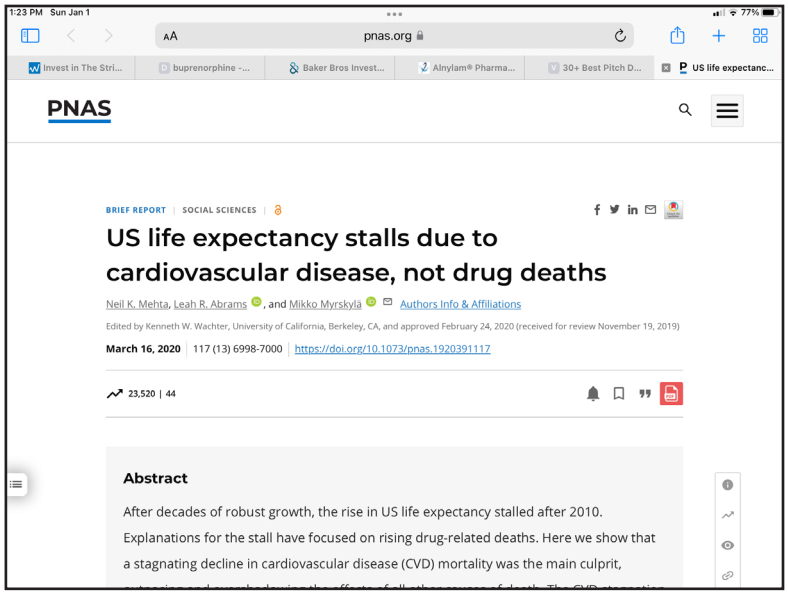
3.7). Literature Review for Evidence of an Epidemic of Cardiovascular Death

The hypothesis had predicted an epidemic of cardiovascular deaths. More specifically, and based upon the severity of the cardiovascular deaths in Lu et al (2020), the hypothesis predicted an epidemic of cardiovascular deaths of such massive proportions that life expectancy in the United States would stall and a stall due exclusively to cardiovascular deaths. As horrendous as the opioid overdose deaths may be, the number of cardiovascular deaths due to catecholamine toxicity would far exceed the number of opioid overdose deaths. As horrendous as the COVID-19 deaths may be, the number of cardiovascular deaths due to catecholamine toxicity would exceed even the COVID-19 deaths. (Figure 19)



(Figure 19) *As seen previously, the data from the American Heart Association cardiovascular deaths pre-COVID-19 had determined an epidemic of cardiovascular deaths as had been predicted by our hypothesis.*

Unfortunately for all, the hypothesis was highly accurate. Data from the American Heart Association details an epidemic of cardiovascular deaths in a parallel timeframe to the opioid crisis and prior to the COVID-19 pandemic.10 At present, the last year available for the American Heart Association data is 2019. By extrapolation of this data, it is our estimate that by the end of 2022, over two million premature, preventable, tragic cardiovascular deaths had occurred.33 This yet to be recognized catecholamine toxicity was a Public Health Crisis unequaled in modern times. And yes, according to Mehta et al (2020), US life expectancy has stalled and due to a surge in cardiovascular deaths. Opioid exposure led to genetic damage. Genetic damage led to a neurotoxicity. A neurotoxicity led to catecholamine toxicity. Catecholamine toxicity led to cardiovascular death. And, again, and based upon available data from the American Heart Association, we are estimating the death toll at two million deaths at the end of 2022. (Figure 20)



(Figure 20) *As further predicted by our hypothesis, US life expectancy fell and due to cardiovascular deaths. This trend will continue until the genetically damaged and vulnerable population either receives an appropriate diagnosis and treatment or begins to move into extinction.*

4). Discussion

Some research uncovers medical breakthroughs. Our research uncovered a medical disaster. Fundamental to the disaster was an inappropriate application of a mental health diagnosis that obscured a deadly genotoxicity. Left undiagnosed and untreated, the genotoxicity turned into a Public Health crisis so large that the life expectancy in the country stalled. The origins of this Public Health crisis were not to be found in nature. This was a disaster completely human made. The pharmaceutical industry pushed out a toxic product onto a trusting public. Despite science to the contrary, the symptoms of the toxicity were wrongfully attributed to a "brain disease". Experts relied upon the confidence of their opinions as opposed to the traditions of science. Yes, it was a human made tragedy. The victims of this tragedy were the unfortunately afflicted. Without permission or warning, their DNA was permanently poisoned. All suffered. All were labeled. Many died. But even while we mourn for those lost, we must move quickly as a nation to rescue the remaining survivors of this national tragedy. As many as possible must be quickly stabilized on the buprenorphine and beginning at 16 mgms per day, the blockade level with 80% opioid receptor saturation. Clinical trials will be necessary to give guidance on the parameters of a tapering of this dose. In addition, the deadly and judgmental term "Opioid Use Disorder" must be stricken from all usage and literature. It is simply an unscientific term that branded all and killed many.

At the risk of stating the obvious, we point out how the two separate groups, the opioid naive and the opioid dependent, behaved differently when confronted with the identical clinical scenario of an opioid abstinence. When confronted with an opioid abstinence, the opioid naive demonstrated no symptoms. However, when confronted with an identical opioid abstinence, the opioid dependent demonstrated symptoms of a neurotoxicity. If we are able to establish that the two groups are genetically separate and distinct, we would have thus defined a new genotoxicity and described a new disease state. This new disease state will need to be named in accordance with the guidelines as set forth by the World Health Organization.

Stabilization of millions of people on buprenorphine in order to stop the catecholamine toxicity epidemic will require a focused effort. It is our belief that this rapid stabilization of the victims of the mass poisoning will be best accomplished via telemedicine and with the use of both synchronous and asynchronous telemedicine. It is noted that as these are victims of a life threatening poisoning, and as an effective and well tolerated antidote exists in buprenorphine, that the provider/patient relationship is thus of an implied nature. This is a bedrock principle for the treatment of life threatening emergencies in this

country. No further action is needed to establish either the diagnosis or a treatment plan. If a victim of the poisoning requests treatment with buprenorphine, then access to the buprenorphine must be supplied. Withholding a known antidote to the victim of a known poisoning is a moral depravity and a violation of the Hippocratic Oath.

Conclusion

In conclusion, the first aim of this clinical trial was achieved. Widespread catecholamine toxicity was found to be a component of opioid withdrawal. Thus, the binary conclusion is that the proper diagnosis for those genetically damaged by the opioids would be the neuroendocrine emergency known as autonomic dysfunction. While we recognize that an underlying substance abuse issue was involved in the methylation of a minority of individuals, the diagnosis known as Opioid Use Disorder must be stricken from the literature and banned from further usage. The term is insulting and damaging to the victims of the mass poisoning. Simply put, continuing the charade continues the harmful and judgmental label. The second aim of the clinical trial was achieved. A clinical test based upon the hypermethylated CpG sites within the promoter region of the OPRM1 gene can be developed. The third aim of the clinical trial was achieved. Buprenorphine was found to be an excellent antidote for the symptoms of the poisoning and resolved both the primary autonomic dysfunction and the secondary opioid craving. The fourth aim of the study, establishing the validity and reliability of the two developed questionnaires was not achieved due to the emergency halt and will require further study.

In addition, the two further predictions of our hypothesis were confirmed. An epidemic of Takotsubo Cardiomyopathy was confirmed in the literature and in a timeframe parallel to the opioid crisis. And lastly, an epidemic of cardiovascular deaths also in a timeframe parallel to the opioid crisis was confirmed and of a magnitude that resulted in a stalling of the U.S. life expectancy. We will continue our research. And we will continue to rescue the suffering victims of the largest epidemic of genetic toxicity in history.

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