BUPRENORPHINE FOR THE TREATMENT OF AUTONOMIC DYSFUNCTION SECONDARY TO OPIOID WITHDRAWAL

ABSTRACT

In the United States, pathologic consumption of an often illegal opioid has resulted in an epidemic of opioid overdose deaths. This pathologic consumption of opioids is driven by an opioid craving. [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19] Understanding this opioid craving as both a symptom and a driver positions opioid craving as a critical treatment target.[20] According to the Brain Disease Model of Addiction, the opioid craving is a consequence of a surge in brain dopamine in response to the administration of an opioid causing "shifting drivers resulting from neuroadaptations".[21] This brain derived craving "impedes self-control and promotes drug-seeking behavior".[20] However, replicating studies have demonstrated no surge in brain dopamine in response to the administration of an opioid.[22, 23] Respected voices have raised this concern.[24] Here we show that opioid craving is not related to a brain process. Rather opioid craving is a sequelae of an unendurable autonomic dysfunction occurring when opioid abstinence is attempted. The human body simply cannot withstand the horror of the autonomic dysfunction and illegal opioids provide a temporary, but risky, relief. We further demonstrate that a single dose of buprenorphine is highly effective at resolving both the primary autonomic dysfunction and the secondary opioid craving. For the first time, widespread catecholamine toxicity is found to be a component of this autonomic dysfunction and may have catastrophic cardiovascular consequences. A DNA methylation due to the opioids is hypothesized to be the underlying culprit. [26] We further show here that this DNA methylation can be measured even within the individual, theoretically allowing the development of a clinical test. DNA methylation from a drug resulting in a toxicity had been previously predicted by others.[25]

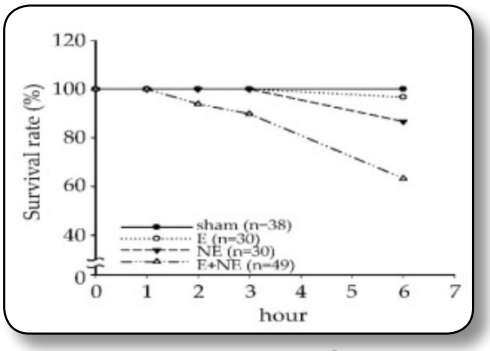
ROOT CAUSE ANALYSIS OF THE OPIOID CRISIS

(According to the Autonomic Dysfunction Hypothesis)

DNA METHYLATION → **Primary** Abnormal *mu-Opioid* Receptor (Autonomic Dysfunction with Opioid Abstinence) → **Secondary** Opioid Craving → **Tertiary** Pathologic Opioid Consumption

This study had a number of endpoints:

- The Primary Endpoint was to determine whether or not catecholamine toxicity could be detected in opioid dependent individuals who have voluntarily stopped taking their opioids and entered into opioid withdrawal. The type, the severity, and the duration of any detected catecholamine toxicity could have profound implications.



(Figure 1 Lu, et al (2020))

Autonomic dysfunction encompasses a multitude of bodily functions and organs. But dysfunction within the Adrenal Gland produces measurable abnormalities within the catecholamines. If detected, such catecholamine toxicity would have the potential for drastic cardiovascular sequela. Furthermore, it is known from prior research with animal models, not all catecholamine toxicity is equal in the ability to produce catastrophic cardiac events and death.[27] Epinephrine toxicity was found to result in a level of cardiac related death. Norepinephrine toxicity had a higher and faster incidence of cardiac related death. But the combination of both epinephrine toxicity and norepinephrine toxicity was the quickest and most lethal in cardiac related deaths producing close to 40% mortality in six hours in mice ***(Figure 1 Lu, et al (2020))***.

• A first Secondary Endpoint was to determine whether or not buprenorphine could alleviate the primary autonomic dysfunction and the secondary opioid cravings.

• A second Secondary Endpoint was to determine if the Autonomic Dysfunction Scale and the Opioid Craving Scale could detect both the level of autonomic dysfunction and opioid cravings and measure the changes in autonomic dysfunction and opioid cravings.

• A first Tertiary Endpoint was to determine if a clinical lab test could be developed to measure the level of DNA methylation damage within any one given opioid dependent individual.

• A second Tertiary Endpoint was to determine if the United States Postal Service (USPS) was adequate for transportation of self-collected saliva samples for DNA methylation analysis.

METHODS

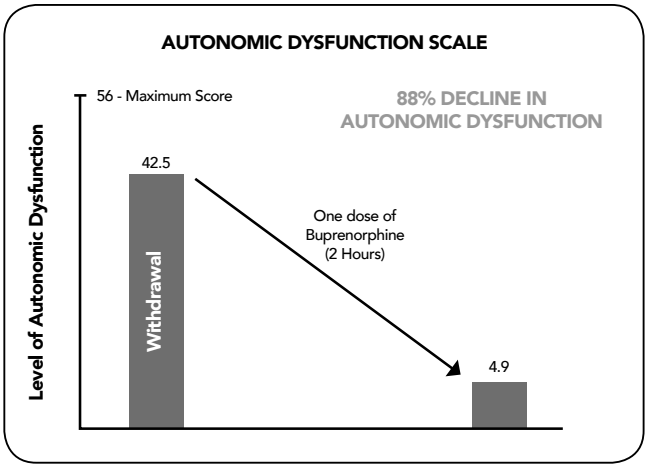
Following IRB approval and under Informed Consent, 25 opioid dependent participants were scheduled to stop all opioids and allow themselves to go into opioid withdrawal. On the third day, participants came into the study site. Vital signs were obtained, both an Autonomic Dysfunction Scale and an Opioid Craving Scale were completed. Blood was drawn for catecholamine levels. Buprenorphine, 16 mgms sublingual, was administered. Two hours after complete absorption of the buprenorphine, the entire process was repeated. This concluded the study requirements. DNA was not collected at this time as the tertiary endpoint was to determine if the DNA samples could be collected at home and transported via USPS. Blood samples for catecholamines were also drawn on an equal number of opioid dependent individuals stabilized on buprenorphine and on an equal number of non-opioid dependent individuals.

RESULTS

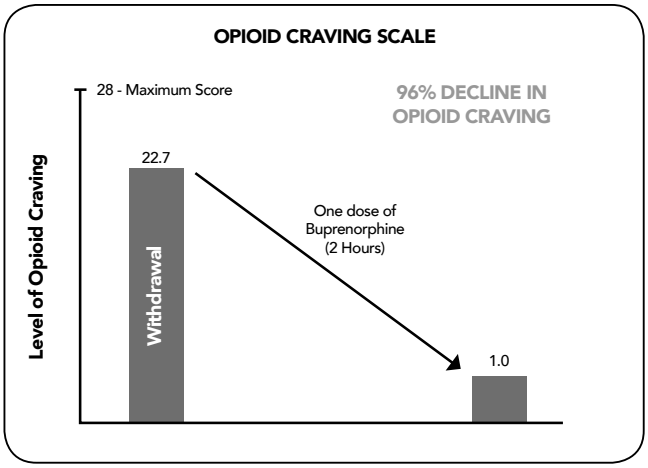
The study was terminated by an emergency halt after 15 participants and the IRB notified as required by HHS Regulation 45 CFR part 46. The catecholamine levels were determined to be a risk to the safety of the participants. Of particular concern were the 2 of the 15 participants with both epinephrine and norepinephrine toxicity. Animal models have demonstrated that both catecholamine toxicities simultaneously can lead to a rapid cardiovascular catastrophe ***(Figure 1 Lu, et al (2020) page 1)***.

Of the first 15 participants, prior to the emergency halt of the study, 13 out of 15 participants had at least one elevated catecholamine. While it is recognized that dopamine toxicity and epinephrine toxicity are associated with death and impairment, it was the norepinephrine toxicities, particularly when combined with epinephrine toxicity, that led us to quickly terminate the study by means of an emergency halt. The risk of a catastrophic cardiovascular event was deemed too great, even under a more extensive Informed Consent process.

During the period of autonomic dysfunction prior to the buprenorphine, the average Autonomic Dysfunction Scale was 42.5 out of a possible 56. The average Opioid Craving Scale was 22.7 out of a possible 28. Two hours after administration of the buprenorphine 16 mgms, the average Autonomic Dysfunction Scale fell 88% to 4.9 out of a possible 56 ***(Figure 2, page 3)***. The average Opioid Craving Scale fell 96% to 1.0 out of a possible 28 ***(Figure 3, page 3)***.



(Figure 2)



(Figure 3)

The catecholamine levels had not fully normalized two hours after the administration of the buprenorphine. This was a concern. Even more concerning, elevated catecholamine levels, while significantly improved on the buprenorphine, still persisted in the control group stabilized on buprenorphine.

CONCLUSIONS

The Primary Endpoint was confirmed. Widespread catecholamine toxicity is a function of the opioid withdrawal. The two Secondary Endpoints were confirmed. Buprenorphine can alleviate the primary autonomic dysfunction and the secondary opioid cravings. Both the Autonomic Dysfunction Scale and the Opioid Craving Scale were effective at measuring the level and the changes in the level of autonomic dysfunction and opioid cravings. The two Tertiary Endpoints were confirmed. A clinical test can be developed to detect DNA methylation in an individual damaged by the opioids. And the USPS is adequate for transportation of self-collected saliva samples for DNA methylation analysis.

At the time of this study, the default diagnosis for this group of individuals is a mental health diagnosis known as Opioid Use Disorder, as found in the current Diagnostic and Statistical Manual, volume 5 (DSM5). The role of the genetic damage, the autonomic dysfunction, and the catecholamine toxicity are not currently recognized. Clearly, this level of dysfunction and toxicity would preclude a mental health diagnosis. The full impact of failing to recognize the dysfunction, particularly the catecholamine toxicity, is not currently realized.

(Study funded by Smith Genetics Research)

1). INTRODUCTION

1.1). SCIENTIFIC EVIDENCE FOR AUTONOMIC DYSFUNCTION DURING OPIOID WITHDRAWAL (Figure 4)

Abundant scientific evidence for an autonomic dysfunction during opioid withdrawal has been evident for decades.[28, 29, 30, 31, 32] In 1963, Gunne noted that in opioid dependent mice, the contents of the adrenal glands were depleted after 48 hours of opioid withdrawal.[28] Akera and Brody (1967) noted increased epinephrine and norepinephrine in the urine of opioid dependent mice during opioid withdrawal.[29] Delle et al (1990) noted a 400% increase in sympathetic nerve activity (SNA) to the adrenal glands in opioid dependent mice and in response to the administration of naloxone.[30] Simultaneous with this 400% increase in sympathetic nerve activity to the adrenal glands was a twenty fold surge in plasma epinephrine levels and a fifteen fold surge in arginine vasopressin. Delle et al (1990) further noted:

Gunne 1963	Depleted Adrenal Glands after two days opioid withdrawal
Akera/Brody 1967	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 following the administration of naloxone to opioid dependent mice
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 following the administration of naloxone to opioid dependent humans
Smith et al 2022	Catecholamine Toxicity widespread in opioid withdrawal in humans

(Figure 4)

"This study shows that a 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."

Chang et al (1990) found that surgical ablation of the adrenal glands prevented the surge in plasma epinephrine in opioid dependent mice in response to the administration of naloxone.[31] Kienbaum et al (1998) had detected a thirty fold surge in plasma epinephrine in opioid dependent humans under anesthesia and in response to the administration of naloxone.[32]

1.2). SCIENTIFIC EVIDENCE FOR DNA TOXICITY BY THE OPIOIDS (Figure 5)

Abundant scientific evidence for a DNA toxicity via methylation and due to the opioids has been evident for over a decade.[33, 34, 35, 36, 37] Nielsen et al (2009) noted an association between opioid dependent subjects and methylation within the promoter region of the OPRM1 gene.[33] Chorbov et al (2011) replicated this association.[34] Wachman et al (2014) showed a correlation between the level of methylation within the promoter region of the OPRM1 gene in an infant born to an opioid dependent mother and the severity of Neonatal Abstinence Syndrome experienced by the infant.[35] Wachman et al (2018) replicated this finding and also demonstrated that the level of methylation within the promoter region of the OPRM1 gene in the mother correlated with the severity of the Neonatal Abstinence Syndrome in the infant.[36] Sandoval-Sierra et al (2020) showed causation between the opioids

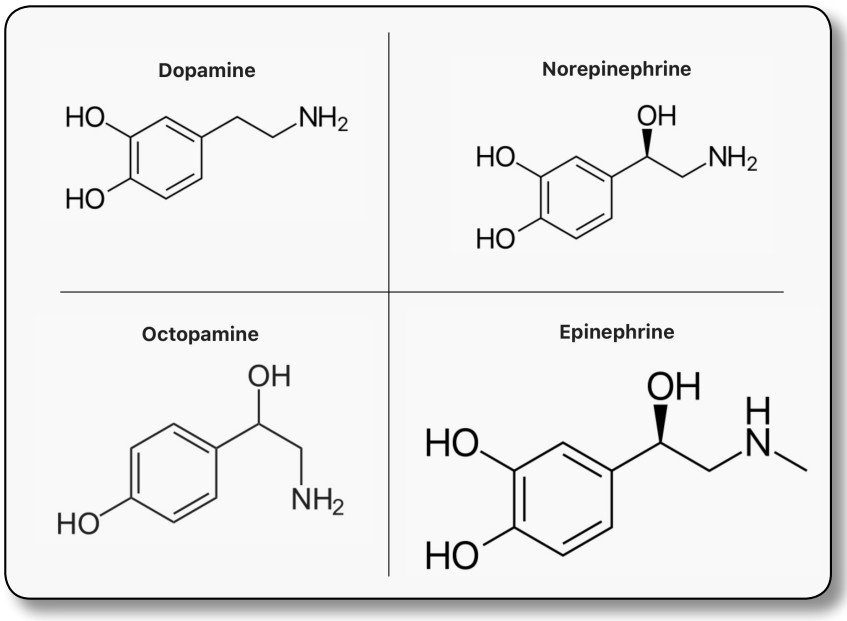
Nielsen 2009 Chorbov 2011	Scientific evidence for an association between opioids and DNA methylation
Wachman 2014 & 2018	Scientific evidence for a correlation between opioids and DNA methylation
Sandoval/Sierra 2020	Scientific evidence for a causation between opioids and DNA methylation (Opioids cause DNA methylation).
Smith 2022	Methylation damage can be measured in individual victims

(Figure 5)

and DNA methylation within the promoter region of the OPRM1 gene. Even a short course of opioids resulted in a measurable methylation within the promoter region of the OPRM1 gene.[37]

1.3). THE ROLE OF THE CATECHOLAMINES

Life has been successful on this planet. Hopefully, life will be discovered elsewhere in the Universe. Part of the success of life on this planet can be attributed to the family of organic chemicals that spur movement and activity. In the vertebrate world, these organic chemicals are known as the catecholamines: epinephrine, norepinephrine, and dopamine. In the invertebrate world, the organic chemical is the structurally similar octopamine ***(Figure 6)***. The premise is simple. If movement and reaction was an advantage, then moving and reacting faster furthered the advantage.



(Figure 6)

While basic in structure, these are some of the most powerful molecules on earth.
The impact within the human body to the catecholamines is profound. The catecholamines drive what is commonly known as the "fight or flight" reaction. The catecholamines are the foundation of our ability to respond to severe medical conditions such as cardiac arrest and septic shock. With the two adrenergic receptors, Alpha and Beta, fundamental parameters such as heart rate, cardiac output, stroke volume, and vascular resistance are positively impacted by the catecholamines. Mother Nature has spent millions of years perfecting this system. And, except for the rare pheochromocytoma/paraganglioma, the system works quite well. Catecholamine toxicity is a rare event. Catecholamine toxicity is within the realm of iatrogenic and human errors in exogenous usage. Mother Nature keeps the half lives of the catecholamines brief at about 2 minutes and stores the catecholamines safely inside the Adrenal Glands perched atop the kidneys and protected by the lower rib cage and abdominal mass. These molecules are simply too powerful to be left to chance.

2). METHODS AND MATERIALS

2.1). SETTING

The clinical trial took place in the medical office setting. After IRB approval and under Informed Consent, the participants stopped all opioids. The participants came into the office on Day #3 after stopping the opioids.

Participants were placed in an examination room and completed the Autonomic Dysfunction Scale and the Opioid Craving Scale. Blood was drawn for the first catecholamine levels. A single dose of buprenorphine at 16 mgms was administered sublingually. After complete absorption of the buprenorphine, a two hour time window elapsed. At the end of the two hours, the Autonomic Dysfunction Scale, Opioid Craving Scale, and blood draw for catecholamine levels were all repeated. This concluded the clinical trial. Of note, the DNA collection devices were back ordered. DNA collection was done at a later date via USPS.

2.2). SUBJECTS

The subjects were volunteers from our Medication Assisted Treatment Program. All subjects were over 18 years of age. All subjects were capable of understanding the Informed Consent. All subjects experienced symptoms of autonomic dysfunction when opioid abstinence was attempted. All subjects had been maintained on Buprenorphine. IRB approval was obtained from Pearl IRB. All subjects gave written consent.

2.3). AUTONOMIC DYSFUNCTION SCALE

The Autonomic Dysfunction Scale was created to meet the needs of this study. 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. 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). AUTONOMIC DYSFUNCTION 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.

Before Buprenorphine

OPIOID CRAVING SCALE / AUTONOMIC DYSFUNCTION SCALE (BEFORE BUPRENORPHINE)																										
PAR-TICIPANT	CATECHOLAMINE			OPIOID CRAVING SCALE							OCS	AUTONOMIC DYSFUNCTION SCALE														ADS
NUMBER	NOREPINEPHRINE	EPINEPHRINE	DOPAMINE	OCS 1	OCS 2	OCS 3	OCS 4	OCS 5	OCS 6	OCS 7	TOTALS	ADS 1	ADS 2	ADS 3	ADS 4	ADS 5	ADS 6	ADS 7	ADS 8	ADS 9	ADS 10	ADS 11	ADS 12	ADS 13	ADS 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	982	55	39	4	3	4	4	4	4	4	27	4	3	4	4	0	1	4	4	4	4	4	4	4	4	48
300	1859	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	971	<15	40	1	0	2	3	2	4	2	14	3	3	3	4	3	3	3	3	3	4	3	2	3	4	44
500	356	70	63	3	3	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	153	58	4	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	4	4	4	3	3	4	4	4	2	4	49
1000	581	26	89	2	1	0	3	4	1	2	13	4	4	4	4	0	3	2	2	1	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	1959	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	4	0	4	4	4	4	4	24	4	4	4	4	0	1	3	4	4	4	4	4	4	4	48
				45	30	44	56	58	53	55	341	51	48	52	54	18	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	1.2	2.53	3	3.26	2.73	3.53	3.66	2.73	2.33	3.8	42.46

See Page 12 for actual OCS questions

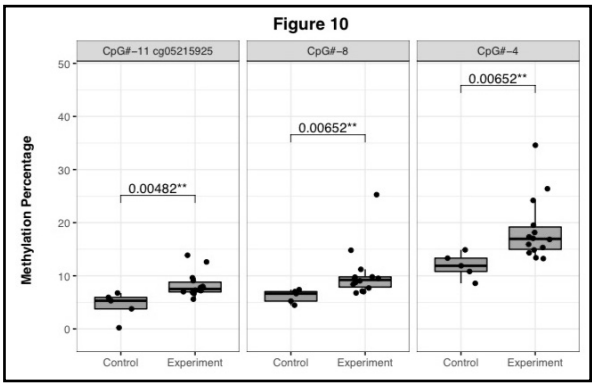
See Page 12 for actual ADS questions

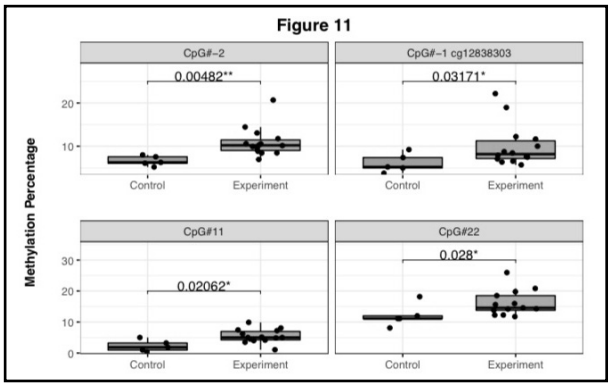
After Buprenorphine

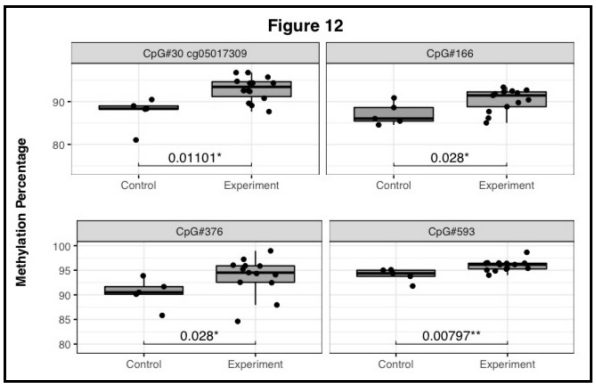
OPIOID CRAVING SCALE / AUTONOMIC DYSFUNCTION SCALE (AFTER BUPRENORPHINE)																										
PAR-TICIPANT	CATECHOLAMINE			OPIOID CRAVING SCALE							OCS	AUTONOMIC DYSFUNCTION SCALE														ADS
NUMBER	NOREPINEPHRINE	EPINEPHRINE	DOPAMINE	OCS 1	OCS 2	OCS 3	OCS 4	OCS 5	OCS 6	OCS 7	TOTALS	ADS 1	ADS 2	ADS 3	ADS 4	ADS 5	ADS 6	ADS 7	ADS 8	ADS 9	ADS 10	ADS 11	ADS 12	ADS 13	ADS 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	1217	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	2	0	0	0	0	2
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	1	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	25	89	0	0	0	0	0	0	0	0	0	1	1	0	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	1	1	0	0	1	0	1	0	1	0	0	0	5
1200	502	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
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	1	1	0	1	0	0	1	1	0	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

See Page 12 for actual OCS questions

See Page 12 for actual ADS questions







3). RESULTS

3.1). THE CATECHOLAMINES

Widespread 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. This catecholamine toxicity continued to a lesser degree after the buprenorphine. It is conjectured this may be indicative of the severity of dysfunction within the mu-opioid receptor and due to the methylation secondary to opioid exposure. Further study will be required.

3.2). AUTONOMIC DYSFUNCTION SCALE

It is unfortunate that an emergency halt was called prior to the completion of the statistical analysis for the validity and reliability of Autonomic Dysfunction Scale. However, despite this setback, the fact that a single dose of buprenorphine lowered the autonomic dysfunction score by 88% can be considered as an indication that buprenorphine is an effective treatment for the autonomic dysfunction.

3.3). 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, given the fact that a single dose of buprenorphine lowered the Opioid Craving Scale by 96% to 1.0 is a strong indication that buprenorphine will be highly impactful on lowering the incidence of both the purchase of illegal opioids and accidental overdose due to the consumption of illegal opioids.

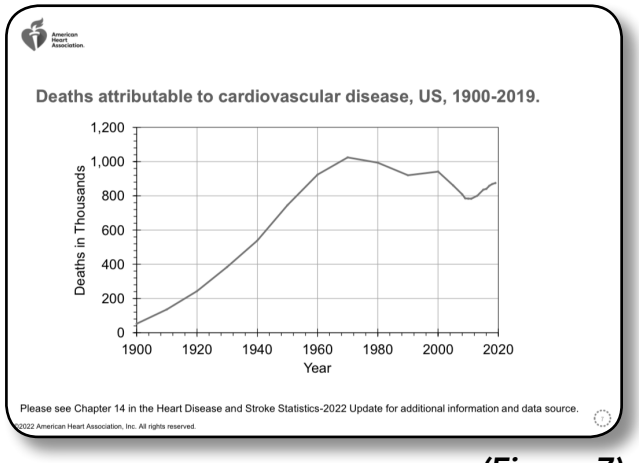
3.4). DNA METHYLATION ANALYSIS

Eight of the 15 study participants collected and submitted a saliva sample for DNA analysis. Another 7 opioid dependent individuals from a treatment program also voluntarily provided saliva samples for DNA analysis. Thus two separate groups of opioid dependent individuals were compared against a group of opioid naive individuals. It is our conclusion that current methodologies to determine levels of DNA methylation within any single individual methylated due to exposure to the opioids are adequate. This will have implications to those individuals who wish to seek legal remedy for the damage to their DNA and due to the opioids.

4). DISCUSSION

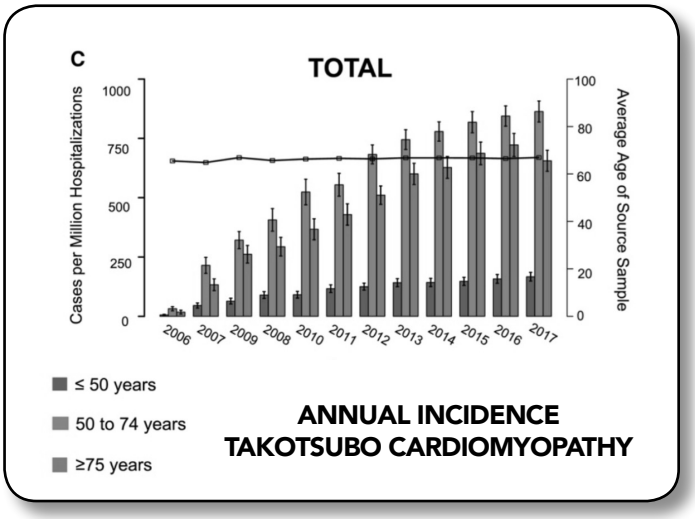
This is the first study to identify catecholamine toxicity as a frequent component of an opioid withdrawal due to opioid abstinence. The catecholamine toxicity raises two immediate concerns. First, this is a direct challenge to the mental health diagnosis of Opioid Use Disorder. Mental health disorders do not present with toxic laboratory levels. Second, catecholamine toxicity, while normally rare, can be deadly. Lu et al (2020) found in mice exposed to catecholamine toxicity, death occurred primarily due to heart failure. This death by heart failure was occurring within hours of catecholamine exposure. This is particularly worrisome when considering that our subjects were coming in for evaluation on Day Three of an opioid abstinence withdrawal. The implication being that not everyone will survive an episode of opioid withdrawal. Furthermore, those that do survive may have permanent heart damage as catecholamine toxicity is also associated with cardiomyocyte necroptosis.[38]

National cardiovascular mortality data has indicated an increase in cardiovascular deaths following decades of declines. It is noted this data is pre-COVID-19 ***(Figure 7)***.[39] This increase in cardiovascular deaths is yet to be explained. But the magnitude of the death rate has stalled US life expectancy.[40] We extrapolated this data from the American Heart Association and estimated that by the end of 2022, approximately 2 million premature cardiovascular deaths had occurred.[41] Furthermore, by the end of 2022, this daily national cardiovascular death rate had exceeded 1,000 deaths per day. At this rate, we believe those afflicted by the methylation to their DNA due to the opioids are moving towards an extinction of the vulnerable population.



(Figure 7)

It is recognized that the cardiovascular mortality data from the American Heart Association is anecdotal evidence as opposed to direct evidence that widespread catecholamine toxicity is resulting in a high incidence of cardiovascular deaths. DNA analysis for methylation in the promoter region of the OPRM1 gene is not a component of the normal autopsy process. However, additional and stronger anecdotal evidence can be found on an analysis of the incidence of the normally rare Takotsubo Cardiomyopathy. Takotsubo Cardiomyopathy is pathognomonic for epinephrine/norepinephrine toxicity. If widespread catecholamine toxicity is occurring in the nation due to the genetic damage from opioid exposure, then an epidemic of the rare Takotsubo Cardiomyopathy would be expected. And this epidemic of Takotsubo Cardiomyopathy is precisely what Pattisapu et al (2021) uncovered in their study ***(Figure 8)***.[42] Again, this is taken as strong anecdotal evidence. More research will be required.



(Figure 8)

The DNA methylation by the opioids is a recognized toxicity or poisoning.[25] The autonomic dysfunction is a consequence of this poisoning. The opioid craving is a consequence of this poisoning. Thus, the use of buprenorphine to treat the manifestations of a poisoning classifies buprenorphine as an antidote.[43] This has significant legal and ethical implications. As the patient is in an emergency situation facing possible loss of life, a provider/patient relationship is implied.[44] No further action is required to establish a provider/patient relationship. It is moral turpitude to deny a known antidote to a known victim of a poisoning.[45]

The primary endpoint of this study was to determine whether or not catecholamine toxicity is a component of opioid withdrawal. Catecholamine toxicity had been predicted by our hypothesis.[26] Catecholamine toxicity in opioid withdrawal was confirmed by this study. The frequency and severity of the catecholamine toxicity was of such a magnitude that an emergency halt to the study was deemed necessary.

The secondary endpoint of this study was to determine whether or not both the Autonomic Dysfunction Scale and the Opioid Craving Scale would be useful tools clinically to determine both the levels of autonomic dysfunction/opioid craving and changes in the levels of autonomic dysfunction/opioid craving. This study seems to confirm this secondary endpoint.

A separate secondary endpoint of this study was to determine the effectiveness and safety of buprenorphine in resolving both the primary autonomic dysfunction and the secondary opioid craving. Buprenorphine was seen to be highly effective at resolving both the primary autonomic dysfunction and the secondary opioid craving. No adverse events to the buprenorphine occurred.

The tertiary endpoint of this study was two fold:

- To determine whether or not the USPS would be a possible venue for the room temperature transportation of self-collected saliva samples for a quality DNA determination of hyper methylation. This proved to be true. Self-collected saliva samples transported at room temperature resulted in an excellent quality of data. Statistically significant hyper methylation was confirmed in the opioid dependent subjects.

- In addition, this was the first study to undertake whether or not bisulfate DNA analysis for hyper methylation could be used to develop a clinical test that could determine with necessary sensitivity and specificity whether or not any one single subject had evidence of the damage to the DNA known to occur secondary to exposure to the opioids. This study would seem to confirm that such testing is within the realm of current technology.

This study confirmed all primary, secondary, and tertiary endpoints. At this point in time, we call for additional research.

BEFORE BUPRENORPHINE

OPIOID CRAVING SCALE

1. If I had an opioid right now, I would take it.	0 1 2 3 4
2. I would not be able to stop myself from taking an opioid right now.	0 1 2 3 4
3. I would feel more in control of things if I could take an opioid right now.	0 1 2 3 4
4. Taking an opioid right now would make me feel better.	0 1 2 3 4
5. If I could take an opioid right now I would feel less restless	0 1 2 3 4
6. I am craving an opioid right now.	0 1 2 3 4
7. Using an opioid right now would make me feel better	0 1 2 3 4

AUTONOMIC DYSFUNCTION SCALE

1. I am yawning more than normal	0 1 2 3 4
2. My eyes are watering more than normal	0 1 2 3 4
3. My nose is running more than normal	0 1 2 3 4
4. I am having stomach cramping	0 1 2 3 4
5. I am vomiting	0 1 2 3 4
6. I have diarrhea	0 1 2 3 4
7. I am sweating more than normal	0 1 2 3 4
8. The hair on my body is standing on end	0 1 2 3 4
9. My heart is beating hard and fast	0 1 2 3 4
10. I feel anxious	0 1 2 3 4
11. I feel hot then cold	0 1 2 3 4
12. I have a tremor (shaking)	0 1 2 3 4
13. I feel like something bad is about to happen	0 1 2 3 4
14. I can't stand feeling this way	0 1 2 3 4

AFTER BUPRENORPHINE

OPIOID CRAVING SCALE

1. If I had an opioid right now, I would take it.	0 1 2 3 4
2. I would not be able to stop myself from taking an opioid right now.	0 1 2 3 4
3. I would feel more in control of things if I could take an opioid right now.	0 1 2 3 4
4. Taking an opioid right now would make me feel better.	0 1 2 3 4
5. If I could take an opioid right now I would feel less restless	0 1 2 3 4
6. I am craving an opioid right now.	0 1 2 3 4
7. Using an opioid right now would make me feel better	0 1 2 3 4

AUTONOMIC DYSFUNCTION SCALE

1. I am yawning more than normal	0 1 2 3 4
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14. I can't stand feeling this way	0 1 2 3 4

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