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Hair Cortisol-A Biomarker for Chronic Stress and Predictor of Disease

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Each year, stress in America continuously rises as work schedules become more intense, college course become consistently more challenging, the job market becomes more competitive and mortgages, student loans, and health care steadily increase in price. From generation to generation, children are expected to accomplish more at younger ages, and adults are working longer hours and retiring at older ages. Everyone experience daily stressors such as a busy day at work, a bad grade on an exam, or a two hour traffic jam. Stress is not something to take lightly or be viewed as minuscule part of everyday life. Stress doesn't diminish after the moment has passed, it manifests itself in the body and releases in the form of hormones. The real definition of stress is, "a process in which environmental demands strain an organism's adaptive capacity resulting in both psychological demands as well as biological changes that could place at risk for illness." [13] Stress is actively involved in the efflux of multiple mental and physical health diseases including but not limited to asthma, rheumatoid arthritis, anxiety disorders, depression, cardiovascular disease, post-traumatic stress disorder, hypertension, ulcers and stroke. While some of these illnesses are only exacerbated by stress, some mental illnesses can be a direct result of the pathway stress takes in the body. One in five adults (43.8 million) experience mental illness each year. One in five youth (21.4%) have suffered from a severe mental illness in their lives. 6.9% of adults suffered for a major depressive episode and 18.1% suffered from an anxiety disorder such as PTSD [8]. Although not all of these illnesses are stress-related, stress is substantially contributes to depression, anxiety disorders, PTSD, and suicidal thoughts.

Finding a biomarker for stress related diseases is the focus of research, specifically cortisol and its method of extraction. Cortisol is a steroid hormone, the molecular structure seen in figure 1, that is released through the adrenal cortex increasingly during stress. Cortisol production is controlled by the hypothalamic–pituitary–adrenal axis, which is directly influenced by the external stressor of an individual's life. Cortisol levels currently are measured through saliva collection, serum extraction, and urine collection. All of these methods have presented results that show the correlation between mental and physical health and the release of cortisol in the body. New research into the relationship between stress and cortisol levels looks into making the process of extraction more efficient and the results more standard and valid.

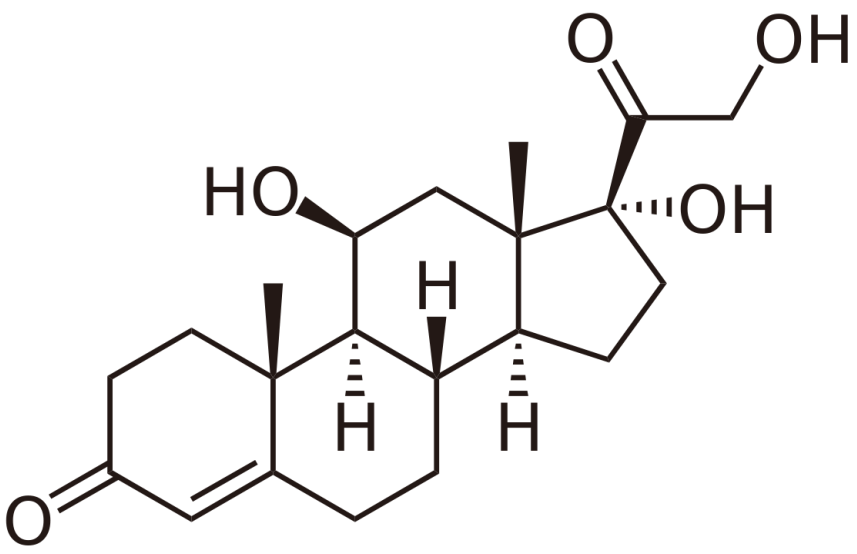

Figure 1. Molecular structure of cortisol

As the levels of stress in the world continue to increase with every generation, the importance of investigating health risks becomes pertinent. The effects that stress has on the body needs to be better understood and negative coping mechanism for stress identified to provide solid evidence that stress is indeed negatively affecting the human body. Finding

biomarkers that will help to diagnose stress based diseases is crucial. Although there is data measuring cortisol levels, most researchers have used blood plasma, saliva, or urine which can be misleading in several ways and inconvenience researchers extensively. Current research looking into hair cortisol extraction strategies that will help to eliminate the problems of other extraction methods and provide new benefits to extracting cortisol levels. Finding the most ideal extraction method for cortisol, will allow for better tracking of chronic stress and identification of the potential risk for disease, such as hypertension, later in life.

Before looking into the physical and mental illnesses that correlate with stress, it's important to understand the significant impact and growth stress has had in America. There are many contributing factors to the amount of stress that American's are experiencing. "Financial worries served as a significant source of stress for 64 percent of adults in 2014, ranking higher than three other major sources of stress: work (60 percent), family responsibilities (47 percent), and health concerns (46 percent)." [14] Money and the economy become an increasing factor of stress as the cost of living continues to rise and minimum wage remains constants or raises just slightly. Many people attested that they have barely enough money, if any at all, to afford only their basic needs. For younger generations, it seems that money becomes an increasing bigger issue along with job responsibilities.

The amount of stress in America is increasingly affecting children at younger ages. According to a study done by the American Psychological Association (APA), millennials had the highest amount of stress in 2015. While stress among all Americans between 2014 and 2015 increased, the most significant increases were among the younger generations, millennials and gen-Xers, which both increased by nearly ten percent. Every generation is seemingly growing up with more stress and responsibilities than the last. When surveyed matures and baby boomers

had 20% less stress then the younger generations, and between years older generation's stress tended to increase at a slower rate. The graph shown in figure 2, from the American Psychological Association, demonstrates the increase in stress among each generation.

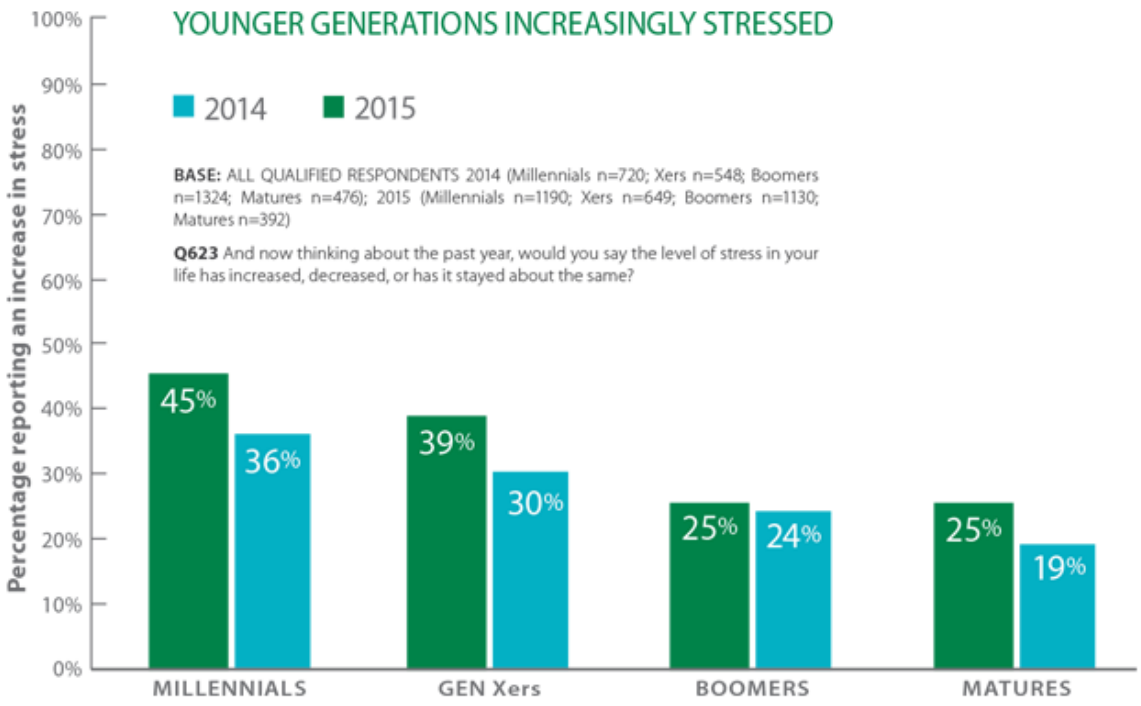

Figure 2. American Psychological Association representing the increase of stress on generations in America [1]

From these studies performed by the American Psychological Association, researchers can view the critical rate at which stress is affecting Americans. "It is alarming that the teen stress experience is so similar to that of adults," says APA CEO Norman B. Anderson, PhD. "It is even more concerning that they seem to underestimate the potential impact that stress has on their physical and mental health." [2] As we continue to put more pressure on kids at a younger age, understanding stress and the affect it has on our bodies becomes pertinent. Stress moves through the body in two pathways that can manifest itself in mental and physical diseases. In

figure 3, a visual illustration of the two pathways stress takes in the body are depicted thoroughly. Through the first pathway stress takes effect through our hypothalamic–pituitary–adrenal (HPA) axis. The HPA system is set up to allow organisms to respond to their external environment, such as outside stressors. As seen in figure three, as an external stressor takes effect on the body, it activates the HPA system. The HPA releases adrenocorticotrophic hormone (ACTH) into the circulatory system where it acts on the adrenal cortex to produce glucocorticoids. The most common glucocorticoids for stress response in humans is cortisol. "Cortisol is a glucocorticoid hormone synthesized from cholesterol by enzymes of the cytochrome P450 family in the zona fasciculata, the middle area of the adrenal cortex." [17] The regulation of production of glucocorticoids is essential to our health. Stress negatively impacts this cycle creating a change in regulation. Pervious research has discovered that a prolonged increase in glucocorticoid production due to increase stress may lead to damage of the hippocampus, the region of the brain which contains multiple cortisol receptors and are pertinent for HPA regulation. This damage can lead to a feed-forward pathway where ongoing stress leads to an indefinite overproduction of cortisol. Increasing the cortisol levels will disrupt cell functions and affect regulation of several physiological processes, including metabolism of fats and carbohydrates, and gluconeogenesis. Although glucocorticoid production is essential for survival, an overproduction can lead to physcological dysfuntion.

The second way stress takes effect in our bodies is through the sympathetic-adrenal-medullary system (SAM). The stress takes effect in the brain stem which flows into the sympathetic nervous system. Finally the pathway ends in the adrenal medulla where catecholamines are released. Stress increases the rate at which the system works and rapidly produces catecholamines, such as norepinephrine. "Catecholamines, which are released in

response to SAM activation, works in concert with the autonomic nervous system to exert regulatory effects on cardiovascular, pulmonary, hepatic, skeletal muscle, and immune systems." [3] SAM is can also be negatively impacted when an individual has too much stress and cause an over-production of catecholamine release. This would directly affect all of the systems mentioned above, leading to health problems later in life.

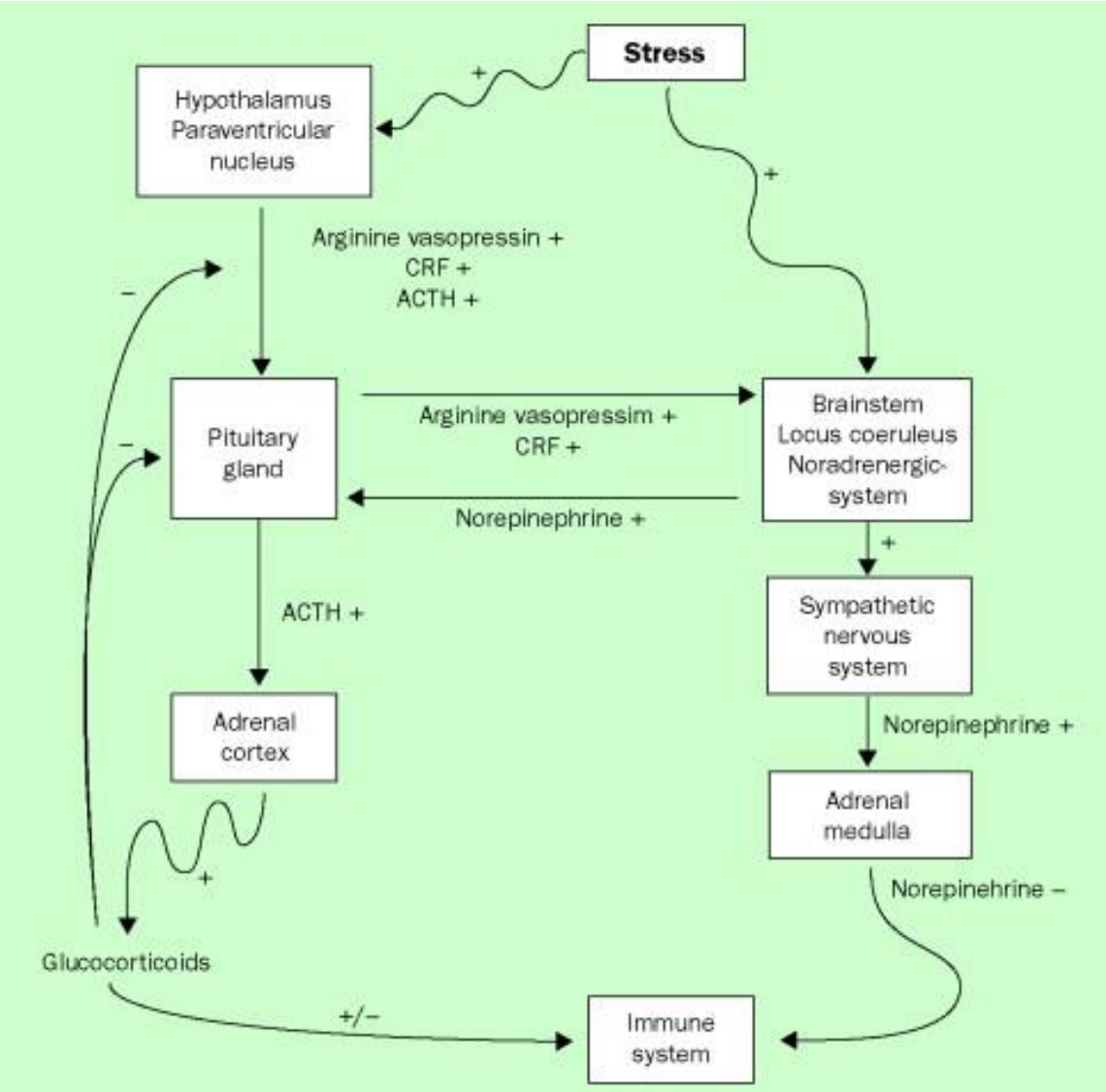

Figure 3. A representation of the pathway stress takes in the body

The overproduction of stress is the body can lead to impacts on both mental and physical health. The inadequate regulation of hormones secreted by the adrenal cortex, such as cortisol, can lead to the formation of pathological states such as anxiety and depression. Since 1955, the percentage of Americans affected by mental illness has increased fivefold. There are 10-20% of children worldwide suffering from mental health disorders. Stress directly causes or exacerbates mental diseases such as anxiety and panic attacks, PTSD, depression, and anger. "Mood disorders are leading causes of both morbidity and mortality. Depressive disorder and bipolar disorder (BD) rank among the leading causes of disability worldwide."[16] In a study completed by the American Psychological Association, all stress-related mental illnesses have increased with the increase in stress in America. The following graph demonstrates the mental-health related symptoms that increased due to stress from 2014 into 2015. These symptoms include anxiety, depression, and anger. In figure 4 it is shown that anxiety and depression both increased by almost 10 %, and constant worrying increased as well. Comparing these results with the rate of stress increase in America data shown in figure 2, show that there is a direct correlation between the two.

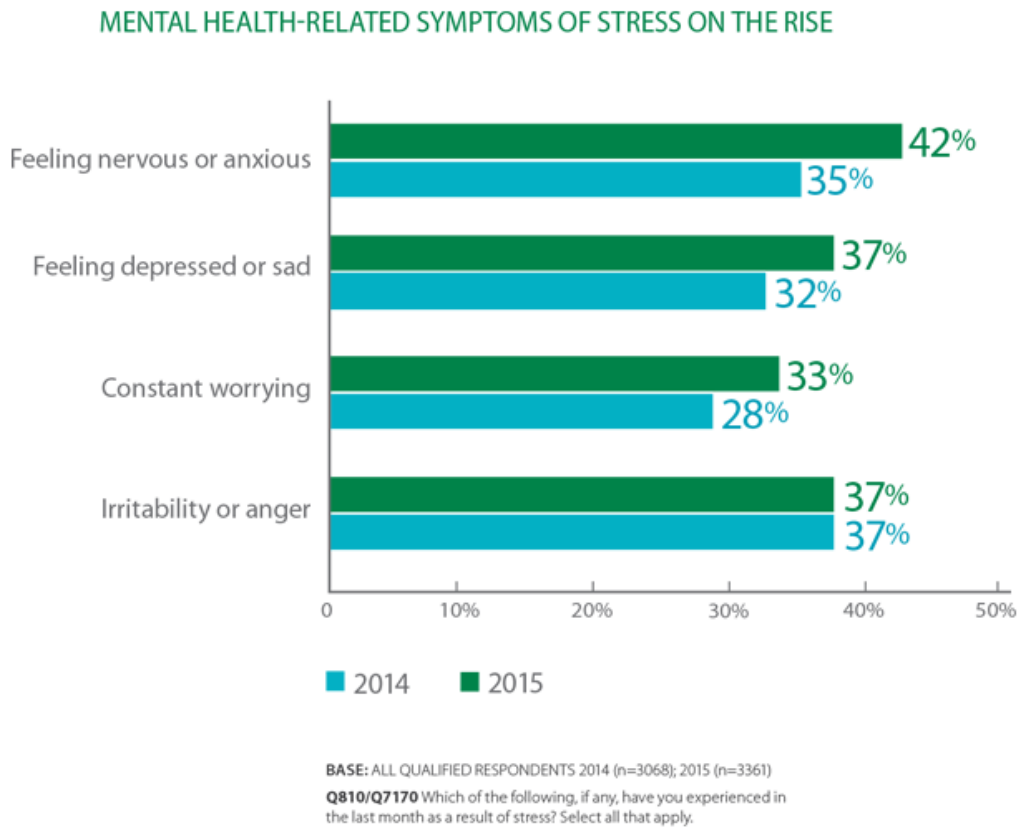

Figure 4. American Psychology Association

statistic of mental health-related symptoms of stress [1]

Many of the disorders mentioned above present similar symptoms. "In the study, researchers compared the results of hundreds of brain imaging studies covering six major psychiatric disorders. They found that most of the disorders were linked to gray matter loss in a network of three brain regions involved in higher cognitive functions." [5] This causes a dilemma when it comes to diagnosing and treating individuals. In the past, psychiatrists have been diagnosing patients based off of a checklist specified in the Diagnostic and Statistical Manual of Mental Disorders. Diagnosing a patient with the wrong illness and prescribing them inaccurate medication can lead to adverse effects. Studying cortisol levels in psychiatric patients can lead to better diagnosing and treating their illnesses. By looking into cortisol levels, the

pathophysiology of stress can be better understood and better more accurate strategies of dealing with immense stress can be developed.

Being able to quantify chronic stress in an individual and then connect the quantities to different diseases is the goal of research into stress biomarkers. While there are connections to both the release of catecholamines and the release of glucocorticoids, the focus will be on the release of the glucocorticoid, cortisol. In previous research done with serum, saliva, and urine, cortisol levels were unique for different psychiatric diseases.

> "For example, a systemic review found that patients with major depression presented with increased cortisol levels, while patients with anxiety disorders (generalized anxiety, panic, and PTSD) presented with lower levels, suggesting that distinct pathways contribute to the dysregulation of the HPA axis in the development and/or maintenance of psychiatric conditions." [19]

Increased levels of cortisol can also be used as a predictor for increased risk of suicide which correlates with the increases cortisol levels in depression. The physiological link between stress and depression is theorized that in increase in uptake of cortisol actually blocks the uptake of serotonin by the synaptic cleft. Cortisol levels give the opportunity to distinguish with biomarkers which mental illness is present in an individual.

Looking into cortisol levels will also help to explain why in the case of PTSD, two individuals who suffer the exact same trauma do not both necessary receive the diagnosis of PTSD. In a study done by the European Child & Adolescent Psychiatry, children in Palestine exposed for military violence were all tested for PTSD and they resulted in "54% of the children suffered from severe, 33.5 % from moderate and 11 % from mild and doubtful levels of

PTSD."[11] Cortisol release at the time of trauma might provide a reasoning for some child presenting PTSD symptoms and others showing none. In previous research, it has also been found that having reduced cortisol levels in PTSD patients at the time of trauma contributes to the increase in catecholaminergic responses, arousal, and anxiety. PTSD patients have also been known to disrupt the HPA axis, elevate levels of catecholamine and also increase inhibition of the negative feedback loop. Being able to look at the cortisol levels directly after the trauma occurs will provide a better understanding of how the stress of a trauma presents differently from individual to individual and help with diagnosing PTSD accurately in the future.

Stress may start in a biological pathway causing mental strain, however individuals with mental disorders can present side effects that lead to physical problems. Stress can cause an individual to develop extremely unhealthy coping methods. Leading habits in 2015 included smoking, drinking alcohol, sleeping less, and mindlessly watching television. These habits can lead to a multitude of diseases including cardiovascular disease, autoimmune disorder, hypertension, and ulcers. While come of these diseases are still being researched to find stress-related connection, hypertension and its relationship to stress has been highly documented. Hypertension is the long-term medical condition associated with consistently high blood pressure. Hypertension can originate from many of the unhealthy practices associated with long-term stress, such as smoking, inactivity, excessive eating and weight gain, and elevated salt levels. Hypertension can also originate the abnormal activation of the nervous system leading to an overproduction of hormones. In the pathways described earlier, the increase in stress leads to an increase in catecholamines and glucocorticoids, which include vasopressin, cortisol, endorphins, and aldosterone. All of these compounds cause an increase in blood pressure. "The combination of sympathetic predominance, vagal withdrawal, and blunted baroreflex sensitivity

might represent a treatable mechanistic link between psychosocial factors and future incidence of hypertension." [6]Hypertension is not the only disease stress-related, and is actually a precursor for other diseases such as cardiovascular disease.

Hypertension may lead to the development of cardiovascular disease which is one of the top causes of death in America. Although stress may not be the main cause of the disease, it is a pathway that exacerbates the system. One analysis found that work stress causes an increase for risk of cardiovascular disease almost 50% [5]. "The INTERHEART study revealed that people with myocardial infarction reported higher prevalence of four stress factors: stress at work and at home, financial stress and major life events in the past year."[13] The behaviours linked with stress alone contribute greatly to cardiovascular diseases. The high cholesterol from over-eating, the sedentary activity level from zoning out on television or the internet, and the negative side effects from over-indulging in alcohol and cigarettes are all increasing one's chance of developing cardiovascular disease.

Another disease which is negatively affected by stress are ulcers. "Approximately 500,000 new cases are reported each year, with 5 million people affected in the United States alone. Interestingly, those at the highest risk of contracting peptic ulcer disease are those generations born around the middle of the 20th century."[18] This correlates with the increase of stress among millennials and gen-Xers shown previously. Stress can increase ulcer formation due to the increase in basal acid secretion during fight-or-flight response which occurs in high time of stress. During high time of stress there is an increase in release of norepinephrine which will affect the capillaries which will cause constriction and break down the protective barrier in the stomach. Without the barrier, the hydrochloric acid present in the stomach can breakdown the tissues forming bleeding ulcers. Ulcers are also exacerbated by smoking, which is a negative

coping method of stress. Smoking increases an individual's susceptibility of ulcers and can also diminish defense factors in the gastric mucosal.

All of the diseases, both mental and physical, are rapidly affecting more American's each day. While coping with stress better may be the answer most are looking for, it is also important to find biomarkers that demonstrate when an individual's stress levels are too high for their body and negatively impacting their health. By looking into the cortisol levels of someone under immense stress, researchers can better diagnose the mental diseases and also look at the risk the individual is at for developing diseases such as hypertension and cardiovascular disease.

The impact of stress in America and the increase of stress-related diseases are well documented in research. Biomarkers for identifying stress in the body, however, remain scarce and inconclusive. Previously, researcher have tested cortisol levels using three main extractions methods, saliva, plasma serum, and urine. While yielding some results to provide comparison, these three methods all had problems some similar and some unique to their extraction. All three methods tend to be influenced by a multitude of factors including pain, medication, and smoking status. According to Urban Knutsson, a contributor to "Circadian Cortisol Rhythms in Healthy Boys and Girls," an article published in the Journal of Endocrinology & Metabolism "The variability of serum cortisol is known to be the result of many influences, including feedback between the pituitary secretion of ACTH and adrenal secretion of cortisol, circadian rhythm, episodic secretion, transcortin levels, wake-sleep patterns and stress factors."[4] While all of the factors mentioned above influence varying results, one specific factor that affects all three extraction methods crucially, is the circadian rhythm. In a research studied done by the Endocrine Society in 1997, a circadian rhythm of both boys and girls was obtained and the results can be viewed below. Consistently among all individuals (no difference in gender), There

is a major peak in cortisol at eight in the morning, usually when an individual would wake up. Then cortisol levels steadily decrease all day until they reach their lowest peak at eleven at night, or a normal time to sleep. This is the typically rhythm for cortisol levels in the average healthy individual. The research showed that circadian rhythms are not correlated with sex, age, height, or weight, and stay consistent throughout the population.

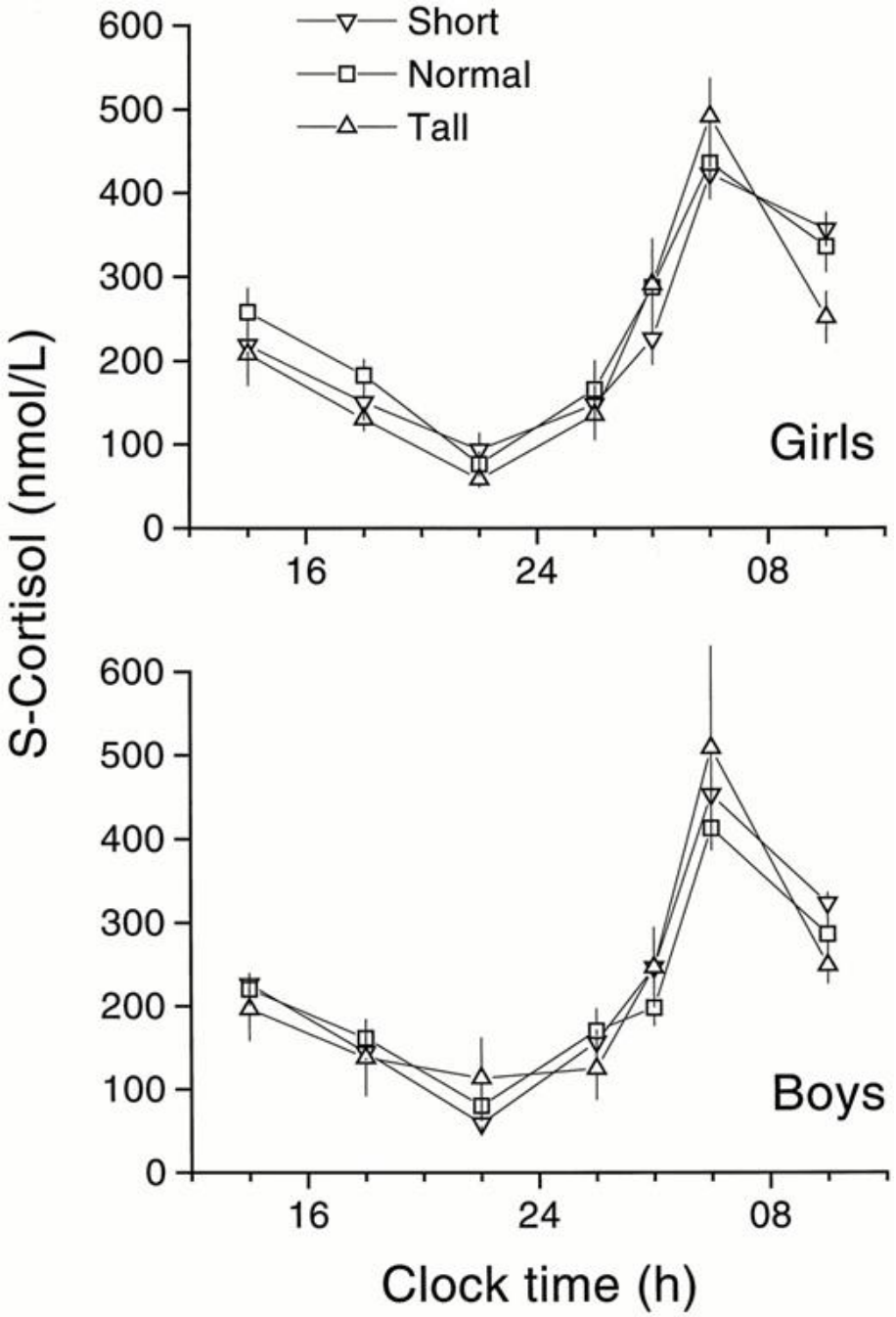

Figure 5. Endocrine Society- Circadian rhythm in boys and girls [4]

The circadian rhythm is a major reason that results stemming from cortisol extraction from serum, urine, and saliva are inconclusive. This rhythm poses a problem for extraction as all three methods only represent a short time lapse. If the serum was extracted at eight in the

morning for the first test and then four in the afternoon for the second test, the results would show a massive decrease in cortisol levels from the previous day. These results would allow for inaccurate conclusions to be made about the individual tested and would provide inconclusive results. To be able to obtain any valid results an individual would have to submit to having the serum extracted multiple times throughout a single day.

Other issues that cortisol extraction through saliva, urine, and serum present are short term illustration of stress, invasive and inconvenient extraction methods, and instability in storage. To start, serum, saliva, and urine all provide short windows of time to view the cortisol level. Saliva and serum only providing a time lapse of a couple minutes and urine providing only a 12-24 hour span. "Given that each of these substrates reflects short-term physiological stress, ranging from minutes (plasma/serum) to several days (faeces), repeated sampling over time is required to obtain a complete temporal picture of physiological stress." [12]The fact that researchers can only view the levels within the time drawn, it is almost impossible to obtain the results of cortisol release at a time or major stress or a traumatic event. The short time lapses each method provides lead directly into the invasiveness of the extraction methods. For salvia and serum the individual would have to submit to giving multiple samples throughout the day to obtain an accurate depiction of cortisol levels that day. Multiple extractions are especially invasive for serum which is taken intravenously. Serum extraction may actually skew data being a stressful situation in itself and raising cortisol levels. Urine is inconvenient in that the individual must submit to a 24 hour urine collection. Lastly, all three methods are also inconvenient when it comes to storage. Urine must be tested within 12 to 24 hours and saliva and serum must be kept at freezing temperatures to keep viable.

Serum, saliva and urine have previously been the only methods used to identify biomarkers for stress in the body. The purpose of continuing research into cortisol levels of individuals is to provide a better and more accurate method, such as hair cortisol. In current laboratory settings hair cortisol is not readily available, however the methodology and technology used in hair cortisol extraction are simple, straightforward, and easily integrated into existing biomonitoring techniques. Hair cortisol reflects the desired changes, provides many advantages, and eliminates many of the undesired methods of pervious extractions. Most importantly, hair cortisol will provide the physiological stress an individual had over an extended period of time. By extracting one hair follicle from an individual, a researcher can view the cortisol levels of multiple months. Each centimeter of hair represents one month of growth. Therefore, by cutting the hair into centimeter sections, starting from the end, not the base, depicts the stress an individual from month to month as seen in the picture below. A research can collect up to five centimeters of growth that will be reliable for data.

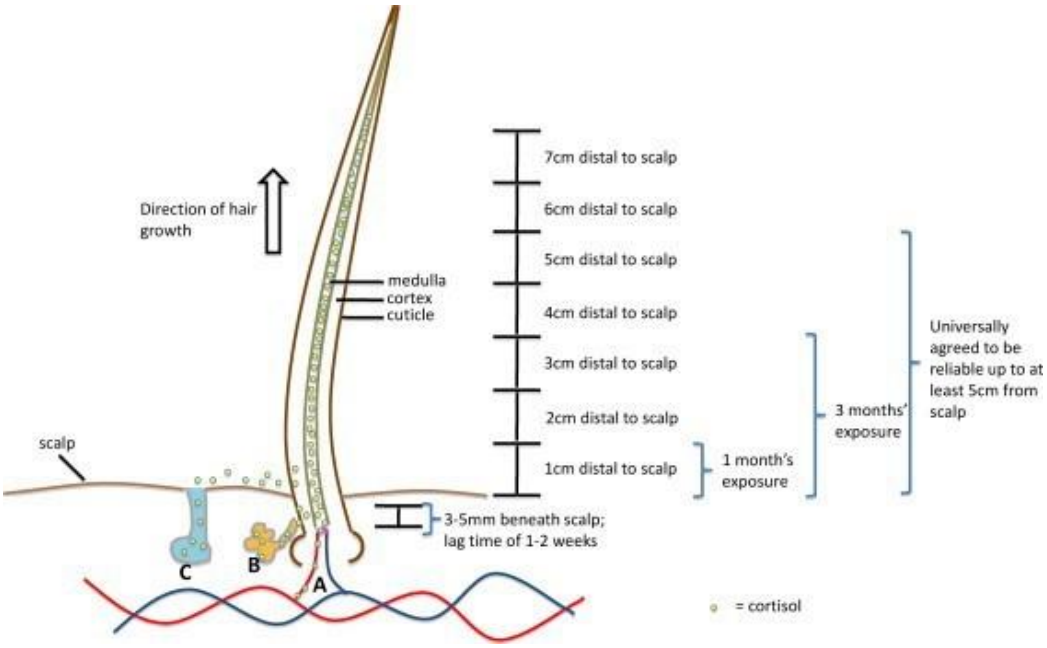

Figure 6. Hair follicle and cortisol representation [12]

This allows for the researcher to identify a time of intense stress and record the cortisol levels at that time point. For individuals who suffer from PTSD or depression, this provides the opportunity to look at the cortisol level at the time of the trauma or triggering event. This will also allow researchers to compare the cortisol levels from a month where trauma or a triggering event occurred to cortisol levels from a month where an individual suffered from little to no stress. No other method of measuring cortisol levels gives researchers a large lapse of time or the ability to identify the times of stress and compare it with a standard when there was no stress present in the individual's life.

Besides providing a long-term physiological stress depiction, hair cortisol provides the deletion of the circadian rhythm from affecting research, the increased stability and storage methods of hair cortisol, and the non-invasive method of the extraction. As previously stated all three other methods are subject to the effects of the circadian rhythm. With hair cortisol, however, the cortisol levels are built up over as month, and therefore are unaffected by circadian rhythm which is a daily influx of cortisol. This correlates with the idea of a more convenient extraction. With the three methods previously used, the extraction required multiple samples throughout the day or with urine a continuous 12 -24 hour collection. Hair eliminates all or this hassle. When it comes to storage, hair is most convenient because it can be stored for months to years after extraction, which is extremely beneficial for researchers. No preventive measures are needed to keep the hair cortisol intact, due to the ability to be stored at room temperature. Finally, extraction of the hair is extremely non-invasive compares to other methods. Hair extraction allows for the researcher to take only two or three follicles of hair with only one collection time. This one collection sample is enough to provide a picture for several months, compared to others that require multiple samples for just one day.

While there are benefits for hair cortisol, there are no experimental set values or previous conclusions to observe. The goal of researching hair cortisol is to create these standards and set an experimental method. Within the lab, a method for washing the hair and pulverizing it into small enough particles has been established. Looking at the extracted cortisol from different individuals along with obtaining their background in stress and life events, will continue to provide more results until standards can be set to use as identifiers in certain diseases. Many trials must be completed to find the most sufficient and valid way of extracting the cortisol from the hair, and then creating assays to view the data. While there is still variability when it comes to the test subjects ability to relay all of the stress factors in their lives, hair cortisol provides the most valid way of obtaining hair cortisol levels.

With research into the disease known as stress only at the start, there is still extensive amount of trails that need to be run and tested to come up with universal standards that can be used in the medical field. Each specific disease will need extensive research. While one lab is looking into the correlation of hair cortisol and PTSD, others will need to look at depression and suicidal thoughts. Furthermore, this can lead to the research into the correlation of cortisol levels and physical health, such as cardiovascular disease. "Further studies need to determine if and to what extent hair cortisol originates from blood, eccrine and/or sebaceous sources, if this is different for medullary versus outer layers, and if hair cortisol is a reflection of total and/or free cortisol exposure."[12] Better understanding the physiological pathways of stress will help to better understand how it develops into different diseases.

The stress levels in America are continuing to rise as more soldiers return home with PTSD, more children develop depression at younger ages, and more young adults and adults can barely afford the cost of living. The statistics for stress rates in America have increased almost

ten percent in younger generations, according to the stress snapshot of 2015 performed by the American Psychological Association. This increase of stress levels is directly related to the increase in deteriorating mental health. In another study by the American Psychological Association, it was found that most mental health disorders are affecting almost ten percent more American's since 2014. Stress has also been connected to physical health conditions, such as hypertension, cardiovascular disease, and ulcers. The connection between stress and mental/physical health has been made statistically, but can also be defined physiologically.

By delving deeper into stress's effect on the body, it is found that stress takes two pathways. It can either stimulate the hypothalamic–pituitary–adrenal axis or the sympathetic-adrenal-medullary system. For purposes of this research, the focus zoned in on the hypothalamic–pituitary–adrenal axis, where the final product is release of glucocorticoids, specifically cortisol. Cortisol releases can become extensive when chronic stress occurs. By stress continuously stimulating the HPA axis, there is a deregulation of the system and can cause an overproduction or lack of production of cortisol. This deregulation can lead to the development of many mental and physical diseases. In previous studies of cortisol levels, it was found that patients with chronic depression tend to have increased levels of cortisol, while patients who experienced anxiety had overall lower cortisol levels. These connections can help when it comes to identifying mental diseases in the future. The cortisol levels have also been connected to physical diseases such as cardiovascular disease, hypertension, and ulcers. Finding a method of cortisol extraction that will provide valid results, will help in identifying the type of mental illness present in an individual and the potential risk of developing physical health problems later in life.

While results from serum, saliva, and urine cortisol extraction have provided a base of data that can be used to further research, none of these methods provided researchers an accurate and convenient way to collect cortisol levels over an extensive period of time. Currently, research into hair cortisol is providing several advantages over the other three methods. By having one extraction time, which provides evidence of cortisol levels over the time period of up to five months is the most beneficial result. However, it also has other advantages including storage at room temperature, the ability to keep a sound hair sample for up to a year, and the ability to compare a standard baseline for a time with little to no stress, with a period of time containing great stress per individual. While hair cortisol provides many benefits, there is still research that needs to be done before standards can be created. Future research will reveal the true value of hair cortisol, and may lead to a more accurate way of identifying chronic stress, mental health, and the risk for physical disease.

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